CYTOPLASMIC MALE STERILITY AND FERTILITY RESTORATION, VARIOUS MECHANISMS - THE SAME EFFECT

ANDROSTERILITATEA CITOPLASMATICĂ ŞI RESTAURAREA FERTILITĂŢII, DIVERSE MECANISME – ACELAŞI EFECT

DUCA Maria[1], MIDONI A.[1], SHESTAKOVA Tatiana[1], PORT Angela[1], NECHIFOR Victoria[1]
e-mail: mduca2000@yahoo.com

Abstract. *This work focused a complex study of various modalities of nucleo – cytoplasmic interaction through the prism of CMS-Rf systems of plants. Were evaluated different suppression pathways of cytoplasmic male sterility phenotype. It was elaborated the hypothetical mechanism of CMS-Rf interaction in plants which highlights various mechanisms but the same effect.*

Key words: cytoplasmic male sterility**,** pollen fertility restoration**,** nucleo – cytoplasmic interaction, *CMS-Rf* systems**.**

Rezumat. *Lucrarea dată reprezintă un studiu complex al diverselor mecanisme de interacţiune nucleu-citoplasmă prin prisma sistemelor ASC-Rf la plante. Analiza diferitelor căi de supresie a androsterilităţii citoplasmatice a permis elaborarea unui model general de interacţiune ASC-Rf la plante.*

Cuvinte cheie: androsterilitate citoplasmatică, restaurarea fertilităţii polenului, interacţiune nucleu – citoplasmă, sistemul ASC-Rf.

INTRODUCTION

Investigations regarding molecular mechanisms of cytoplasmic male sterility phenomenon has seen a significant development since the 70s of the last century. The first core assumptions about nucleo-cytoplasmic interaction were exposed by Turbine N.V. and Palilova A.N. (Turbine N.V., Palilova A.N., 1970). According to these authors, cytoplasmic male sterility (CMS) is the result of mitochondrial mutations which affect intracellular communication and determines expression of some repressor proteins that target the mitochondria and are coded by the nucleus. Alteration of mitochondrion biochemical activity triggers apoptotic mechanisms in the male gametophyte (Turbine NV, Palilova AN, 1970).

Recent data show specific features of nucleo-cytoplasmic interaction at different species (Koizuka N. et al., 2003; Duca M., 1998; Leipner J., Horn R., 2002; Mackenzie S.A., McIntosh L., 1999) which can not be completely explained by the general model reported by Palilova A.N. and Turbine N.V. Conducted research represents an analysis of data stored in the NCBI - The National

[1] University of the Academy of Sciences of Moldova, Republic of Moldova

Center for Biotechnology Information [www.ncbi.nlm.nih.gov] regarding proteins associated with the CMS and Rf aimed to highlight a general mechanism of interaction between cytoplasmic and nucleus genes using bioinformatics software.

MATERIAL AND METHOD

Different softwares and Web Links (Protein Workbench 5.0.1. [www.clcbio.com], PFAM [www.pfam.sanger.ac.uk / family.acc], ClustalW2 [www.ebi.ac.uk/Tools/clustalw2], PhyloDraw Ver. 0.8 [Graphics Application Lab., Pusan National University]) were applied for analysis of protein domains and motifs.

Gene analysis included: BLAST analysis [http://blast.ncbi.nlm.nih.gov/Blast.cgi] for identification of ESTs presenting high similarity index with the gene of interest; ESTs alignment using CAP3 program [http://www.pbil.univ-lyon1.fr/cap3.php] and translation of the obtained secquence in protein sequence via DNA-RNA-Protein translator. [http://www.attotron.com/cybertory/analysis/trans.htm].

Investigated protein and RNA sequences are summarized in table 1 and table 2.

Table 1

Analysed RNA and protein sequences

Species	Accesion number [www.ncbi.nlm.nih.gov] of the protein associated with:	
	CMS	**Rf**
Petunia spp.	CMS 402 (A.A.A96602)	*Rf*–PPR591 (A.A.M52340) and *Rf*–PPR592 (A.A.M52339)
Brassica napus	*ORF 222* (A.A.B41354)	*Rfo* (ACJ70132)
Raphanus sativus	*ORF 125* (BAB21870)	*orf687* (CAD61285)
Oryza sativa	*ORF 79* (A.A.18902)	*Rf1a*, *Rf1b* and *Rf1c* (ABC42330, ABC42331 şi BAD13711)
Zea mays CMS–T	–	*ALDH2*, *ALDH2b* (AAK58370, NP_001105576)
Zea mays CMS–S	*ORF 77*(A.A.N38288) and *ORF 355* (A.A.N38287)	–
Helianthus annuus CMS PET1	*ORFH 522* (CAA39429)	–

