Theor Appl Genet (2000) 101:977–983

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Identification of molecular markers linked to *Rdr1*, a gene conferring resistance to blackspot in roses

Received: 23 December 1999 / Accepted: 25 March 2000

Abstract Blackspot resistance in the tetraploid rose genotype 91/100–5 had been characterised previously as a single dominant gene in duplex configuration. In the present study a tetraploid progeny (95/3) segregating for the presence of the blackspot resistance gene *Rdr1* were screened with 868 RAPD and 114 AFLP primers/primer combinations. Seven AFLP markers were found to be linked to *Rdr1* at distances between 1.1 and 7.6 cM. The most closely linked AFLP marker was cloned and converted into a SCAR marker that could be screened in a larger population than the original AFLP and was linked at a distance of 0.76 cM. The cloned fragment was used as an RFLP probe to locate the marker on a chromosome map of diploid roses. This is the first report of markers linked to a resistance gene in roses, and the possibilities of using them for a marker-assisted selection for blackspot resistance as well as for map-based cloning approaches are discussed.

Key words Blackspot · *Diplocarpon* · Molecular marker · Resistance gene · *Rosa*

Introduction

One of the most severe diseases of field-grown roses is blackspot, caused by the host-specific facultative fungal pathogen *Diplocarpon rosae* (Horst 1995). Successful infection of susceptible rose genotypes leads to the typical dark spots on infected leaves, surrounded in many cases by heavy chlorotic areas. This results in very early defoliation of the whole plant which, after several successive infections, is severely weakened and eventually dies (Bhaskaran et al. 1974). Due to legal restrictions and increasing consumer concerns about the application of fungicides as the only control measure against blackspot, breeding for resistance has become one of the major goals of current rose breeding programmes. Despite the great economic importance of roses as ornamental plants, relatively little success has been achieved in breeding varieties with resistance against blackspot or other major pathogens and pests. As major genes for the respective traits are lacking in the gene pool of present-day rose varieties, they have to be introgressed from wild rose species. As a means of accelerating this time-consuming process, molecular markers tightly linked to the resistance genes may be a promising tool for marker-assisted selection, as has already been demonstrated for several crop species (Kelly and Miklas 1998; Lawson et al. 1997; Ordon et al. 1998). In roses, very little is known about the inheritance of important characters. Molecular markers have been applied to investigate phylogenetic relationships (Millan et al. 1996) and genetic variation between rose varieties (Ben-Meir and Vainstain 1994; Debener et al. 1996; Hubbard et al. 1992; Torres et al. 1993) and to construct a genetic linkage map (Debener and Mattiesch 1999). The characterisation of five physiological races of the blackspot fungus (Debener et al. 1998) and the monogenic inheritance of *Rdr1*, a gene conferring resistance to all five races in roses (Malek and Debener 1998), has been also described previously.

It has been shown that by combining bulked segregant analysis (Michelmore et al. 1991) with high-resolution marker systems, markers tightly linked to a target gene can be identified even in complex genomes (Bendahmane et al. 1997; Brigneti et al. 1997). Following these strategies it should also be possible to detect closely linked markers to any target gene in tetraploid roses.

The purpose of the study presented here was the identification of molecular markers linked to *Rdr1* in order to

Communicated by F. Salamini

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develop improved breeding strategies based on marker-assisted selection for blackspot resistance in roses and to set up the conditions for the molecular isolation of the gene by map-based cloning.

Materials and methods

Plant material

The rose genotypes used in the present study are part of the genotype collection of the Institute for Ornamental Plant Breeding in Ahrensburg. The origin of the resistant breeding line 91/100–5 has been described previously (Drewes-Alvarez 1992; Malek and Debener 1998). The genotype 91/100–5 served as a parent for crosses with the susceptible cultivars Caramba (progeny 95/1), Pariser Charme (progeny 95/2) and Heckenzauber (progeny 95/3). The resulting F_1 plants segregating in a 5:1 ratio have been characterised previously as resistant or susceptible to the race 5 isolate DortE4, suggesting a monogenic dominant inheritance of *Rdr1*, the corresponding resistance gene (Malek and Debener 1998).

