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BREAKING CYTOPLASMIC MALE STERILITY IN INBRED CORN LINES (*Zea mays* L.): A REVIEW

Simona COMAN, Lizica SZILAGYI, Costică CIONTU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: simona_coman1989@yahoo.com

Abstract

Cytoplasmic male sterility (CMS) in corn is an important aspect of hybrid seed production, enabling efficient crossbreeding by eliminating the need for mechanical and manual detasseling. However, breaking sterility is a phenomenon that appears often. Research highlights that the breaking of sterility is governed by genetic factors as restorers of fertility (Rf genes), interactions between nuclear and mitochondrial genomes, environmental factors such as temperature and light condition, physiological and biochemical factors, or anthropogenic interventions. While these mechanisms have advanced hybrid crop production, they can also introduce challenges such as reduced genetic adaptability and heightened susceptibility to environmental stressors. A detailed understanding of the factors implications in breaking sterility is vital to optimizing corn production systems while mitigating potential risks. This study consolidates information from the literature on studies of the factors that lead to the sterility breakage of CMS lines in corn.

Key words: *corn, sterility breakage, seed production, cytoplasmic male sterility.*

INTRODUCTION

Corn is considered a multifunctional agricultural crop around the world. Seed production is a very important activity for agricultural production as the quality of the cultivated biological material largely depends on the level of production achieved. Hybrid corn seed is produced in hybridization fields using two important methods. One method involves the use of normal inbred lines, while the other takes advantage of cytoplasmic male sterility.

In corn there are two general types of male sterility: genic and cytoplasmic.

Genic male sterility occurs when lesions in nuclear-encoded genes disrupt normal male gametogenesis. Genic male sterile mutants can be either dominant or recessive and typically exhibit Mendelian inheritance. (Skibbea & Schnablea, 2005). Cytological examination of a subset of these male sterile mutants showed that the lesions in the nuclear genes affect nearly all stages of another development, ranging from pre-meiosis to fully engorged pollen (Albertsen, 1997; Chaubal et al., 2003).

Cytoplasmic Male Sterility (CMS) in corn is a genetic characteristic that stops plants from producing fertile pollen. It is a consequence of changes in mitochondrial DNA and is a trait that is transmitted maternally (Williams, 1995). So male sterility is characterized by non-functional pollen grains, while female gametes function normally (Sunidhi & Versha, 2018).

CMS is reported to over 150 plant species existing as a spontaneous mechanism (Hanson and Bentolila, 2004) or can be created by experimental means such as induced mutations, hybridization, protoplasmic fusion, broad/inter-specific, or genetic engineering (Singh et al., 2015; Wang et al., 2013).

Thoroughly understanding the nucleocytoplasmic interactions between genes linked to fertility restauration and cytoplasmic male sterility offers valuable insights for agriculture and developing corn hybrids.

In corn hybrids, fertility restoration is crucial for the effective production of hybrid seeds, which are essential for achieving higher yields and improving crop performance, however, the same does not apply to the use of sterile lines in seed production.

There are situations in which the process of sterility breakage occurs in the case of certain CMS lines. This process of breaking sterility is very dangerous and can contaminate the entire field if it is not noticed in time. It is very important to understand this process of breaking sterility and the factors that influence it, in order to avoid the contamination of hybrid production fields. Additionally, it is crucial to implement measures that prevent this phenomenon from occurring.

THE ADVANTAGES OF USING CMS LINES IN HYBRID SEED PRODUCTION

The production of corn hybrid seed requires a directional cross to be performed between two inbred lines, where pollen from one inbred line (the male parent or pollen donor) is used to fecundate the silk from the second inbred line (the female parent or pollen receiver). Since corn is a monoecious plant is needed to perform the removal of female plant tassel manually or mechanized and in most cases performing both operations.

The use of CMS forms in corn seed production eliminates the need for castration operations. In the case of fertile maternal forms, this process is delicate, involves a special machine for detasseling, as well as manual labor for verification, making it very important from an economic perspective meaning that this phenomenon of male sterility is of great significance to produce cost effective hybrid seeds (Sunidhi &Versha, 2018).

