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Development of SCAR markers linked to the gene *Or5* conferring resistance to broomrape (*Orobanche cumana* Wallr.) in sunflower

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Abstract A consensus molecular linkage map of 61.9 cM containing the *Or5* gene, which confers resistance to race E of broomrape *orobanche cumana*, five SCAR markers (three dominant, two codominant) and one RAPD marker were identified based on segregation data scored from two F_2 populations of susceptible×resistant sunflower line crosses. Bulked segregant analysis was carried out to generate the five SCAR markers, while the single RAPD marker in the group was identified from 61 segregating RAPD markers that were directly screened on one of the two F_2 populations. The five SCAR markers, RTS05, RTS28, RTS40, RTS29 and RTS41, were significantly (LOD≥4.0) linked to the *Or5* gene and mapped separately at 5.6, 13.6, 14.1, 21.4 and 39.4 cM from the *Or5* locus on one side, while the RAPD marker, UBC120_660, was found at 22.5 cM (LOD=1.4) on the opposite side. These markers should facilitate the efficient transfer of the resistance gene among sunflower breeding lines. As the first report on molecular markers linked to a broomrape resistance gene, the present work provides a starting point to study other genes and to examine the hypothesis of the clustering of broomrape resistance genes in sunflower.

Key words SCAR · RAPD · Bulked segregant analysis · Marker-assisted selection · *Orobanche cumana* · *Helianthus annuus*

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Introduction

Broomrapes (*Orobanche* spp.) are destructive holoparasitic angiosperms infecting roots of many economic crops (Parker 1994). One broomrape species, *Orobanche cumana* Wallr. (syn. *Orobanche cernua* Loefl.), causes severe yield losses in sunflower in Eastern Europe and some Mediterranean countries (Vranceanu et al. 1986). In Turkey, due to the broomrape problem, the sunflower production area was reduced by as much as 50% during the period 1956–1982 (Bulbel et al. 1991). In Spain, broomrape has become one of the most dangerous phytopathological problems that threatens the survival of sunflower as a commercial crop in the most important cropping areas (Dominguez 1996).

Control of this parasite remains extremely difficult because the throusands of tiny seeds produced by one single broomrape plant can be easily dispersed by wind, water, animals, humans, machinery or soil attached to agricultural products. The seeds may remain viable for 15–20 years and capable of germination in the presence of the host plant (Pustovojt 1975; Skoric 1988). Several control methods have been investigated including the use of herbicides (García-Torres et al. 1994, 1995), soil solarization (Sauerborn et al. 1989), crop rotation with pepper as a catch crop (Hershenhorn et al. 1996), and modifying sunflower planting time (Aydin and Mutlu 1996).

Breeding for resistant sunflower cultivars is considered the most effective and feasible method to control this parasitic weed. The first sunflower cultivars resistant to broomrape were developed in the USSR between 1912 and 1917 against the local broomrape, which was designated as race A (Vranceanu 1977). Several years later, these resistant cultivars became susceptible to a new, more virulent race, race B, which caused a 7–9-fold reduction in seed yield until new resistant cultivars were developed (Pustovojt 1975). In the early sixties, these cultivars resistant to races A and B were infected by a broomrape population which included a few more-virulent physiological races (Skoric 1988). Vranceanu et al.

(1980) studied broomrape populations from the Braila area in Romania. They identified five physiological races, A, B, C, D and E, of the parasite and suggested the presence of five different resistance genes, *Or1–Or5*, based on the five different hosts. Acimovic (1980) reported the occurrence of seven races in a broomrape population collected from Vojvodina in Yugoslavia. Bulbul et al. (1991) confirmed the presence of races A to E in the Thrace region of Turkey. Several physiological races of broomrape (including the most-virulent races D and E) were also identified in Spain, but the racial composition was different from that of Eastern Europe (González-Torres et al. 1982; Melero-Vara et al. 1989; Saavedra del Río et al. 1994a; Refoyo and Fernández 1994).

Breeding for resistance to broomrape is, however, laborious, time-consuming and dependent on environmental conditions. Since broomrape populations are commonly used for virulence studies, and more than one race may occur in a population, results can lead to confusing conclusions. This could explain the different observations concerning the heredity of broomrape resistance in sunflower, namely: a single dominant gene (Vranceanu et al. 1986; Ish-Shalom-Gordon et al. 1993; Sukno 1997), a single recessive gene (Ramaiah 1987), two independent dominant genes (Domínguez 1996), modifier genes (Ssavedra et al. 1994b), quantitative factors (Pustovojt 1966), or cytoplasmic factors (Russell 1981), depending on the resistance source studied. However, monogenic dominant inheritance seems to be the most-likely case.

To confront the continual appearance of new virulent broomrape races, sunflower breeders have to search for new resistance genes and introgress them into current breeding materials without losing the existing resistance genes. Molecular markers linked to broomrape resistance genes would permit a better understanding of the genetic determinism of broomrape resistance in sunflower and facilitate early selection by simply screening the presence of a few specific DNA markers.