Sixty plants of diploid population 94/1 (derived by a cross between 93/1–119 and 93/1–117) were used to localise the markers linked to *Rdr1* on a linkage map of roses. The origin of this mapping population and the construction of the linkage map have been described previously by Debener and Mattiesch (1999).

DNA extraction

Genomic DNA was isolated using the procedure described by Suhl and Korban (1996) with the following modifications. After incubation of the ground leaf material in extraction buffer (2% CTAB, 3% PVP, 1.4 *M* NaCl, 20 m*M* EDTA, 100 mM TRIS/HCl pH 8.0) for 30 min at 65°C, 0.5 vol. of 8 *M* LiCl was added. The samples were further incubated for 15 min at 65°C, extracted three times with chloroform, precipitated with ethanol and resuspended in TE. Any remaining carbohydrates were digested with Driselase (5 mg/ml in 10 m*M* MES, Sigma Aldrich, Deisenhofen, Germany) for 3 h at room temperature, followed by a treatment with RNAse A (30 µg/ml, Boehringer, Mannheim, Germany) for 60 min. After a phenol/chloroform extraction, the DNA was precipitated with ethanol and resuspended in TE.

RAPD analyses

The RAPD reactions were performed with 20 ng of genomic DNA in a total volume of 25 µl using primers of 10, 15 and 20 bases. The primers, the composition of the reaction buffer and the amplification profiles were as described by Debener and Mattiesch (1998).

Amplified fragment length polymorphism (AFLP) analyses

AFLP analyses were performed as described by Vos et al. (1995) with the following modifications. A 250-ng aliquot of genomic DNA was digested overnight with the restriction enzymes *Hin*dIII (Life Technologies, Eggersheim, Germany) and *Mse*I (Biolabs, Schwalbach, Germany). After ligation of the corresponding adaptors preamplification reactions were conducted with primers carrying one selective nucleotide at the 3'end. The preamplification was followed by selective amplification with primers carrying three additional nucleotides at the 3'end. Three instead of two selective nucleotides were used because they resulted in better resolved fragment patterns. The selective *Hin*dIII primers used were end-labelled with the IRD 800 dye (MWG Biotech, Ebersberg, Germany). The amplified fragments were separated on 6% denaturing polyacrylamide gels and analysed on a Licor-DNA-Analyser Gene ReadIR 4200 (MWG Biotech).

Cloning and sequencing of an AFLP fragment

The AFLP marker M10 was cloned from DNA of the resistant bulk according to Rouppe van der Voort et al. (1997) with minor modifications. To obtain the appropriate fragment amplified with the IRD-labelled primer, different fractions surrounding the supposed location of the fragment were cut out of the polyacrylamide gel and transferred to an Eppendorf tube. DNA was eluted overnight by diffusion. One microliter of the resulting supernatant was reamplified using the selective AFLP primer combination. The amplification products were again separated on a polyacrylamide gel to isolate the fraction containing the appropriate fragment. To reduce the amount of additional undesired bands we repeated the steps taken to isolate the marker fragment. The product amplified from the fraction containing the smallest number of additional fragments was loaded onto an 3% agarose gel. The band was excised from the gel, purified with the biotrap BT1000 system according to the instructions of the manufacturer (Schleicher und Schuell, Dassel, Germany), extracted with phenol/chloroform, precipitated with ethanol and resuspended in 5 µl of distilled water. The AFLP fragment was then cloned into the vector pGEM-T (Promega, Madison Wis.) according the recommendations of the manufacturer. Competent *E. coli* DH10B cells were transformed with 1 µl of ligation mix by electroporation, and recombinant clones were identified by blue/white selection. To identify clones corresponding to the desired fragment, we used each clone as a template for further amplification, using the same selective primers and polymerase chain reaction (PCR) conditions that had revealed the initial AFLP marker.

Sequencing of cloned fragments was carried out using a thermo sequenase cycle sequencing kit with fluorescently labelled M13 primers (Amersham Pharmacia, Freiburg, Germany). Sequence analysis was performed on 6% denaturing polyacrylamide gels using a Licor DNA-Analyser Gene ReadIR 4200 (MWG Biotech).

