Positioning of CAPS Markers for Resistance to Downy Mildew on Linkage Maps as Determined in Three Sunflower Mapping Populations

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Abstract

The inheritance of resistance to downy mildew of sunflower was investigated in three mapping populations. From each cross 73 to 75 F_3 families were produced. The resistance of plant material to downy mildew was evaluated by the whole seedling inoculation technique. F_3 families were studied using several PCR and two CAPS markers for resistance gene analogues (RGAs) at *Pl6* locus. Seven haplotypes, in coupling or repulsion phase with resistance, five dominant and two co-dominant, were mapped for each mapping population. The results of heterogeneity test between recombination fractions for each marker pair among three families, permitted the construction of composite map. Mapping results are consistent, regardless on the parental lines used as sources of resistance genes.

Key words: *Helianthus annuus* L., *Plasmopara halstedii,* marker-assisted selection (MAS), cleaved amplified polymorphic sequences (CAPS)

Pozicioniranje CAPS biljega za otpornost na plamenjaču na kartu vezanosti ustanovljenu u tri populacije za kartiranje kod suncokreta

Sažetak

Nasljeđivanje otpornosti na plamenjaču kod suncokreta je istraživano u tri populacije za kartiranje. Od svakog križanja je proizvedeno 73 do 75 F_3 obitelji. Otpornost biljnog materijala na plamenjaču je procijenjena tehnikom inokulacije cijelih klijanaca. F_3 obitelji su proučavane pomoću nekoliko PCR i dva CAPS biljega za analoge gena za rezistentnost. (RGAs) na *Pl6* lokusu. Sedam haplotipova, u seriji spajanja ili razdvajanja s otpornošću, pet dominantnih i dva kodominantna, su kartirana u svakoj populaciji. Rezultati testa heterogenosti između rekombiniranih frakcija za svaki par biljega između tri obitelji, su omogućili konstruiranje sastavljene karte. Rezultati kartiranja su konzistentni, bez obzira na roditeljske linije korištene kao izvor gena za otpornost.

Ključne riječi: *Helianthus annuus* L., *Plasmopara halstedii,* selekcija pomognuta biljezima (MAS), rascijepljene amplificirane polimorfne sekvence (CAPS)

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Introduction

Downy mildew is a sunflower disease caused by the oomycetes of *Plasmopara halstedii*, with at least 15 races reported so far (Roeckel-Drevet, 2003). The disease is controlled by major *Pl* genes (Bouzidi et al., 2002).

Resistance genes *Pl1* and *Pl2* were first shown to confer resistance to race 100, and races 100 and 300, respectively (Gedil et al. 2001). However, *Pl6*, *Pl7* and *Pl8*, introduced from wild *Helianthus*, confer resistance to four races (100, 300, 700 and 730) of *P. halstedii* (Miller and Gulya 1991). *Pl1*, *Pl2, Pl6* and *Pl7* were shown to be grouped into clusters on linkage group 8 of the SSR map (Gedil et al., 2001). Several dominant (Bouzidi et al., 2002; Slabaugh et al., 2003) and co-dominant markers (Panković et al., 2007) for downy mildew resistance were positioned in this region.

As known for other crop-pathogen interactions durability of downy mildew resistance would most usefully combine as wide a range of *Pl* genes as possible.

Several lines, sources of *Pl6* and *Pl7* resistance genes are available. Among them Ha336, a donor of *Pl6* gene introduced from wild *H. annuus*, and Ha338, a donor of *Pl7* gene inroduced from *H. praecox* (Miller and Gulya, 1991). According to the pedigree, and IFLP haplotyping (Slabaugh et al., 2003) of these lines, *Pl6* and *Pl7*, though clustered and conffering resistance to the same races, are different genes. We have crossed the sunflower line Ha26S, a susceptible parent, with lines Ha26R and JM8, donors of *Pl6* resistance gene, and Ha338 donor of *Pl7* resistance gene. From these crosses three mapping populations, consisting of 73 to 75 F_3 families, were formed. The resistance of plant material to downy mildew was evaluated by the whole seedling inoculation technique. F_3 families were also studied using several PCR and two CAPS markers for resistance gene analogues (RGAs) at *Pl6* locus. The objective of this study was to compare the similarity of marker content and relative marker orders, on maps of three mapping populations, particularly in the case of co-dominant CAPS markers developed previously for MAS of downy mildew resistance (Panković et al., 2007).