Using the RAPD technique (Williams et al. 1990) in conjunction with bulked segregant analysis (BSA, Michelmore et al. 1991), molecular markers tightly linked to resistance genes have been identified for various plants, such as the genes of resistance to downy mildew in lettuce (Michelmore et al. 1991), those conferring resistance of barley to *Rhynchosporium secalis* (Barua et al. 1993) and to leaf rust (Borovkova et al. 1997), and to powdery mildew in wheat (Hu et al. 1997), among many others. The gene-linked RAPD markers can be converted to sequence-characterized amplified regions (SCARs, Paran and Michelmore 1993), also named sequence-tagged sites (STSs, Olson et al. 1989), which give more-reproducible markers and are more suitable for a marker-assisted selection (MAS) procedure (Paran and Michelmore 1993; Naqvi and Chattoo 1996; Barzen et al. 1997).

In sunflower, following the construction of a consensus RFLP map (Gentzbittel et al. 1995), BSA strategy was used to identify RFLP markers linked to the downy mildew resistance genes *Pl1*, *Pl2* and *Pl6* (Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Vear et al. 1997). All three genes were found to be linked to the same set of RFLP markers and formed a cluster on linkage group 1 (Vear et al. 1997). The present study, using the RAPD-BSA approach, reports the development of SCAR markers linked to the sunflower *Or5* locus conferring resistance to a highly virulent Spanish population of broomrape that belongs to race E. These markers could be used either in a MAS procedure for the selection of new resistant lines or as a starting point to study the molecular genetics of broomrape resistance genes in sunflower.

Materials and methods

Plant materials

Two F_2 populations of sunflower were used in this study: the first one consists of 230 F_2 plants derived from RPG01×AEHC1, named C3, and the second one consists of 204 F_2 plants derived from RPG01×R300V, named C4. RPG01 is a sunflower resistant line while AEHC1 and R300V are lines susceptible to race E of broomrape. In addition to the three parental lines, nine other inbred lines, namely AA724, AO1750, HA89, HIR 1734.3, PAC2, RHA147, RHA271, CG01 and CG02, from the collection of Rustica Prograin Génétique (Mondonville, France), were also used to characterize the SCAR markers developed.

Resistance testing

Tests on broomrape susceptibility were carried out in two different growth chambers in Spain, one at Arlesa-Semilla (Sevilla) and the other at IAS-CSIC (Córdoba). Broomrape seeds (population SE194 collected from central Andalucía, southern Spain, essentially consisting of race E) were homogeneously mixed with peat previously screened through a 2-mm sieve (1.5 g seed: 500 g peat). Sunflower seeds were planted in this mixture (approximately 10 g in each tube containing one sunflower seed). After irrigation, the tubes were incubated in growth chambers at 25±3°C with a 16-h photoperiod. The evaluations of resistance were performed from 25 to 35 days after planting.

The genotype of each F_2 plant was determined by segregation analysis of the resistance in its F_3 family. Twenty F_3 seedlings from every F_2 plant were examined for their susceptibility to the broomrape infection, half of them in each of the two growth chambers. The results were grouped and the F_2 plants from each cross were divided into three classes according to the following reactions of the F_3 plants tested: homozygous resistant (RR), no susceptible plants; heterozygous (Rr), at least one plant but not all were susceptible; homozygous susceptible (rr), all the plants tested were susceptible. A few data sets, which were not complete, were excluded from the analysis.

DNA extraction and RAPD analyses

DNA was isolated from the young leaf tissue of F_2 plants and 12 sunflower lines using the CTAB method as described by Gentzbittel et al. (1995). The concentration of DNA was adjusted to 5 ng/µl for PCR amplification use.

RAPD primers were purchased from Operon Technologies (Alameda, Calif.) and the University of British Columbia Biotechnology Center (Vancouver, B.C.). PCR reactions were carried out in a 20-µl volume containing 67 mM TRIS-HCl, pH 8.0, 16.6 mM $(NH_4)_2SO_4$, 2.0 mM $MgCl_2$, 100 µM of each dNTP, 0.6 µM of

primers, 1% DMSO (Sigma), 4 µg of bovine serum albumin, 0.05% W1 (Gibco BRL), 1.0 U of *Taq* DNA polymerase (Gibco BRL), and 20 ng of template DNA. DNA amplification was performed in microtitre plates in a PTC-100 Programmable Thermal Controller (MJ Research, Inc.). The PCR profile consisted of one cycle of 4 min at 94°C, followed by 45 cycles of 1 min at 93°C, 1 min at 36°C, and 2 min at 72°C, then one cycle of 6 min at 72°C for final extension. Ten microliters of PCR products were electrophoresed for 3 h at 300 V on a 10% vertical polyacrylamide gel (20×15 cm) using 1×TBE buffer. After staining with ethidium bromide, gels were visualized under UV.