Primer design and amplification of the sequence-characterised amplified region (SCAR) marker

Based on the sequence of the cloned AFLP fragment oligonucleotide primers were designed with the OLIGO™ PRIMER ANALYSIS software (NBI, Plymouth, UK) for specific amplification of the locus identified by AFLP marker M10. Primers were synthesised by MWG Biotech, Ebersberg, Germany. The PCR reactions were performed in a total volume of 25 µl containing the same components as described for the random amplified polymorphic (RAPD) reactions in Debener and Mattiesch (1998) except for the primer concentration and the addition of 50 m*M* TMAC (Sigma Aldrich, Deisenhofen, Germany). The amplification profile for the primer pair finally used consisted of 1 cycle at 94°C for 5 min, followed by 35 cycles of 1 min at 95°C, 1 min at 60°C and 3 min at 72°C, with a final extension of 10 min at 72°C.

Southern hybridisation

Five micrograms of genomic rose DNA was digested with 300 U of the restriction enzyme *Eco*RI in a total volume of 500 µl, fractionated on 0.8% agarose gels and blotted onto Hybond N membrane (Amersham Pharmacia, Freiburg, Germany) following standard procedures (Sambrook et al. 1989). The cloned AFLP fragment M10 was labelled by random priming with 0.74 µBq µCi of α–[^{32}P]dCTP (Amersham Pharmacia, Freiburg, Germany) followed by a purification over spin columns of Sephadex G50 (Amersham Pharmacia, Freiburg, Germany).

Hybridisation was performed over night at 65°C following 2 h of prehybridisation in 1 *M* NaCl, 1% SDS, 10% dextran sulfate and 70 µg/ml denatured salmon sperm DNA at 65°C. Membranes were washed twice with 1% SSC, 0.1% SDS and twice with 0.5% SSC, 0.1% SDS at 65°C. Filters were exposed to Kodak XOMAT films for 5–7 days.

Linkage analyses

Linkage analysis in the tetraploid populations 95/1–95/3 was performed in the following way. First χ^2-analysis for chromosomal segregation for each locus (expected ratio is 5 : 1) and for homogeneity of the 2×2 contingency table for *Rdr1* and each marker separately was performed to test for the presence of linkage. Since the χ^2-value was highly significant in all cases, recombination frequencies *p* were estimated using the following formula for one crossover event *x*:

$$X = \frac{3a+13(b+c)+10}{2(a+b+c+d)} - \sqrt{\left[\frac{3a+13(b+c)+10}{2(a+b+c+d)}\right] - \frac{30(b+c)}{a+b+c+d}}$$

with *a* = the number of plants with the dominant phenotype at both loci, *b* and *c* the number of plants with the dominant phenotype at one or the other locus and *d* the number of plants with the recessive phenotype at both loci.

Since chromosomal segregation can be assumed and chromatid segregation be rejected in all cases, we consider that for the locus *A* closer to the centromere pure chromosome segregation occurs. In the case of a rare crossover event a chromosome segregation can no longer be assumed for the second locus, *B*, closer to the telomeric end. Since all markers were dominant and in the F_1 in a biallelic state and in coupling phase, a ratio of

17 *A.B.* : 3 *A.bb* : 2 *aaB.* : 2 *aabb*

is expected for the gametes in the case of one crossover event, indicating the 5 : 1 ratio for *A.* : *aa*, but 19 : 5 for *B.* : *bb*.

Since in the biallelic case only two-thirds of the chromosome pairs are involved in the crossover event, the total ratio is :

$$\frac{2}{3}(17\ A.B.:3\ A.bb:2\ aaB.:2\ aabb) + \frac{1}{3}(20\ A.B.:4\ aabb)$$

yielding 27 *A.B.* : 3 *A.bb* : 2 *aaB.* : 4 *aabb* in the case of one crossover (probability *x*) and 30 *A.B.* : 6 *aabb* in the case of no crossover (probability 1–*x*).

x can be estimated by the ML-Method [for example see Bailey (1961) or Weber and Wricke (1994)] inserting the observed frequencies *a*, *b+c* and *d*. This yields the equation given above. Since only two out of eight chromatids are involved in one crossover event, the recombination frequency is $p = 0.25\ x$.