Table 2

EST and polynucleotide-phosphorilase sequences used in investigation

Species	Model gene and accesion number [www.ncbi.nlm.nih.gov]
Arabidopsis thaliana	polynucleotide-phosphorilase (AK117900)
Pisum sativum	polynucleotide-phosphorilase (AAC50039.1)
Oriza sativa	polynucleotide-phosphorilase (BAF20896.1)
ESTs (Expressed sequence tags), NCBI [www.ncbi.nlm.nih.gov]	
(DY912504.1; GE494064.1; EL415574.1 and EL443928.1)	

RESULTS AND DISCUSSIONS

Amino acid sequence analysis revealed *transmembrane* and *cytoplasmic motifs*, protein domains like *Major Facilitator Superfamily, Retroviral aspartyl protease* etc. for CMS proteins and cytoplasmic motifs such as "*RRM_1 - RNA recognition motif*" and protein domains of "*ALDH - Aldehyde Dehydrogenase*" for proteins associated with Rf. Integration of these data reveals some general interaction principles of CMS-Rf system (Fig. 1).

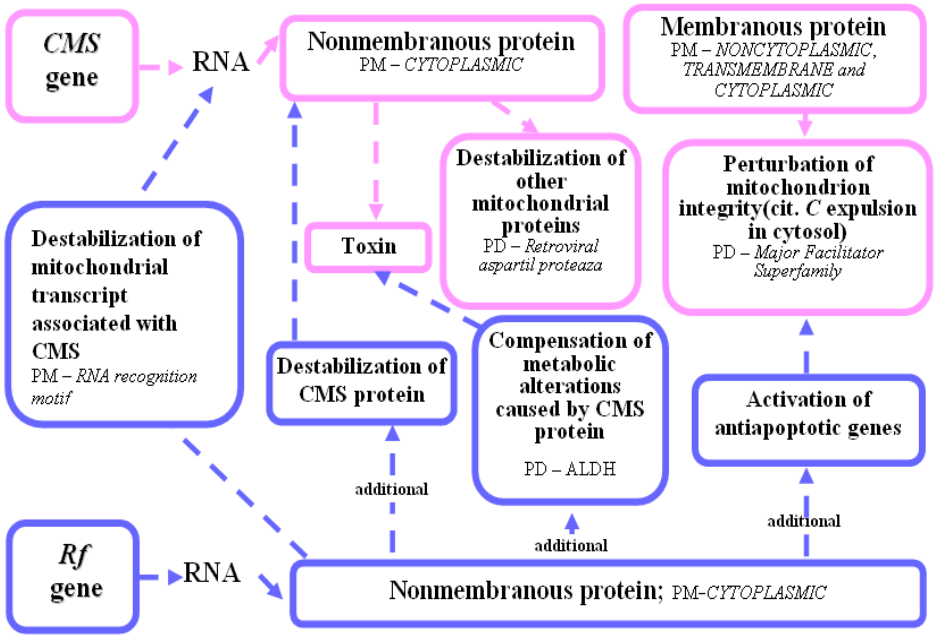

Fig. 1 – General mechanism of interaction between components of CMS-Rf system (PD – protein domain, PM – protein motif) (Midoni A., 2010)

According to bioinformatics data cytoplasmic male sterility is determined by the synthesis of mitochondrial proteins which in most cases contain *transmembrane motif* and domains involved in transmembrane transport of substances, leading to destabilization of mitochondrial membrane integrity and thus alterating its function.

In other cases, cytoplasmic male sterility could be the result of proteins with "retroviral aspartyl protease" domain that involved in degradation of core protein with energy metabolism function or cytoplasmic proteins with cytotoxic effect.

Cytoplasmic localization of nuclear Rf gene expression products with motifs involved in RNA recognition of CMS genes represented in figure 2 reveals that the effect of pollen fertility restoration is achieved by specific posttranscriptional modifications changes of CMS precursor.

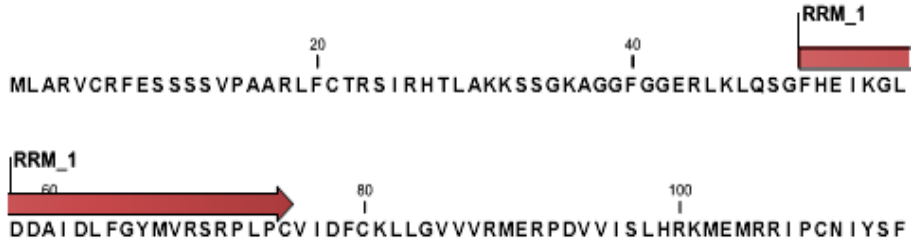

Fig. 2 – *RRM_1– RNA recognition motif.*

Fertility restorer genes from other plant species (rice, maize, tobacco, etc.) may also have additional role determined by the presence of protein domains of "*ALDH - Aldehyde Dehydrogenase*", which regulates the cellular content of acetic aldehyde, thus exhibiting a compensatory effect of altered mitochondrial function.