BSA and SCAR analyses

Bulked segregant analysis was carried out as described by Michelmore et al. (1991). One homozygous-resistant and one homozygous-susceptible bulked segregant progeny DNA pool was constructed for each of the two crosses. For the C3 cross, an equal quantity (volume) of DNA from ten homozygous-resistant and ten homozygous-susceptible F_2 individuals was pooled to form respectively the resistant and susceptible bulks. For the C4 cross, eight homozygous-resistant and seven homozygous-susceptible F_2 plants were used for form the contrasting DNA bulks. A total of 456 random primers were screened against the two bulk pairs. The polymorphism observed within each DNA bulk pair was then analysed both on eight F_2 resistant plants as well as on eight susceptible plants.

The RAPD fragments potentially associated with resistance to broomrape were excised from the gel, and squashed with the micropipette tip just before injecting 100 µl of TE buffer into an Eppendorf tube. Five microliters of this dilution were used in a second 20-µl PCR reaction employing the same RAPD primer (0.2 µM) and performing 30 cycles. The amplified DNA was run on a 10% polyacrylamide gel to verify the purity of PCR products. The RAPD fragments were cloned into an AT vector (LigATor cloning kit, R&D System) according to manufacturer's specifications. The DNA inserts were amplified directly from bacterial cultures of different clones by a PCR reaction using a pair of vector-specific primers: 5´-CACAGGAAACAGCTATGACC-3´ and 5´-GCCAGGGTTTTCCCAGTCAC-3´. Positive clone identification was done by a size comparison between amplified inserts and the original RAPD fragments. The successfully cloned DNA fragments were sequenced from PCR products by Eurogentec (Seraing, Belgium).

Using DNA sequence data, SCAR (Paran and Michelmore 1993) primers F1 and R1 were designed and synthesized. For 4 of 5 *Or5*-linked SCAR markers, a second primer pair (named F2 and R2) located more internally and being compatible with the first pair were designed using the software program OLIGO version 5 (Rychlik et al. 1990). The SCARs obtained were named RTS (Rustica Tournesol STS) followed by a number designating the original RAPD fragment.

PCR-amplification of SCARs was performed using the RAPD conditions with the following modifications: 5% of DMSO, 0.8 µM of each primer, an annealing temperature of 60°C and 30 cycles of amplification instead of 45. PCR products were electrophoresed either on a 1.2% agarose gel in 0.5×TBE buffer or on a 10% vertical polyacrylamide gel using 1×TBE buffer. When two SCARs were co-amplified in a single reaction, the concentration of each primer remained at 0.8 µM. For the monomorphic SCAR markers, 5 µl of PCR products were first monitored in an agarose gel and the remainder was digested overnight with one unit of enzyme *Taq*I at 65°C. The digested products were resolved in a 10% polyacrylamide gel for 1 h 30 min at 300 V.

Linkage analysis

Chi-square (χ^2) tests were performed to examine the goodness-of-fit between the expected Mendelian ratio and the segregation data scored for RAPD and SCAR markers and the resistance trait. Linkage analysis was first performed using MAPMAKER/EXP version 3.0b (Lander et al. 1987) for each population. The software JOINMAP version 1.4 (Stam 1993) was then used to generate a consensus linkage group based on the data from the two F_2 populations. In all cases, the Kosambi mapping function (Kosambi 1944) was used to convert recombination frequency into genetic map distance (in centimorgans).

Results

The sunflower line RPG01 was fully resistant to the Spanish broomrape population SE194 (race E) that attacks genotypes carrying genes *Or1–Or4* (data not shown). All tested F_1 plants of the crosses C3 and C4 were resistant. Ninety nine out of 230 F_3 families of C3 and 84 out of 204 F_3 families of C4 were successfully characterized. The distribution of F_2 plants was

