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ORIGINAL PAPER

# Molecular markers from a BAC contig spanning the *Rdr1* locus: a tool for marker-assisted selection in roses

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**Abstract** We constructed a BAC contig of about 300 kb spanning the *Rdr1* locus for black spot resistance in *Rosa multiflora* hybrids, using a new BIBAC library from DNA of this species. From this contig, we developed broadly applicable simple sequence repeat (SSR) markers tightly linked to *Rdr1*, which are suitable for genetic analyses and marker-assisted selection in roses. As a source for the high molecular weight DNA, we chose the homozygous resistant *R. multiflora* hybrid 88/124-46. For the assembly of the BAC contig, we made use of molecular markers derived from a previously established *R. rugosa* contig. In order to increase the resolution for fine mapping, the size of the population was increased to 974 plants. The genomic region spanning *Rdr1* is now genetically restricted to 0.2 cM, corresponding to a physical distance of about 300 kb. One single-stranded conformational polymorphism (SSCP) and one SSR marker cosegregate with the *Rdr1*-mediated black spot resistance, while one SSR and several cleaved amplified polymorphic sequence or SSCP markers are very tightly linked with one to three recombinants among the 974 plants. The benefits of the molecular markers developed from the *R. multiflora* contig for the genetic analysis of roses and the integration of rose genetic maps are discussed.

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## Introduction

The black spot disease caused by the hemibiotrophic fungus *Diplocarpon rosae* is a major problem in the production of field-grown roses, as it causes significant losses each year (Horst 2007). *Rdr1*, the single dominant resistance gene against black spot disease in roses, was discovered and characterised by Von Malek and Debener in 1998. Since then, several molecular markers for the gene have been developed (Von Malek et al. 2000; Kaufmann et al. 2003). Except for one cleaved amplified polymorphic sequence (CAPS) marker linked to *Rdr1* at a distance of 0.93 cM, all other markers were single-stranded conformational polymorphism (SSCP) or amplified fragment length polymorphism (AFLP) markers. The multilocus AFLP markers are not directly suitable for marker-assisted selection or positional cloning, and the SSCP technique is quite laborious and time-consuming. Therefore, the development of closely linked, robust molecular markers suitable for high-throughput analyses, such as simple sequence repeats (SSRs), also known as microsatellites, would be desirable for further genetic analysis of the *Rdr1* locus in roses. SSRs suitable across the genus *Rosa* also represent valuable anchor markers for the combination of different rose chromosome maps.

Kaufmann et al. (2003) constructed a BAC library from *Rosa rugosa* covering 5.2 genome equivalents. The choice of *R. rugosa* as a source of DNA to construct the library was based on information regarding the small genome size of this species. Its 1 C DNA content is estimated to be 0.5 pg (Yokoya et al. 2000), corresponding to 489 Mb (Dolezel et al. 2003), while, for example, the *R. multiflora* genome is almost twice as large with a 1 C content of 0.83 pg or 812 Mb (Dickson et al. 1992). From the *R. rugosa* library, a contig was assembled around the black spot resistance gene *Rdr1* comprising six BAC clones and

spanning a physical region of about 400 kb. The genetic region was restricted to 0.18 cM on each side of the gene by one recombinant among 538 plants. To identify the corresponding genomic region in *R. multiflora*, we made use of markers derived from the *R. rugosa* contig to screen a genomic library made from a homozygous resistant *R. multiflora* hybrid with reduced insert sizes in a BIBAC vector that also allows plant transformation.

In the present study, we report the assembly of a new minimal contig from *R. multiflora* clones spanning the *Rdr1* locus. SSR, CAPS and SSCP markers tightly linked to or cosegregating with *Rdr1* and suitable for the genetic analysis in the rose genus are presented. The usefulness of the newly developed SSR markers as anchor points on different rose linkage maps as well as for genetic analyses will be discussed.

## Materials and methods

### Plant materials

All roses used in the present study are part of the genotype collection of the Department of Molecular Plant Breeding, Institute for Plant Genetics, Leibniz University of Hannover, Germany. In addition to the original mapping populations 97/7, 97/8 and 97/9 comprising 538 plants, five new populations were generated by back-crossing one heterozygous resistant individual of the F1 population 95/13 to the homozygous susceptible genotype 82/78-1 (Kaufmann et al. 2003, Table 1). All populations are diploid.

### Construction of a *R. multiflora* BIBAC library

The *Rosa multiflora* hybrid line 88/124-46, homozygous for the black spot resistance gene *Rdr1* (Von Malek and Debener 1998), was used to construct a large insert genomic DNA library. The binary vector pCLD04541 (Jones et al. 1992), which was designed for *Agrobacterium*-mediated plant transformation and is capable of stable maintenance of large DNA inserts (Tao and Zhang 1998), was selected as a cloning vector. Isolation of high molecular weight DNA, partial restriction with *Hin*dIII, size selection, ligation and transformation were performed as described by Kaufmann et al. (2003), with a modification in the fragment size range. We selected DNA fragments of 50–150 kb for cloning.

About 60,000 clones were robotically arrayed in 384-well microtiter plates and were gridded in duplicate on 22.5 × 22.5 cm Hybond N+ membranes (GE Healthcare, Freiburg, Germany) in a 5 × 5 format at the Max-Planck-Institut für Züchtungsforschung (Cologne, Germany) using a Microgrid Robot (Biorobotics, Cambridge, UK). Filters were processed and hybridised using standard protocols (Sambrook and Russell 2001).

