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Identification and validation of breeder-friendly DNA markers for Pl_{arg} gene in sunflower

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Abstract Downy mildew is a fungal disease of sunflower that can lead to severe yield losses. The damage caused by the pathogen can be controlled by growing resistant sunflower varieties. Gene Pl_{arg} was introgressed into cultivated sunflower from the wild species *Helianthus argophyllus* and provides resistance against all known downy mildew races. In this study, we used a mapping population from the cross-RHA 419/RHA-N-49. We identified a new co-segregating simple sequence repeat marker ORS675 and confirmed the co-segregation of markers ORS716 and ORS662 with Pl_{arg} gene. The markers were validated on two registered resistant inbred lines RHA 443 and RHA 464, as well as on twenty inbred lines RH 1–20 obtained through methods of classical breeding. Molecular marker ORS716 was assessed for usefulness in selecting resistant progeny in 12 BC populations. Markers were found to be valuable for molecular breeding in diverse genetic backgrounds and enabled transfer of the resistance gene in different sunflower genotypes.

Keywords Downy mildew · Pl_{arg} · Sunflower · SSR · Validation · MAS

Introduction

Downy mildew, caused by *Plasmopara halstedii* (Farl.) Berlese et de Toni, is a common sunflower disease that can cause significant yield reduction. Infection of the susceptible varieties is enhanced by warm and humid conditions (Sakr 2010). Damage caused by pathogens can be reduced with the use of fungicides. However, their application increases production costs, leads to environmental problems and enhances the risk of the fungicide tolerance in the fungus population (Albourie et al. 1998). Growing resistant varieties is an effective way of ensuring stabile yield. Therefore, development of resistant cultivars is an important objective in sunflower breeding programs (Kaya et al. 2012), especially in regions where the disease is severe.

At least 37 races of downy mildew have been identified worldwide (Gulya et al. 1991; Gulya 2007; Liu et al. 2012). Extensive research on downy mildew has resulted in identification of a number of different resistance genes in germplasm of wild and cultivated sunflower. To date, at least 23 downy mildew

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resistance genes have been reported to exist ($Pl_{1–16}$, *Plv*, *Plw*, *Plx–z*, *Mw*, *Mx* and Pl_{arg}) and 11 of them have been mapped (Jocic et al. 2012). Genetic mapping and segregation studies have shown that some loci consist of clusters of resistance genes with different specificities (Vear et al. 1997). Genes Pl_1, Pl_2, Pl_6, Pl_7 and Pl_{15} were mapped on LG8 (Bert et al. 2001; Bouzidi et al. 2002; Gentzbittel et al. 1998; Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Vear et al. 1997; Radwan et al. 2008; Slabaugh et al. 2003; de Romano et al. 2010), whereas Pl_5 and Pl_8 were mapped on LG13 (Bert et al. 2001; Radwan et al. 2004). Recently, four new resistance genes were placed on LG1 (Pl_{arg}, Pl_{13}, Pl_{14} and Pl_{16}) (Bachlava et al. 2011; Dußle et al. 2004; Radwan et al. 2004; Mulpuri et al. 2009). Resistance gene Pl_{arg} was mapped at the upper end of LG1 (Dußle et al. 2004; Wieckhorst et al. 2010) and Pl_{13} with Pl_{14} at the lower end of the LG1 (Mulpuri et al. 2009).

As the *Pl* genes are race-specific, new races of downy mildew tend to overcome them. For a long-time Pl_6 was efficient, hence this gene was introgressed into the majority of commercial sunflower hybrids. Race 304, which was found in France in the year 2000, was the first to overcome Pl_6 (Vear et al. 2007). In the next 8 years, six more "hot" races were identified in France (307, 314, 334, 704, 707, 714). In 2009, race 374 overcame Pl_6 in the USA (Gulya et al. 2011). Genes Pl_8 and Pl_{arg} derived from the wild specie *Helianthus argophyllus* are currently the only resistance genes for which there are no records of downy mildew races overcoming them (Gulya 2007; Gulya et al. 2011). Dußle et al. (2004) mapped Pl_{arg} in the telomeric region of LG1 using 126 F_2 individuals of cmsHA342/Arg1575-2 and identified flanking markers. Wieckhorst et al. (2010) remapped Pl_{arg} on LG1, enriched the target region with closely linked markers ORS509, HT244 and HT446 and identified co-segregating simple sequence repeat (SSR) and SNP markers ORS716, HT211 and HT722. They reported three co-segregating NBS–LRR class resistance gene candidates (RGC151, RGC52a and RGC52b) and provided groundwork for map-based cloning of this resistance locus.

