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Cytoplasmic male sterility in plants with special emphasis on sugar beet

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ABSTRACT

The phenomenon of cytoplasmic male sterility (CMS) in plants is characterized by a disturbance in the development of functional pollen. The cause of this disorder is the incompatibility of the mitochondrial and nuclear genome. Determinants of CMS are found in the mitochondrial genome and are inherited maternally. Nuclear *Rf* genes are responsible for restoring male fertility in CMS plants most often by posttranscriptional and/or posttranslational activity on mitochondrial gene expression. Two fertility restoring genes have been identified in sugar beet plants, of which *Rf1* is the best characterized. It is concluded that the translation product of this gene acts as a molecular chaperone leading to inactivation of a specific mitochondrial protein that is a marker for the CMS trait in sugar beet. The CMS phenomenon is applied in the commercial breeding of hybrid varieties of many crop species. Exploiting heterosis is easier with the knowledge of cytoplasmic (mitochondrial) determinants of sterility and corresponding restorer genes.

KEYWORDS: *Beta vulgaris* L., cytoplasmic male sterility, mitochondrial DNA, open reading frame, fertility restorer genes

Introduction

The phenomenon of cytoplasmic male sterility (CMS) has been described in more than 150 species of monocotyledonous and dicotyledonous plants (Sofi *et al.*, 2007), including sugar beet (*Beta vulgaris* L.) (Kubo *et al.*, 1999).

CMS includes a number of processes leading to disturbances in the microsporogenesis process, resulting in formation of non-functional microspores or pollen grains (Majewska-Sawka and Sadoch, 2003). Disturbances in stamen production have also been observed (Andersen, 1965; Rohrbach, 1965). These disorders result from mitochondrial and nuclear genome incompatibility (Chase, 2007). CMS is a trait inherited in the female line – the determinants of this trait are in the mitochondrial genome, which is

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transferred to the embryo by the egg cell (Zhiwen *et al*., 2017). For masking of CMS fertility restorer genes are responsible for, which are present in the nuclear genome.

Cytoplasmic male sterility has found wide application in breeding of many crops. CMS forces plants to pollinate with foreign pollen, which promotes the increase in heterozygosity and genetic recombination in the offspring and, as a result, leads to an increase in its vigour (Kaul, 1988; Święcicki *et al*., 2011).

CMS symptoms

Cytoplasmic male sterility manifests itself at the level of flower morphology, flower tissues, the structure of mitochondria and the process of microsporogenesis. Changes may also affect the structure of the flower itself (Ivanov and Dymshits, 2007). The literature describes cases of the transformation of stamens into petals or carpels and stigmas (Laser and Lersten, 1972). Cases of complete absence of inflorescences are also known (Chase, 2007). Structural changes were found in stamen filaments (primarily in the vascular bundle), as well as in anthers, which was associated with abortion of microspores in plants characterized by CMS (Wasiak, 2019). It has been shown that the filaments of normal beet (*Beta vulgaris*) stamens were characterized by elongated cells with a large amount of cytoplasm as opposed to the cells in the filaments of sterile plant stamens (Rohrbach, 1965).

Cytoplasmic male sterility also affects mitochondria. In tapetum and microspores of CMS plants, it was observed that mitochondria are often much smaller and also characterized by a smaller area of the internal membrane forming the crest system (Majewska-Sawka and Sadoch, 2003). Cell division and differentiation are processes that require large amounts of ATP. In the tapetum of CMS plants, a reduced number of mitochondria was observed, and thus also production of ATP at a lower level (Jańska and Wołoszyńska, 1996; Majewska-Sawka and Sadoch, 2003). As a result of recombination in mtDNA, abnormal open reading frames encoding transmembrane proteins may be created that alter the permeability and potential of the internal mitochondrial membrane (Zhiwen *et al*., 2017; Wasiak, 2019). Based on numerous studies of enzyme activity present in mitochondria and structural analyses of mitochondrial DNA, mitochondria were identified as organelles responsible for phenotypic changes observed in CMS plants' pollen (Majewska-Sawka and Sadoch, 2003).

