Maize restorers of fertility loci polymorphism

H.I. Slischuk, **N.E. Volkova**

Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation, Ukraine, 65036, Odessa, str. Ovidiopols'ka doroga, 3

geoncrfqg@gmail.com

Introduction

Cytoplasmic male sterility (CMS) – complex feature, which is a result of specific mutation of mitochondrial genome, which leads to microspore degeneration, and, ultimately, to inability to produce fertile pollen. Specific nuclear loci, which are called *Restorers of fertility* (*Rf*), nullify the action of CMS-associated mitochondrial open reading frames, leading to full restoration of male fertility in genotypes with dominant *Rf* loci.

There are at least three types of CMS in maize, each caused by specific mitochondrial mutation, and restored by specific *Rf* loci. T-type CMS is caused by T-urf open reading frame, and restored by *Rf1* and *Rf2* loci. S-type CMS is caused by *orf355-orf77*, and restored by *Rf3* locus. And CMS-C is caused by chimerical *atp6-atp9* open reading frame, and restored by *Rf4*, *Rf5* and *Rf6* loci [1]

CMS is widely used in hybrid maize breeding programs, especially in Ukraine, so there is great demand in stable and reliable marker system, allowing quick and rapid maize lines differentiation according to their CMS and fertility restoring status. Given the fact that CMS is widely used in maize breeding programs, aim of our work was devoted to the restorers of fertility loci polymorphism investigation and molecular markers system creation.

Materials and Methods

86 maize lines, differing in their CMS-associated loci and *Rf* allele spectra, were used as plant material. DNA was isolated from one week old seedlings according to [2]. Nucleotide and amino acid alignment was performed, using Needleman-Wunsch [3] and Smith-Waterman [4] method, using BLAST and MEGA software [5].

Primer design, as well as *in silico* PCR was performed, using FastPCR software [6].

Results and discussion

Rf1 gene nucleotide sequences polymorphism was investigated. Polymorpic sites, differing within dominant and recessive alleles were found. PCR primer pair VSGIRf1 (5' – 3'): sense – ctcagaaggtttctttgtgc, antisense – cggagacgacgcggcagagg. Designed primers allowed both *in silico* and *in vitro* differentiation of *Rf1* and *rf1* alleles (fig. 1)

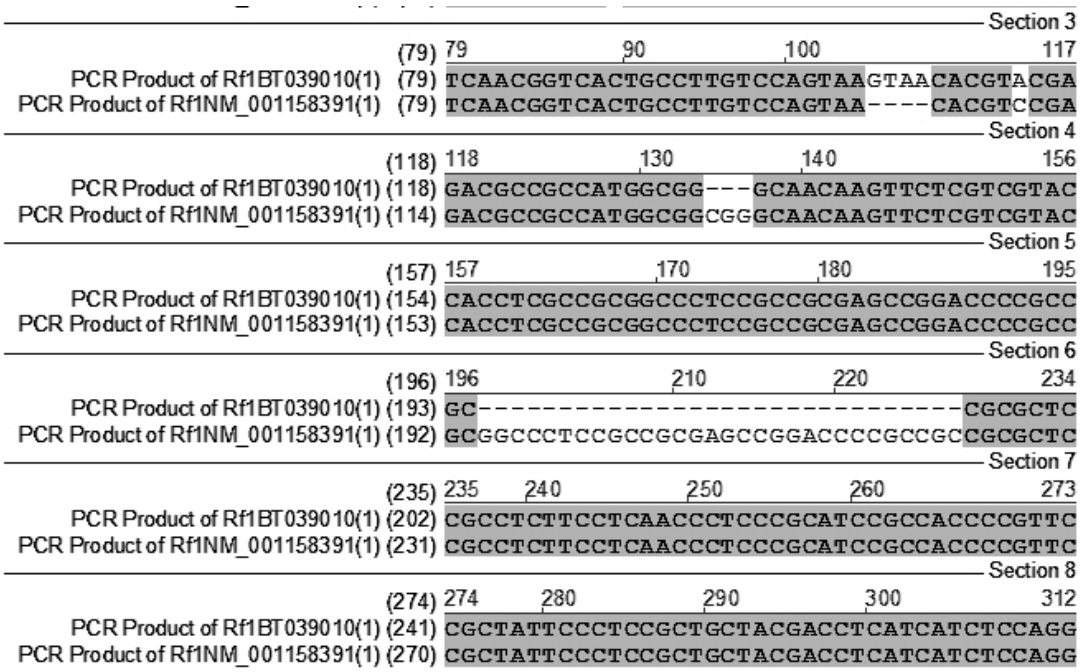

Fig. 1. PCR products nucleotide alignment, conservative sites highlined by grey

Translated *Rf1* sequences were aligned against protein database. Homologs of RF1 protein, belonging to pentatricopeptide family, were found (fig. 2).