Resistant cultivars have become one of the most effective methods for controlling downy mildew, and backcross method is used in plant breeding to transfer desirable alleles from a donor parent, which usually has poor agronomic traits, into a recipient elite genotype (Grewal et al. 2008). As an alternative to time-consuming and unreliable phenotypic screening methods, molecular markers can be used as a diagnostic tool to trace the presence of target genes in successive backcross generations (Frisch and Melchinger 2001). For the markers that are used in marker-assisted selection (MAS), conformation is necessary to ensure that they are informative in different crosses (Grewal et al. 2008). Markers with limited application have been reported in different crops, including common bean (Namayanja et al. 2006), soybean (Rani et al. 2011), rice (Sheeba et al. 2009) and wheat (Conaway-Bormans et al. 2003; Sharp et al. 2001). Various reasons as to why the markers have limited application have been reported, including the linkage not being tight enough, or varying widely across different genetic backgrounds, the gene not being expressed in certain genetic backgrounds and the marker being difficult to assay in certain genetic backgrounds (Miklas 2002). The present study was carried out to (1) identify and verify locus-specific molecular markers closely linked to the gene Pl_{arg} that could be used for MAS; (2) validate the linkage between the gene and the markers in genotypes with different genetic background; and (3) validate their potential in marker-assisted selection.

Materials and methods

Plant material

Plant material used for mapping

Inbred lines RHA 419 and RHA-N-49 were used as parental lines for mapping population. RHA 419 is a F_4-derived F_6 fertility restorer created by pedigree selection from the cross-RHA 373/ARG 1575-2. The line is homozygous resistant to downy mildew races 300, 700, 730 and 770 (Miller et al. 2002). RHA-N-49 is a restorer created by pedigree selection from the cross-RHA-ANN-65/RHA-SEL. RHA-N-49 has good combining abilities; however, it is susceptible to all downy mildew races (Table 1).

Plant material used for marker validation

Two registered lines RHA 443 and RHA 464 (provided by USDA, Fargo, North Dakota) were used for validation of the selected markers. RHA 443 is an

Table 1 Lines that were used and their haplotypes

Line	Reference	*Pl* genes	Line description	Molecular profile with SSR markers		
				ORS662	ORS716	ORS675
PC-GR-252	NS breeding material	None	Restorer line resistant to broomrape race E	310	325	245
RHA-N-49	NS breeding material	None	Restorer line resistant to broomrape race E	310	325	245
RHA-UK	NS breeding material	None	Restorer line resistant to broomrape race E	317	325	237
RHA-PL-1	NS breeding material	None	Restorer line	317	325	237
RHA-ANN-65	NS breeding material	None	Very early restorer line	310	325	236
RHA-SP	NS breeding material	None	Restorer line resistant to broomrape race E	310	325	236
SU-RF-49	NS breeding material	None	Restorer line resistant to broomrape race E, tolerant to tribenuron-methyl	310	325	236
NIMI-RF-UK	NS breeding material	None	Restorer line tolerant to imidazolinone herbicides, resistant to broomrape race E	325	325	241
NIMI-RF-72	NS breeding material	None	Early restorer line tolerant to imidazolinone herbicides	325	325	243
HA-26-OR	NS breeding material	None	Female (B) line resistant to broomrape race E	280	323	237
BT-VL-17	NS breeding material	Pl_6	Female (B) line resistant to downy mildew	280	323	243
HA-26-OL-SOL	NS breeding material	Pl_6	Female (B) line resistant to downy mildew race 730 with high oleic content (>80 %)	280	323	241
RH 1–20	NS breeding material	Pl_{arg}	Resistant restorer inbred lines obtained by classical breeding	303	302	–
RHA 419	Miller et al. (2002)	Pl_{arg}	Resistance source	302	303	–
RHA 443	Miller et al. (2006)	Pl_{arg}	Resistance source	302	303	–
RHA 464	Hulke et al. (2010)	Pl_{arg}	Resistance source	302	303	–