Material and methods

Plant materials

The first mapping population was formed as described previously (Panković et al., 2007). Shortly, sunflower inbred line Ha26S, with a high general combining ability was used as the recipient of the *Pl6* gene from the initial cross with Ha336 (Miller and Gulya 1991). After several cycles of backcrosses Ha26S was converted to a resistant NIL Ha26R. Lines were crossed and 73 F_3 families produced. Other two mapping populations were produced after initial cross of Ha26S with JM8 and Ha338, donors of *Pl6* and *Pl7* resistance genes, respectively. In continuation 73 and 75 F_3 families were produced.

Resistance tests

The resistance to downy mildew race 730 was determined using the whole seedling inoculation technique. The details of the resistance test procedure and plant growth are given in Panković et al. (2007). At least 50 seedlings were examined for each F_3 family. Susceptible line L1 was sown in each tray as a control. When the first pair of true leaves emerged on plants, they were transferred to an incubation chamber with 100% relative humidity for 48 h in the dark to induce sporulation on the cotyledons of susceptible seedlings. Resistant seedlings showed no symptoms.

Molecular markers

The details on sampling, DNA extraction, primer sequences for RGA (Bouzidi et al., 2002), PCR conditions, and conditions for restriction digestions for CAPS markers are the same as in Panković et al. (2007). PCR reactions were replicated 3-5 times. Gel images were stored with the BIO-Print system (Vilber Lourmat, France), and fragment size was determined with the BIO-CAPT V.97 program (Vilber Lourmat, France).

Comparative mapping

Goodness-of-fit of each marker to the dominant (3:1) or co-dominant ratio (1:2:1) was tested by ^{2} analysis. Recombination fractions between markers were calculated from data of each mapping population separately and the heterogeneity of estimates was tested using maximum likelihood approach proposed by Morton

(1956). Linkage maps of each mapping population as well as the composite map based on pooled data were constructed by MAPMAKER Version 2.0 (Lander et al. 1987) using a LOD score of 4.0 and a maximum recombination fraction of 0.40 as thresholds for considering significant linkage. The heterogeneity of marker orders was tested by comparing log-likelihood values of the most likely marker order in each mapping population and the log-likelihood value of the most likely marker order of the pooled data. Moreover, the heterogeneity among linkage groups as constructed from different mapping populations was tested using Morton's approach extended to multipoint linkage maps with the same set of loci (Beavis and Grant, 1991; Williams et al., 1995). Nominal significance level (p) has been adjusted by Bonferroni correction taking into account the number of marker intervals tested simultaneously. Recombination fractions were converted to centiMorgans (cM) using the mapping function of Kosambi (1944).

Results and discussion

Resistance of the sunflower parental lines and their F_3 progeny to race 730 of *Plasmopara halstedii* was examined. At least fifty plants of each inbred line or F_3 family, were evaluated using the whole seedling inoculation technique. Lines Ha26R, JM8 and Ha338 were 100% resistant (R), while Ha26S was 100% susceptible (S). The results of the χ^2 test confirmed that the segregation ratio of resistant to susceptible F_3 families fit a 3:1 ratio in all examined mapping populations (Table 1).

Dominant haplotype patterns revealed after amplification with HAP1, HAP2 and HAP3 primers, in coupling (R) or repulsion (S) phase with resistance, as well as co-dominant CAPS markers (HhaI, RsaI) completely resembled the ones reported by Panković et al. (2007).

Linkage maps of each mapping population and the composite map are shown on Fig. 1. Out of 28 pairwise estimates of recombination fractions in three mapping populations, none was proven significantly heterogeneous ($p > 0.05$) among different genetic backgrounds. The log-likelihood values of the most likely marker order in each family were not significantly higher than the log-likelihood of the most likely marker order of the pooled data as shown in Table 2. Consequently, linkage groups as constructed from different mapping populations were homogeneous ($p_{CORR} = 0.014$) indicating that the composite recombination fractions were accurate estimators of target gene proximity.

