VALIDATION OF SCAR-MARKER FOR RESTORATION FERTILITY GENE IN UKRANIAN INITIAL MATERIAL OF SUNFLOWER

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ABSTRACT

The breeding lines, lines of mutant origins, samples of interspecific hybrids and varieties of sunflower (in general 105 sample) for the presence of HRG01 locus, which linked with the *Rf1* gene have been analyzed. The HRG01 locus has always identified when this gene is present in the plant material. In the result of amplification band 426 bp has been synthesized. In the absence of the *Rf*1 gene the specific band in the samples of sunflower was not found. Therefore, amplified product in male sterility restoration lines was not synthesized, while its synthesis was in male restoration lines. Percentage of samples with HRG01 locus in samples obtained from interspecific hybrids was 62. It was found that the allele of HRG01 locus occurred in 30 % of sunflower varieties. Its frequency was varied from 0.053 to 0.263.

Key words: Sunflower, SCAR marker, *Rf* gene

INTRODUCTION

The different types of DNA markers widely used in genetic and breeding research of sunflower. A significant amount of information about variability of markers is accumulated by RAPD, AFLP, SSR and SNP analyses. It has been constructed detailed genetic maps of sunflower including resistance genes to pathogens, morphological and biochemical traits, which are relative to a particular type of molecular markers (Jan et al., 1998; Lai et al., 2005; Heesacker et al., 2008; Tang et al., 2002). It was possible not only with the development of molecular methods, but detailed studies of genetics of resistance, morphological and biochemical traits of sunflower that studying the effects of most genes (Sharypina et al., 2008). Therefore, information on linkage marker and gene can be used in marker-assisted selection, which is widely used in many countries to intensify the breeding process (Popov & Kirichenko, 2010).

Restoration of pollen fertility in sunflower is controlled by *Rf1* gene with the possible interaction between at least two or three *Rf* genes. Features of genetic control of pollen fertility restoration are summarized in review articles and monographs (Gavrilova & Anisimova, 2003; Vedmedeva & Tolmachev, 2006; Popov & Kirichenko, 2010). The main step of creation of the sunflower restorer lines is determination of the ability of these lines to fully restore fertility of pollen in their crosses with male sterile lines. This selection process is laborious and time consuming (Popov & Kirichenko, 2010). Therefore, the creation of inbred sunflower lines should involve different DNA markers for screening the presence of *Rf* genes in various initial material that optimizes the breeding process.

Currently, the details of mapping of restoring fertility pollen gene *Rf1* has been gathered (Jan et al., 1998; Horn et al., 2003; Kusterer et al., 2005; Schnabel et al., 2008). Thus, based on polymorphic of RAPD fragments two SCAR markers – HRG01 and HRG02 were developed (Horn et al., 2003). One of the closely linked markers TRAP was converted to STS marker (Yue et al. 2010). Distance between STS and *Rf1* was 0.4 cM. Construction of a genetic map based on SSR-markers revealed that the *Rf1* gene is located in 13 linkage group (Tang et al., 2002). Also the molecular mechanisms of interaction between mitochondrial and nuclear genes were clarified (Moneger et al., 1994; Horn et al., 1999).

For efficient use of molecular markers in the breeding process their initial material of various origins should be validated. The purpose of this study was to establish the presence of SCAR-marker (HRG01) linkage with the gene *Rf1* in various breeding material of sunflower.

MATERIAL AND METHOD

Thirty seven inbred lines of sunflower created in the laboratory of breeding and genetics of sunflower of the Plant Production Institute named after V. Ya. Yuriev of NAAS (Kharkiv, Ukraine) were involved, including 11 male sterile lines, 19 male sterility restoration lines and 7 mutant lines. In addition, the study used 29 sunflower samples that obtained from interspecific hybrids. Also we involved 39 sunflower varieties of different origins.

SCAR-marker identification was performed by PCR with a pair of primers that flank certain areas of genomic sunflower DNA. The nucleotide sequences of primers to locus HRG01 were as follow: F: TATGCATAATTAGTTATACCC and R: ACATAAGGATTATGTACGGG (Horn et al., 2003).