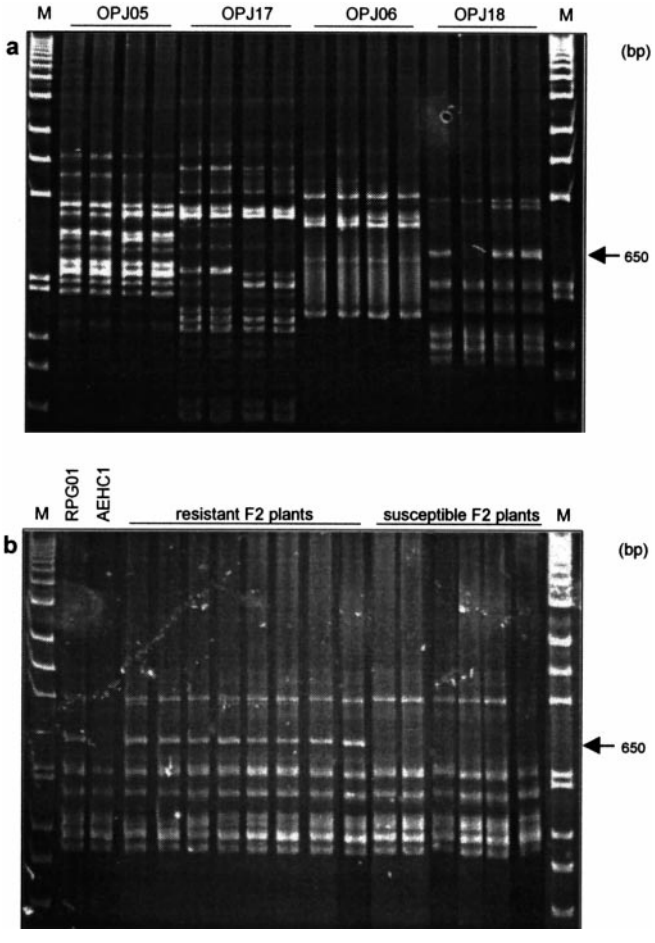

Fig. 1a, b Ethidium bromide-stained polyacrylamide-gel electrophoretic profiles of RAPD products. **a** RAPD screening using four different random primers against two pairs of alternate resistant/susceptible DNA bulks (*from left to right*) respectively from populations of crosses RPG01×AEHC1 and RPG01×R300V. A polymorphism (indicated by an *arrow*) was observed with the OPJ18 primer. **b** RAPD analysis of individual F_2 plants in the resistant/susceptible bulks from the cross RPG01×AEHC1 using the OPJ18 primer. *Lane M* shows a molecular-weight marker 1-kb DNA ladder (Gibco BRL)

Table 1 Five *Or5*-linked SCAR loci and a monomorphic SCAR marker, their original RAPD fragments (primer name_fragment size), and their primer sequences. The underlined nucleotides are derived from RAPD primers

SCAR marker	Initial RAPD fragment	Primer	Primer sequence (5´ to 3´)	Polymorphism[a] on	
				RPG01× AEHC1	RPG01× R300V
RTS05	OPJ18_650	F1	TGGTCGCAGATGGACGTGTGGGTG	Dominant	
		R1	GTCGCAGAGAGTGAGAGAGAGTGT		
		F2	AAGTTTGAATCCGTAGTTGATG		
		R2	GAGTGTTCTAGGGTTTCTAAAATG		
RTS28	UBC111_520	F1	AGTAGACGGGCAAAGCGAAAGGAT	Dominant	Dominant
		R1	AGTAGACGGGTTGAATATGTTGAA		
		F2	CGGAGGTAATTGGGATGAACATT		
		R2	AAGCAGGAAAAGAAGATGGTTAGC		
RTS29	UBC138_330	F1	GCTTCCCCTTAATGATCCGGAAGA	Codominant	Codominant
		R1	GCTTCCCCTTGGCTAGAAGATGAA		
		F2	CGACCGTTTAATGGCAACAGTAGC		
		R2	GTTTTCCGGTTCCTCTTCAGCATA		
RTS40	UBC264_370	F1	TCCACCGAGCTACCAGTTCCGGAG		Dominant
		R1	TCCACCGAGCGAGCATATTCCGAG		
		F2	CGATAAGCGACGAGCGATTTG		
		R2	GAGCTACCAGTTCCGGAGAAAACC		
RTS41	UBC293_440	F1	TCGTGTTGCTGATCGGAAAGGAAC	Codominant	Codominant
		R1	TCGTGTTGCTCAACAGTGGAGAAT		
RTS43	UBC318_350	F1	CGGAGAGCGAGCATAGGGTCAGGT		
		R1	CGGAGAGCGACGACATCATTCCAA		

[a] A polymorphis was observed after the digestion of the monomorphic PCR product by the enzyme *Taq I*

20(RR):43(Rr):36(rr) (χ^2=6.88, P>0.025) for C3 and 10(RR):48(Rr):26(rr) (χ^2=7.81, P>0.01) for C4.

Among the 456 random primers screened on the two pairs of resistant and susceptible bulks, 15 gave no amplification at all, while the others amplified from 5 to 15 fragments each (Fig. 1a). Forty seven RAPD fragments amplified by 44 random primers seemed to be polymorphic between the contrasting DNA bulks in at least one of the two populations. Six fragments were simultaneously polymorphic in both populations, while 16 were polymorphic specifically to the C3 population and 25 were specifically to the C4 population.

The forty seven polymorphic fragments were then analysed on DNAs of the parental lines and the individual F_2 plants used for the bulk constructions (Fig. 1b). Out of them, 30 fragments were reproducible and showed obvious differences in frequency between the two contrasting bulks. These fragments were then cloned and sequenced. Analysis of sequencing data showed the presence of two pairs of allelic fragments as indicated by an internal deletion/insertion and the base mutations observed between sequences. In each case, the terminal ten bases matched the original RAPD primer sequence.

