

**APPLICATION OF MOLECULAR MARKERS
IN SUNFLOWER BREEDING**

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Pankovic-Saftic D. (2007): *The application of molecular markers in sunflower breeding*. – Genetika, Vol. 39, No. 1, 1 - 11.

The results of the application of molecular markers in sunflower breeding obtained in the Institute of Field and Vegetable Crops in the last decade are reviewed. Our results on genetic distance (GD=7-75%) between sunflower inbred lines obtained with RAPD and SSR markers, indicate large variability and provide important information for the selection of parental lines for future crosses. Interspecific hybridization is often used in sunflower breeding. As only some populations of *H. giganteus* and *H. maximiliani* are resistant to sunflower diseases, the investigation of genetic variability in/between two species is of interest. The results obtained with SSR markers are presented. The successful hybridization between *H. rigidus* and *H. annuus* was confirmed with RAPD markers, and the variability between F₁ and BC₁F₁ plants is discussed. Desirable alleles and haplotypes can be detected with molecular markers both in early phases of plant development and in early

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phases of the production of improved lines, which reduces or completely eliminates the large number of testing cycles for desirable phenotypes. CAPS markers for resistance to downy mildew, that can be used in marker assisted selection are presented.

Key words: genetic variability, inbred lines, wild species, RAPDs, SSRs

INTRODUCTION

Recent advances in biotechnology, particularly in molecular marker techniques, have led to the development of novel tools that offer the promise of making plant breeding more precise and faster (VARSHNEY et al., 2004). Plant breeding in its simplest form consists of 1) generation of genetic variation through recombination and 2) selection to identify superior recombinants for advancement in the breeding program (LAMKEY and LEE, 1993). Molecular markers may complement plant breeding in both areas. Historically, the assessment of genetic diversity was done with morphological and biochemical markers. Due to the strong environmental effects on the expression of these markers, experiments had to be conducted in various environments. The discriminating power of molecular markers, in comparison to morphological and biochemical markers is higher, therefore they are generally superior for assessment of genetic diversity due to better sensitivity and efficiency (LAMKEY and LEE, 1993).

GENETIC VARIABILITY OF INBRED LINES

In allogamous crops, particularly maize, heterotic groups have been established to optimize hybrid performance by choosing promising parental combinations and thus avoiding inferior testcrosses. MELCHINGER (1999) showed that inter-group hybrids in maize had greater parental genetic distance and midparent heterosis, when compared to intra-group hybrids. In sunflower the genetic diversity of inbred lines was investigated with RAPD (LAWSON et al., 1994), RFLP (BERRY et al., 1994; GENTZBITTEL et al., 1994), AFLP (HONGTRAKUL et al., 1997) and more recently with SSR markers (TANG and KNAPP, 2003; PANIEGO et al., 2002; YU et al., 2002). Firstly, all of these investigations indicate that genetic diversity of cultivated sunflower was much wider than previously thought (RIESEBERG and SEILER, 1990). Our first results on genetic variability of sunflower inbred lines developed in Novi Sad (PANKOVIĆ et al., 1997) confirmed that RAPD methodology provides an efficient tool for the analysis of sunflower genome in breeding programs (LAWSON et al., 1994; TEULAT et al., 1994). Both RAPD and other PCR markers that we used revealed that the genetic distance between examined NS sunflower genotypes varied between 7 and 75% (Fig. 1) (PANKOVIĆ et al., 2004a; b) as obtained for other

germplasm of sunflower and other marker systems (BERRY et al., 1994; YU et al., 2002; PANIEGO et al., 2002).