Analysis of phylogenetic trees, protein domains and motifs, spatial structure etc. highlighted similar peculiarities of different systems. For example, phylogenetic clustering of proteins associated with fertility restoration shows a high degree of similarity between the systems described in *Brassica napus* and *Raphanus sativus*. Also protein domains analysis indicates a similarity in the physiological expression of CMS-Rf system in corn with the T type and *Oryza sativa* (Boro II) where cytoplasmic protein with cytotoxic effect is destabilized postranscriptionally.

Unlike the CMS-Rf system of maize with Texas cytoplasm, S-type cytoplasm closely resembles sunflower PET1 at both structural and functional levels: transmembrane localization, disruption of mitochondrial integrity associated with the expulsion of cytochrome C and other apoptotic signals, CMS transcripts destabilization and pleiotropic effects on specific microsporogenesis genes.

In sunflower was found that decreasing of the stoichiometric content of *ATPase-orfH522* transcript is determined by polyadenylation that targets substrate for RNAase2 degradation. These molecular and biochemical processes is achieved by forming an enzymatic complex including polynucleotide phosphorylase and RNAase.

Complete amino acid sequence of proteins with polynucleotide phosphorylase function were described for a few plants (*Arabidopsis thaliana* - AK117900, *Pisum sativum* - AAC50039.1 and *Oriza sativa* - BAF20896.1). Since this enzyme represents one of the key components of the fertility restoration physiological events it was analyzed *in silico*.

Thus, the study of polynucleotide phosphorylase from model plants and ESTs (Expressed Sequence Tags) from sunflower revealed four sequence with maximum sequence similarity. Through the program CAP3 and DNA-RNA-protein translator was modeled *in silico* polynucleotide phosphorylase protein (fig. 3).

>Contig1
ESLTPTPPPSYSSRHHRGSLNLIPNALQPPASMEDRTLTSHIQQPPDTTNPTSALQNLIPHL
LFALFFHREDPQIASQLGLIVMTSLVLRKKLSRFFLTSIRLRFQSEIDLLLKLVKEDKLVDL
LLEMERLLSTRQFVCLMFQANLLTFFPMSVHYQERFSAAGRTSGGFFKREGRTKDHEVL
ICRLIDRPLRPTMLKGFYHETQILSWVLSYDGLHSPDSLAVTAAGIAVALSEVPNTNTVA
GVRIGLIGDKFVVNPTTMEMEDSKLDLLVAGSETGILMIEGYCDFLPEEKLLEAIEVGQD
AVRAICKEVDNLVKICGKPKMLDSIKLPPPELYKHVEEIAGDVLVDVLQIKNKVPRRKAL
SLLEEKVLSILTEEGYVSKSESCVGAEITPDMLEDEDEEEEVVVDGEYDEGDVQIKPVFK
KPTPTFYSEVDVKLVFKSVSSKYLRNRIVKGGKRSDGRTSEEIRVIDAECGLLPRVHGSA
LFTRGETQALAVVTLVINNG

Fig. 3 – Hypotetic sequence of sunflower polynucleotide phosphorylase. *ESTs used for in silico analysis (DY912504.1; GE494064.1; EL415574.1 and EL443928.1).*

This hypothetical protein shows high degree of similarity with polynucleotide phosphorylase from other plant species: 78% - *Ricinus communis*, 77% - *Spinacia oleracea*, 75% - *Pisum sativum* and 72% - *Arabidopsis thaliana*, which confirms the reliability of obtained results.

CONCLUSIONS

1. Proteins associated with CMS in various plant species have domains such as: *Retroviral aspartyl protease*, *Major Facilitator superfamily* etc., demonstrating their involvement in cell apoptosis and those associated with fertility restoration - *RNA recognition motif, Radical SAM superfamily*, etc. These data allowed to ascertain a hypothetical mechanism of CMS-Rf system in plants.

2. Similar features have been highlighted for following CMS-Rf systems: sunflower (PET1) and maize (CMS-S); *Brassica napus* and *Raphanus sativus*; maize T type and *Oryza sativa* (Boro II).

3. It was *in silico* modeled enzyme *polynucleotide phosphorylase* from sunflower that potentially could be involved in polyadenylation of orfH522 transcript with subsequent digestion by RNAase, thus restoring pollen fertility.

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