A total of 28 pairs of SCAR primers (24-mer, including ten bases of the initial RAPD primer sequence) were determined. The sequence of six SCAR primer pairs (F1/R1) are presented in Table 1. Genomic DNA from 12 sunflower lines including the three parents were used for PCR-amplification with each pair of SCAR primers. Apart from one that failed to amplify any band under the conditions employed, all the other 27 pairs of SCAR primers amplified the expected fragments (same size in comparison with the initial RAPD fragment) on at least one of the three parents. Supplementary or non-specific fragments were co-amplified with some primer pairs. Ten SCAR primer paris gave monomorphic amplification profiles, while 17 others revealed polymorphisms (ten dominants, four codominants, three dominant/codominant mixed) among the 12 inbred lines tested. Figure 2a shows five polymorphic SCAR amplification patterns obtained on 12 sunflower lines. Sixteen of twenty seven amplified-SCARs were polymorphic (ten dominant, six codominant) on at least one of two crosses: four (two dominants, two codominant) were polymorphic only on the cross C3, four (three dominants, one codominant) on the cross C4, and eight (five dominant, three codominant) on both crosses. Two additional codominant polymorphic SCARs were obtained on both crosses after restriction of monomorphic PCR-products with the enzyme *Taq*I.

The possibility to co-amplify two SCARs in a single PCR reaction was also investigated. Seven pairs of SCARs were tested against eight F_2 plants from the cross C3, of which five gave successful co-amplification of both two SCARs while two others failed to correctly amplify the expected cumulative profiles. As an example, co-amplification of a codominant (RTS26) and a dominant (RTS08) marker is shown in Fig. 2b. The dominant

