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Original scientific paper

POTENTIALLY A NEW SUBTYPE OF THE CYTOPLASMIC MALE STERILITY S-TYPE IN MAIZE

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In gene-bank maize collection of Maize Research Institute Zemun Polje (MRI) two samples with untypical mtDNA profile for cytoplasmic male sterility (cms) were identified. These two samples showed typical multiplex polymerase chain reaction (PCR) band for cms-S, but also an additional band of unknown nature. It is assumed that the additional band is the result of a rearrangement of the two mitochondrial episomes characteristic for the cms-S in maize or a duplication of the part of cms-S mitochondrial genome. Additional field and laboratory experiments are necessary in the further lightening of this phenomenon.

Key words: cytoplasmic male sterility, episom, multiplex PCR reaction, MRI gene-bank

INTRODUCTION

In maize (*Zea mays* L.) three types of cytoplasmic male sterility (cms) are known: C, T and S-type. Among them, S-type is the most abundant (BECKETT, 1971, VANCETOVIC *et al*., 2010) and with the highest number of sources (BECKETT, 1971). Also, this type is the least stabile one (WEIDER *et al*., 2009), i.e. it shows the largest degree of the late break of sterility. This is probably the consequence of the presence of higher number of alleles on the restorer-of–fertility *Rf3* loci or the 2L chromosomal region tightly linked with *Rf3* loci (GABAY-LAUGHNAN *et al*., 2004) as well as of spontaneous nuclear and cytoplasmic mutations on some other loci that cause reversion of sterile to fertile plants (GABAY-LAUGHNAN *et al*, 1995).

The known sources of cms-S type of maize are: B, CA, D, EK, F, G, H, I, IA, J, K, L, M, ME, ML, MY, PS, R, S, SD, TA, TC, VG and W (BECKETT, 1971; Cornell's collection of cms sources of maize, Urbana, Illinois). Sorting newly discovered sources to the particular type of cms can be done by crossing these sources with the carriers of the restorer-of-fertility (*Rf*) genes

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for the three types of cms (BECKETT, 1971). This procedure, however, requires a lot of field work, time, and the obtained data are not completely reliable. However, a method of multiplex polymerase chain reaction (PCR) developed for this purpose by LIU *et al*. (2002) is more reliable and much faster (IGNJATOVIĆ-MICIĆ *et al*., 2006).

In the MRI maize gene-bank additional 85 new sources of cms-S among local varieties from the Ex Yugoslav territories were revealed by the method of multiplex PCR. Apart from these, two new potential sources of cms-S were discovered in two varieties. They were characterized with a regular PCR band for cms-S and with an additional band not characteristic for either known type of cms in maize (VANCETOVIC *et al*., 2010). For the confirmation that this is a truly new phenomenon, it was necessary to analyze all known sources of cms-S together with these two samples by the multiplex PCR method. If the expected differences were revealed, they would make an argument for potential existence of a new cms-S subtype in maize.

The aim of this research was to confirm the existence of a new cms-S subtype in two local varieties from Ex Yugoslav teritory.

MATERIALS AND METHODS

In this work 24 sources of cms-S (provided by the courtesy of Marty Satch, the director of the Maize Genetics Cooperation Stock Center, Illinois, Urbana) and two sources from Ex Yugoslav collection of MRI, that previously have shown an extra band in the PCR analysis, were analyzed (Table 1). The sterility rating, i.e. LB-late break of sterility according to the Cornell Cytoplasms stocks data, for both standard and Ex Yugoslav samples according to field experience are also given in Table 1.

The sterility rating scale used was according to BECKETT's (1966):

1 = Completely male sterile, no anthers exerted
2 = Male sterile, sterile anthers exerted
3 = Partially fertile, subnormal anthers, some pollen shed, number of anthers highly variable
4 = Slightly subnormal anthers, more than 75% of the anthers exerted, pollen shed
5 = Normal anthers exerted at or before silking time

The first Ex Yugoslav variety was represented by the progeny of one ear (cytoplasm is inherited only maternally, so in the next generations it is uniform), and the second variety by the progenies of two ears. Sources from the Ex Yugoslav collection were found during the experiment for search of hypothetical restorer cytoplasm for *ms10* gene in maize (VIDAKOVIC *et al*, 2002). They were revealed by crossing of single plants from the varieties, and than backcrossing of progenies from these crosses with heterozygous *ms10*/*Ms10* tester. For the final backcross progenies with more than 1/3 of sterile plants it was supposed that they contain some type of cms, since expected frequency of *ms10/ms10* sterile plants was 1/8. (For the progenies with eventual restorer cytoplasm, expectation was that they would be a 100% fertile).