$F_{6:7}$ imidazoline herbicide-resistant restorer selected from the cross-RHA426/RHA419//RHA377/AS4379 (Miller et al. 2006). RHA 464 is an $F_{6:7}$ restorer line advanced by pedigree selection from the cross-RHA 418/RHA 419/3/RHA 801//RHA 365/PI 413047 (Hulke et al. 2010) (Table 1). In addition, markers were tested on twenty F_{14} restorer lines resistant to downy mildew, designated RH 1–20. The lines were created by classical pedigree selection from the cross-RHA 419/RHA 583. During selection process, each of the 14 generations was tested for resistance to downy mildew race 730 using whole seedling immersion (WSI) method (Rahim et al. 2002), and resistant plants were further backcrossed. Results of laboratory testing showed inbred lines RH 1–20 to be homozygous resistant to downy mildew.

Plant material used for marker-assisted selection

For backcrossing, RHA 419 and RHA 443 were used as resistance sources, whereas recipient lines are listed in Table 1. The recipient lines were highly divergent and included both restorer and female lines. Apart from having good general and specific combining abilities for seed and oil yield, high tolerance to biotic stresses, each of the recipient lines is characterized by specific significant agronomical traits.

Mapping population

Mapping population was obtained from the cross-RHA 419/RHA-N-49. From the 83 plants that had sufficient amount of seed, corresponding F_3 and F_4 families were developed using the head-row pedigree method.

Downy mildew resistance testing

The parents and the F_4 progeny were evaluated for downy mildew resistance with the WSI test (Rahim et al. 2002). Approximately 120 plants of each of the 83 $F_{2:4}$ families were tested with downy mildew race 730. Seedlings with visible root hairs were submerged in 4×10^4 zoosporangia/ml suspension of *P.*

halstedii. Plants were then grown for 8–14 days in a mixture of perlite and sand (3:2 v/v) in growth chambers under controlled environmental conditions at 18 ± 2 °C. In each tray, four rows were sown including susceptible control L1. When the first pair of true leaves emerged, trays were covered with PE cover to maintain 100 % RH for 48 h, and sporulation on the susceptible seedlings was induced. Seedlings were considered susceptible when sporulation was present on the cotyledons and resistant when no sporulation or only spurious sporulation was observed. Resistance of F_2 plants was evaluated, and goodness of fit of observed segregation ratios was calculated based on the phenotypes of $F_{2:4}$ families.

DNA extraction and PCR assay

Fresh leaf samples were collected and immediately frozen in liquid nitrogen. DNA was isolated using the modified CTAB method (Permingeat et al. 1998). PCR was performed in Eppendorf Gradient Mastercycler. Reaction mixture of 15 µl was used that contained 1× PCR buffer, 3 mM $MgCl_2$, 0.2 mM of dNTPs, 0.3 µM each of 3′- and 5′-end primers, 1 *U* of DNA polymerase, 25 µg BSA and 40 ng of genomic DNA. To reduce non-specific amplification, a touchdown program was used as in Dimitrijevic et al. (2010). PCR products were separated on 2 % metaphor agarose gels using 1× TBE buffer. The gels were stained with ethidium bromide 10 mg/ml and visualized with the BIO-Print system (Vilber Lourmat, Marne la Vallee, France). After closely linked markers were identified, allele length of markers ORS662, ORS716 and ORS675 in the resistant and recipient lines was determined using an ABI PrismTM 3730 sequencer (Applied Biosystems). For this purpose, PCR was carried out with fluorescently labeled primer pairs, and allele calling was carried out with GeneMapper version 4.0 (Applied Biosystems) (Table 1).

