Mapping Downy Mildew Resistance in Sunflower

Lutz BRAHM, Thomas RÖCHER, Renate HORN, Markus PRÜFE, Heike KÖHLER and Wolfgang FRIEDT

Institute of Crop Science and Plant Breeding, Justus-Liebig University, Giessen, Germany

Abstract

RAPD and AFLP analysis of near isogenic lines, AS110/AS110*Pl2* and S1358/S1358*Pl2*, differing in the *Pl2* locus and Bulked Segregant Analysis of F_2 populations, HA 89 (cms) x AS 110 *Pl2* and HA 342 (cms) x ARG1575-2, segregating for race 7 and 9 resistance originating from AS 110 *Pl2* and race 5 resistance derived from *Helianthus argophyllus*, respectively, were used to identify molecular markers for resistance against downy mildew of sunflower. On the basis of these markers, linkage maps for both populations were constructed representing the genomic region carrying the respective resistance locus, which can now be used for further investigations.

Introduction

Downy mildew of sunflower caused by *Plasmopara halstedii* is one of the major problems in most of the sunflower growing areas of the world. The incidence of the disease in the field ranges from traces up to 90 % under extreme conditions, depending on the inoculum potential, the variety used, and the occurrence and intensity of rainfall during the planting period (Viranyi 1992). Up to now resistance against downy mildew as well as chemical seed treatments with metalaxyl have been used for plant protection. Corresponding to results of Oros & Viranyi (1984), recent reports of metalaxyl resistant downy mildew pathotypes in field collections from France (Bervillé, pers. comm.) demonstrate the adaptability of the pathogen. Therefore, the use of resistant varieties will be the major task in plant protection of sunflower for the next years.

A number of major resistance genes have been either identified in cultivated sunflower or were introduced from wild *Helianthus annuus* or other wild *Helianthus* species (Miller 1992, Korell et al. 1996). 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). Former genetic studies suggested that the different genes providing resistance to a varying number of downy mildew races were inherited independently. However, segregation analyses by Mouzeyar et al. (1992) followed by integration of the genes *Pl1*, *Pl2* and *Pl6* by Mouzeyar et al. (1995), Reockel-Drevet et al. (1996) and Vear et al. (1997) in the RFLP consensus linkage map of sunflower (Gentzbittel et al. 1995) showed that these genes share the same map position. Moreover, Vear et al. (1997) were able to show that the «locus» *Pl6* providing resistance against all known races consists of at least two genes or groups of genes conferring resistance against French races A, B and C (= race 3) and 1 and D (= race 2), respectively, which show a

very close linkage of 0,6 cM between the two groups. These results indicate that *Pl* genes giving resistance to a number of downy mildew races are a cluster of closely linked loci, each conferring resistance against one single race.

However, particular aspects of the resistance genetics remain unclear. The construction of linkage maps using different sources of resistance against downy mildew of sunflower will help to get a better understanding of the genetics of *Plasmopara* resistance as was demonstrated by the authors mentioned above. This report deals with resistance against a field isolate comparable with races 7 and 9 and resistance against race 5. Resistant genotypes originate from inbred AS 110 *Pl2* (*Pl2*[+?]) and from line ARG1575-2 (race 5 resistance). Since colocation of different *Pl* genes has been proofed for three genes, all derived from *H. annuus* (*Pl1*, *Pl2*, *Pl6*), it will be of special interest whether resistance genes originating from interspecific crosses to other Helianthus species, e.g. race 5 resistance derived from *H. argophyllus* in ARG1575-2, also share the same location.

Materials and Methods

• *Sunflower genotypes*

An overview of the crosses, which were carried out at our Institute by using cytoplasmic male sterile (cms) inbred lines as females and inbred restorer lines as males is given in Table 1. In addition, two sets of near isotonic lines AS 110/AS 110 *Pl2* and S1358/S1358 *Pl2* were used for the identification of molecular markers. Both sets were originally developed by Dr. V.A. Vranceanu (Fundulea, Romania) with special reference to the *Pl2* locus, conferring resistance against downy mildew races 1 and 2.