Disorders of microsporogenesis result in the formation of non-functional microspores or pollen grains (Jańska and Wołoszyńska, 1996). Abnormalities may appear at different stages of differentiation – during meiotic division of the pollen stem cell or just after the end of meiosis (Bino, 1985), at the tetrad stage (Horner and Rogers, 1974), as well as during other phases of development of haploid microspores (Majewska-Sawka *et al*., 1993). The most common cause of CMS is cytological disorders in pollen stem cells and tapetum cells (Majewska-Sawka and Sadoch, 2003). Symptoms of abnormal tapetum development include excessive vacuolation, loss of cellular character of tissue and formation of multicellular syncytia, and premature or delayed death (Scoles and Evans, 1979). Electron microscopy techniques have shown structural abnormalities of some cellular organelles in pollen stem cells and tapetum. In the tapetum structure of sterile anthers of sugar beet (*Beta vulgaris* L.) plants, fusion of cells into multi-nucleus syncytia and concentric forms of endoplasmic reticulum was observed (Majewska-Sawka and Sadoch, 2003).

Fertility restorer genes

In many plant species, it is possible to restore male fertility with one or more nuclear *Rf* genes (Tan *et al*., 2015), which are responsible to varying degrees for the variability of the trait (Wang *et al*., 2013). These genes compensate or inactivate the effect of sterilizing mitochondrial factors (Touzet and Meyer, 2014), correcting thus disturbed pollen development (Ivanov and Dymshits, 2007). Many studies show that most fertility restoring genes act posttranscriptionally and/or posttranslationally (Jańska and Wołoszyńska, 1996). As a result of the action of these genes, transcript ends of regions correlated with CMS (Kennell and Pring, 1989) and/or changes occur in quantitative relationships between transcripts (Pruitt and Hanson, 1991). Consequently, a reduction (Nivison and Hanson, 1989) or absence (Song and Hedgcoth, 1994) of proteins characteristic of male sterile cytoplasms is observed.

Most fertility restorer genes belong to the PPR (pentatricopeptide repeat protein) family. Genes of the PPR family are present in the nuclear, mitochondrial and chloroplast genomes (Nakamura *et al*. 2012), and their expression products are responsible for regulating the transcription process (Schmitz-Linnerweber and Small, 2008). PPR-type RF proteins contain 35-amino acid repeating motifs, and by binding specifically to the mitochondrial transcripts that condition the CMS feature, they promote their degradation (Barkan and Small, 2014). This results in a strong reduction in the production of mitochondrial CMS-inducing proteins (Chen and Liu, 2014).

The phenomenon of cytoplasmic male sterility in sugar beet (*Beta vulgaris* L.) was discovered by Owen in 1945 and is now used in breeding (McGrath and Panella, 2018). CMS in sugar beet is linked to a specific hydrophobic mitochondrial protein with a molecular weight of 39 kDa, preSATP6, which forms a 250 kDa complex (Matsuhira *et al*., 2012). Two restorers have been identified in *B. vulgaris* – *X* (*Rf1*) and *Z* (*Rf2*), the latter having little effect on fertility restoration (Honma *et al*., 2014). The use of genetic mapping allowed the localization of these genes – on chromosomes III and IV respectively (Owen, 1945). The *Rf1* gene is best characterized. Analysis of the nucleotide sequence of the *Rf1* gene revealed a cluster consisting of four copies of an open reading frame encoding a yeast metallopeptidase-like protein OMA1, which is involved in quality control of mitochondrial inner membrane proteins (Matsuhira *et al*., 2012; Arakawa, 2020). One of the genes forming the cluster, *bvORF20*, showed partial ability to restore fertility in an experiment using transgenic sugar beet plants (Matsuhira *et al*., 2012). When *bvORF20* was expressed in sugar beet suspension cells characterized by CMS, binding of the expression product of this gene to the preSATP6 protein and formation of a new 200 kDa complex was observed (Arakawa *et al*., 2019). The presence of bvORF20-preSATP6 complex was also detected in the anthers of sugar beet plants with previously restored fertility (Kitazaki *et al*., 2015). Together with the appearance of the bvORF20-preSATP6 complex in the anthers of plants with restored fertility, it was observed that the amount of the preSATP6 complex was significantly reduced, while the total amount of the monomeric protein preSATP6 remained almost unchanged. This phenomenon has been interpreted as a modification of the higher order structure of preSATP6 by the chaperone-like molecular activity exerted by bvORF20 (Matsuhira *et al*., 2012; Arakawa, 2020).

