MOLECULAR MARKERS AS A TOOL IN BREEDING FOR RESISTANCE AGAINST SUNFLOWER DOWNY MILDEW

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Summary

Sunflower production is endangered by several diseases, necessitating a sophisticated disease management. Downy mildew of sunflower incited by *Plasmopara halstedii* belongs to the major sunflower diseases. Recent reports of pathotypes resistant to metalaxyl showed the necessity to breed for durable resistance of future hybrids. In order to develop molecular markers for different resistance genes, bulks and near isogenic lines were analysed employing RAPD and AFLP techniques. Segregation analysis of disease resistance was performed using the whole seedling immersion method. Markers useful in indirect selection for resistance genes Pl_2 (e.g. RHA325), Pl_6 (HA335), and Pl_{arg} (ARG1575-2) were identified. Crosses between several resistant genotypes were used in segregation and marker analyses to study the genetics of the genes involved. These experiments confirm the allelic relationship of loci Pl_2 Pl_6 and Pl_7, whereas Pl_6 and Pl_{arg} as well as Pl_6 and Pl_5 seem to be inherited independently. Pyramiding of the different resistance genes may result in an extended useful lifetime of the individual genes. Therefore, the markers are now employed in marker assisted selection experiments to combine for instance genes Pl_6 and Pl_{arg}, both giving resistance to all known races so far. But also the combination of already defeated genes (like Pl_1 or Pl_2) with one of those genes giving multiple resistances may provide more durable resistance than this complex loci alone. Hence, the management of resistance against downy mildew in sunflower should employ different combinations of *Pl* genes, even if some of them seem to be not very effective at the moment.

Introduction

A number of major resistance genes have been either identified in cultivated sunflower or were introduced from primitive *H. annuus* or other wild *Helianthus* species (Korell et al. 1996; Miller 1992). These dominant resistance genes have been designated as *Pl* genes. Some of them provide resistance to a single race of downy mildew, whereas others impart resistance against two or more races (Miller 1992). At the moment most commercial hybrids are resistant to race 2 of downy mildew (Gulya 1998), which was the predominant race in the USA until 1981 (Gulya et al. 1991). Six hybrids were resistant to the most virulent pathotype (race 5). Most breeders acknowledged that the resistance was obtained from public USDA lines HA335 through HA339 that are resistant to all races (Gulya 1998). It may be very attractive for practical breeding to use, for example, the Pl_6 gene which provides resistance to all known races. However, it is not advisable to use only one resistance gene. Strong resistance genes effective against all known races could be overcome soon by new pathogen races if used alone (Kelly and Miklas 1998).

Breeding for resistance against *P. halstedii* involving different genetic sources could be accelerated through the use of marker assisted selection methods; RFLP markers linked to genes Pl_1, Pl_2, and Pl_6 are already available (Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Vear et al. 1997). Recently, the application of a candidate gene approach revealed cloned RFLP markers which are located in the *Pl* region, including Pl_6 and Pl_2 (Gentzbittel et al. 1998), and may therefore detect parts of the clustered genes themselves. PCR based markers for genes Pl_2, Pl_6 and Pl_{arg} useful for marker assisted selection were recently reported (Brahm et al. 1998, 1999, 2000). However, a detailed knowledge of the genetics of the target resistances is essential for pyramiding strategies. Therefore, marker as well as classical segregation analyses have been conducted to proof the genetic relationship of resistance loci Pl_2, Pl_6, Pl_5, Pl_{arg} and Pl_7.

Materials and Methods

Plant Material

An overview of the crosses which we carried out for this work is given in Table 1. Either cytoplasmic male sterile (cms) inbred lines as females and inbred restorer lines as males (I and IV), or an emasculated male fertile line as a female and a maintainer as a male (II, III, V and VI), were used in the different cross combinations. In addition, two sets of near isogenic lines (NIL pairs: P7/PEM77 and CR2/CEM22) differing in the Pl_6 locus were used for marker analyses.

Segregation analysis

Resistance of F_2 plants was reinvestigated by testing 20-24 F_3 seedlings per F_2 individual as described by Brahm et al. (1998). Populations I, II and III were screened by using a field isolate (GG-F5), collected at our breeding station of Gross-Gerau near Frankfurt/Main. Populations II and IV, V and VI were tested with race 5, and population III was screened with race C of downy mildew.

