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ORIGINAL PAPER

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# $Pl_{Arg}$ from *Helianthus argophyllus* is unlinked to other known downy mildew resistance genes in sunflower

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**Abstract** The $Pl_{Arg}$ locus in the sunflower (*Helianthus annuus* L.) inbred line Arg1575-2 conferring resistance to at least four tested races (300, 700, 730, 770) of downy mildew (*Plasmopara halstedii*) was localized by the use of simple sequence repeat (SSR) markers. Bulked segregant analysis (BSA) was conducted on 126 individuals of an $F_2$ progeny from a cross between a downy mildew susceptible line, CmsHA342, and Arg1575-2. Twelve SSR markers linked to the $Pl_{Arg}$ locus were identified. All markers were located proximal to $Pl_{Arg}$ on linkage group LG1 based on the map of Yu et al. (2003) in a window of 9.3 cM. Since $Pl_{Arg}$ was mapped to a linkage group different from all other *Pl* genes previously mapped with SSRs, it can be concluded that $Pl_{Arg}$ provides a new source of resistance against *P. halstedii* in sunflower.

## Introduction

Downy mildew, caused by *Plasmopara halstedii* (Farl.) Berlese et de Toni, is one of the most important world wide fungal diseases in cultivated sunflower (*Helianthus annuus*) causing serious economic losses. Typical symptoms in seedlings include dwarfing and yellowing (chlorosis) of the leaves and the appearance of white cottony masses (fungal mycelium and spores) on the lower and sometimes on the upper leaf surface during periods of high humidity or dew.

The first genetic studies of resistance to downy mildew have shown that dominant major genes, denoted *Pl*, control resistance to different races of *P. halstedii*. So far, 11 *Pl* genes have been described (Rahim et al. 2002) from both the cultivated sunflower (Vranceanu and Stoenescu 1970) and wild *Helianthus* species (Miller and Gulya 1991). $Pl_6$, $Pl_7$, and $Pl_8$, found in wild *Helianthus* species, confer resistance to almost all races of *P. halstedii* (Bert et al. 2001; Bouzidi et al. 2002). $Pl_6$ was obtained from wild *H. annuus*, whereas $Pl_7$ came from *H. praecox* and $Pl_8$ from *H. argophyllus*. Vear et al. (1997) showed that the $Pl_6$ locus could be split into at least two genetically distinct regions, each conferring resistance to different *Plasmopara* races. Furthermore, Bouzidi et al. (2002) assumed at least 11 functional *Pl* genes at the $Pl_6$ locus since the sunflower lines that contained this locus were resistant to 11 races of downy mildew.

The inbred line Arg1575-2 carries the *P. halstedii* resistance locus $Pl_{Arg}$, introgressed from the wild species *H. argophyllus*, which confers resistance to all known races of the fungus (Seiler 1991; Gulya 2000; G.J. Seiler, personal communication). The objective of the present study was to map the $Pl_{Arg}$ locus within $F_2$ individuals of a cross between the lines CmsHA342 (susceptible) × Arg1575-2 (resistant) using simple sequence repeat (SSR) markers, in order to elucidate the genetic fine structure of the $Pl_{Arg}$ locus.

## Material and methods

### Plant materials

Phenotypic resistance evaluation was conducted in a subset of 126 $F_{2:3}$ families of the cross between CmsHA342 × Arg1575-2, after immersion of the whole seedlings in a suspension of *P. halstedii* spores (race 730) (Gulya 1996). 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 fungal sporulation was evident on cotyledons and true leaves, and resistant if no sporulation was observed on true leaves (Mouzeyar et al. 1993, 1994). Plants with sporulation only on the cotyledons were considered as resistant, but progenies with many such plants were re-tested to confirm the classifi-

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cation as resistant, susceptible or segregating. After infection with *P. halstedii*, $F_2$ plants corresponding to the $F_3$ families were then classified as homozygous susceptible, homozygous resistant, or heterozygous. SSRs were screened for polymorphisms by bulked segregant analysis (BSA, Michelmore et al. 1991) in the susceptible inbred line CmsHA342, the resistant inbred line Arg1575-2, a resistant (Br), and a susceptible bulk (Bs) with 15 resistant and 15 susceptible $F_2$ individuals of the cross between CmsHA342 × Arg1575-2.