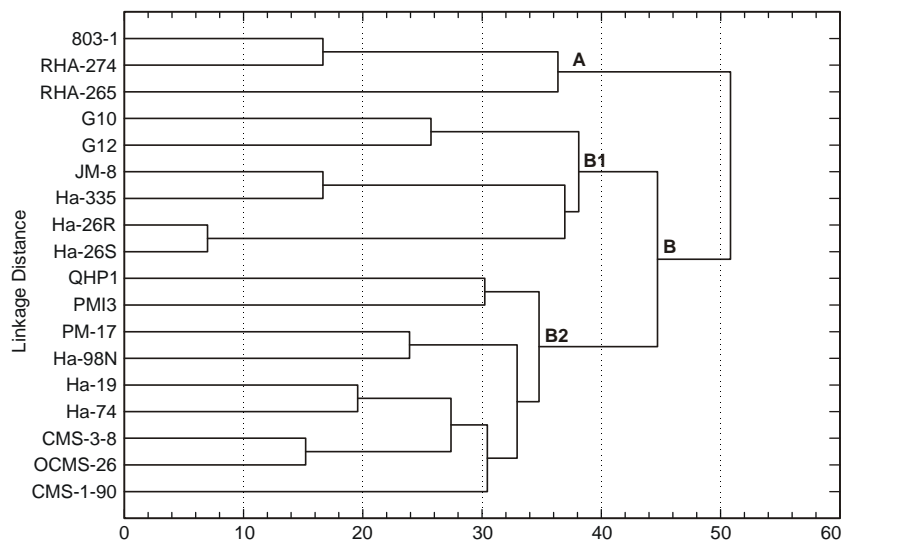


Figure 1. Dendrogram obtained with UPGM analysis (Statistica for Windows, v.5.0) of genetic distances (GD) between all pairs of 18 sunflower inbred lines. GD was calculated based on presence/absence of polymorphic PCR markers in genomic DNA (Panković et al., 2004).

Secondly, the practical aspect of these descriptive phylogenetic analyses is the definition of heterotic groups and patterns in sunflower that have been much less studied than in corn and should have utility for predicting hybrid performance (HONGTRAKUL et al., 1997; CHERES and KNAPP, 1998; CHERES et al., 2000). Sixty two elite oilseed sunflower inbreds were analyzed by RFLPs (BERRY et al., 1994; GENTZBITTEL et al., 1994) or AFLPs (HONGTRAKUL et al., 1997) and strongly separated into maintainer (B) or restorer (R) lines. This grouping is a consequence of sunflower breeding history (B x R) and three breeding bottle-necks: oilseed traits, self-compatibility and self-pollination and hybrid seed-production traits. HONGTRAKUL et al. (1997) and CHERES and KNAPP (1998) proposed a series of heterotic subgroups within these groups for public inbred lines of sunflower developed in the US.

DIAS et al. (2004) have reviewed 54 articles dealing with a prior choice of hybrid parents based on parental distances by means of agronomic and molecular data. Positive divergence-heterosis association was detected in 28 articles, whereas 26 articles revealed negative association or inconclusive results. The authors conclude that divergence and heterosis do not always associate linearly, which explains erratic results in many studies. In sunflower genetic distance estimated from AFLP fingerprints, although significantly correlated with seed yield, was a poor predictor of hybrid performance (CHERES et al., 2000). DNA markers should

be carefully chosen, since genetic distance does not accurately predict hybrid performance, unless markers used in the analysis are associated with genes affecting the trait.

GENETIC VARIABILITY OF WILD SUNFLOWER SPECIES AND IDENTIFICATION OF INTERSPECIES HYBRIDS

Interspecies hybridization is often used in sunflower breeding. Wild perennial sunflower species are generally regarded as important sources for disease resistance (ŠKORIĆ, 1992). TANG and KNAPP (2003) have used SSRs to find extraordinary diversity in native American landraces and wild populations of cultivated sunflower. Due to the high discriminating power of SSRs they were able to uncover the possibility of multiple domestication origins in sunflower, previously thought to be single domestication origin (RIESEBERG and SEILER, 1990).

Only some populations, i.e. clones of *H. giganteus* and *H. maximiliani*, are resistant to for example white rot (HENN et al., 1997; CERBONCINI et al., 2002), therefore the investigation of genetic variability in/between these two species has already attracted attention (MILJANOVIĆ et al., 2000; VASIĆ et al., 2003). Our results on genetic variability between 15 populations of each *H. giganteus* and *H. maximiliani*, obtained with SSR markers, confirm clustering pattern between examined populations obtained after analysis of 30 morphological traits (SAFTIĆ-PANKOVIĆ et al., 2005). Also, some populations of *H. giganteus* grouped in the cluster with *H. maximiliani*, which raised the question of correct determination and/or speciation in *H. giganteus* in our collection.