Molecular analysis of the RHA 419 × RHA-N-49 population

Twelve SSRs flanking the Pl_{arg} gene (Dußle et al. 2004; Wieckhorst et al. 2010) were selected from LG1 of the public sunflower genetic map (Yu et al. 2003; Tang et al. 2002) (Supplement 1). Chosen markers were preliminary screened on RHA 419 and RHA-N-49, bulk F_1 sample and five randomly chosen F_2 individuals. Based on those results, markers ORS543, ORS662, ORS716 and ORS675 were selected for analysis of the RHA 419/RHA-N-49 population. ORS543 is a marker located in the proximal region of LG1 (Yu et al. 2003) that revealed intense polymorphic bands in the preliminary study. ORS662 and ORS716 are SSR markers that were closely linked to Pl_{arg} in the work of Dußle et al. (2004) and Wieckhorst et al. (2010) and were also polymorphic between the samples that we tested. ORS675 was not used in the previous studies, but showed clearly scorable polymorphism between susceptible and resistant genotypes in our preliminary screening. This marker has been mapped in the same locus with ORS662 and ORS716 on the referent genetic map (Yu et al. 2003) and is hence presumably located near the resistance gene.

Data analyses

Chi-squared analyses were carried out on a RHA 419/RHA-N-49 F_2 population segregating for the Pl_{arg} gene to detect deviations from the expected Mendelian ratios for co-dominant (1:2:1) or dominant (3:1) markers. Genetic map was constructed with Joinmap Version 4.1 (Van Ooijen 2006) using an LOD score of 4.0. Recombination fractions were converted to centiMorgans (cM) using the mapping function of Kosambi (1944). Goodness of fit to expected 1:1 segregation ratio in the BC_1 populations was also tested with Chi-squared analyses.

Marker confirmation and marker-assisted selection

In order to validate marker–gene linkage, downy mildew resistant lines RHA 443, RHA 464 and RH 1–20 were tested with markers ORS662, ORS716 and ORS675. Markers ORS662 and ORS716 were used due to the fact that these markers were closely linked to Pl_{arg} in research of other authors (Dußle et al. 2004; Wieckhorst et al. 2010), whereas ORS675 was selected because it is closely linked marker which has not been used before.

Twelve different genotypes were chosen as recipient lines for marker-assisted selection, with aim of investigating applicability of the marker in different genetic backgrounds. Crossings were made as indicated in Table 3. Prior to MAS, the lines used for backcrossing were genotyped with ORS662, ORS716

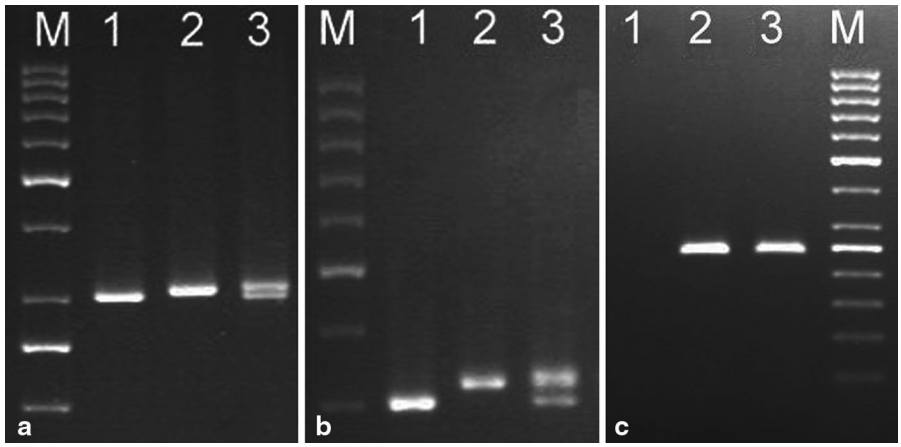

Fig. 1 Genotyping results using SSR markers ORS662 (**a**), ORS716 (**b**) and ORS675 (**c**) for lines RHA 419 (*1*), RHA-N-49 (*2*) and F_1 sample from the cross-RHA 419/RHA-N-49 (*3*). *M*: DNA size marker

and ORS675. ORS716 was more suitable for allele calling on agarose gels and was therefore chosen for testing progeny of the 12 BC_1 populations.

Results

Resistance testing and phenotypic segregations

When tested with *P. halstedii* race 730, parental lines consistently displayed differences in response: RHA 419 was 100 % resistant, while RHA-N-49 was 100 % susceptible. Out of 83 $F_{2:4}$ families that were used for phenotypic evaluation, 20 were non-segregating resistant, 39 were segregating resistant and 24 were non-segregating susceptible. The results of the chi-square test confirmed that the segregation ratio of resistant to susceptible families fitted a 3:1 ratio ($\chi^2 = 0.69$; $P = 0.70$).