### SSR analyses

A total of 180 SSRs (Tang et al. 2002) were screened for polymorphisms between CmsHA342 and Arg1575-2. Those SSRs showing polymorphisms between the resistant and the susceptible parents were analyzed by BSA. SSRs identifying polymorphisms between Br and Bs were mapped with the 126 $F_2$ individuals used for phenotypic evaluation of downy mildew. SSR analyses were performed as described by Tang et al. (2002) on an ALFExpress DNA Sequencer (Amersham Pharmacia, Freiburg, Germany).

### Statistical analyses

Marker orders and map distances for the CmsHA342 × Arg1575-2 population were calculated with JOINMAP 3.0 (Stam 1993) using a LOD threshold of 4.0 and the mapping function of Kosambi (1944).

## Results

The downy mildew resistance tests of the (CmsHA342 × Arg1575-2) $F_3$ families, derived from 126 $F_2$ plants, resulted in 16 homozygous resistant, 82 segregating, and 28 homozygous susceptible progenies ($\chi^2$=13.6, Table 1). Assuming one dominant resistance gene conferring resistance to *P. halstedii* race 730, the segregation showed a significant distortion from the expected 1:2:1 Mendelian ratio, as indicated by $\chi^2$ analysis. An excess of heterozygotes could be observed at the expense of the number of homozygous resistant progenies while the number of homozygous susceptible progenies fitted the expectation of one quarter. Additionally, the number of individuals carrying the marker alleles of Arg1575-2 in the homozygous state was reduced in the same way (Table 1).

Of the 180 SSRs, 66 were polymorphic between the resistant parent Arg1575-2 and the susceptible parent CmsHA342. Analysis of these 66 SSRs using the susceptible and the resistant bulks identified 12 polymorphic SSRs linked to the *Plasmopara* resistance gene $Pl_{Arg}$. All of these SSRs mapped on the same linkage group, namely LG1, as based on the map of Yu et al. (2003), spanning a maximum distance of 9.3 cM (Fig. 1). The closest linked SSR mapped within a distance of 1.9 cM to $Pl_{Arg}$. All SSR mapped proximal to $Pl_{Arg}$, whereas no SSR could be identified distal to the resistance gene.

**Table 1** Segregation ratio of nine codominant SSR markers analysed within the set of 126 $F_2$ individuals of the cross between CmsHA342 × Arg1575-2

| Locus | Map position | Segregation observed | $\chi^2$ | Df |
|---|---|---|---|---|
| $Pl_{Arg}$ | 0.0 | 28:82:16 | 13.6 | 2 |
| ORS 662 | 1.9 | 27:63:10 | 12.5 | 2 |
| ORS 1039 | 2.9 | 29:72:13 | 12.4 | 2 |
| ORS 543 | 2.9 | 33:72:12 | 13.8 | 2 |
| ORS 1128 | 2.9 | 33:74:12 | 14.5 | 2 |
| ORS 610 | 3.0 | 34:71:11 | 14.9 | 2 |
| ORS 1182 | 3.0 | 34:70:13 | 12.1 | 2 |
| ORS 710 | 3.0 | 31:75:14 | 12.3 | 2 |
| ORS 959 | 6.2 | 31:72: 9 | 17.8 | 2 |
| ORS 371 | 9.3 | 33:69:13 | 11.6 | 2 |

## Discussion

All SSR markers that were polymorphic between the resistant parent Arg1575-2 and the susceptible parent CmsHA342, as well as between Bs and Br, mapped to LG1 with a maximum marker distance of 9.3 cM between $Pl_{Arg}$ and SSR ORS-371. Therefore, it can be concluded that a single region, $Pl_{Arg}$, on LG1 of Arg1575-2, confers the resistance against all tested races of *P. halstedii*. Nevertheless, $Pl_{Arg}$ confers resistance to all known races of