Another major advantage of using CMS lines in production is that the plants are not stressed by the castration operations, resulting in significantly higher yields. For a fertile female form, by castration, losing part of the plant, automatically will decrease the production potential (Figure 1).

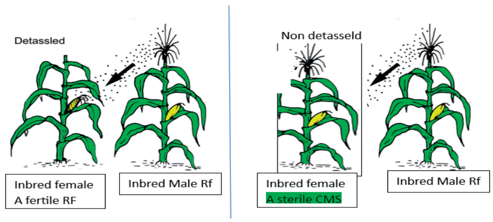

Figure 1. Influence of detasseling process on plant vs non-detasseling (original)

The quality of fecundation on CMS forms is much better, resulting in a higher percentage of large seeds, with a high TKW, and a very good germination and at the same time with a reduction in the percentage of waste (broken or small round seeds) in the seed lots after the final processing.

CMS LINES

The detailed study of CMS inbred lines before their introduction into the production process, as well as the identification of factors that lead to sterility breakage is an important objective for the research departments within the companies producing hybrid corn seed.

Utilization of male sterility involves following steps:

- Identification and obtaining the CMS form.
- Integration of the male sterility trait into desired variety through recurrent backcrossing, transforming it into female parent for hybridization.
- Multiplication of male sterile lines by developing maintainer line.
- Hybrid seeds production in open pollination under controlled conditions or via manual cross-pollination techniques. (Sunidhi&Versha, 2018).

Obtaining a cytoplasmic male sterility line (CMS) involves several steps that combine plant breeding techniques with knowledge of genetics.

Developing cytoplasmic male sterility (CMS) lines in corn involves a focus on two critical phases: discovery and isolation of CMS donors, which are typically identified within cultivated corn or related wild species.

The plants selected as sources of cytoplasmic male sterility exhibit an inability to produce viable pollen due to genetic alterations in the cytoplasmic (mitochondrial) genome. CMS genes are chimeric in nature and originate from recombination events that occur between mitochondrial genes and the flanking sequences (He et al., 2020). While these recombination events contribute toward complexity of the genomic structures, they also help maintain genomic stability along with increasing genetic variation (Tuteja et al., 2013).

A second step consists of the initial crossing between a fertile female elite line (also called the "A" line) with a plant that carries the sterile cytoplasm (the "S" line), to transfer the sterile cytoplasm to the A line.

The third stage is that of recurrent crossing (backcross), so the F1 line is repeatedly crossed with the original A line for several generations. This process aims to maintain the nuclear genetic background of the A line, but with sterile cytoplasm. CMS can be transferred easily to a given strain by using that strain as a pollinator (recurrent parent) in the successive generations of backcross programme (Sunidhi & Versha, 2018). After 6-7 backcrosses, the nuclear genotype of the male sterile line would be almost identical to the recurrent pollinator strain (A line) but contains sterile cytoplasm (Figure 2). “A sterile” line is the male-sterile female line with “S” cytoplasm and recessive fertility nuclear alleles (frfr).

The 4th stage continues with the confirmation of male sterility. Line A is tested to ensure that it does not produce viable pollen. This may involve morphological examination of the anthers and pollen testing for viability.

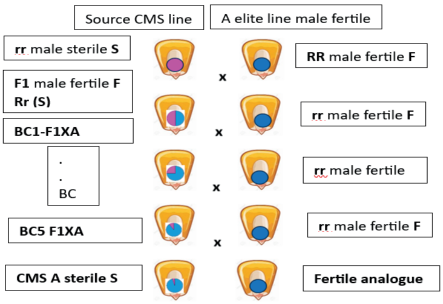

Figure 2. Obtaining a CMS line after crossing a CMS source with an Elite line (original)

Once obtained the CMS line, to complete hybrid system, involves another 2 distinct genotypes:

- “B” line is a maintainer of the female line, and it has fertile “F” cytoplasm and recessive nuclear alleles (frfr). When this line is crossed with “A” line, the entire progeny is male sterile.
- Third parent is designated as “R” line, and it contains dominant fertility-restoring gene (FrFr). This line can restore the male fertility of the hybrid plants produced by crossing with “A” line. (Saxena & Anupama, 2015).