Molecular analysis of the RHA 419/RHA-N-49 population

Initial molecular analyses were carried out on a small subset of samples, and polymorphism between parental lines RHA 419 and RHA-N-49, heterozygous F_1 sample and five randomly chosen F_2 individuals was tested. Preliminary screening showed that all of the 12 SSR markers from LG1 were polymorphic between RHA 419 and RHA-N-49, suggesting association of the markers with Pl_{arg} gene. Depending on the profiles of F_1 sample, markers were classified as dominant or co-dominant. Five markers (ORS1128, ORS509, ORS710, ORS371 and ORS675) were dominant, and seven (ORS610, ORS543, ORS1039, ORS1182, ORS662, ORS053 and ORS716) exhibited a co-dominant pattern amplifying alleles from both parents.

Based on their location on the genetic map (Dußle et al. 2004; Wieckhorst et al. 2010) and results of preliminary screening, 4 of the closest markers (ORS543, ORS662, ORS716 and ORS675) that showed intense and clearly scorable polymorphic bands were chosen for analyzing mapping population, as described in Materials and methods. With ORS543, 1,400- and 260-bp-long fragments were obtained in resistant genotype, whereas one fragment of 265 bp was amplified in susceptible genotype. Markers ORS662 and ORS716 (Fig. 1a, b) amplified 302- and 303-bp-long fragments in resistant line and 310- and 325-bp fragments in susceptible line RHA-N-49, respectively. ORS675 amplified a 245-bp fragment only in susceptible line, having no homologous site at the locus from the resistant parent (Fig. 1c).

Table 2 Segregation analysis for molecular markers in the F_2 population from the cross-RHA 419/RHA-N-49

DNA marker	Expected segregation ratio	Observed segregation	χ^2
ORS543	1:2:1	18:45:20	0.69
ORS662	1:2:1	20:39:24	0.69
ORS716	1:2:1	20:39:24	0.69
ORS675	1:3	20:63	0.04

Linkage analyses of the selected markers and genetic map construction

Markers ORS543, ORS662 and ORS716 segregated in a 1:2:1 ratio which fits the segregation of the co-dominant marker. Segregation of ORS675 was 1:3, as expected for the marker with null allele in the resistant line (Table 2). Based on the obtained results, partial genetic linkage map flanking Pl_{arg} was constructed (Fig. 2). Eight recombination events were recorded with ORS543. The recombinants were confirmed by repeating marker amplification profiles of the F_2 individuals. The marker ORS543 mapped proximally to Pl_{arg}, with a map distance of 5.2 cM (Fig. 2). No recombination was recorded between ORS716, ORS662, ORS675 and the Pl_{arg} gene, indicating that the three SSR markers co-segregate with Pl_{arg} gene.

Marker confirmation and marker-assisted backcrossing

The specificity of the markers ORS662, ORS716 and ORS675 to the gene Pl_{arg} was tested on RHA 443, RHA 464 and RH 1–20. The sizes of the amplified fragments in all of the resistant lines obtained with ORS662 and ORS716 corresponded to the 302 and 303 bp fragment in RHA 419, respectively, which indicated that this allele length is specific for the Pl_{arg} locus (Table 1). With ORS675, null allele was detected in the resistant lines as in RHA 419.

ORS662, ORS716 and ORS675 were polymorphic between the parents of all the 12 backcross populations. The recipient lines had different profiles than the resistance sources, but also differed in haplotype among themselves (Table 1). Bigger difference in the length of allele observed in resistance source and alleles in recipient lines was obtained with co-dominant marker ORS716 than with ORS662. Due to that fact, this marker was chosen for marker-assisted backcrossing. ORS716 identified only homozygous susceptible and heterozygous resistant genotypes in each BC population, and the segregation fitted the expected Mendelian ratio of 1:1 (Table 3).