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

F_2 populations	Cross (female x male)	Segregating for resistance against
I	HA 89 (cms) x AS 110 *Pl2* (*Pl2*[+?])	races 1, 2, 7 + 9
II	HA 342 (cms) x ARG1575-2 (?)	races 1 to 6

? = responsible gene not known

• *Resistance tests*

Downy mildew resistance was determined by application of the whole seedling immersion test as described by Gulya et al. (1991*a*). Resistance of F_2 plants was reinvestigated by testing 20-24 F_3 germlings per individual. Symptoms were observed two weeks after inoculation, following 72h under a saturated atmosphere. Population I was screened by using a field isolate (GG-F5), collected at the breeding station of our Institute at Gross-Gerau near Frankfurt/Main. This isolate reacts similar to American races 7 and 9, according to tests of USDA lines HA 821, RHA 266, RHA 274, DM 2, 799-2, and HA 335 as differentials (Spring & Rozynek, unpubl.). Population II was tested with race 5 donated from Prof. F. Viranyi (Gödöllö, Hungary).

Depending on the host-pathogen-interaction, resistance was defined as absence of sporulation (population I) and absence or slight sporulation on cotyledons (population II).

• *Molecular analysis*

DNA was extracted from leaf material collected at flowering according to Doyle & Doyle (1990). Bulked Segregant Analysis (BSA) of populations I and II was performed according to Michelmore et al. (1991). For both populations, two resistant and two susceptible bulks were compiled consisting of 10 homozygous resistant and homozygous susceptible plants. Molecular markers for resistance against field isolate GG-F5 (population I, *Pl2*[+?]) were developed by analyzing near isogenic lines (RAPDs) and bulks (RAPDs, AFLPs), respectively. Markers for race 5 resistance (population II) were identified by investigating bulks only. RAPD analysis was performed as described by Brahm & Friedt (1996). AFLP markers were generated using AFLP Analysis System I (Gibco BRL/Life Technologies). *Eco* RI specific primers were labeled with [γ-^{33}P]dATP (NEN Life Science). Amplification and fragment analysis were carried out according to the suppliers protocol.

• *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). Linkage groups were identified with a minimum LOD score of 3.0 and a maximum distance of 37.2 cM. Initial map orders were determined with the "compare" command following three point analysis. Additional markers were integrated into the maps using the "build" command with a strict exclusion threshold of LOD 3.0. Final map orders were tested with the "ripple" command.

Results

• *Resistance tests*

Population I derived from the cross of HA 89 (cms) x AS 110 *Pl2* was segregating for resistance against the field isolate GG-F5, which is similar to sunflower downy mildew races 7 and 9 (Spring & Rozinek, unpubl.). Segregation pattern in both F_2 populations fitted a 1 : 2 : 1 ratio as is expected for a single dominant trait following the test of F_3 families of each F_2 plant. The same could be observed for population II, derived from the cross HA 342 (cms) x ARG 1575-2, when tested with downy mildew race 5 (Table 2).

Table 2 : Segregation ratios of F_2 generations for resistance to downy mildew of sunflower

Population	Cross	Segregation (RR:RS:SS)		X^2 -	P
		observed	tested	value	(DF = 2)
I	HA 89 (cms) x AS 110 Pl_2	43 : 63 : 35	1 : 2 : 1	2,50	0,29
II	HA 342 (cms) x ARG1575-2	32 : 63 : 33	1 : 2 : 1	0,04	0,97

• *Analysis of near isogenic lines*

A total of 380 RAPD primers were screened using the near isogenic line pairs AS 110/ AS 110 *Pl2* and S 1358/S 1358 *Pl2*. Only 21 primers which generated fragments polymorphic between both sets of near isogenic lines were considered for further analysis. Three of those 21 primers generated a fragment with linkage to the resistance locus. Two markers, A2-630 and B8-730 are linked in coupling phase (Brahm & Friedt 1996), whereas the third (O4-486) is linked in repulsion.

• *Bulked Segregant Analysis*

Bulks of Population I (HA 89 (cms) x AS 110 *Pl2*) were tested with an additional set of 500 RAPD primers. Five primers generated fragments with linkage to resistance locus *Pl2*(+?). Moreover, 23 Eco/Mse primer combinations were used in an AFLP analysis, and two markers for the resistance gene were identified.

Bulked Segregant Analysis of population II (HA 342 (cms) x ARG 1575-2) was carried out with 400 RAPD primers. Four fragments generated by four primers showed linkage to race 5 resistance.