Cytoplasmic Male Sterility (CMS) corn lines are of three main types, depending on differences in mitochondrial DNA:

All these three types of CMS are classified based on specific nuclear restorer genes (Rf), which can neutralize the CMS trait and restore fertility in the first generation of plants (F1). (Christophe Weider et al., 2009).

1.Type T (Texas cytoplasm, CMS-T) was widely used in the 1970s, but its use declined due to its susceptibility to *Helminthosporium maydis*, race T, which causes corn leaf blight disease (Levings, 1993). The T-cytoplasm was removed from breeding germplasm after a widespread and serious epidemic of Southern Corn Blight, which drastically reduced maize yields in 1969 and 1970 (WISE et al., 1999)

2. Type C (Charrua cytoplasm, CMS-C) is the most used source of sterility in corn seed production. Is less susceptible to disease compared to type T and is used as an alternative to avoid problems related to disease susceptibility. It has been discovered in maize lines in South America, and this form is caused by specific changes in the mitochondrial genome that affect pollen fertility (Laughnan, 1983).

3. Type S (USDA cytoplasm CMS-S) is used for its stability and resistance to diseases, being another option in maize breeding programs. However, plants with CMS-S show a moderate resistance to pathogens and have varied performances depending on environmental conditions and agronomic practices (Beckett, 1973).

These types are chosen according to the specific needs of the hybridization programs and the local growing conditions.

CMS lines are very important in hybrid corn production also because they increase productivity, ensure a controlled and more uniform pollination, which contributes to obtaining high-quality hybrids with desired genetic characteristics and minimizes the risk of unwanted or accidental pollination, ensuring the genetic purity of the hybrids and increasing the success rate of production (Saxena et al., 2013). The use of CMS lines has revolutionized the seed industry and significantly advanced modern agriculture, providing farmers with access to superior quality seeds that enhance production and contribute to global food security. However, the use of these lines is not without its challenges.

Problems reported by the production teams are sometimes related to the height of the plants, which is sometimes significant compared to the male form, and then the pollination of the central rows of mother is poor, the pollen not being able to pass between the rows of mother especially for sowing ratio (6Ax2Rf rows).

Another problem that may arise is related to the restoration of fertility in F1 for hybrids obtained with CMS lines due to the recessiveness of sterility genes respectively mitochondrial genes mutations that disrupt normal pollen development (Schnable &Wise, 1998), with the risk that a significant percentage of plants will exhibit androsterility and not produce pollen. In this case, the hybrids obtained with these female CMS forms are checked and tested very well before moving on to their commercial production.

The most important problem is the sterility breakage of CMS lines. If this breakage is not noticed in time, the seed production field is totally compromised because biological contamination with pollen from the female line form takes place.

If this phenomenon is observed in time, the field can be saved by urgently performing castration. In this case, the farmer must intervene quickly with the castration operation that requires the available machine, manual labour to pass and remove any tassels from the smaller plants that have not been removed by the castration machine and thus the costs increase significantly and unforeseen.

Breaking sterility factors

Breaking male sterility refers to the phenomenon where plants that are expected to remain male-sterile begin producing functional pollen.

This is a unique male sterility system where the expression of male sterility and fertility of the plants is controlled by environmental factors. Under this system the male sterility, gene expresses only under specific environment such as low or high temperature, short or long photoperiod, variable light intensity, different soilborne stresses, or their specific combinations (Kaul, 1988). This situation can arise both in genetic as well as cytoplasmic nuclear male sterility systems. The reversion of sterility is influenced by cytoplasmic rather than nuclear genetic factors, and loss of such factors is correlated with reversion of male sterility to male fertility (Levings et al., 1980). The conversion of male sterility to fertility and its reversal is a complex genetic phenomenon, and more research is required at genomics and physiological levels to understand it better. Fertility restoration occurs through mechanisms that suppress the activity of CMS-related genes or mitigate their harmful effects. Like CMS itself, fertility restoration is a highly intricate process, taking place at multiple molecular levels.