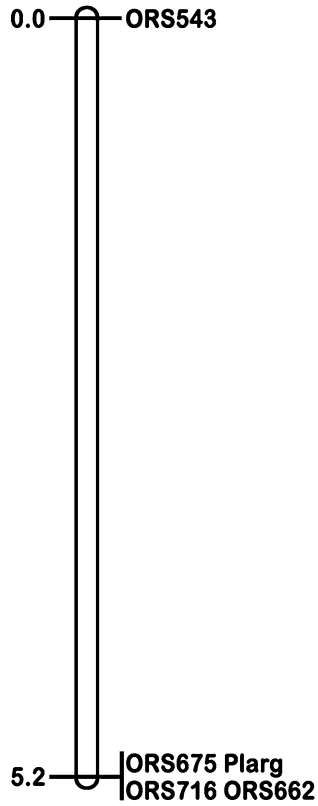

Fig. 2 Genetic map of the Pl_{arg} locus of sunflower showing the localization of SSR markers for the resistance to downy mildew

Table 3 Segregation analysis for molecular marker ORS716 in the BC_1 populations

Population	χ^2	P
RHA-N-49/RHA419/RHA-N-49	0.60	0.44
PC-GR-252/RHA419/PC-GR-252	0.11	0.74
RHA-UK/RHA419/RHA-UK	0.60	0.44
RHA-PL-1/RHA419/RHA-PL-1	0.59	0.28
RHA-ANN-65/RHA419/RHA-ANN-65	0.33	0.56
RHA-SP/RHA419/RHA-SP	0.08	0.78
SU-RF-49/RHA419/SU-RF-49	0.82	0.36
HA-26-OR/RHA419/HA-26-OR	0.81	0.36
BT-VL-17/RHA419/BT-VL-17	0.07	0.80
HA-26-OL-SOL/RHA419/HA-26-OL-SOL	0.82	0.36
NIMI-RF-UK/RHA443/NIMI-RF-UK	0.60	0.44
NIMI-RF-72/RHA443/NIMI-RF-72	1.14	0.28

Discussion

In our work, we found 12 markers that were polymorphic between resistant line RHA 419 and susceptible line RHA-N-49. We constructed a partial linkage map of LG1 that comprised 5 loci. Four markers were SSRs and one was a phenotypic marker

corresponding to the Pl_{arg} gene mapped as a Mendelian trait. We identified a new co-segregating marker ORS675 and confirmed the co-segregation of ORS716 and ORS662 with Pl_{arg} gene, which were used by Dußle et al. (2004) and Wieckhorst et al. (2010). Additionally, we demonstrated application of marker ORS716 in molecular breeding on 12 diverse backcross populations.

Validation of the selected markers

Various resistant lines that have Pl_{arg} were used to test the reproducibility of markers and confirmed marker–gene linkage. Confirmation of markers over different genotypes was also done by Gupta et al. (2006) in wheat, who tested the markers linked to *Lr19* on different resistant and susceptible lines, as well as in Fesenko et al. (2012) where a comparative analysis of the *I2* locus nucleotide sequences of tomato genotypes resistant and susceptible to Fusarium wilt was performed. Our results showed that ORS662, ORS716 and ORS675 are specific to the resistance gene Pl_{arg} in different genotypes. Markers had the same amplification pattern in lines RHA 443, RHA 464 and RH 1–20 as in RHA 419. Genotyping of RHA 419 and RHA 443 was previously done in Wieckhorst et al. (2010), whereas lines RHA 464 and RH 1–20 were genotyped in our work for the first time. All of the lines were homozygous resistant and will be used in breeding programs as a resistance source.

Suppressed recombination in RHA 419 × RHA-N-49 population

According to the referent genetic map of sunflower (Tang et al. 2002; Yu et al. 2003), the distance between ORS716 and ORS543 is 22.4 and 22.8 cM, respectively, whereas in our research, the same markers mapped 5.2 cM apart. This indicates a highly suppressed recombination rate in RHA 419/RHA-N-49 population. Wieckhorst et al. (2010) have also pointed out a lack of recombination in their work and reported that chromosomal rearrangements are not likely the reason for suppressed recombination, rather reduced homology may be a plausible explanation. Suppressed recombination that occurred due to lower chromosome homology has previously been found in populations with wild genome introgression in sunflower (Rieseberg et al. 2000; Ortiz-Barrientos et al. 2002), maize (McMullen et al. 2009) and wheat (Gupta et al. 2006). In a study that included several sunflower mapping populations, Bowers et al. (2012) found that the cross that showed both the largest regions of reduced recombination and lowest rate of recombination was the only cross that involved wild sunflower ANN1238. A recombination rate of 5.2 cM in our study was higher than in the work of Dußle et al. (2004) and Wieckhorst et al. (2010), who found that the distance between ORS716 and ORS543 was 2.4 cM and 0.3 cM, respectively. This could be due to a reduced proportion of wild sunflower genome in line RHA 419 compared to line ARG1575-2 which was used as a resistance source in Dußle et al. (2004) and Wieckhorst et al. (2010). Higher chromosome homology between RHA 419 and RHA-N-49 in our study resulted in higher recombination rate, bringing our result closer to a genetic distance of 22.8 cM mapped on the public genetic map of Yu et al. (2003). All this can be explained by the fact that, even though wild genome introgression reduces recombination, chromosome block sizes derived from wild species tend to decrease in selfing and sib-mating breeding populations over time (Rieseberg et al. 2000) leading to higher homology between the genomes.

