

Fine mapping of the downy mildew resistance locus *Pl_{ARG}* in sunflower

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ABSTRACT

The oomycete *Plasmopara halstedii* is the causal agent of downy mildew and can cause high yield losses in sunflower (*Helianthus annuus* L.). Several *Pl* loci from different sources were introduced into breeding material to control the pathogen. One of it is *Pl_{ARG}* which originates from the wild sunflower species *Helianthus argophyllus* and protects sunflower against all known races of *P. halstedii*. The aim of our project was to investigate the fine structure of the *Pl_{ARG}* locus to answer the question, whether the *Pl_{ARG}* locus is a single resistance gene or a cluster of resistance genes effective against different races of *P. halstedii*. *Pl_{ARG}* was mapped on linkage group (LG) 1 in a population from cross cmsHA342 x ARG1575-2 comprising more than 1,000 F₂ plants. In comparison with the intraspecific *H. annuus* reference map, recombination on LG 1 of our cross was suppressed. Several flanking markers and a few which completely cosegregate with *Pl_{ARG}* could be identified. ARG1575-2, a backcross-derived resistant inbred line, carries *H. argophyllus* (donor) alleles throughout LG 1 and no alleles from the recurrent parent (HA89); thus, LG 1 in ARG1575-2 originated from *H. argophyllus*.

Key words: fine mapping – *H. annuus* – *H. argophyllus* – *Plasmopara halstedii* – suppressed recombination.

INTRODUCTION

Plasmopara halstedii (Farl.) Berl and de Toni, a seed-, soil- and wind-born pathogen, is one of the major diseases in cultivated sunflower (*Helianthus annuus* L.) and can cause yield losses of up to 95%. The disease can be controlled with fungicides (e.g. metalaxyl) and resistant hybrids. *Pl₁* was the first known resistance locus against downy mildew, pathotype 100, discovered by Vrănceanu and Stoiculescu (1970). Later, additional *Pl* genes, originating from different species of *Helianthus*, were described and conferred resistance against one or more pathotypes (Zimmer and Kinman, 1972; Miller and Gulya, 1991; Rahim et al., 2002). Mapping of *Pl* genes and studies on the inheritance of resistance to different races of *P. halstedii* showed that some of the *Pl* genes are clustered and are not single genes as initially reported (Mouzeyar et al., 1995; Roeckel-Drevet et al., 1996; Vear et al., 1997; Bert et al., 2001; Bouzidi et al., 2002; Radwan et al., 2003).

The question remains unsolved whether the *Pl_{ARG}* locus is a single gene which can cause a non-race-specific complete resistance against several pathotypes or whether this is a complex locus containing several closely linked *Pl* genes, each of which provides resistance to one single pathotype. Clusters of resistance genes have been identified in different plant species including lettuce, a member of the *Compositae*, where resistance genes against the downy mildew fungus *Bremia lactucae* exist and are organized in multigene families (Meyers et al., 1998).

The aim of this project is to explore the structure of the *Pl_{ARG}* locus, which was introgressed from the wild species *H. argophyllus* (Seiler et al., 1991). *Pl_{ARG}* confers resistance to all known races of *P. halstedii* (Seiler et al., 1991; G.J. Seiler, personal communication). The *Pl_{ARG}* locus is unlinked to other known downy mildew resistance genes in sunflower and has been mapped to LG 1 (DuBLE et al., 2004).

MATERIALS AND METHODS

Sunflower genotypes

The sunflower line ARG1575-2 carries the *Pl_{ARG}* locus (Seiler et al., 1991) and is resistant to all known races of *P. halstedii*. ARG1575-2 arose by backcrossing the wild species *H. argophyllus* (Acc. 1575) three times with cmsHA89 followed by five selfing generations (Seiler et al., 1991). The isogenic

sunflower lines cmsHA342 and HA342 are susceptible to all known races of *P. halstedii*. ARG1575-2 was crossed to each of the susceptible lines and several flower heads were used for generating two populations for fine mapping of the Pl_{ARG} locus. Population 1 (cmsHA342 x ARG1575-2) contains 1063 F_2 individuals and population 2 (HA342 x ARG1575-2) includes 1,084 F_2 individuals.