Research indicates that this phenomenon is influenced by both genetic and environmental factors as is mentioned above.

Genetically, fertility restoration can happen at various stages, including genomic, post-transcriptional, translational, post-translational, or metabolic levels. Interestingly, all identified restorer genes are located within the nuclear genome. In corn, restorer genes such as Rf2 have been successfully cloned (Cui et al., 1996).

The restorer allele at Rf2 encodes a functional mitochondrial aldehyde dehydrogenase, suggesting that the restoration of fertility can occur through metabolic compensation. This compensation offsets the effects caused by the mitochondrial CMS-determining gene (Chase, 2007). From our perspective, this mechanism highlights the adaptability of plant genetic systems, as they develop complex strategies to ensure reproductive success even when challenged by cytoplasmic sterility.

In corn, the three major CMS types - T-CMS, S-CMS, and C-CMS - are defined by their unique restorer genes. For T-CMS, fertility restoration depends on the combined action of two restorer genes, Rf1 and Rf2, which must function together to fully restore the plant’s ability to produce viable pollen (Duvick, 1965). However, particularly fascinating is that these genes are ineffective in restoring fertility in other CMS systems, such as S-CMS and C-CMS. This specificity underscores the nuanced relationship between nuclear restorer genes and mitochondrial CMS systems.

For S-CMS, fertility restoration is managed by a single major gene, Rf3, which is critical for enabling pollen fertility (Duvick, 1965). According to our understanding, the simplicity of the S-CMS system compared to T-CMS reflects the diversity of evolutionary adaptations in corn, each tailored to meet specific environmental or genetic challenges. This insight not only emphasizes the intricacies of nucleocytoplasmic interactions but also provides a foundation for targeted breeding strategies aimed at optimizing hybrid seed production.

Compared to the restoration mechanisms of T-CMS and S-CMS, the process of restoring fertility in C-CMS is far more complex and remains largely unexplored. Evidence suggests the involvement of at least three nuclear restorer genes, known as Rf4, Rf5, and Rf6, which are believed to interact in a complementary manner. Interestingly, this trigenic system may also be duplicated elsewhere in the genome, adding another layer of complexity to the restoration process (Vidakovic, 1988; Vidakovic et al., 1997). In our opinion, this intricate interplay between multiple genes reflects the incredible sophistication of plant genetic systems and highlights the challenges researchers face in unravelling these mechanisms.

Complementary interactions between paternal and maternal factors appear to play a significant role in the fertility restoration of C-CMS. This conclusion was drawn from experiments involving crosses between C-CMS lines and maintainer lines with normal cytoplasm, where the progeny exhibited partial or even full restoration of fertility (Vidakovic, 1988; Sotchenko et al., 2007). This finding underscores the importance of studying the dynamic interactions between nuclear and cytoplasmic components, as they seem to be pivotal in determining the stability of CMS systems. A unique feature of C-CMS is its tendency toward "partial restoration", which often includes a phenomenon called the "late-break of sterility". In such cases, pollen shedding can still occur several weeks after the silks have emerged (Tracy et al., 1991; Kheyr-Pour et al., 1981; Vidakovic, 1988). This delayed response is fascinating, as it may point to underlying regulatory mechanisms that are not yet fully understood. Exploring these mechanisms could provide valuable insights for improving hybrid seed production and mitigating risks associated with unintended fertility restoration.

The restoration of CMS sometimes occurs spontaneously. This was found for T- and S- (Smith & Chowdhury, 1989).