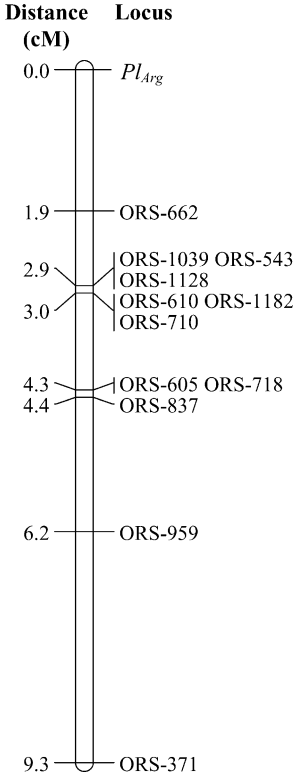


**Fig. 1** SSR map of sunflower LG 1 containing the $Pl_{Arg}$ locus for resistance to *Plasmopara halstedii*. Map distances (in centiMorgan) are based on the analysis of 126 $F_2$ plants derived from the cross between the susceptible line CmsHA342 and the resistant line Arg 1575-2

*P. halstedii* including the Metalaxyl-resistant races (Brahm et al. 2000a; G.J. Seiler, personal communication), and the existence of $Pl_{Arg}$ as a cluster of different but closely linked resistance genes therefore can not be ruled out. Similarly, Vear et al. (1997) reported a complex resistance locus with at least two very tightly linked genes, $Pl_6$ and $Pl_2$, conferring resistance to most races of *P. halstedii*. Brahm et al. (2000b) concluded that the $Pl_2$ locus might be a part of the $Pl_6$ cluster including several closely linked genes, whereas $Pl_{Arg}$ did not belong to the multi-allelic locus $Pl_6$ (Brahm et al. 2000a).

The development of SSR markers for cultivated sunflower (Yu et al. 2002; Tang et al. 2002) has supplied the critical mass of DNA markers needed to create a public map, unify independently developed molecular genetic linkage maps, and establish an universal linkage group nomenclature (Yu et al. 2003). Hitherto, *Plasmopara* resistance genes, such as $Pl_1$, $Pl_2$, $Pl_5$, $Pl_6$, and $Pl_8$ were mapped using marker systems such as RFLPs, RAPDs, and AFLPs (Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Vear et al. 1997; Bert et al. 2001). Although $Pl_5$ and $Pl_8$ were mapped to LG6 in the RFLP-based consensus linkage map of Gentzbittel et al. (1995), the SSRs have confirmed their position on LG13 (Yu et al. 2003). Furthermore, $Pl_1$, $Pl_2$, $Pl_6$, and $Pl_7$, which were mapped to LG1 on the same RFLP map, were placed in LG8 using SSRs (Yu et al. 2003). Additionally, Radwan et al. (2003), have pointed out that linkage groups 1 and 6 of the map of Gentzbittel et al. (1999) correspond to linkage groups 8 and 13 respectively of the map of Yu et al. (2003). Hence, as the map positions of the *Pl* genes are comparable, it can be concluded that $Pl_{Arg}$ is different from $Pl_8$, although both resistance genes were introgressed from *H. argophyllus*. $Pl_8$ was derived from *H. argophyllus* 415 (Miller and Gulya 1991), whereas $Pl_{Arg}$ originated from *H. argophyllus* 1575 (Seiler 1991). Moreover, $Pl_{Arg}$ is most likely different from all the other *Pl* genes because none of them were identified on LG1 with publicly available SSRs (Tang et al. 2002; Yu et al. 2003).