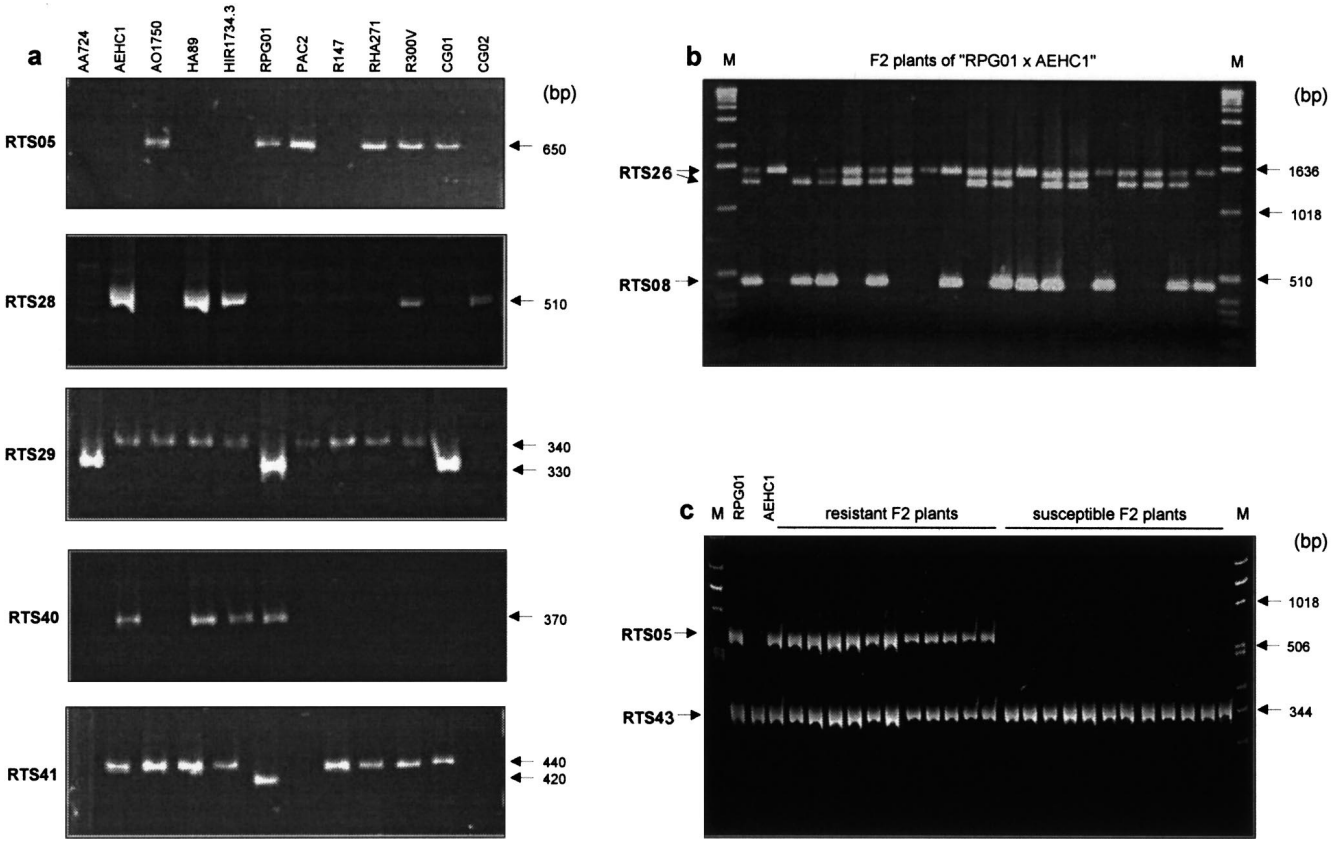

Fig. 2a–c Ethidium bromide-stained polyacrylamide-gel electrophoretic profiles of SCAR markers. **a** Analysis of five *Or5*-linked SCARs among 12 sunflower lines. **b** Intra-PCR simultaneous co-amplification of a codominant SCAR marker, RTS26, and a dominant SCAR marker, RTS08, on 19 F_2 plants of the cross RPG01×AEHC1. **c** Intra-PCR co-amplification of the SCAR marker RTS05 and a monomorphic SCAR marker RTS43 on 12 resistant and 12 susceptible F_2 plants of the cross RPG01×AEHC1

SCAR marker RTS05, tightly linked to the *Or5* gene (see the following paragraph), was successfully co-amplified with the monomorphic marker RTS43 (Fig. 2c).

In addition to 14 SCAR markers, 61 polymorphic RAPD bands revealed by 31 random primers were analysed on the C3 population at the beginning of the programme. For the C4 population, only 14 polymorphic SCAR markers were screened. A linkage group containing the *Or5* locus was identified from each population, and a consensus map of 61.9 cM was constructed using the segregation data from the two populations (Fig. 3). Five SCAR markers, RTS05, RTS28, RTS40, RTS29 and RTS41, were located at the same side of *Or5*, and one RAPD marker was found on the opposite side. All five SCAR markers were significantly linked to the *Or5* gene with a LOD score varying from 4.0 to 18.7, while the RAPD marker UBC120_660 was mapped 22.5 cM distal from *Or5* with a LOD=1.4 (Fig. 3). Four SCAR markers have a distance interval ≤20.3 cM from the *Or5* locus. The nearest marker, RTS05, was mapped at 5.6 cM from *Or5* on the consensus map.

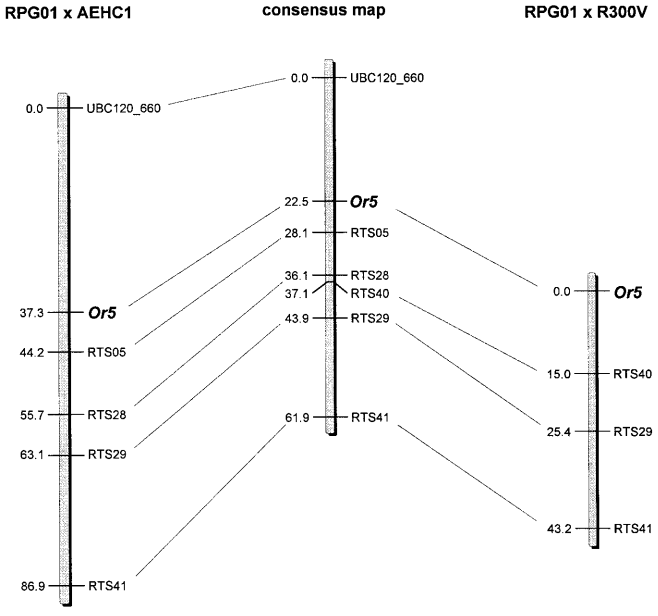

Fig. 3 Molecular maps of sunflower linkage group containing the *Or5* locus conferring resistance to race E of broomrape. Two individual maps were first obtained from the analysis of 230 F_2 progenies of RPG01×AEHC1 and 204 F_2 progenies of RPG01×R300V by MAPMAKER. A consensus map was constructed by JOINMAP using segregation data from the two populations. In both cases, the Kosambi function was used for mapping. 'RTS' loci designate SCAR markers derived from RAPD fragments and 'UBC120_660' refers to a RAPD marker of 660 bases revealed by the UBC120 primer. The cumulative map distances in centimorgans are shown at the left of each map