In T-CMS, the CMS-inducing gene, urf13, can be lost through recombination, resulting in restored fertility (Fauron et al., 1990). In S-CMS, spontaneous reversion to fertility arises either through reorganization within the mitochondrial DNA (Small et al., 1988) or through mutations of the nuclear DNA resulting in new restorer genes (Gabay-Laughnan et al., 2004). Apart from the restorer genes, which fully restore the fertility, some genes lead to a partial restoration of fertility (Duvick, 1965). Partially restored plants bear fewer anthers than male-fertile plants, and the emergence of the anthers is usually delayed.

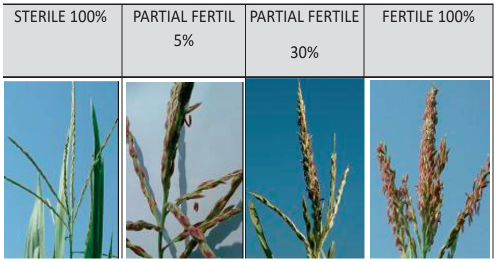

Figure 3. Partial restoration of fertility for tassel comparing with complete sterile and complete fertile

The anthers may be misshapen and have less pollen than a fully restored type (Tracy et al., 1991). The genetic basis of partial restoration is still unclear. It may be governed by multiple genes, which may have an effect in the absence of fully restoring genes (Tracy et al., 1991). Furthermore, partial restoration might result from incomplete homozygosity if the inbred line has been converted into CMS by backcrossing rather than maternal induction of haploidy (Gontarovskii, 1974).

Regarding the environmental factors, several scientific studies have been done to investigate the influence of environmental factors on the stability of male sterility in corn.

Wind, air temperature, solar radiation, and water evaporation appeared to influence, positively or negatively, the expression of male sterility 2 to 3 weeks before anthesis (Sarvella, 1966; Marshall et al., 1974). In another research 22 CMS variants of corn hybrids has been examined across 17 different environments in countries as France, Bulgaria and Switzerland. The results revealed significant variability in the stability of male sterility across the three CMS types (T, S, and C) (Weider et al., 2009). Among these, T-cytoplasm hybrids show the best stability, while S-cytoplasm hybrids were more susceptible to partial fertility restoration. The study highlighted that the environmental factors, particularly temperature, evapotranspiration, and water vapor levels during the critical 10-day period before and during anthesis, were closely linked to partial reversion to male fertility (Weider et al., 2009). These findings emphasize the interaction between genetic factors and environmental conditions in determining CMS stability.

Another study examines how deficiencies in the Dicer-like 5 (DCL5 protein) gene render corn plants sensitive to temperature changes during meiosis. This genetic deficiency leads to stop tapetal development and subsequent male sterility under specific temperature conditions. (Teng et al., 2020).

Although these studies did not specifically address the impact of sowing density or water availability, the results strongly suggest that environmental conditions are a critical component in maintaining stable male sterility. In our opinion, this raises an important point: factors such as water stress and improper sowing densities, while not explicitly studied, likely play a role in influencing the expression of Cytoplasmic Male Sterility (CMS). Such insights are invaluable for breeding programs, as they highlight the need to consider a wide range of environmental parameters when developing and testing CMS lines for hybrid seed production.

CONCLUSIONS

Cytoplasmic male sterility (CMS) is an inherited trait passed through the maternal line, characterized by the plant's inability to generate functional pollen.

CMS lines are valuable instruments for hybrid seeds production used to facilitate the production of hybrid seeds and to minimize costs as detasseling operation is not needed.

Using CMS line in hybrids production increase not only yield but also the quality of hybrids seeds.

CMS breakage is a very dangerous phenomenon translated into the restoration of fertility and therefore the emission of viable pollen.

Male sterility breakage in corn can be associated with various factors, leading to unintended fertility and affecting hybrid seed production.

The most important factors studied linked to sterility breakage are genetic factors and environmental factors.

Genetic factors that lead to sterility breakage are Restauration of Fertility (Rf) genes, Mitochondrial Gene Mutation and Interaction between nuclear and cytoplasmic genes.

The environmental factors that influence breakage sterility are air temperature, evapotranspiration but the drought or inadequate sowing density are not excluded either. Understanding these factors is crucial for maintaining stable male sterility in corn breeding programs, ensuring the production of hybrid seeds without unintended fertility restoration.