Table 1: Resistant and susceptible inbred lines used as parents for the development of segregating populations

F_2 population	Cross female x male	Segregation for resistance gene(s)
I	HA89(cms) x AS110*Pl*$_2$	*Pl2*
II	AS110*Pl*$_2$ x HA335	*Pl2, Pl6*
III	AS110*Pl*$_2$ x DM2	*Pl2, Pl5*
IV	HA342(cms) x ARG1575-2	*Plarg*
V	HA335 x ARG1575-2	*Pl6, Plarg*
VI	HA335 x HA337	*Pl6, Pl7*

Molecular analysis

DNA was extracted from leaf material collected at flowering according to Doyle & Doyle (1990). RAPD and AFLP analyses were performed according to Brahm and Friedt (1996) and Brahm et al. (1999). Markers for genes Pl_2, Pl_6 and Pl_{arg} developed by Brahm et al. (1998, 1999, 2000) were mapped using Populations I, II and IV. Additional markers for the Pl_6 locus were developed in a bulked segregant analysis of population II (Michelmore et al. 1991). Marker assisted selection was conducted in Population V to combine genes Pl_6 and Pl_{arg}.

Linkage analysis

Linkage maps for the specific *Pl* regions in all populations were constructed using Mapmaker 3.0 software (Lander et al. 1987). Map units were computed by applying the Kosambi function (Kosambi 1944).

Results and Discussion

The results of the segregation analyses are presented in Table 2. Populations I and IV segregate for the genes Pl_2 and Pl_{arg}, respectively. Hence, both populations show the expected 1:2:1 segregation ratio of a single (dominant) resistance gene. These populations were used to develop molecular markers for the Pl_2 and the Pl_{arg} locus and for mapping both resistance loci (Brahm et al. 1998, 1999, 2000; see Fig. 1). Population II segregates for the Pl_2 and Pl_6 gene. The resistance test with race 5 results in the segregation of a single gene (Pl_6) in this population, whereas the analysis using isolate GG-F5 to which both genes give resistance shows that Pl_2 and Pl_6 are two allels of a single locus or two closely linked loci, as suggested by Mouzeyar et al. (1996). Correspondingly, population VI shows no segregation when tested with race 5 indicating that the Pl_7 gene may be a third allele of a multi-allelic locus or closely linked to the Pl_2/Pl_6 region. On the other hand, resistance against race 5 of downy mildew segregated in a 11:4:1 ratio in population V where downy mildew resistance is ruled by Pl_6 and Pl_{arg}. The resistance screening of population III with isolate GG-F5 and race C revealed a 1:2:1 segregation of resistance against each pathotype. In this population resistance to GG-F5 is governed by the Pl_2 gene from line AS110*Pl2*, whereas resistance to race C is given by the Pl_5 gene from DM2. Linkage analysis revealed that resistance against GG-F5 is not linked to resistance against race C in this population. Thus, the genes Pl_2 and Pl_5 seem to be inherited independently.

Table 2: Segregation ratio of investigated F_2 populations regarding downy mildew resistance following the test of F_3 progenies

Popula-tion	cross	Inoculum	Segregation (RR:Rs:ss) observed	tested	χ^2	P (FG=2)
I	HA89(cms) x AS110*Pl2*	GG-F5	43:63:35	1:2:1	2,50	0,29
II	AS110*Pl2* x HA335 (*Pl*$_6$)	Race 5	28:54:30	1:2:1	0,21	0,90
		GG-F5	103:2:0	11:4:1	41,2	ns*
III	AS110*Pl2* x DM2 (*Pl*$_5$)	Race C	41:80:59	1:2:1	5,82	0,05
		GG-F5	42:70:33	1:2:1	1,30	0,52
IV	HA342(cms) x ARG1575-2 (*Pl*$_{arg}$)	Race 5	32:63:33	1:2:1	0,04	0,97
V	HA335 (*Pl*$_6$) x ARG1575-2 (*Pl*$_{arg}$)	Race 5	79:28:11	11:4:1	1,91	0,38
VI	HA335 x HA337	Race 5	173:0:0	11:4:1	78,64	ns*

*ns = not significant

RAPD and AFLP markers identified by Brahm et al. (1998, 1999, 2000) were used to map the resistance genes *Pl*$_2$, *Pl*$_6$ and *Pl*$_{arg}$ in populations I, II, and IV (Fig. 1). Eight RAPD and three AFLP markers covered an interval of approximately 20 cM enclosing the *Pl*$_2$ locus. The closest flanking markers OP-AA14$_{750}$ and OP-BC05$_{480}$ are located in 0.3 and 0.8 cM distance to *Pl*$_2$ (Fig. 1A). Besides RAPD marker AS12$_{280}$ and AFLP E41M62_2, which are linked in repulsion phase to the resistance locus, all markers generate an additional fragment in resistant genotypes carrying the *Pl*$_2$ gene. Since Roeckel-Drevet et al. (1996) and Mouzeyar