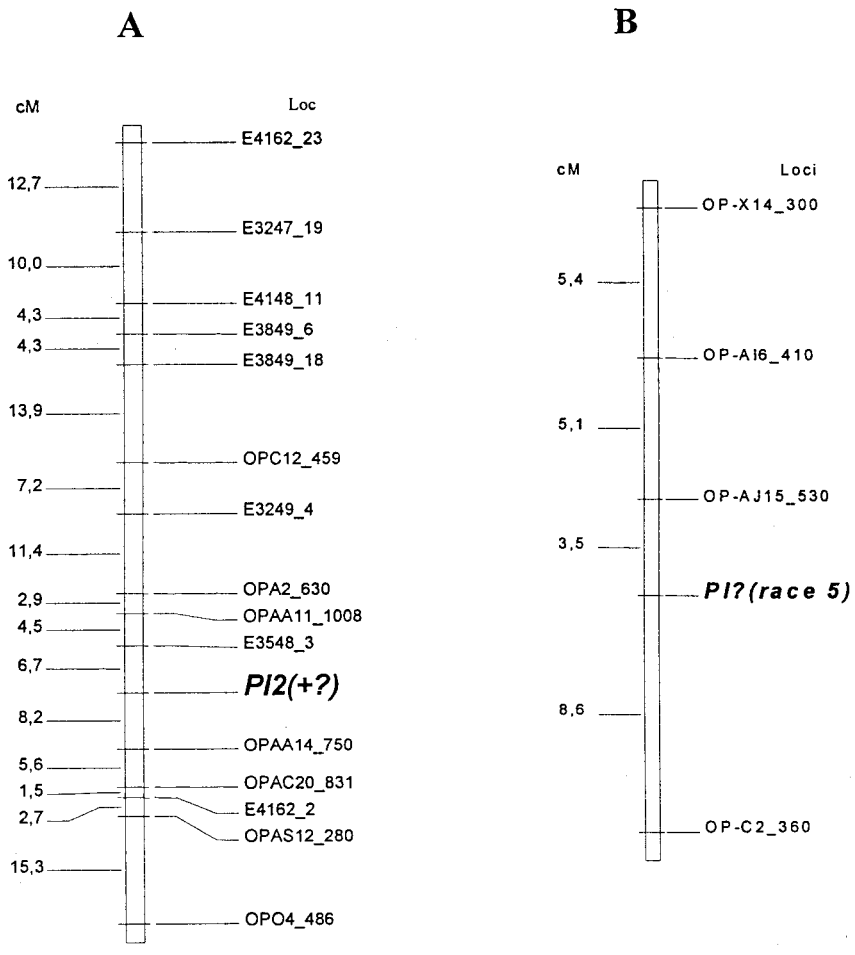
• *Mapping downy mildew resistance*

Markers for *Pl2*(+?), conferring resistance against downy mildew races 7 and 9, respectively, in population I, were used to construct a linkage map of that particular region of the sunflower genome using 132 F2 plants (Köhler et al. 1996). Subsequently, this partial linkage map was integrated into an AFLP linkage map which was constructed for population I with a set of 90 F2 individuals (data not shown). The resistance locus is flanked by AFLP marker E35M48_3 and RAPD marker AA14_750 in 6.7 and 8.2 cM distance, respectively, on linkage group 1 of that AFLP map (Fig. 1A). RAPD markers AA11_1008 and AA14_750, both flanking the *Pl2*(+?) locus, also distinguish between seven inbreds carrying gene *Pl2* (RHA 325, RHA 345, RHA 348, CM 587, CM 592, CM 610, CM 596) and nine (HA 350, HA 342, HA 850, HA-R2, HA 323, HA 291, CM 603, CM 594, CM 591) susceptible to downy mildew (Korell et al. 1991). Therefore, both markers may be useful also in populations derived from other crosses segregating for the *Pl2*(+?) locus.

For population II (HA 342 (cms) x ARG 1575-2) segregating for race 5 resistance, a linkage map of the sunflower genomic region carrying the resistance locus was constructed using the four RAPD markers which were identified in Bulked Segregant Analysis (Fig. 1B). A total of 123 F2 plants were analyzed. The resistance locus is flanked by markers AJ15_530 in a distance of 3.5 cM, and C2_360, 8.6 cM in distance.

Figure 1A : Linkage group 1 of the AFLP map constructed using 90 F2 plante of the cros HA 89 (cms) x AS 110 *Pl2* with integrated RAPD markers.