The study of male sterility and factors that leads to breakage of sterility holds great promise for agriculture, biotechnology, as well as basic science.

REFERENCES

Albertsen, M.C. (1997). Male sterile mutants. p.313. In: M.G. Neuffer, E.H. Coe, S.R. Wessler (Eds.), *Mutants of Maize*. Cold Spring Harbor Lab. Press, Plainview.

Beckett, J.B. (1973). Classification of male-sterile cytoplasms in maize (*Zea mays* L.). *Crop Sci. 11*. 724–727.

Chase, C.D. (2007). Cytoplasmic male sterility: A window to the world of plant mitochondrial–nuclear interactions. *Trends Genet. 23*.81–90.

Chaubal, R., Anderson, J.R., Trimnell, M.R., Fox, T.W., Albertsen, M.C., Bedinger, P. (2003). The Transformation of Anthers in the Msca1 Mutant of Maize. *Planta 216*. 778–788.

Cui, X., Wise, R., Schnable, P. (1996). The Rf2 restorer gene of male sterile T-cytoplasm in maize. *Science 272*. 1334–1336.

Duvick, D.N. (1965). Cytoplasmic pollen sterility in corn. *Adv. Genet. 13*. 1–56.

Fauron, C.M.R., Havlik, M., Brettell, R.I.S. (1990). The mitochondrial genome organization of a maize fertile CMS-T revertant line is generated through recombination between 2 sets of repeats. *Genetics 124*. 423–428.

Gabay-Laughnan, S., Chase, C.D., Ortega, V.M., Zhao, L.M. (2004). Molecular-genetic characterization of CMS-S restorer-of-fertility alleles identified in Mexican maize and teosinte. *Genetics 166*. 959–970.

Gontarovskii, V. (1974). Role of supplementary genes in control of Texas type of CMS in maize. *Genetika 10*. 15–24.

Hanson, M.R., Bentolila, S. (2004). Interactions of mitochondrial and nuclear genes that affect male gametophyte development. *Plant Cell 16*. S154–S169. *https://doi.org/10.1105/tpc.015966*

He, W., Chen, C., Adedze, Y.M., Dong, X., Xi, K., Sun, Y., Dang, T., Jin, D. (2020). Multicentric origin and diversifcation of atp6-orf79-like structures reveal mitochondrial gene fows in *Oryza rufpogon* and *Oryza sativa. Evol Appl 13(9)*. 2284–2299. *https://doi.org/10.1111/eva.13022*

Laughnan, J.R., Gabay-Laughnan, S. (1983). Cytoplasmic male sterility in maize. *Ann. Rev. Genet. 17*. 27–48. *https://doi.org/10.1146/annurev.ge.17.120183.000331*

Kaul, M.L.H. (1988*). Male sterility in higher plants.* Springer, Berlin.

Kheyr-Pour, A., Gracen, V.E., Everett, H.L. (1981). Genetics of fertility restoration in the C-group of cytoplasmic male-sterility in maize. *Genetics 98*. 379–388.

Levings, C.S. (1993). Thoughts on cytoplasmic male sterility in cms-T maize. *Plant Cell 5*. 1285–1290.

Levings, C., Kim, B., Pring, D., Conde, M., Mans, R., Laughnan, J., Gabay Laughnan, S. (1980). Cytoplasmic reversion of CMS-S in maize: association with a tRNA positional event. *Science 209*. 1021–1023.

Marshall, D.R., Thomson, N.J., Nicholls, G.H., Patrick, C.M. (1974). Effects of temperature and day length on cytoplasmic male sterility in cotton (Gossypium). *Aust. J. Agric. Res. 25*. 443–450.

Sarvella, P. (1966). Environmental influences on sterility in cytoplasmic male-sterile cottons. *Crop Sci. 6*. 361–364.