For the computation of the local map around *Rdr1* in the tetraploid background, recombination frequencies between all marker-pairs were used to compute the most likely order with the JoinMap™ computer package version 2.0 using the following parameters: LOD = 0.001, REC = 0.499, mapping function = Kosambi, ripple = 1 jump = 3, triplet = 6 (Stam and van Oijen 1995).

The localisation of the restriction fragment length polymorphism (RFLP) marker SCM10 on the linkage maps of diploid roses was performed as described previously (Debener and Mattiesch 1999). RFLP fragments were treated both as individual dominant markers as well as a codominant marker and located onto the two parental maps with JoinMap™ 2.0. Drawing of the maps was conducted with the Drawmap computer programme (Van Ooijen 1994).

Results

Identification of markers linked to *Rdr1*

As no previous marker data were available for the tetraploid populations segregating for *Rdr1*, a single dominant resistance gene to blackspot (Malek and Debener 1998), bulked segregant analysis (Michelmore et al. 1991) was applied to identify linked markers. In a first step 392 RAPD primers and primer combinations of 10, 15 and 20 bases were analysed on bulks of 20 resistant and 10 susceptible plants from progeny 95/3. As none of the detected polymorphic DNA fragments were linked to *Rdr1* the bulks were reduced to 7 resistant and susceptible plants, respectively, and the analysis was continued with 476 additional primers and primer combinations. Again none of the polymorphic fragments were found to be linked to *Rdr1*. In total, 868 primers and primer combinations were used to amplify about 6100 DNA fragments between 100 and 2000 bp in size with an average of 7 fragments per primer and primer combination. While 24 polymorphic fragments were detected between the bulks, these were not linked to *Rdr1* based on the analyses of individual plants from the bulks.

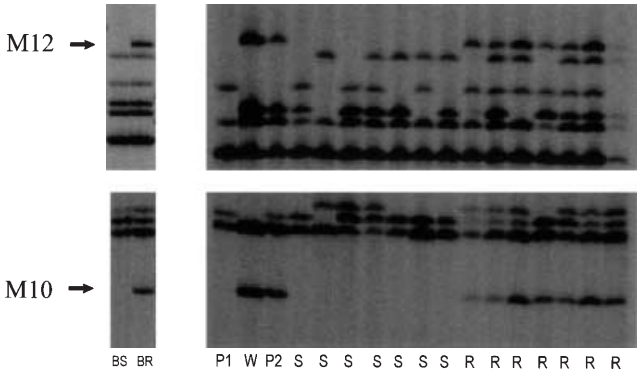

Fig. 1 Fragment pattern of the AFLP markers M10 and M12. The *left portion* of the figure shows the susceptible and resistant bulks (*BS* and BR); the right portion shows the parental genotypes and the individual genotypes of the bulks. P1 Susceptible parent Heckenzauber, *W* 88/124–46 (resistant parent of 91/100–5), *P2* resistant parent 91/100–5, *S* susceptible plant, *R* resistant plant

The reduced bulks were subsequently screened with AFLP markers. Selective amplifications with 114 *Hin*d III/*Mse*I primer combinations generated approximately 10 500 distinguishable DNA fragments, with on average 92 discrete fragments per primer combination. Of the primer combinations 35 revealed at least 1 polymorphic fragment. The subsequent analysis of the single plants belonging to the bulks indicated the linkage of 7 AFLP fragments to *Rdr1*. All seven markers were inherited from the resistant parent 91/100–5. As an example, segregation of the markers M10 and M12 is shown in Fig. 1.

To estimate the distance between the seven markers and *Rdr1*, we carried out linkage analysis with 247 plants belonging to the progenies 95/1, 95/2 and 95/3. The results from the three progenies were merged as the same source of resistance had been used in these crosses; previously performed inoculation experiments had not shown any difference in the segregation ratio (Malek and Debener 1998), and the order of linkage to *Rdr1* for all seven markers was identical in the three populations (data not shown).