Resistance tests

Phenotypic resistance evaluation was conducted in a subset of 185 $F_{2,3}$ families of population 1 (cmsHA342xARG1575-2), using the whole seedling immersion test (Gulya, 1996) with a suspension of *P. halstedii* spores of race 730. The resistance of F_2 plants was investigated by testing 16-40 F_3 seedlings per F_2 individual. Seedlings were scored as susceptible if a high fungal sporulation was evident on cotyledons. Seedlings were scored to be resistant if no or only spurious sporulation was observed on cotyledons. Progenies with unclear evaluation were re-tested and 4-10 $F_{3,4}$ families were tested additionally. According to the segregation in the corresponding F_3 or F_4 families, F_2 plants were then classified as homozygous susceptible, homozygous resistant or heterozygous.

Marker analysis

Around 180 simple sequence repeat (SSR) markers (Tang et al., 2002) were screened for polymorphisms between cmsHA342 and ARG1575-2. SSRs showing polymorphisms between the resistant and the susceptible parents were analysed with bulked segregant analysis (BSA) (Michelmore et al., 1991). Each phenotypic bulk contained 15 homozygous susceptible or 15 homozygous resistant F_2 individuals, respectively. Codominant SSRs exhibiting differences between the resistant and the susceptible bulks were mapped. Further markers like single nucleotide polymorphism (SNP) markers (Lai et al., 2005a) and resistance gene candidate (RGC) markers were screened with BSA using the single strand conformation polymorphism (SSCP) method or analysed as CAPS (cleaved amplified polymorphic sequences) markers.

Statistical analysis

Goodness-of-fit test and maps were constructed with JOINMAP 3.0 (Stam 1993) using a LOD threshold of 4.0 and the mapping function of Kosambi (1944).

RESULTS

Resistance tests with race 730 were carried out on the F_3 progenies derived from a subset of 185 F_2 individuals from population cmsHA342 x ARG1575-2. The distribution of the F_2 subset was 26 homozygous resistant, 114 heterozygous-resistant and 45 homozygous-susceptible. We observed a significant distortion from the expected 1:2:1 segregation ratio for all markers and for Pl_{ARG} ($\chi^2=13.9$, $DF=2$, $p=0.001$).

Sixteen codominant SSR markers all mapping to LG 1 were found with BSA. Thus, marker analysis focused on this linkage group. Eleven SNPs (Lai et al., 2005a) and three RGCs from LG 1 were screened with BSA. For SNP marker HT211 and one RGC cleaved amplified polymorphic sequences (CAPS) markers were developed and were mapped into the target interval.

A cluster of markers including ORS610, ORS543, ORS1128, CRT272, ORS1039, ORS1182 and ORS710 cosegregated at the distal end of LG 1. Between these markers and HT211, ORS662 which completely cosegregated with Pl_{ARG} in population 1, we found only a few recombinations. Most recombinations were proximal to Pl_{ARG} where we mapped markers ORS053, ORS959 and ORS371. In comparison with the intraspecific *H. annuus* map of LG 1 of Tang et al. (2002) our map is ten times shorter for the interval ORS610-ORS959. Therefore we screened a second F_2 population from the same cross to find more recombinants. In the second population the susceptible parent HA342 does not carry the CMS plasma. Here, we found the expected segregation ratio of 1:2:1 for all markers. Marker order and map length are the same as in population 2. Phenotyping is still in progress and thus Pl_{ARG} has not been mapped in population 2. In both populations, the order of the markers was the same as in the reference map of Tang et al. (2002).

The comparison of the marker alleles of 13 markers evenly distributed along LG 1 showed that the resistant parent ARG1575-2 carries only alleles from the wild species *H. argophyllus* and no allele from the backcrossing parent cmsHA89. This indicates that no recombination occurred on LG 1 during the backcrossing process.

DISCUSSION

Several reports indicate the wide spread of existing pathotypes and the discovery of new downy mildew races (Gulya et al., 1991; Molinero-Ruiz et al., 2002). Some races developed tolerance to the fungicide metalaxyl (Albourie et al., 1998). Therefore, it is necessary to search for new resistance sources against *P. halstedii* and to investigate the structure and functionality of the *Pl* loci in order to use them effectively and durably in plant breeding.