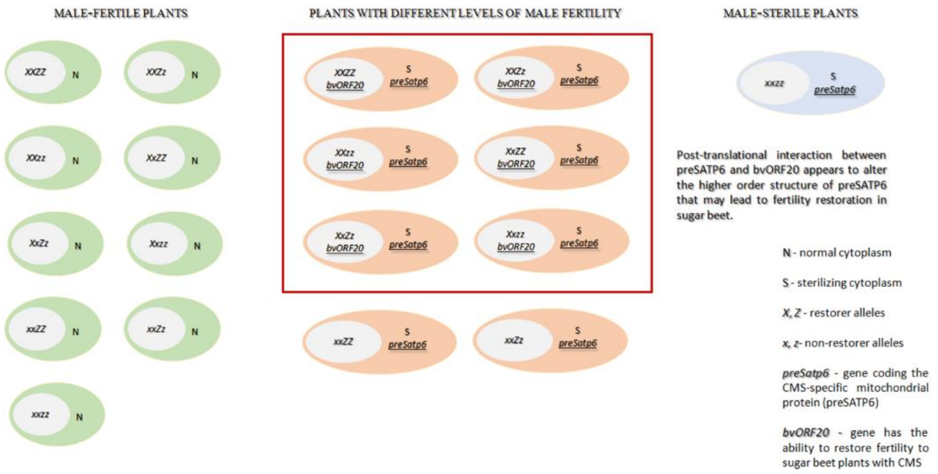

Figure 1. Genetics of cytoplasmic male sterility (CMS) in sugar beet (Owen, 1945; Matsuhira *et al*., 2012; Kitazaki *et al*., 2015)

Practical application of the CMS

The production of hybrid varieties is one of the methods that allows to increase the yield of plants. Such varieties are obtained by pollination of inbred lines with foreign pollen, and the resulting F1 generation shows heterosis (Tsaftaris, 1995). Currently widely used in commercial breeding of hybrid varieties of many crop species is the phenomenon of cytoplasmic male sterility (Stojałowski *et al*., 2019).

The phenomenon of heterosis is not fully understood (Wasiak, 2019). Heterosis is manifested by increased biomass, increased growth rate, increased fertility and better resistance to pests and diseases (Wolko *et al*., 2019), thanks to which hybrid forms give better breeding results than parent forms (Hochholfinger and Hoecker, 2007).

The cultivation of sugar beet owes much to cytoplasmic male sterility, since all current varieties are hybrids produced using CMS (Bosemark, 2006). Production of hybrid seeds using CMS requires three lines – maternal, complementary and paternal (Chen and Liu, 2014). Maternal lines must have a cytoplasm inducing male sterility (S) and so-called complement alleles (*rf*) (Schnable and Wise, 1998). The complement line differs from the CMS line in the presence of normal cytoplasm (N) that determines male fertility. Such cytoplasm should also be present in the paternal line. Restorer alleles (*Rf*) that condition male fertility despite the presence of S cytoplasm and occur in the same locus as the complement alleles (*rf*) make it difficult to obtain CMS and complement lines.

Conclusions

Cytoplasmic male sterility (CMS) favours crosspollinations and is widely used to obtain hybrid seeds. The phenomenon of CMS is masked by fertility restorer genes (Rf) genes located in the nuclear genome, therefore it is important to identify mitochondrial determinants of sterility and responsible Rf genes. The molecular characterization of these sequences also includes studies of their expression at the protein level.

References

Andersen, W.R. 1965. Cytoplasmic male sterility in hybrids of *Lycopersicon esculentum* and

Solanum pennelli. Journal of the Minnesota Academy of Science, 32, 93–94.

Arakawa, T., Ue, S., Sano, C., Matsunaga, M., Kagami, H., Yoshida, Y., Kuroda, Y., Taguchi, K., Kitazaki, Kazuyoshi, K., Kubo, T. 2019. Identification and characterization of a semi-dominant restorer-of-fertility 1 allele in sugar beet (*Beta vulgaris*). Theoretical and Applied Genetics, 132, 227–240.