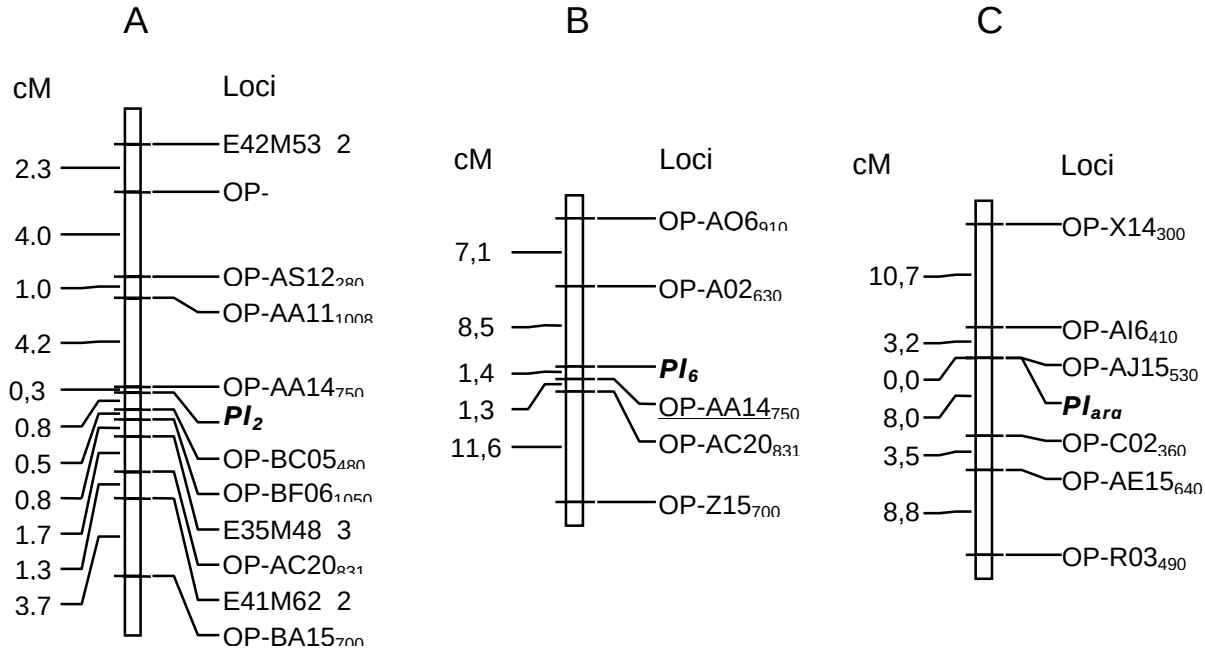

Figure 1:Mapping of resistance loci. A: Localisation of locus *Pl*$_2$ in population I. B: Map position of gene cluster *Pl*$_6$ in population II; markers which were used to map *Pl*$_2$ und *Pl*$_6$ are underlined. C: Localisation of resistance locus *Pl*$_{arg}$ from ARG1575-2 in population IV.

et al. (1996) mapped resistance genes *Pl*$_1$, *Pl*$_2$ and *Pl*$_6$ in the same marker interval of the sunflower RFLP linkage map constructed by Gentzbittel et al. (1995), we decided to test if the RAPD markers for *Pl*$_2$ are also useful to map the *Pl*$_6$ gene. In a bulked segregant analysis the markers shown to be linked to resistance against isolate GG-F5 were now detected as an additional fragment in bulks susceptible to race 5 of downy mildew. In the linkage analysis the markers that were linked to resistance allele of the *Pl*$_2$ locus were linked to the susceptibility allele of the *Pl*$_6$ locus. Roeckel-Drevet et al. (1996) and Mouzeyar et al. (1996) concluded from the colocation of the resistance genes *Pl*$_1$, *Pl*$_2$ and *Pl*$_6$ that *Pl*$_1$ and *Pl*$_2$ represent smaller parts of the complex locus *Pl*$_6$. In opposite, marker and segregation analysis presented here indicate that *Pl*$_2$ and *Pl*$_6$ are alleles at the same resistance locus. Since there was no segregation between *Pl*$_6$ and *Pl*$_7$ detected in the resistance test of population VI with downy mildew race 5, there may be another allele *Pl*$_7$.