All of the markers were observed to segregate in a 5:1 ratio (band present:band absent) and to be linked to *Rdr1* (Tables 1 and 2). As the dominant nature of AFLP markers does not allow the detection of all recombination events in tetraploid populations, the calculated recombi-

Table 1 Segregation analyses of AFLP markers linked to *Rdr1*

Marker	*n*	Marker+[a]	Marker–[a]	χ^{2} [b]	p-value
M10 (*Hin*d+AGA/*Mse*+AGT)	171	146	25	0.52	0.4708
M16 (*Hin*d+ACG/*Mse*+AAG)	247	212	35	1.12	0.2899
M15 (*Hin*d+ACA/*Mse*+AGA)	247	211	36	0.79	0.3741
M18 (*Hin*d+ACA/*Mse*+AGA)	247	209	38	0.29	0.5902
M11 (*Hin*d+AGA/*Mse*+ACC)	247	208	39	0.14	0.7083
M12 (*Hin*d+AGA/*Mse*+AGG)	247	208	39	0.14	0.7083
M1 (*Hin*d+ATA/*Mse*+AAG)	247	208	39	0.14	0.7083
SCM10	247	211	36	0.79	0.3741

[a] Marker +/–, Presence or absence of marker
[b] The χ^{2}-analysis was performed for a segregation ratio of 5:1 (marker+ : marker–)

Table 2 Segregation of AFLP and SCAR markers and test for linkage to *Rdr1* by contingency χ^{2}-analysis

Marker	*n*	R+M+[a]	R+M–	R-M+	R–M–	χ^{2}	p-value	Distance[b] (cM)
M10 (*Hin*d+AGA/*Mse*+AGT)	171	146	1	0	24	163.0	<0.001	1.1
M16 (*Hin*d+ACG/*Mse*+AAG)	247	211	1	1	34	230.8	<0.001	1.5
M15 (*Hin*d+ACA/*Mse*+AGA)	247	210	2	1	34	223.3	<0.001	2.3
M18 (*Hin*d+ACA/*Mse*+AGA)	247	208	4	1	34	209.4	<0.001	3.8
M11 (*Hin*d+AGA/*Mse*+ACC)	247	206	6	2	33	189.0	<0.001	6.1
M12 (*Hin*d+AGA/*Mse*+AGG)	247	206	6	2	33	189.0	<0.001	6.1
M1 (*Hin*d+ATA/Mse+AAG)	247	205	7	3	32	175.5	<0.001	7.6
SCM10	247	211	1	0	35	239.0	<0.001	0.8

[a] R+/–, *Rdr1* present/absent; M+/–, marker present/absent, R+M– and R–M+, recombinant class

[b] Map distances were computed from the recombination frequencies with the JoinMap™ computer programme and represent direct distances between locus pairs

nation frequencies are higher than the observed ones. The map distances ranged from 1.1 for the marker M10 to 7.6 cM for marker M1 (Table 2). All were located on the same side of *Rdr1* and linked in coupling, with M10 being the most closely linked AFLP marker. However, this marker could only be analysed on the basis of 171 plants belonging to the progenies 95/1 and 95/3 because a fragment of identical size was amplified from the DNA of Pariser Charme, the susceptible parent of progeny 95/2.

Conversion of an AFLP fragment to a SCAR marker

To develop a rapid and reliable PCR-based marker for marker-assisted selection as well as a probe for a map-based cloning of *Rdr1*, we cloned and sequenced M10.