2B : Linkage map of markers and race 5 resistance in the F2 population HA 342 x ARG 1575-2



Discussion

Earlier publications concluded from segregation analysis of populations derived from crosses between different sources of resistance, that resistance against downy mildew of sunflower is controlled by independent single dominant genes *Pl*. On the contrary the results of Mouzeyar et al. (1992) and Mouzeyar et al. (1996) indicate that the *Pl* genes are rather closely linked than independent. Mapping experiments of Mouzeyar et al. (1995), Roeckel-Drevet et al. (1996) and Vear et al. (1997) which integrated the genes *Pl1*, *Pl6* and *Pl2* into the RFLP linkage map of sunflower (Gentzbittel et al. 1995) gave evidence for the colocation of these three *Pl* genes in the same region of the sunflower genome. These results lead to

the hypothesis, that those Pl loci, conferring resistance to more than one race of downy mildew, do not represent single genes but are a cluster of a number of closely linked genes (Mouzeyar et al. 1996). Subsequently, Vear et al. (1997) were able to show that the *Pl6* gene, providing resistance to all known races so far, indeed consists of at least two different loci, one giving resistance to the French races A, B and C (similar to American races 4, 3, and indistinguishable from race 3) and one to races 1 and 2 (French race D) and which are linked with 0.6 cM.

The linkage map of the *Pl2*(+?) region presented in this paper should represent the same part of the sunflower genome as the map of Vear et al. (1997), since both maps contains the *Pl2* locus. This locus was originally identified in the inbred line HA61 (Zimmer & Kinman 1972) and used for the development of several resistant genotypes. Some inbreds carrying the *Pl2* gene, e.g. inbred lines RHA 274 and RHA 325, are not only resistant against races 1 and 2 but also against races 6,7 and 9, and 7 and 9 (Gulya et al. 1991*b*), respectively. These complexes could not be separated from the *Pl2* locus so far (Seiler & Jan, pers. comm.). Hence, the resistance against races 7 and 9 mapped in this paper, originating from the resistant inbred AS 110 *Pl2*, may be derived from a "*Pl2*" cluster conferring resistance to races 1, 2, 7 and 9.

Since the different functions of the *Pl6* "gene" are very closely linked and at least some of the *Pl* genes share the same genomic region, a marker developed for the *Pl* region using a particular source of resistance may also be useful in investigations of *Pl* genes different from the one used to detect the marker itself, as shown for *Pl1*, *Pl2* and *Pl6* (Mouzeyar et al. 1996, Roeckel-Drevet et al. 1996, Vear et al. 1997). Therefore, the markers developed in this investigation should also be helpful in studies of other *Pl* donors. The markers AA14_750 and AA11_1008 identified for the *Pl2*(+?) locus are at least useful to distinguish between different lines carrying the *Pl2* gene and a number of susceptible lines, although it is possible that RAPD fragments of the same size may not share the same sequence. However, this problem can easily be overcome by converting the respective markers into a SCAR/STS. Such markers are able to distinguish specific alleles (Paran & Michelmore 1993) and are therefore as reliable as RFLP markers but more powerful in screening large numbers of individuals in actual breeding programs.

On the other hand, evidence of the colocation of the *Pl* genes was given by consensus mapping of genes *Pl1*, *Pl2* and *Pl6* (Mouzeyar et al. 1996, Roeckel-Drevet et al. 1996, Vear et al. 1997) which were identified in cultivated sunflower or obtained from hybridization with wild *H. annuus* (Vranceanu & Stoenescu 1970, Zimmer & Kinman 1972, Miller & Gulya 1991). It may well be that *Pl* genes derived from interspecific hybridization with other *Helianthus* species like *Pl7* from *H. preacox* ssp. *runyonii* or *Pl8* from *H. argophyllus* (Miller & Gulya 1991) do not share the same location in the sunflower genome. Although, segregation analysis of a testcross between HA 335 (*Pl6*) and HA 338 (*Pl7*) by Mouzeyar et al. (1996) showed no segregation in a test with French race A, indicating that genes *Pl6* and *Pl7* are not independent. It will be of special interest to compare map positions of genes originating from different wild *Helianthus* species. Therefore, we will extend the map for population II (HA 342 (cms) x ARG 1575-2) presented here and subsequently use the markers identified for both, Pl2(+?) and race 5 resistance of ARG 1575-2, to compare the map position of both resistance loci.

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