All these samples were analyzed by multiplex PCR method (LIU *et al*, 2002). Three samples that had known types of cms (S, T and C) were included as controls. DNA extraction was done from six seeds per genotype with CTAB buffer. The PCR reaction mix contained 1 x PCR buffer, 1.5 mM $MgCl_2$, 0.8 mM dNTP, 50 pmol of CMSSF, CMSSR, CMSTF, CMSTR, CNSCF and CMSCR primers, 1 U of *Taq* polymerase and 50 ng of DNA. The PCR amplification consisted of 40 cycles (steps 2, 3 and 4), as follows: 1) initial denaturation 94°C/2 min, 2) denaturation 94°C/1 min, 3) annealing 55°C/1 min, 4) elongation 72°C/1.30 min, and 5)

final elongation 72°C/5 min. The amplified fragments were separated on 1.5% agarose gels, stained with ethidium-bromide and photographed.

Table 1. List of analyzed genotypes, their classification (according to BECKETT, 1966) and sterility rating

#	Genotype	Type of cms	Sterility rating (indicating LB-late break of male sterility)
1	Control for cmsS	S	1
2	Control for cmsT	T	1
3	Control for cmsC	C	1
4	B-14AHt ML	S	3-4
5	B-37Ht CA	Probably S	1
6	B-37Ht D	Unclassified	1-2
7	B-37Ht EK	Probably S	1
8	B-37Ht G	S	1
9	B-37Ht J	S	1
10	B-37Ht K	Probably S	1
11	B-37Ht ME	Unclassified	1
12	B-37Ht MY	Probably S	1
13	B-37Ht PS	Probably S	1
14	B-37Ht S	S	1
15	B-37Ht SD	Probably S	1
16	A-619 B	Unclassified	5
17	A-619 F	Probably S	3
18	A-619 H	Probably S	3
19	A-619 R	S	3-4
20	A-619 VG	S	4
21	A-619 W	Probably S	4
22	A-632 I	S	4
23	A-632 IA	Probably S	3-4
24	A-632 M	S	1-4
25	A-632 TA	Probably S	3-5
26	A-632 TC	Two sources of cytoplasm	4
27	Co-220 L	Probably S	4
28	OP Ex Yu 1/1	?	1
29	OP Ex Yu 2/1	?	1
30	OP Ex Yu 2/2	?	1

RESULTS

All the sources of cms-S obtained from the Maize Genetics Cooperation Stock Center, Urbana, Illinois, have shown the characteristic multiplex PCR band for cms-S (Figure 1). On the other hand, the two Ex Yugoslav sources have shown another band, non-characteristic for any type of cms in maize. In the second Ex Yugoslav source, the progeny of the first ear did not give the additional band, implying the existence of cytoplasmic variability in this variety. It should be

noted that the typical S-band in the known sources, as well as in the progeny of the first ear of the second Ex Yugoslav variety, was much stronger than in the two potentially new sources.

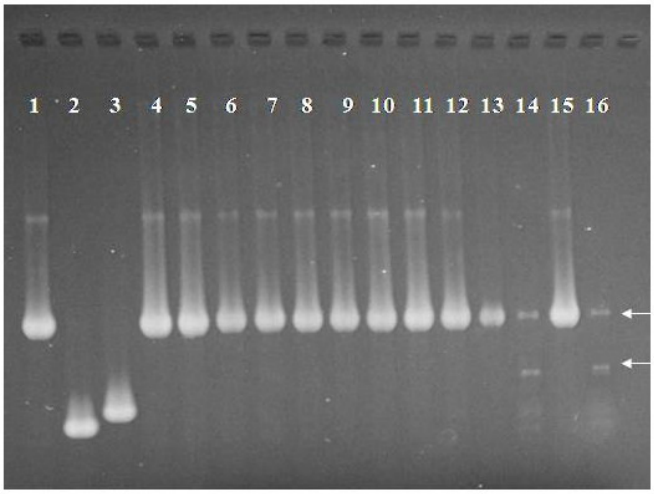

Figure 1. PCR amplification of CMS types with specific primers for C, T and S cytoplasm. Lanes 1, 2 and 3 – controls for CMS_S, CMS_C and CMS_T; Lanes *4 to* 13 - CMS-S genotypes from Maize Genetics Cooperation Stock Center (A-619 H, A-619 R, A-619 VG, A-619 W, A-632 I, A-632 IA, A-632 M, A-632 TA, A-632TC, Co-220 L); Lanes 14, 15 and 16 – genotypes from MRI gene bank (OP Ex Yu 1/1, OP Ex Yu 2/1, OP Ex Yu 2/2). Amplification products giving a new profile with two bands (a new CMS-S sub- type?) are shown in lines 14 and 16.