PCR was performed using reagent kit GenePak PCR Core of LLC "Laboratory Izogene" (Russia). The final volume of the reaction mixture was 20 µl and contained 20 ng of genomic DNA with the addition of 0.2 mM of each primer. In test tubes of reaction mixture 20 µl of mineral oil was added. PCR was performed in thermocycle "Tertsyk" (Russia) using program according (Kusterer et al., 2005).

PCR products were run on 2 % agarose gel with high resolution and the addition of ethidium bromide in the low-molarity buffer. The amplified products were visualized using photography in UV light with photosystem NikonD50. For determination of the lengths of PCR products DNA ladders 50 bp and Mcombi (LLC " Laboratory Izogene", Russia) were used.

RESULT AND DISCUSSION

Cytoplasmic male sterility (CMS) always is in use for creation of high yields sunflower hybrids. On the base of CMS the inbred lines of three types – male-sterile lines ($cyt^S rf_1 rf_1$), sterility fixing lines ($cyt^N rf_1 rf_1$) and male sterility restoration lines ($cyt^N Rf_1 Rf_1$) create. The creation of inbred lines of sunflower is based on the interaction between classical cytoplasm PET1 with gene *Rf*.

At the first stage of the testing of marker HRG01 the inbred lines of sunflower (male-sterile and male sterility restoration lines) from collection of initial material of the laboratory of sunflower breeding and genetics of Plant Production Institute named after V. Ya. Yuriev of NAAS were involved. These lines are used to create single cross and three-way cross hybrids of sunflower in Plant Production Institute (Kirichenko et al., 2014). According to literature PCR product with size 426 bp indicates the presence of HRG01 locus and as a consequence of *Rf1* gene presence in sunflower genotypes. Involvement of inbred lines of Kharkiv breeding in the research allowed us to conduct the validation of HRG01 markers. Thus, absence of amplicon has been observed in all male-sterile lines, while in male sterility restoration lines

were identified PCR product with size of 426 bp. These results confirm the diagnostic ability of the marker to identify gene Rf_1 in the plant genotypes (fig.1).

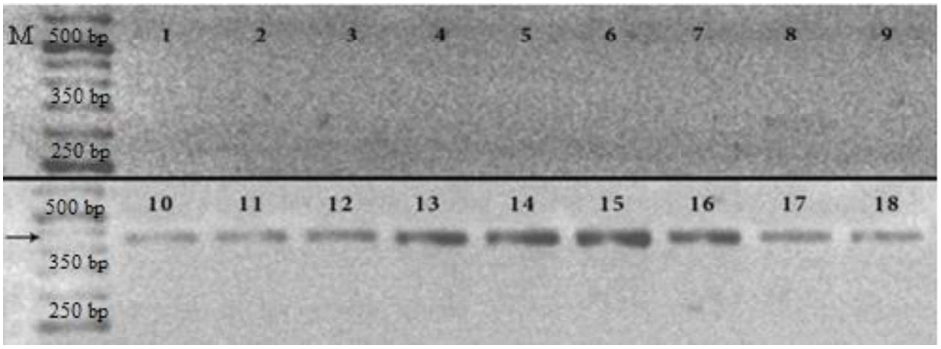

Fig.1 Electrophoregram of separation of amplified products of SCAR-marker HRG 01. 1–9 – male-sterile lines; 10–18 – male sterility restoration lines. The arrow shows the PCR product of the size of 426 bp.; M – DNA ladder «Mcombi».

Six lines of mutant origin were also tested with a pair of primers to HRG01. In the three lines of sunflower – Mkh1829, Mkh4 and Mkh42 the amplified product with size of 426 bp was found. It was absent in the lines Mkh2122, Mkh108, Mkh1091.

For the molecular genetic analysis samples obtained from interspecific hybrids of different origin have been involved. They were created with using annual wild species of sunflower *H. annuus*, *H. argophyllus* and *H. debilis*. It should be noted, that these samples are not analyzed for the presence of Rf_1 genes using classical plant breeding methods. As a result, molecular analysis revealed that 17 samples had specific PCR product. The size of this product was 426 bp, which corresponds with male sterility restoration lines, in which the presence of Rf_1 gene has been clearly identified by hybridization with male-sterile lines. The frequency of such lines was 0.586. In 12 samples obtained from interspecies hybrids PCR product with size of 426 bp (frequency 0.414) was not observed. The results allow to differentiate experimental material into two groups – the samples male-sterile lines type (absence of amplicon 426 bp) and the samples of male sterility restoration lines type (presence of amplicon size 426 bp).