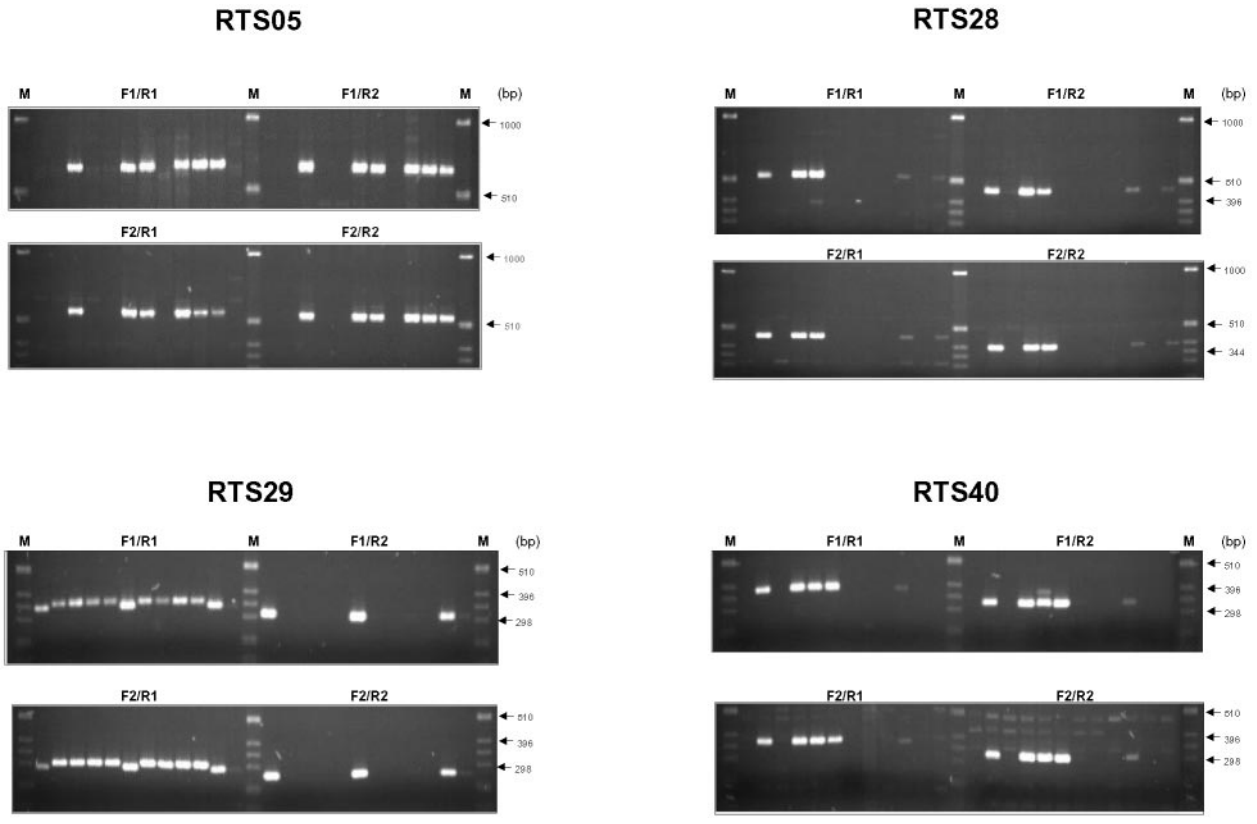

Fig. 4 Ethidium bromide-stained agarose-gel electrophoretic profiles of PCR products from 12 sunflower lines (same lines and order as indicated in Fig. 2a) using four different primer-pair combinations (F1/R1, R1/R2, F2/R1 and F2/R2) for each of four *Or5*-linked SCAR loci (RTS05, RTS28, RTS29 and RTS40). For a single SCAR locus, F1 and R1 refer to the first designed primer pair while F2 and R2 are a more internally located second primer pair

With the objectives of transforming dominant markers into codominant and improving the existing polymorphism of *Or5*-linked SCAR markers (Fig. 2a, Table 1), a second pair of primers (F2 and R2), located more internally and compatible with the first pair (F1 and R1), were designed for RTS05, RTS28, RTS40 and RTS29. Each SCAR can be amplified by four possible primer combinations: F1/R1, F1/R2, F2/R1 and F2/R2. The amplification patterns obtained from the 12 inbred lines using different primer combinations of the four SCARs are shown in Fig. 4. For RTS05, RTS28 and RTS40, all four possible primer pairs gave identical segregation patterns indicating that the observed polymorphism in 12 lines probably resulted from chromosomal insertion/deletion events instead of point mutations. For RTS29, the use of primer R2 transforms the codominant marker into a dominant marker suggesting that R2 hybridizes to a site where an insertion/deletion (of about ten bases) occurred among sunflower lines (Fig. 2a).

Discussion

RPG01 is one of several sunflower lines that carry the *Or5* gene conferring resistance to race E of *O. cumana*. The objective of the present study was to identify molecular markers, essentially SCAR markers, linked to broomrape resistance gene *Or5* that may be useful for breeding resistance to broomrape race E.

Two F_2 populations generated from the crosses RPG01×AEHC1 and RPG01×R300V showed a distorted Mendelian segregation ratio for the character of resistance ($P>0.025$ for C3, $P>0.01$ for C4) in favor of the susceptible class. This could be due to the residual heterogeneity of the resistance gene *Or5* in RPG01, or to the presence of a small fraction of other physiological race(s) in the broomrape population SE194.