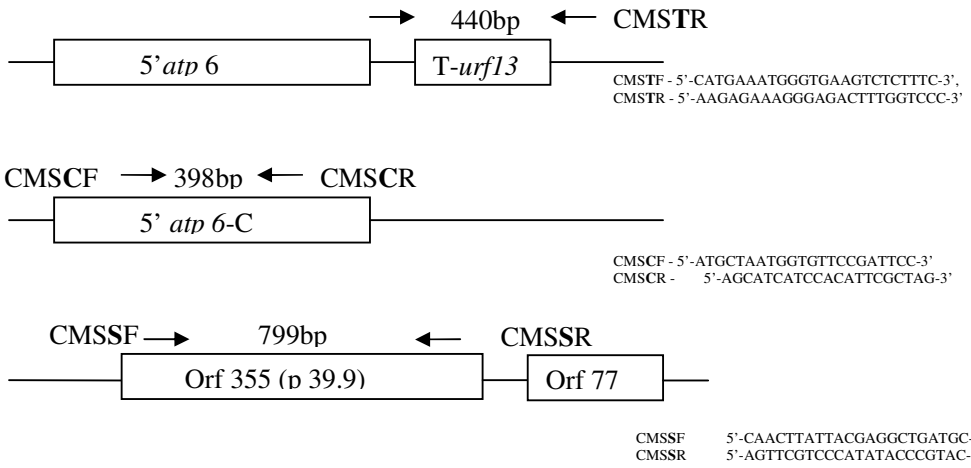

Figure 2. mtDNA recombinant regions of T, C and S cytoplasm types and the binding sites of the specific primers (taken from LIU *et al*., 2002)

DISCUSSION

Since cms in plants is coded by genes that are located on the mitochondrial DNA (SCHNABLE and WISE, 1998), different PCR product profiles with specific cms-S primers in two varieties indicate changes in mitohondrional genome that could have caused occurrence of a new source of cms-S. The maize CMS-S mitochondrial genome appears to exist mostly as linear molecules (SCHARDL *et al,* 1984; ALLEN *et al,* 2007). In cms-S mitochondria, the genome structure is complicated by the existence of two linear double-stranded DNA episomes, S1 (6397 bp) and S2 (5453 bp) (LEVINGS and SEDEROFF, 1983; PAILLARD *et al*, 1985). These episomes have identical terminal inverted repeats (IRs) of 208 bp (LEVINGS and PRING, 1979). Recombination between the episomal and integrated IRs results in the rearrangement of the mitochondrial genome that can cause the reversion of sterile to fertile plants (SCHARDL *et al*, 1984). A similar process could be the reason for additional PCR band in the two varieties. The explanation includes the mode of PCR reaction of the two progenies of the second variety. Namely, the first ear progeny had the profile characteristic for the cms-S, while the second ear progeny profile was modified with additional band that could arise from the rearrangement of the mitochondrial genome in this variety.

Typical S-band was much stronger in the known sources compared to the S band in the two varieties. At the same time the S band in the two varieties was about the same strength as the additional band. It was shown that the repeated region R contains two himeric open reading frames (ORFs), i.e. ORF355 and ORF77 in cms-S (ZABALA *et al*, 1997). Expression analysis identified R region and ORF77 as the most important candidate gene for S-CMS (ZHANG *et al*., 2003; MATERA *et al*., 2011). The binding sites of the specific primer pairs are located within ORF355 sequence (Figure 2). Since the primer binding sites are highly specific and the band intensities are similar it could be assumed that the PCR profile is the consequence of the duplication of a smaller part of the cms-S mitochondrial genome. More ever, as the additional band is not characteristic for C or T-type of cms, we exclude the possibility that this result is the consequence of the mixture of two known types of cms. In the work of LIU *et al*. (2002), minor molecular differences between cms subtypes did not affect primer specificity or PCR product lengths, as all tested lines matched the pattern of the respective B37 reference material. These results support our speculation on some more severe genetic rearrangements within mtDNA.

In any case, it is necessary to test these potentially new sources in the field with the *Rf* genotypes for cms-T, S and C, in order to confirm affiliation of the two sources to cms-S. If this would be the case, it would be the confirmation of a new subtype within the cms-S. On the other hand, if these genotypes do not belong to any known type of cms in maize, there is a possibility that a new cms type is discovered, although this assumption is less probable. Besides, additional molecular research on the mitochondrial genome of these two sources is necessary.

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NOVI PODTIP CMSS KOD KUKURUZA?

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Izvod

U banci gena kukuruza (*Zea mays* L.) Instituta za kukuruz Zemun Polje otkrivena su dva uzorka koja sadrže netipičan mitohondirjalni genom koji uzrokuje citoplazmatičku mušku sterilnost (cms). Ova dva genotipa pokazuju tipičnu multipleks *polymerase chain reaction* (PCR) traku za S-tip citoplazme, ali i dodatnu traku, čije je poreklo za sada nepoznato. Smatra se da je ona proizvod ili rearanžiranja dva mitohondrijalna epizoma karakteristična za cmsS kukuruza ili do sada još nesekvencioniranih gena koji uzrokuju cms. Za dodatnu potvrdu da je ovo zaista nov fenomen, uradili smo analizu svih poznatih izvora cmsS zajedno sa ova dva uzorka. Nijedan od analiziranih podtipova nije pokazao dodatnu traku koja je nađena u našim uzorcima. Dodatna poljska i laboratorijska istraživanja neophodna su u daljem razjašnjavanju ovog fenomena.

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