Sunflower varieties were further involved to test the marker HRG01. The varieties of sunflower are the source of initial material for a complex of traits, including genotypes with genes Rf_1. However based on the genetic structure the most varieties are fixers of sterility. This means that the populations consists mainly of genotypes $cyt^N rf_1 rf_1$. Therefore, for intensification of creation of male fertility restorer lines from sunflower varieties DNA markers linked to the gene Rf_1 are need to use. Using pairs of primers to HRG01 locus amplified product with size of 426 bp was obtained. Results of separation of amplified products are shown on figure 2. It should be noted that amplified product 426 bp was not identified in all varieties-populations because most genotypes of these varieties are fixers of sterility.

In the studies of the molecular genetic structure of Ukrainian varieties the 426 bp allele of HRG01 locus were identified only in four varieties – Zaporiz'kii konditerskii, Mistsevii 1, Mistsevii 2, Mistsevii 15, ChaS. The frequency of allele 426 bp for these varieties was 0.105, 0.263, 0.579, 0.684 and 0.316, respectively. In other varieties this band was not detected.

However 426 bp allele of HRG01 locus was not identified in a sample of sunflower varieties of Russian breeding. In the varieties of sunflower breeding of Greece only variety Rodopi detected allele of 426 bp size with frequency 0.053. In other varieties allele of this size is not identified.

In French variety Nain noir and varieties Slovenska siva and Bucianska olejna from Czechoslovakia we also not found band 426 bp.

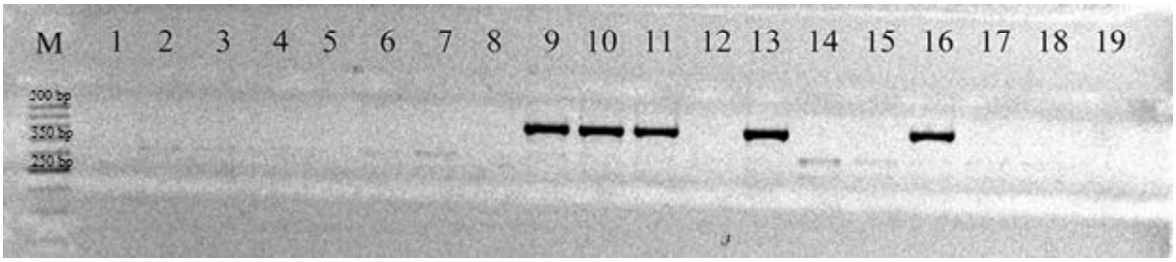

Fig. 2. Electrophoregram of separation of amplified product of SCAR-marker HRG 01 in sunflower variety Mennonite (Canada). 1–19 – genotypes of variety Mennonite; M – DNA ladder 50 bp.

Analysis of the distribution of frequency of allele 426 bp of HRG01 locus for the Hungarian varieties revealed that allele was present only in varieties Mezoeheguesi and Lovaszpatonai. The allele frequency was 0.053 in both varieties. In two other varieties of Hungarian breeding allele was not detected.

According to the analysis of HRG01 locus allele 426 bp was identified in three out of four USA varieties. These are the varieties Untitled (PI 432515), Arrowhead, Ghray Mammoth. The frequencies of allele in these varieties were 0.105, 0.158 and 0.211 respectively. In variety Mingren allele 426 bp is not detected.

In varieties of Canadian breeding the allele of size 426 bp of HRG01 locus was identified only in variety Mennonite. Its frequency was 0.263. In other varieties allele is not detected.

In general, it should be mentioned that the 426 bp allele of HRG01 locus in 12 (30 %) of sunflower varieties is distributed and in 27 varieties (70 %) is not identified.

CONCLUSION

SCAR-marker (locus HRG01) clearly identified in breeding lines, which are shown of the presence or absence of a gene *Rf1*. Using samples of sunflower, created with the involvement of annual wild species and varieties has proved the presence in their genotypes of PCR products with size of 426 bp, which indicates also the presence of the *Rf1* gene. The results make it possible to conduct targeted selection of male lines from interspecific hybrids and varieties.

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