Arakawa, T., Matsunaga, M., Matsui, K., Itoh, K., Kurodaa, Y., Matsuhira, H., Kitazaki, K., Kubo, T. 2020. The molecular basis for allelic differences suggests *Restorer-of-fertility 1* is a complex locus in sugar beet (*Beta vulgaris* L.). BMC Plant Biology, 20, 503.

Barkan, A., Small, I. 2014. Pentatricopeptide repeat proteins in plants. Annual Review of Plant Biology, 65, 415–442.

Bino, R.J. 1985. Ultrastructural aspects of cytoplasmic male sterility in *Petunia hybrida*. Protoplasma, 127, 230–240.

Bosemark, N.O. 2006. Genetics and breeding. (In:) Draycott, A.P. (ed.), Blackwell, Oxford, pp. 50 –88.

Chen, L., Liu, Y.G. 2014. Male Sterility and Fertility Restoration in Crops. Annual Review of Plant Biology, 65, 579–606.

Hochholdinger, F., Hoecker, N. 2007. Towards the molecular basis of heterosis. Trends in Plant Science, 12, 427–432.

Honma, Y., Taguchi, K., Hiyama, H., Yui-Kurino, R., Mikami, T., Kubo, T. 2014. Molecular mapping of *restorer-of-fertility 2* gene identified from a sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) homozygous for the non-restoring *restorer-of-fertility 1* allele. Theoretical and Applied Genetics, 127, 2567–2574.

Horner, H.T., Rogers, M.A. 1974. A comparative light and electron microscopic study of microsporogenesis in male-fertile and cytoplasmic male-sterile pepper (*Capsicum annuum*). Canadian Journal of Botany, 3, 435 –441.

Ivanow, M.K., Dymshits, G.M. 2007. Cytoplasmic male sterility and restoration of pollen fertility in higher plants. Russian Journal of Genetics, 43, 354–368.

Jańska, H., Wołoszyńska, M. 1996. Molekularne podstawy cytoplazmatycznej męskiej sterylności u roślin wyższych. (In:) Postępy biochemii. Zielińska Z. (ed.), Polskie Towarzystwo Biochemiczne, Warszawa, 253 –259.

Kaul, M.L.H. 1988. Male sterility in higher plants. Monographs on Theoretical and Applied Genetics, vol. 10. Springer-Verlag, Berlin-Heidelberg-New York.

Kennel, J.C., Pring, D.R. 1989. Initiation and processing of *atp6*, *T-urf13* and *ORF221* transcripts from mitochondrial of T cytoplasm maize. Molecular Genetics and Genomics, 216, 16–24.

Kitazaki, K., Arakawa, T., Matsunaga, M., Yui-Kurino, R., Matsuhira, H., Mikami, T., Kubo, T. 2015. Post-translational mechanisms are associated with fertility restoration of cytoplasmic male sterility in sugar beet (*Beta vulgaris*). The Plant Journal, 83, 290–299.

Kubo, T., Nishizawa, S., Mikami, T. 1999. Alterations in organization and transcription of the mitochondrial genome of cytoplasmic male sterile sugar beet (*Beta vulgaris* L.). Molecular and General Genetics, 262, 283–290.

Laser, K.D., Lersten, N.R. 1972. Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms. The Botanical Review, 38, 425–454.

Majewska-Sawka, A., Rodriguez-Garcia, M.I., Nakashima, H., Jassem, B. 1993. Ultrastructural expression of cytoplasmic male sterilityin sugar beet (*Beta vulgaris* L.). Sexual Plant Reproduction, 6, 22–32.

Majewska-Sawka, A., Sadoch, Z. 2003. Cytoplazmatyczna męska sterylność roślin – mechanizmy biologiczne i molekularne. Kosmos, 52, 413–423.

Matsuhira, H., Kagami, H., Kurata, M., Kitazaki, K., Matsunaga, M., Hamaguchi, Y., Hagihara, E., Ueda, M., Harada, M., Muramatsu, A., Yui-Kurino, R., Taguchi, K., Tamagake, H., Mikami, T., Kubo, T. 2012. Unusual and typical features of a novel restorer-of-fertility gene of sugar beet (*Beta vulgaris* L.). Genetics, 192, 1347–1358.

McGrath, J.M., Panella, L. 2018. Sugar Beet Breeding. (In:) Plant Breeding Reviews. Golgman I. (ed.), Wiley, USA, 167–218.