Wild species are often used as a source for resistance genes in different cultivars, because they represent a rich source of untapped R genes (Slabaugh et al., 2003). For example, sunflower rust resistance genes were introduced from wild *Helianthus* species into the cultivated sunflower (Quresh et al., 1993). *Pl_{ARG}*, introduced from *H. argophyllus*, is an outstanding source of resistance because of its wide range of efficacy against all known races of *P. halstedii*. However, the genetic structure of the locus is still unclear. Vear et al. (1997) showed that *Pl₆* is not a single gene, conferring resistance to all downy mildew races, but rather a cluster of genes, each providing resistance to one or few downy mildew races. Slabaugh et al. (2003) gave the physical evidence for a large cluster of resistance genes in the *Pl₁-Pl₂-Pl₆* region. Therefore, it is an obvious assumption that *Pl_{ARG}* could be a cluster of resistance genes too and thus the goal of our project is to investigate the fine structure of the *Pl_{ARG}* locus. First *Pl_{ARG}* was mapped in a subset of lines to identify flanking markers for the *Pl_{ARG}* locus. Only the flanking markers were used to screen the whole population (cmsHA342 x ARG1575-2) to identify F₂ individuals with recombination events in the target region. This strategy allows to restrict phenotyping work with different races of *P. halstedii* to genetically informative lines (Bauer and Graner, 1995).

On LG 1 recombination was suppressed as concluded from comparing the map distances with published intraspecific maps. Several flanking markers and a few which completely cosegregated with *Pl_{ARG}* could be detected. To investigate the reason for the suppressed recombination, the marker alleles of LG 1 were compared between cmsHA342, ARG1575-2 and cmsHA89, the recurrent backcrossing parent. Marker alleles of ARG1575-2 and cmsHA89 differed for all markers tested along LG 1. It can be concluded that LG 1 was derived from the original *H. argophyllus* accession used as the donor and that during backcrossing no recombination occurred between linkage group 1 in *H. annuus* and the homologous *H. argophyllus* chromosome. Thus, with respect to LG 1, HA342 x ARG1575-2 can be regarded as a cross between the wild species *H. argophyllus* and cultivated sunflower.

Suppressed recombination often occurs in populations with gene material from wild species and sometimes is accompanied by negative side-effects such as undesirable linkage drag. The root-knot nematode resistance gene (*Mi*) was introgressed into the cultivated tomato from the wild species, *Lycopersicon peruvianum*. In crosses containing the *Mi* gene, suppressed recombination occurred and the map was five times shorter than in crosses without the *Mi* gene (Messeguer et al., 1991). The authors assumed that the suppressed recombination may be due to reduced homology between the *L. esculentum* DNA and the *L. peruvianum* DNA containing the *Mi* gene.

In sunflower, comparative genetic linkage maps of *H. annuus*, *H. petiolaris*, *H. anomalus*, *H. deserticola* and *H. paradoxus* were established to study karyotypic evolution. It became apparent that chromosomal rearrangements had the highest rate reported for any taxonomic group (Burke et al., 2004; Lai et al., 2005b). Heesacker et al. (2006) extended comparative mapping to *H. argophyllus*, because silver-leaf sunflower is a source of novel alleles for the improvement of common sunflower. They showed that nine *H. argophyllus* and *H. annuus* linkage groups are collinear and the other *H. argophyllus* linkage groups carry translocations, inversions, or both when compared to *H. annuus* linkage groups. LG 1 is one of the collinear linkage groups compared with *H. annuus*, and, therefore, chromosomal rearrangements are not a likely reason for suppressed recombination. Recombination on LG 1 could be suppressed, because of reduced homology between *H. annuus* and the wild *H. argophyllus*. In the intraspecific cross of *H. argophyllus* recombination was suppressed per se in the region distal to *Pl_{ARG}* (A. Heesacker, personal communication) which could be an alternative explanation for our findings or it could be an interplay of both mechanisms.

Further work is in progress to investigate the fine structure of *Pl_{ARG}*. A large population which does not segregate for *Pl_{ARG}* is being developed to map additional markers with a higher genetic resolution and to explore the relationship between genetic and physical distances in the target region. With cosegregating markers, a BAC library will be screened and BAC-ends will be sequenced and remapped into the mapping populations. cDNA-AFLPs are used to identify differentially expressed transcripts between the susceptible and resistant parent, each non inoculated and upon inoculation with different races of *Plasmopara halstedii*. Interesting fragments will be sequenced and remapped to the segregating populations to further enrich the target region with potential resistance candidate genes.

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