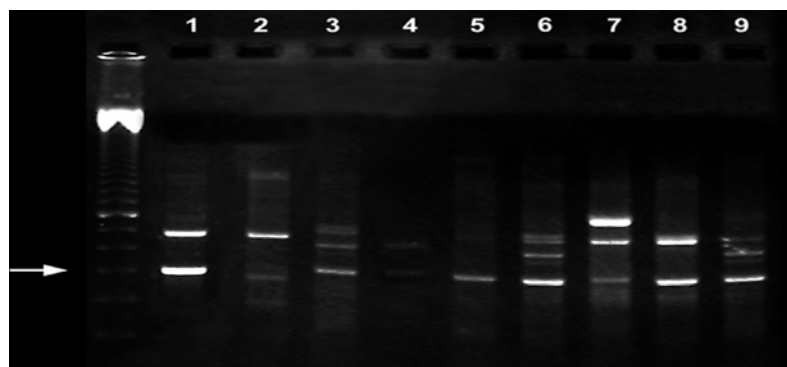


Figure 2. RAPD fragments amplified with primer UBC-39. Arrow indicates marker specific for cultivated *H. annuus* (1), and some interspecies hybrid plants (3, 5) with *H. rigidus* (2), also present in some plants of BC₁F₁ generation (6,8,9) (Atlagić et al., 2003).

Molecular markers are also useful tools in the identification of interspecies sunflower hybrids (RIESEBERG et al., 1995; NATALI et al., 1998; BINSFELD et al., 2001). We have confirmed the hybrid nature of progeny from cross between *H. rigidus* and *H. annuus* by RAPD markers, which also revealed considerable variability between F₁ and BC₁F₁ plants (Fig. 2) (ATLAGIĆ et al., 2003). The genetic distance between *H. rigidus* and *H. annuus* was 83%. After the first backcross, the genetic distance between interspecies progeny and cultivated sunflower decreased from 74% for F₁ plants to 62% for BC₁F₁ plants, indicating that the relative proportion of the genome from cultivated sunflower increased. SSR markers are particularly useful for identification of interspecies hybrids between cultivated sunflower and diploid species from the genus *Helianthus*. We analyzed the progeny of interspecies cross between 28 populations of *H. annuus* and cultivated sunflower, to find that the changes of phenotypic traits of F₁ plants were not always significant. Also, pairing of chromosomes was regular and pollen vitality was high (TERZIĆ et al., 2006). At the same time most of the successful interspecies hybrids were identified with only three SSR primers, indicating that SSR markers are efficient tools for identification of interspecies hybrids between closely related species.

MOLECULAR MARKERS FOR GENES OF AGRICULTURAL IMPORTANCE AND MARKER-ASSISTED SELECTION

The most important precondition for the application of molecular markers for genes of agricultural importance and marker-assisted selection (MAS) are well developed molecular genome maps (VARSHNEY et al., 2004). Among crop plants rice genome map is considered the most saturated containing more than 2200 markers (VARSHNEY et al., 2004). The first molecular genetic linkage maps of cultivated sunflower were developed by means of RFLP (BERRY et al., 1995), RAPD (RIESEBERG et al., 1993) and AFLP (GEDIL et al., 2001) markers. TANG et al. (2002) constructed the first dense public genetic linkage map on the basis of single- or low-copy SSR markers that was 1368.3 cM long with the mean density of 3.1 cM per locus. Three more maps, recently constructed by YU et al. (2003) supply a dense genome-wide framework of sequence-based DNA markers for molecular breeding and genomics research in sunflower. Saturated linkage maps enabled mapping of genes for qualitative as well as for quantitative traits. For example, gene for fertility restoration (HORN et al., 2003) and two QTLs for grain oil content (MOKRANI et al., 2002) were mapped in sunflower.

Though sunflower is considered to be tolerant to drought in comparison to some other crops, we found considerable variability in physiological parameters of drought tolerance among different genotypes (PLESNIČAR et al., 1993; PANKOVIĆ et al., 1999). We have measured these parameters and bulk segregant analysis approach (BSA) was applied to identify RAPD markers linked to genes for drought

tolerance in sunflower (PANKOVIĆ et al., 2000b). We also used RAPD markers in the investigation of mutations in NS high-oleic sunflower lines, as well as in lines with different content of tocopherols (PANKOVIĆ et al., 2000a). As RAPDs are dominant markers they are not useful in molecular marker-assisted selection (MAS).