Successful cloning of the desired fragment of 250 bp was confirmed by hybridisation of an AFLP reaction of the parents and several resistant and susceptible plants of the progeny 95/3 using the cloned fragment as a probe (data not shown). Three pairs of oligonucleotide primers were designed for specific amplification of the locus. Only one of the three primer pairs designed for the development of a SCAR marker led to polymorphisms between all parental genotypes of the progenies 95/1–95/3. A 209-bp DNA fragment was amplified from the resistant parent 91/100–5 but was absent in the susceptible genotypes Heckenzauber, Pariser Charme and Caramba. Linkage analysis in progenies 95/1–95/3 showed that the SCAR marker SCM10 segregated in a 5:1 ratio and was linked to *Rdr1* (Tables 1 and 2). Only 1 recombinant plant was detected among the 247 individuals under investigation leading to a map distance of 0.76 cM to *Rdr1*. Therefore, as determined in the present study, SCM10 is the marker with the closest linkage to *Rdr1*. The calculation of recombination frequencies between all markers and *Rdr1* was used to calculate a local map around *Rdr1* with the JoinMap™ computer programme (Stam and van Oijen 1995) (Fig. 2). As SCM10 is derived from M10 and cosegregates with it, both markers were placed to the same locus with an intermediate distance of 0.9 cM to Rdr1.

Localisation of M10 on the rose linkage map

To determine the position of *Rdr1* on a linkage map of diploid roses (Debener and Mattiesch 1999) we analysed the parental genotypes of the diploid mapping population 94/1, 93/1–117 and 93/1–119, for polymorphisms of markers linked to *Rdr1*. Mapping *Rdr1* directly in this population was not possible because no segregation of blackspot resistance could be observed (there were no clear differences in the reaction phenotypes against blackspot for the parents). Because none of the AFLP markers could be detected in the parental lines 93/1–117 and 93/1–119 and since the SCAR marker SCM10 did not show any polymorphism between both genotypes, the amplification products of SCM10 were digested with nine frequent-cutting restriction enzymes to produce CAPS markers (cleaved amplified polymorphic sequence,