Bulked segregant analysis (Michelmore et al. 1991) was first carried out to identify several RAPD markers potentially linked to the *Or5* locus. Twenty seven of these RAPD markers were cloned, sequenced and converted into SCAR (Paran and Michelmore 1993) markers. Eighteen were polymorphic on at least one of the two crosses and were analysed on the appropriate F_2 population(s). Linkage analysis has permitted the identification of a molecular linkage group containing the *Or5* locus along with five SCAR markers on one side and one RAPD marker on the opposite side (Fig. 3). This is the first report of molecular markers linked to a broomrape resistance locus in sunflower.

All five SCAR markers were significantly linked to the *Or5* locus. The nearest marker, RTS05, was estimat-

ed at 5.6 cM from the *Or5* locus. Three other markers, RTS28, RTS40 and RTS29, were located within a distance interval of about 20 cM from the resistance gene. UBC120_660 is the only marker located on the opposite side of the *Or5* locus. This marker was included in the linkage group by a significant association with the SCAR marker RTS28 (pairwise distance=30.3 cM, LOD=4.0). The conversion of UBC120_660 into a co-dominant/dominant SCAR marker would permit a more accurate characterization of the F_2 plants and a more reliable position of this marker on the linkage map.

The identified SCAR markers, especially RTS05, are being successfully used for introgression of the *Or5* gene from RPG01 into other advanced sunflower lines in backcross programs. The dominant marker RTS05 (650 bp) was co-amplified together with the monomorphic marker RTS43 (340 bp) in a single PCR reaction. This monomorphic marker serves as an internal control for the dominant marker RTS05 and permits one to distinguish the absence of PCR amplification due to a genetic factor with that due to a technical problem. The optimal PCR conditions are being researched for a simultaneous amplification of all five SCAR markers in a single reaction.

One of the hypotheses in the broomrape resistance genetic studies is the clustering of genes, as has been shown for resistance to downy mildew in sunflower (Roeckel-Drevet et al. 1996; Vear et al. 1997). This can be examined by checking the association between our markers and other resistance genes (*Or1–Or4*) in an appropriate population, as has been shown for the downy mildew resistance genes *Pl1, Pl2* and *Pl6* which were found to be linked to the same set of RFLP markers and formed a cluster on the linkage group 1 (Vear et al. 1997).

An important consideration in using MAS is the ability of a marker to reveal a polymorphism, and the type of polymorphism (dominant/codominant) involved. SCAR markers, while very easily used, may be less-efficient in detecting polymorphism in comparison with microsatellite markers. Two or three alleles (null alleles included) were observed with each of the five *Or5*-linked SCAR markers among the 12 sunflower lines (Fig. 2a). The polymorphism of SCAR markers can be enhanced by the digestion of monomorphic PCR products with restriction enzymes (Paran and Michelmore 1993). The remaining 9 of the 28 BSA-derived SCARs which were monomorphic in the two crosses should be digested with a number of restriction enzymes to identify new *Or5*-linked SCAR markers. The cloned RAPD fragment which is used for generating SCAR markers could also be used as a DNA probe for RFLP analysis to give more polymorphic alleles, as shown by Salentijn et al. (1995) in sugar beet, by Burow et al. (1996) in peanut and by Mestre et al. (1997) in citrus. In a recent study in rapeseed, we found that the monomorphic PCR products of certain STS markers could give a polymorphism when analysed by the SSCP technique (Orita et al. 1989) (unpublished data).

Of the five *Or5*-linked SCAR markers, two (RTS29, RTS41) are codominant while the three most closely linked markers, i.e. RTS05, RTS28 and RTS40, are dominant. Dominant markers are as efficient as codominant markers when used in a back-cross breeding program. The dominance condition may be changed to codominance when another cross is considered. Primer re-designations have not permitted a change in the dominance of RTS05, RTS28 and RTS40, while the codominant marker RTS29 was converted to a dominant marker by using a re-designed primer pair (Fig. 4).

Three molecular linkage maps have been developed in sunflower using RFLP markers (Berry et al. 1995; Gentzbittel et al. 1995; Jan et al. 1988). They have been shown to be useful tools for locating agronomically important traits in sunflower breeding, such as resistance to downy midlew (Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Vear et al. 1997) and oil content (Leon et al. 1995). The integration of our linkage group to these RFLP maps would permit us to locate the *Or5* gene to a reference sunflower chromosome and to find other *Or5*-linked molecular markers.

The present *Or5* linkage map can be used to conduct other BSA-based work by saturating the *Or5* region with more new molecular markers (Giovannoni et al. 1991). The *Or5*-linked SCAR markers, as well as the resistance trait, can serve to generate more-efficient DNA bulks.

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