The significant negative distortion of the segregation ratio for the phenotypic resistance evaluation and the SSR markers might be due to the selection of the families. Phenotypic evaluation was conducted for only those $F_3$ families with more than 10 g seed available from the respective $F_2$ individuals. By selecting based on the amount of seeds produced, more homozygous susceptible and segregating families were observed than homozygous resistant families from the approximately 1,500 $F_2$ individuals tested in total. This fact suggests a higher fertility of individuals with a small donor region from the inbred line Arg1575-2. Segregation distortion has been observed in wide crosses of tomato and other species (Zamir and Tadmor 1986), presumably reflecting the effects of competition among gametes or a selection at postzygotic stages. Chetelat et al. (2000) observed a significant segregation distortion at several loci on four chromosomes in a $BC_1$ population of a wide cross between tomato (*Lycopersicon esculentum*) and a wild nightshade (*Solanum lycopersicoides*). The authors also supported the assumption of competition of alleles in wide crosses. Additionally, a 1:2:1 segregation ratio ($\chi^2_{0.05;2}$=0.04) of a single dominant resistance gene were found in 128 independent $F_2$ individuals of the cross between CmsHA342 × Arg1575-2 by Brahm et al. (2000a). Therefore, the segregation distortion in the present study seems to be based on the selection of individuals with a high number of seeds.

Comparisons of LG1 from the $F_2$ population of CmsHA342 × Arg1575-2 and the maps published by Tang et al. (2002) and Yu et al. (2003) uncovered similar SSR marker orders but different map lengths for LG1. The length of LG1 between SSR markers ORS-610 and ORS-371 spanned about 40 cM on the maps for population RHA280 × RHA801 (Tang et al. 2002; Yu et al. 2003) and about 45 cM for population HA370 × HA372 (Yu et al. 2003). In the present study, the distance between the two SSRs ORS-610 and ORS-371 measured about 6 cM and therefore spanned only 15% of the size of the publicly available SSR maps. This reduction of the map length might be due to suppressed recombination in the population CmsHA342 × Arg1575-2. Suppressed recombination has already been observed around plant disease resistance loci that have been introgressed into cultivated crops from their wild relatives. In barley, it has been shown that recombination was suppressed in a 240-kb interval around the *Mla* (powdery mildew) resistance cluster introgressed from *Hordeum spontaneum*, a wild relative of *H. vulgare* (Wei et al. 1999). The *Mla* resistance gene cluster was positioned next to the telomere of the barley chromosome 5 (1H), which is comparable to the position of $Pl_{Arg}$ which we have mapped to the telomeric region of LG1 in sunflower. Suppressed recombination has been observed in other introgressed regions, such as the *Mi* nematode resistance gene (van Daelen et al. 1993) and the *Tm-2a* virus resistance gene (Ganal et al. 1989) in tomato. Both resistance loci were introgressed into cultivated tomato from the distantly related tomato species *L. peruvianum*. *Mi* and *Tm-2a*, however, were physically located near the centromere, where suppressed recombination has been demonstrated in eukaryotic genomes (Copenhaver et al. 1999).

The phenomenon of suppressed recombination and the fact that no publicly available SSR marker could be mapped distal to $Pl_{Arg}$, obscure the elucidation of the fine structure around the $Pl_{Arg}$ locus. Therefore, alternative marker techniques such as sequence-related amplified polymorphisms (SRAPs, Li and Quiros 2001), resistance gene analogs (RGAs, Leister et al. 1996), or RAPDs (Brahm et al. 2000b) are currently being applied to saturate the region distal to $Pl_{Arg}$ with closely linked markers. Identification of markers closely linked to both sides of $Pl_{Arg}$ using a high-resolution mapping strategy (Bauer and Graner 1995) will be essential for the selection of recombinants for the $Pl_{Arg}$ locus and can answer the question of whether $Pl_{Arg}$ harbors a single gene or a gene cluster like $Pl_6$ (Bouzidi et al. 2002). However, the results reported here are of importance for breeders since Arg1575-2 provides a new source of resistance to *P. halstedii*. Miller et al. (2002) registered two fertility restorer sunflower germplasms RHA419 and RHA420 derived from the cross RHA 373 × Arg1575-2 expressing resistance against *Plasmopara* races 300, 700, 730, and

770. Combined with other known *Pl* resistance loci, $Pl_{Arg}$ should supply a multigenic resistance protecting sunflower cultivars against the spread of new *Plasmopara* pathotypes.

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