MAS involve scoring for the presence or absence of a desired plant phenotype indirectly based on DNA banding pattern of linked markers (VARSHNEY et al., 2004). MAS increase the screening efficiency in breeding programmers due to several advantages. For example the segregants can be scored at the seedling stage for traits that are expressed late in plant development (grain quality, male sterility, photoperiod sensitivity). Also, traits that are difficult, expensive or time consuming to score and measure can be screened in MAS (drought tolerance, resistance to diseases). Finally, heterozygotes are easily identified and distinguished from either homozygotes without progeny testing. MAS for two rust resistance GENES IN SUNFLOWER WAS REPORTED USING RAPD-SCAR MARKERS (LAWSON et al., 1998).

To fulfill one of the goals of NS-breeding sunflower, conversion of susceptible inbred lines with good combining abilities to lines resistant to downy mildew, we used PCR markers (PANKOVIĆ et al., 2004a; b). We confirmed that the resistance to downy mildew race 730 is controlled by single gene, i.e. several resistance gene analogues (RGAs) are clustered at Pl_6 locus. Two co-dominant markers (CAPS) were developed and mapped (Fig. 3) (PANKOVIĆ et al., in press). They provide means for selecting homozygous resistant plants already in the F_2 generation during the conversion of susceptible lines to resistant lines. Therefore efficient MAS for sunflower resistance to downy mildew can be performed (SAFTIĆ-PANKOVIĆ et al., 2006).

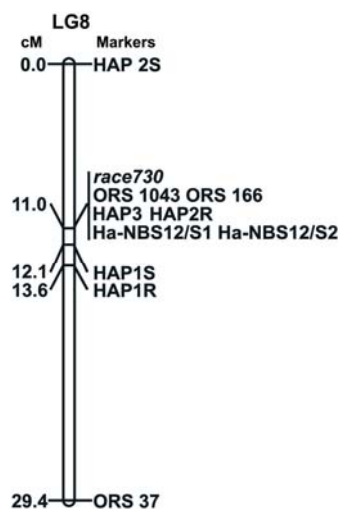


Figure 3. Genetic map of the Pl_6 locus of sunflower showing the localization of CAPS markers for resistance to *P. halstedii* race 730.

CONCLUSION

We have successfully applied molecular markers to investigate diversity of sunflower inbred lines, wild sunflower species, as well as to identify interspecies hybrids. We also obtained markers for some important agronomic traits such as drought tolerance and resistance to downy mildew. CAPS markers for resistance to downy mildew provide production of lines with resistance gene in homozygous state in only two generations, therefore greatly increasing efficiency of traditional sunflower breeding.

Received June 22nd, 2006
Accepted December 18th, 2006

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KORIŠĆENJE MOLEKULARNIH MARKERA U OPLEMENJIVANJU SUNCOKRETA

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Izvod

U radu je dat pregled rezultata koji se odnose na primenu molekularnih markera u oplemenjivanju suncokreta dobijenih u toku poslednjih deset godina u Institutu za ratarstvo i povrtarstvo.

Naši rezultati u ispitivanju genetičke udaljenosti ($GD= 7-75\%$) samooplodnih linija suncokreta primenom RAPD i SSR markera, ukazuju na visoku varijabilnost i predstavljaju jednu od važnih informacija pri izboru roditeljskih linija za ukrštanja.

Interspecies hibridizacija se često koristi u oplemenjivanju suncokreta. Samo neke populacije *H. giganteus* i *H. maximiliani* su pokazale otpornost prema bolestima suncokreta, pa je ispitivanje genetičke varijabilnosti unutar/između ove dve vrste aktuelno. Prikazani su rezultati dobijeni sa SSR markerima.

Primenom RAPD markera potvrđena je uspešnost interspecies hibridizacije između *H. rigidus* i *H. annuus* i diskutovana varijabilnost između F_1 i BC_1F_1 biljaka.

Molekularni markeri omogućuju detekciju poželjnih alela i haplotipova u ranim fazama razvića biljke, a takođe i u ranim fazama stvaranja poboljšanih linija, što redukuje ili potpuno eliminiše veliki broj ciklusa u kojima se testiraju poželjni fenotipovi. Prikazani su rezultati o CAPS markerima koji mogu da se koriste u MAS za otpornost prema plamenjači.

Primljeno 22. VII 2006.

Odobreno 18. XII 2006.