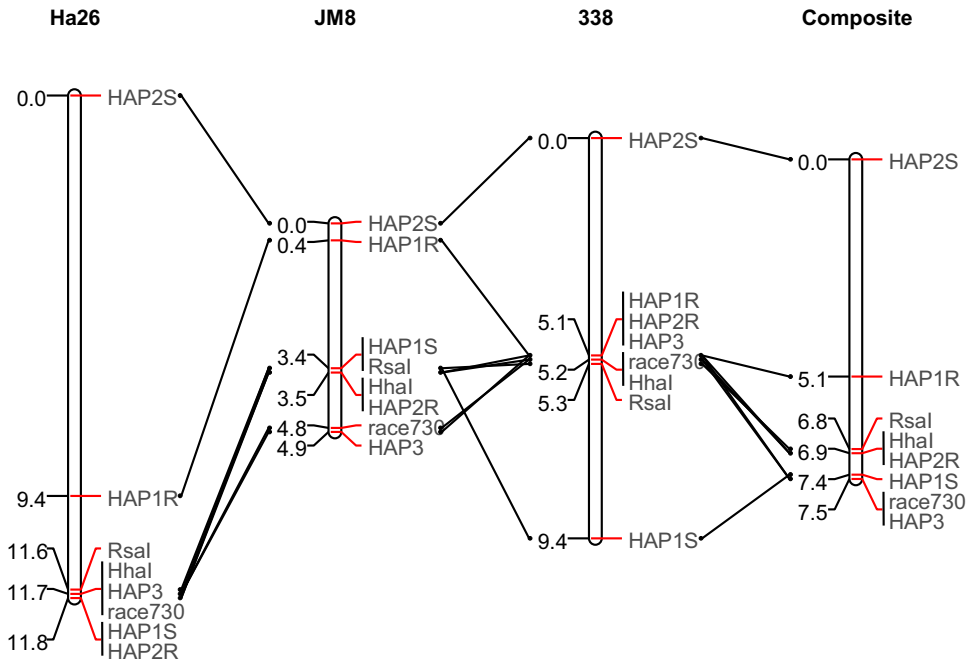

Figure 1.
Linkage maps of the three sunflower families (Ha26, JM8, 338) and the composite map

Table 1. The resistance to race 730 of *Plasmopara halstedii* in three mapping populations.

Susceptible parent	Resistant parent	No. of F3 families	Observed segregation	Expected ratio	χ^2	P
Ha26S	Ha26R	73	54 : 19	3 : 1	0.04	0.84
Ha26S	JM8	73	52 : 21	3 : 1	0.55	0.46
Ha26S	Ha338	75	56 : 19	3 : 1	0.00	0.95

Table 2. Log-likelihoods and test statistics for heterogeneity of marker orders and linkage groups among three families

Family	L_{MAX}	L_{ORDER}	χ^2_{ORDER}	p
Ha26	-56.87	-56.87	0.00	1.00
JM8	-47.67	-47.68	0.05	0.83
338	-47.42	-47.91	2.26	0.13
Total	-151.96			
Pooled	-154.66			
χ^2_{MAP}	12.43			
p	0.002			
p_{CORR}	0.014			

L_{MAX} - log-likelihood value of the most likely marker order in each family (Ha26, JM8, 338), total log-likelihood summed over families and log-likelihood value of pooled data

L_{ORDER} - log-likelihood value of the most likely marker order of the pooled data

χ^2_{ORDER} - test statistics for homogeneity of marker orders $(2\ln 10)[L_{MAX} - L_{ORDER}]$ that follows a ^{2} distribution with 1 degree of freedom

χ^2_{MAP} - heterogeneity test for linkage groups $(2\ln 10)[L_{TOTAL} - L_{POOLED}]$ that follows a ^{2} distribution with n-1 degrees of freedom, n being number of analysed families. Nominal significance level (p) and adjusted (pCORR) by Bonferroni correction $p_{CORR} = 1-(1-p)^n$, where n is a number of marker intervals tested simultaneously (n = 7).

Waycott et al. (1999) have found that map intervals between dominant markers can vary greatly on maps in different mapping populations of lettuce. In our case despite the differences that exist between maps, the loci order and linkage distances are well maintained between maps. The differences might be caused by relatively small number of F_3 families that were analysed and therefore the artefacts arising from the statistical analysis of the mapping data. But as the differences between recombination fractions were not significant we may conclude that the mapping results are consistent, regardless of the parental lines used as sources of resistance genes, both *Pl6* and *Pl7*.

Conclusions

Co-dominant CAPS markers for the resistance of sunflower to downy mildew race 730 can be used in MAS for this trait regardless of the parental lines used as sources of resistance genes, both *Pl6* and *Pl7*.

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