Saxena, K.B., Anupama, J.H. (2015). *Plant Biology and Biotechnology: Volume I: Plant Diversity, Male Sterility Systems in Major Field Crops and Their Potential Role in Crop Improvement p. 642.*

Saxena, K.B., Kumar, R.V., Tikle, A.N. et al. (2013). ICPH2671—the world's first commercial food legume hybrid. *Plant Breed 132*. 479–485.

Schnable, P.S., Wise, R.P. (1998). The molecular basis of cytoplasmic male sterility and fertility restoration. *Trends Plant Sci 3(5)*. 175–180. *https://doi.org/10.1016/S1360-1385(98)01235-7*

Singh, S.P., Pandey, T., Singh, R.K., Sawant, S.V. (2015). A novel male sterility-fertility restoration system in plants for hybrid seed production. *Sci Rep 5*. 11274.

Skibbea, D.S., Schnablea, P.S. (2005). Male sterility in maize. *Maydica 50*. 367–376.

Smith, R.L., Chowdhury, M.K.U. (1989). Mitochondrial DNA polymorphism in male sterile and fertile cytoplasms of pearl millet. *Crop Sci 29*. 809–81.

Small, I.D., Earle, E.D., Escotecarlson, L.J., Gabay-Laughnan, S., Laughnan, J.R., Leaver, C.J. (1988*). A comparison of cytoplasmic revertants to fertility from different CMS-S maize sources. Theor Appl Genet 76*. 609–618.

Sotchenko, V., Gorbacheva, A., Kosogorova, N. (2007). C-type cytoplasmic male sterility in corn. *Russian Agricultural Sciences 33*. 83–86.

Sunidhi, M., Versha, K. (2018). A review on Male Sterility-Concepts and Utilization in Vegetable Crops. *Int.J.Curr.Microbiol.App.Sci. 7(2)*. 3016–3034. https://doi.org/10.20546/ijcmas.2018.702.367

Teng, C., Zhang, H., Hammond, R., Huang, K., Meyers, B.C., Walbot, V. (2020). *Dicer-like5 deficiency confers temperature-sensitive male sterility in maize. Nature Communications, volume 11, Article number 2912.* https://doi.org/10.1038/s41467-020-16634-6

Tracy, W.F., Everett, H.L., Gracen, V.E. (1991). Inheritance, environmental effects, and partial male-fertility in C-type CMS in a maize inbred. *J Hered 82*. 343–346.

Tuteja, R.E., Saxena, R.K., Davila, J.A., Shah, T.R., Chen, W.E., Xiao, Y.L., Fan, G.U., Saxena, K.B., Alverson, A.J., Spillane, C.H., Town, C.H. (2013). Cytoplasmic male sterility-associated chimeric open reading frames identified by mitochondrial genome sequencing of four Cajanus genotypes. *DNA Res 20(5)*. 485–495.https://doi.org/10.1093/dnares/dst025

Vidakovic, M., Vancetovic, J., Vidakovic, M. (1997). The existence of a duplicated or parallel genetic system for fertility restoration in cmsC of maize (*Zea mays* L.). *Maydica 42*. 313–316.

Vidakovic, M. (1988). *Genetics of fertility restoration in cytoplasmic male-sterility of the c-type (cmsC) in maize (Zea mays* L.). *Maydica 33*. 51–64.

Wang, J., Jiang, J., Wang, Y. (2013). Protoplast fusion for crop improvement and breeding in China. *Plant Cell Tissue Organ Cult 112*. 131–142. https://doi.org/10.1007/s11240-012-0221-y

Weider, C., Stamp, P., Christov, N., Hüsken, A., Foueillassar, X., Camp, K.-H., Munsch, M. (2009). Stability of Cytoplasmic Male Sterility in Maize under Different Environmental Conditions. *Crop Science, vol. 49.*

Williams, M.E. (1995). Genetic engineering for pollination control. *Trends Biotechnol., 13*. 344–349.

Wise, R.P., Bronson, C.R., Schnable, P.S., Horner, H.T. (1999). *The genetics, pathology, and molecular biology of T-cytoplasm male sterility in maize. Adv. Agron. 65*. 79–130.