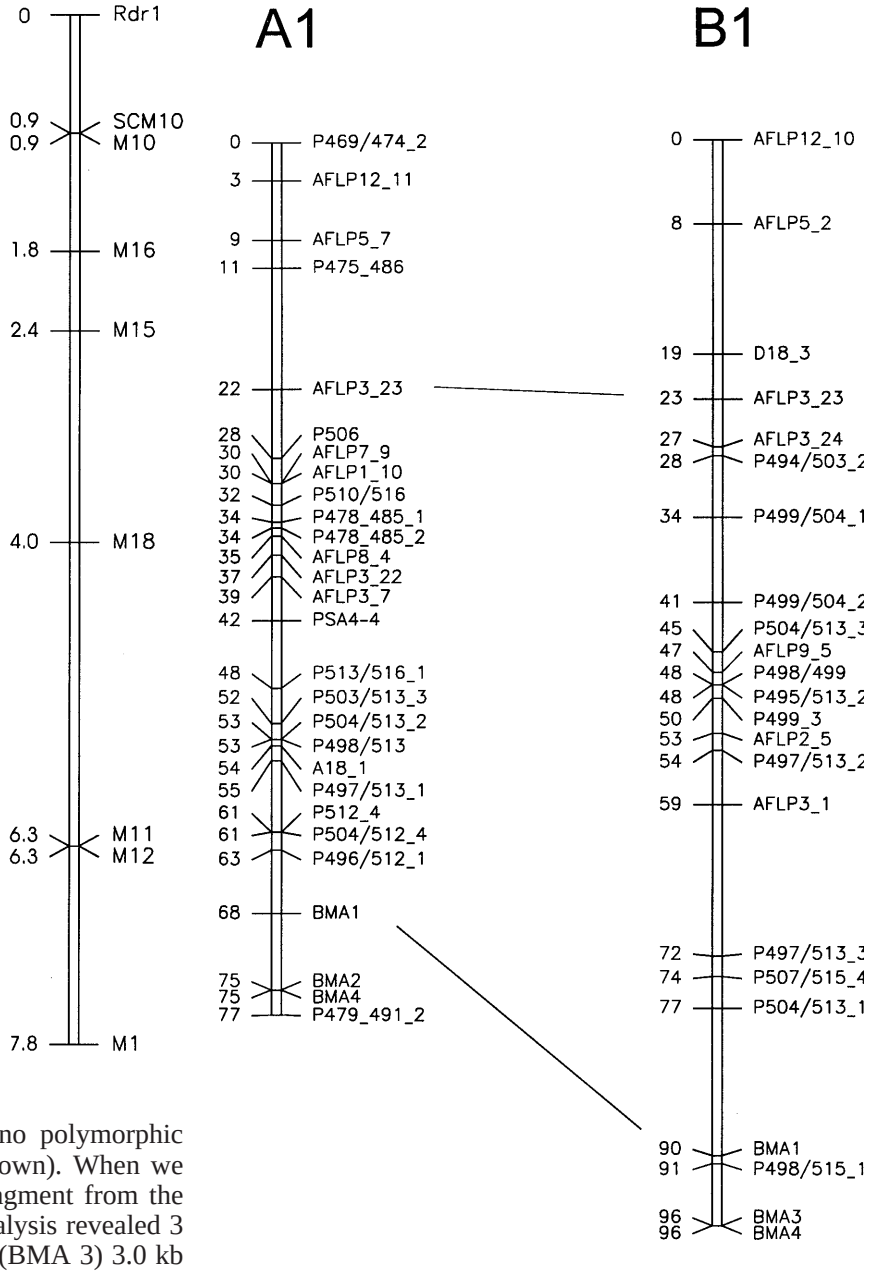

Fig. 2 Local map around *Rdr1*. The map was constructed by providing the JOINMAP™ computer programme with the estimated recombination frequencies between locus pairs. The map distances displayed in centiMorgans at the *left side* of the chromosome are rounded off

Fig. 3 Position of markers BMA1–4 on linkage groups A1 and B1 of diploid roses. Linkage groups were calculated separately for the parents 93/1–117 (*A1*) and 93/1–119 (*B1*) using the JOINMAP™ computer package. Two markers (AFLP 3_23, BMA1) shared by both groups were used to determine the relative orientation of both linkage groups and are connected by a *line*

Konieczyn and Ausubel 1993). Again no polymorphic fragment could be detected (data not shown). When we subsequently used the cloned marker fragment from the AFLP marker M10 as a probe, RFLP analysis revealed 3 polymorphic *Eco*RI fragments of 2.3 kb (BMA 3) 3.0 kb (BMA 2) and 3.3 kb (BMA 1) segregating in the mapping population. Each of two fragments (BMA2, BMA3) were present in one of the parental genotypes but absent in the other, whereas the third fragment was present in both parental genotypes (BMA1). These 3 fragments were treated individually as single dominant markers and in addition were merged to one codominant marker (BMA4). Adding these data to the map dataset of the diploid population enabled us to localise all four markers to the distal ends of linkage groups A1 and B1 of 93/1–117 and 93/1—119, respectively (Fig.3).

Discussion

In the current study we identified seven AFLP markers linked to *Rdr1*, a gene conferring race-specific resistance to blackspot in roses. The marker M10 with the closest linkage was converted into SCAR and RFLP markers and localised on linkage group 1 of the chromosome map of diploid roses. These results are the first report of linkages between molecular markers and a resistance gene in roses.

Initially, 868 RAPD primer and primer combinations were screened without any fragment linked to blackspot resistance being detected. The identification of markers linked to *Rdr1* in the tetraploid populations may have

been hampered by the fact that the resistant bulk consisted of individuals of the simplex and duplex marker genotype (Rrrr or RRrr), therefore diluting the target sequences below the concentration usually encountered in diploid homozygous F_2 individuals, for which the method had been originally developed (Michelmore et al. 1991). However, reducing the number of individuals in each bulk to 7 during the screening procedure failed to overcome this problem. Thus, the identification of markers was more probably hindered by the lower reproducibility of RAPD reactions, which has been observed in several other investigations (Ellsworth et al. 1993; Weeden et al. 1992).

The efficient use of the bulked segregant strategy in tetraploid roses to identify markers closely linked to a target gene was demonstrated with the application of the more informative and more reproducible AFLPs (Vos et al 1995). This also underlines the effectiveness of the AFLP technique for the bulked segregant analysis, as has already been shown for numerous other species, e.g. *Populus* (Cervera et al. 1996), soybean (Kasuga et al. 1997), tomato (Thomas et al. 1997) and potato (Brigneti et al. 1997).