Nakamura, T., Yagi, Y., Kobayashi, K. 2012. Mechanistic insight into pentatricopeptide repeat proteins as sequence-specific RNA-binding proteins for organellar RNAs in plants. Plant and Cell Physiology, 53, 1171–1179.

Nivison, H.T., Hanson, M.R. 1989. Identification of a mitochondrial protein associated with cytoplasmic male sterility in petunia. The Plant Cell, 1, 1121–1130.

Owen, F.V. 1945. Cytoplasmically inherited male sterility in sugar beet. Journal of Agricultural Research, 71, 423–440.

Pruitt, K.D., Hanson, M.R. 1991. Transcription of the *Petunia* mitochondrial CMS-associated *Pcf* locus in male sterile and fertility-restored lines. Molecular Genetics and Genomics, 227, 348 –355.

Rohrbach, U. 1965. Beitrage zum Problem der Pollensterilitat bei *Beta voulgaris* L. Untersuchungen uber die Ontogenese des Phanotyps. Zeitschrift fur Pflanzeuchtung, 52, 105–104.

Schmitz-Linnerweber, C., Small, I. 2008. Pentatricopeptide repeat proteins, a socket set for organelle gene expression. Trends in Plant Science, 13, 663–670.

Schnable, P.S., Wise, R.P. 1998. The molecular basis of cytoplasmic male sterility and fertility restoration. Trends in Plant Science, 3, 175–180.

Scoles, G.J., Evans, L.E. 1979. Pollen development in male-fertile and cytoplasmic male-sterile rye. Canadian Journal of Botany, 57, 2782–2790.

Sofi, P.A., Rather, A.G., Wani, S.A. 2007. Genetic and molecular basis of cytoplasmic male sterility in maize. Communications in Biometry and Crop Science, 2, 49–60.

Song, J., Hedgcoth, C. 1994. A chimeric gene (orf256) is expressed as protein only in cytoplasmic male-sterile lines of wheat. Plant Molecular Biology, 26, 535–539.

Stojałowski, S., Orłowska, M., Bienias, A., Myśków, B., Tomczak, P., Wesołowski, W., Szklarczyk, M., Brukwiński, W., Banaszak, K., Hanek, M., Krysztrofik, R., Zając, M. 2019. Poszukiwanie wspólnych mechanizmów dziedziczenia płodności roślin z cytoplazmą CMS-C oraz cytoplazmą CMS-Pampa. Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin, 286, 151–154.

Święcicki, W.K., Surma, M., Koziara, W., Skrzypczak, G., Szukała, J., Bartkowiak-Broda, I., Zimny, J., Banaszak, Z., Marciniak, K. 2011. Nowoczesne technologie w produkcji roślinnej – przyjazne dla człowieka i środowiska. Polish Journal of Agronomy, 7, 102–112.

Tan, Y., Xu, X., Wang, C., Cheng, G., Li, S., Liu, X. 2015. Molecular characterization and application of a novel cytoplasmic male sterility-associated mitochondrial sequence in rice. BMC Medical Genetics, 16, 45.

Tsaftaris, A.S. 1995. Molecular aspects of heterosis in plants. Physioligia Plantarum, 94, 362–370.

Wang, Z.W., Wang, C., Gao., L., Mei S.Y., Zhou, Y., Xiang, C.P., Wang, T. 2013. Heterozygous alleles restore male fertility to cytoplasmic male-sterile radish (*Raphanus sativus* L.), a case of overdominance. Journal of Experimental Botany, 64, 2041–2048.

Wasiak, M. 2019. Genetyczne podstawy cytoplazmatyczno-jądrowej męskiej sterylności (CMS) u roślin oraz jej wykorzystanie w hodowli. Agronomy Science, 74, 15–30.

Wolko, J., Dobrzycka, A., Bosianowski, J. 2019. Ocena efektu heterozji cech struktury plonu mieszańców pojedynczych i mieszańców trójliniowych rzepaku (*Brassica napus L.*). Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin, 287, 21–22.

Zhiwen, C., Nan, Z., Shuangshuang, L., Corrinne, E. G., Hushuai, N., Wendel, J.F., Jinping, H. 2017. Plant mitochondrial genome evolution and cytoplasmic male sterility. Critical Reviews in Plant Sciences, 36, 55–69.