Null alleles in interspecific crosses

Most of the SSR markers developed for common sunflower amplify alleles from closely related sunflower species and should have broad utility for comparative mapping in *Helianthus* (Heesacker et al. 2008; Pashley et al. 2006). However, in our study, marker ORS675 failed to amplify fragments in the lines with the resistance gene that was introgressed from wild species *H. argophyllus*. Hence, in lines RHA 419, RHA 443, RHA 464 and RH 1–20, null allele was detected. This phenomenon was also observed in other crosses with wild species (Gupta et al. 2006; Doebley et al. 2006; Muller et al. 2011). As a marker with null allele in resistant genotypes, ORS675 is significant for identifying homozygous resistant genotypes in final stages of backcrossing. Additionally, marker ORS675 could become a valuable resource for genetic purity analyses of parental inbred lines. The main source of genetic contamination in foundation seed production is pollen from other sunflower fields, as well as pollen from volunteer and weedy sunflower (Miklic et al. 2011). By testing

genetic purity with molecular markers, the risk of contamination could be significantly reduced. Marker ORS675, which amplifies only susceptible allelic form, could provide a much needed tool for detection of contamination even in bulk samples.

Marker-assisted backcrossing

Particular marker is defined relative to the parental lines of interest (Hassan et al. 2011), and it is often the case that the marker identified through the process of fine mapping may not be polymorphic in different populations (Perez-Vich and Berry 2010). Utility of markers in selecting resistant genotypes needs to be determined outside the original mapping populations. Validation of marker in different populations was done by Mudge (1999), who tested utility of SSR marker Satt309 linked to soybean cyst nematode resistance gene *rhg1*. The marker was tightly linked to SCN resistance in two populations, but linkage was loose in the third. Similarly, Sharp et al. (2001) reported loose linkage between a molecular marker and wheat rust resistance gene *Lr28* when the gene was present in different genetic backgrounds. In our study, we used 12 different populations for purpose of testing the SSRs linked to Pl_{arg} in various genetic backgrounds. When tested on recipient lines, markers ORS662, ORS716 and ORS675 showed high level of polymorphism, which is particularly important for SSR markers used in MAS. Conaway-Bormans et al. (2003) reported that the utility of a marker DP1 linked to bacterial blight resistance in rice *Pi-z* was limited because many susceptible varieties contained an allele of DP1 that is not linked to *Pi-z* but which gives a band of exactly the same size and sequence. In our research, marker alleles in all the recipient lines differed from the ones linked to resistance gene, indicating that these markers could be used for Pl_{arg} detection in the wide range of genotypes used in breeding.

From the aspect of breeding, the most significant was ORS716. This marker was useful for screening large amounts of material using agarose gels and successfully identified the heterozygous resistant individuals in each of the backcross populations which were used for further crossings. ORS716 will serve as an important tool for rapid transfer of the resistance gene in other sunflower genotypes and will greatly increases the efficiency of backcrossing by identifying resistant plants without infecting them.

This is the first report of successful use of molecular markers linked to gene Pl_{arg} in MAS. The markers that were identified and confirmed in this study have shown to be of great use in molecular breeding. Resistance gene pyramiding is gaining considerable importance as it could improve the efficiency of plant breeding, and the molecular markers presented herein will be very valuable for this purpose. Combined with molecular markers tagged to other *Pl* genes, the confirmed SSRs which co-segregate with Pl_{arg} will be used for large-scale marker-assisted selection, thereby shortening the length of the breeding programs.

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