However, the segregation analysis of populations III and V (Table 2) showed that the resistance genes *Pl*$_5$ and *Pl*$_{arg}$ do not belong to this potential multi-allelic locus. This corresponds to the report of Vear et al. (1998): They could not assign resistance genes *Pl*$_4$ and *Pl*$_5$ to the *Pl*$_2$/*Pl*$_6$ region in their experiments. Therefore, it can be concluded that there a at least two distinct genomic regions controlling resistance to downy mildew in sunflower.

Subsequently, loci of these distinct regions can now be combined to breed for lines with a more durable resistance. Hence, we used markers OP-AQ6$_{910}$ for resistance gene *Pl*$_6$, and OP-

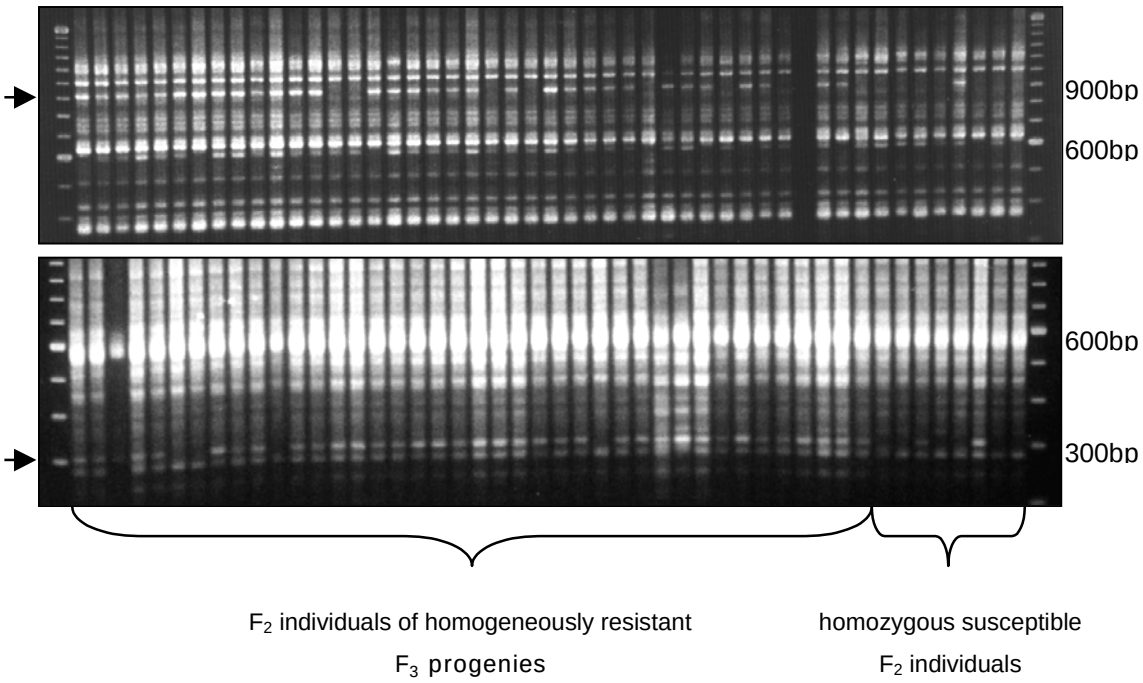

Fig. 2: Screening of F_2 individuals of population V with RAPD primers OP-AQ6 (A) and OP-X14 (B). Fragments OP-AQ6$_{910}$ and OP-X14$_{300}$ are marked with

X14$_{300}$ as well as OP-AJ15$_{530}$ for gene *Pl*$_{arg}$ to analyse population V, which segregated for both loci. OP-AQ6$_{910}$ and OP-X14$_{300}$ are linked with the corresponding resistance allele, whereas OP-AJ15$_{530}$ is linked with the susceptibility allele of *Pl*$_{arg}$. All other markers did not segregate in population V. Plants showing markers OP-AQ6$_{910}$ and OP-X14$_{300}$ and missing marker OP-AJ15$_{530}$ were considered for further breeding generations. The selected genotypes should be either homozygous or heterozygous for the resistance allele of the *Pl*$_6$ locus and homozygous for resistance controlled by the *Pl*$_{arg}$ locus. Thus, sunflower genotypes

combining resistance against all races of downy mildew governed by the distinct resistance genes Pl_6 and Pl_{arg} are already available.

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