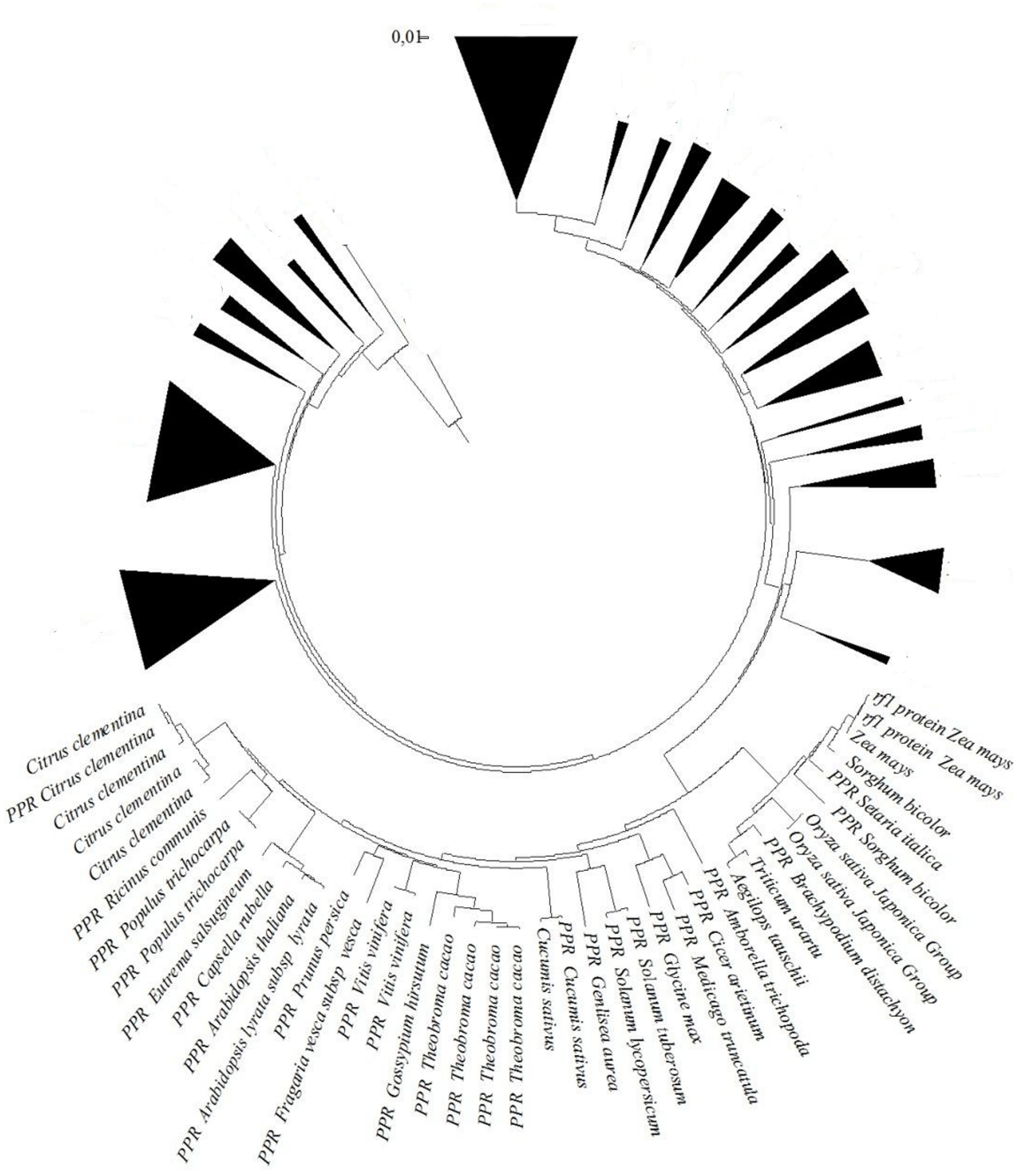

Fig. 2. Phylogenetic dendrogram, representing evolutional relationship between RF1 homologs

Rf2 gene polymorphism was being researched, using nucleotide alignment. Several polymorphisms, including numerous SNP, InDel mutations, as well as MS polymorphisms were detected. Primer pair VSGI4SP: sense gtcgtgactgcatccaagta, antisense VSGI4ASP: cttgcattttgatggtggta, specific to the exons 8-10 of *Rf2* gene was designed. PCR products differed between *Rf2* and *rf2* lines – only *rf2* lines produced 216 bp long PCR product, while *Rf2* lines didn't produced anything.