The dominant inheritance of the 7 AFLP fragments in a ratio of 5:1 and their linkage to *Rdr1* in coupling confirms earlier data on the monogenic dominant inheritance of *Rdr1* in duplex configuration from the resistant parent 91/100–5 (Malek and Debener 1998).

Although the resistance gene *Rdr1* could not be bracketed between two closely related markers, the current results represent an important first step in marker-assisted selection for blackspot resistance in roses. The markers can be applied in model breeding programmes for a more efficient development of resistant breeding lines, which can then be used in the breeding of rose cultivars with resistance to blackspot.

Although efficient screening methods for blackspot resistance are available for laboratory purposes (Debener et al. 1998), tests in the field and in the greenhouse are often difficult. In particular, coinfection with other blackspot races and other pathogens in the field and pesticides applied in the greenhouses often interfere with these assays. Furthermore, the introgression of resistance genes from wild rose species, especially into Tea hybrids, has been avoided in the past because of the co-transmission of unwanted characters. Thus, marker-assisted selection would be of advantage in breeding programmes aiming at the introgression of particular resistance genes into the genetic background of modern roses.

However, the applicability of molecular markers in a breeding programme depends on a fast and reliable detection method with a high specificity in diverse genetic backgrounds (Schachermayr et al. 1997). Therefore, the AFLP marker M10 was converted to a SCAR marker. With a distance of 0.76 cM based on 1 recombinant plant among 247 genotypes under investigation, the linkage between SCM10 and *Rdr1* is close enough for marker-assisted selection purposes, as distances of 1 cM or less are considered to be sufficient for marker-assisted selection in plant breeding programmes (Mohan et al. 1997). The use of SCM10 is only limited by the fact that amplification products must be separated on polyacrylamide gels in order to be able to distinguish between resistant and susceptible plants. A solution to this problem would be the development of allele-specific primers which additionally would allow the utilisation of detection systems without gel separation of DNA fragments; for example genetic bit analysis (Alcala et al. 1997).

Molecular markers tightly linked to a gene of interest can also be used for an efficient pyramiding of several resistance genes to create a more durable resistance against one or more pathogens. This has been demonstrated for bacterial blight resistance genes in rice (Huang et al. 1997) and suggested, for example for common bean (Kelly and Miklas 1998) and wheat (Schachermayr et al. 1997).

Though a newly occurring fungal race overcame *Rdr1* resistance in 1998, the breeding line 91/100–5 is still displaying resistance against the five fungal races (Debener et al. 1998) as well as several isolate mixtures of different origins collected in Germany and other European countries. Therefore, it is still an interesting source of resistance against blackspot and is currently being combined with resistance genes from wild rose species that have been identified in another study (Malek and Debener, in preparation).

All markers map to the same side of *Rdr1*, suggesting a position at the end of a chromosome or distorted recombination around the gene of interest due to the introgression of *Rdr1* from the wild rose species *Rosa multiflora* (Malek and Debener 1998). The localisation of BMA1–4 near the end of linkage group A1 and B1 of the chromosome map of diploid roses is in support of a position of *Rdr1* near the telomere.

The localisation of BMA1–4 on the linkage map has further advantages. Additional markers like P479_491_2 and P498/515_1 (Fig.3) from the linkage map can be tested as RAPD, SCAR or RFLP markers in populations segregating for *Rdr1*, thus providing a chance to find markers more closely linked than some of the markers in the present study and ones that may be located telomeric to *Rdr1*.

An additional use of the markers presented here is the cloning of *Rdr1*. The small genome size of diploid roses – for example 0.98 pg DNA per nucleus for *Rosa rugosa* (K. Yokoya and A. Roberts, personal communication), which is about 2–2.5-fold the size of the *Arabidopsis* genome – will facilitate the positional cloning of genes from roses. A large insert BAC-library of *R. rugosa* is currently constructed and already covers 3.1 genome equivalents. In addition, diploid rose populations of more than 400 plants segregating for *Rdr1* in a 1:1 ratio are available. The markers presented here will serve as a starting point for the high-resolution mapping of *Rdr1* in these populations. This will, as a by-product, yield new markers which may be more effective for marker-assisted selection.

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