Gene *whp1*, linked to *Rf3* locus, was analyzed. Numerous SNPs, InDel mutations and SSR polymorphisms were found, including complex MS $(tcc)_2(gac)_2tactag(ct)_2cgcttgctcc(gac)_n$ in promoter region and $(cta)_2gt(cta)nc(cccta)_4(cta)_2$ in intronic region. Six primer pairs, specific to different whp1 regions, were designed (fig. 3).

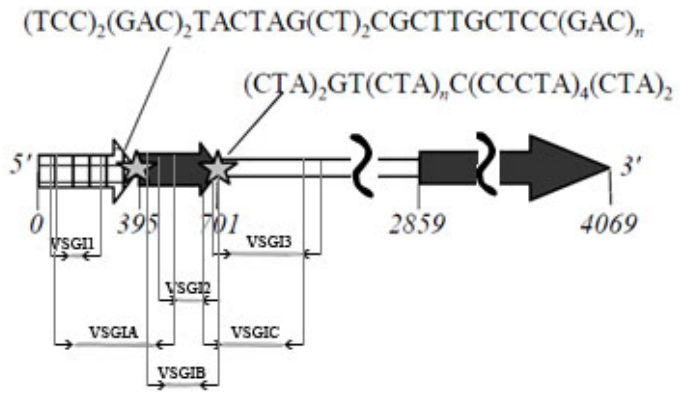

Fig. 3. *whp1* gene, priming sites shown by tiny arrows. Dashed arrow is promoter, gray arrows are exons 1 and 2 respectively

Primer pairs VSGIA and VSGIC allowed differentiation of *Rf3* and *rf3* lines.

As a result of our investigation, designed primer pairs VSGIRf1, VSGIRf2. VSGIA and VSGIC could be used as molecular markers to differentiate loci, associated with CMS-T and CMS-S respectively.

Resume

Rf1, Rf2 and *whp1* gene, associated with restoration of T-type and S-type CMS were researched, using nucleotide and aminoacid alignment for phylogenetic analysis, Numerous polymorphic sites were found. Primer pairs, specific for polymorphic sites were designed. Designed primers allowed differentiation of recessive and dominant alleles of these genes in *in silico* PCR.

86 maize lines, differing by their CMS-associated mitochondrial loci, as well as their restorer of fertility loci allelic status were investigated using designed primers. Maize genotypes were successfully differentiated, according to their *Rf1, Rf2* and *Rf3* allelic status, using designed primers. Potential marker status of designed PCR primers was proven.

References

1. J. O. Allen, C. M. Fauron, P. Minx, L. Roark, S. Oddiraju, G. N. Lin, L. Meyer, H. Sun, K. Kim, C. Wang, F. Du, D. Xu, M. Gibson, J. Cifrese, S. W. Clifton, and K. J. Newton, “Comparisons among two fertile and three male-sterile mitochondrial genomes of maize.,” *Genetics*, vol. 177, no. 2, pp. 1173–1192, Oct. 2007.

2. M. G. Murray and W. F. Thompson, “Rapid isolation of high molecular weight plant DNA.,” *Nucleic Acids Res*, vol. 8, no. 19, pp. 4321–4325, Oct. 1980.

3. S. B. Needleman and C. D. Wunsch, “A general method applicable to the search for similarities in the amino acid sequence of two proteins.,” *J Mol Biol*, vol. 48, no. 3, pp. 443–453, Mar. 1970.

4. T. F. Smith and M. S. Waterman, “Identification of common molecular subsequences.,” *J Mol Biol*, vol. 147, no. 1, pp. 195–197, Mar. 1981.

5. K. Tamura, M. Nei, and S. Kumar, “Prospects for inferring very large phylogenies by using the neighbor-joining method.,” *Proc Natl Acad Sci U S A*, vol. 101, no. 30, pp. 11030–11035, Jul. 2004.

6. R. Kalendar, D. Lee, and A. H. Schulman, “FastPCR software for PCR, in silico PCR, and oligonucleotide assembly and analysis.,” *Methods Mol Biol*, vol. 1116, pp. 271–302, 2014.