**Table 1** Crossing parents and population sizes of the new mapping populations

| Population | Crossing parents (female × male) | Number of individuals |
|---|---|---|
| 97/10 | 95/13-94 × 82/78-1 | 260 |
| 06/1 | 82/78-1 × 95/13-90 | 92 |
| 06/2 | 82/78-1 × 95/13-39 | 17 |
| 06/3 | 95/13-39 × 82/78-1 | 45 |
| 06/4 | 95/13-90 × 82/78-1 | 27 |
| Sum | | 441 |

### BIBAC library screening procedure and contig assembly

The *R. multiflora* BIBAC library was screened with a set of *R. rugosa* BAC end probes spanning the *Rdr1* genomic region and one resistance gene analogue (RGA) probe. End probes were amplified in a vectorette PCR (Matallana et al. 1992) as previously described in Kaufmann et al. (2003). The RGA probe was prepared from a 388-bp PCR product amplified under standard PCR conditions and at an annealing temperature of 56°C with the following RGA specific primers: help1 5′-TTTGCGTCTTTCTTGCTAA-3′ and help2 5′-CTTCTTCAGGCTCACATTTC-3′.

The DNA of the positive clones was restricted with *Hin*dIII and *Eco*RI, separated on agarose gels and analysed in Southern blot hybridisations. Positive clones were confirmed by hybridisation to *R. rugosa* probes, and overlaps were analysed with probes generated from clone ends.

### Development of molecular markers from clone end sequences

The clone ends were sequenced by Agowa (Berlin, Germany) or GATC Biotech (Konstanz, Germany). Molecular markers for the end sequences of the contig clones were developed by designing specific primers with Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) using the default parameters. PCR assays were performed with 20 ng of genomic DNA or 1 ng of BAC-DNA in a total volume of 25 µl containing 10 pmol forward and reverse primers (Eurofins MWG Operon, Ebersberg, Germany), 1 unit of *Taq* polymerase (Bioline, Luckenwalde, Germany), 0.2 mM deoxy-nucleotide triphosphates and 2.5 mM $MgCl_2$ in the buffer provided by the manufacturer. PCR conditions were as follows: initial denaturation for 3 min at 94°C, 30 cycles of 45 s at 94°C, 45 s at 56–68°C (Table 2), 1 min 72°C, followed by a final extension step for 10 min at 72°C. To generate

polymorphisms between the rose genotypes, PCR products were digested with different restriction enzymes and separated by standard agarose gel electrophoresis on 2% gels or by nondenaturing polyacrylamide gel electrophoresis on 0.5 × mutation detection enhancement (MDE; Biozym, Hessisch Oldendorf, Germany) gels. Restriction fragments were visualised by ethidium bromide staining in agarose gels or silver staining in MDE gels for SSCP analyses (Orita et al. 1989).

### Analysis of SSR markers

SSR–PCR reactions were performed with 10 ng of genomic DNA in a total volume of 7 µl containing 4 pmol forward and reverse primers, one of which was labelled with IRD700 or 800 (Eurofins MWG Operon), 1 unit of *Taq* polymerase, 0.2 mM deoxy-nucleotide triphosphates and 1.4 mM $MgCl_2$ in the buffer provided by the manufacturer. PCR conditions were as follows: initial denaturation for 3 min at 94°C, 30 cycles of 30 s at 94°C, 30 s at 58 or 60°C (Table 3), 45 s at 72°C. After PCR, 100–250 µl of formamide loading dye (98% formamide, 10 mM EDTA, 0.05% pararosaniline) was added, and 0.5 µl was separated in a 6% denaturing acrylamide gel by means of an automatic LI-COR sequencer under the following conditions: 1,500 V, 31.5 W, 34 A, 45°C.

### SSR data analysis and integration of SSR markers into rose chromosome maps

A measure of the information content of a given DNA marker is the polymorphic information content (PIC) value. According to Anderson et al. (1993), the PIC values for the SSR markers were calculated as follows:

$$\mathrm{PIC} = 1 - \sum_{i=1}^{k} P_i^2$$

where $k$ is the total number of alleles detected for a given marker locus and $P_i$ is the frequency of the $i$th allele in the set of genotypes investigated.

Map construction and integration of SSR markers into the map were performed as described by Linde et al. (2006) with Version 3.0 of JoinMap (Stam 1993; Van Ooijen and Voorrips 2001) using a LOD threshold of 10.

## Results

### Construction and characterisation of the *R. multiflora* BIBAC library and contig assembly

We constructed a BIBAC library from the homozygous resistant *R. multiflora* hybrid 88/124-46 comprising 59,136 clones. One hundred and thirty clones were randomly selected and analysed by *Not*I digestion and pulsed field gel electrophoresis. The average insert size was estimated to be 48 kb. Twenty clones (15%) had no insertion or inserts smaller than 10 kb. The genome size of *R. multiflora* is estimated to be 812 Mb (Dickson et al. 1992); therefore, our *R. multiflora* library contains 3.2 genome equivalents.

To identify clones matching the *R. rugosa* contig from the *R. multiflora* library, we used BAC end-derived markers defining the telomeric and the centromeric end of the *R. rugosa* contig as well as an RGA fragment of the TIR-NBS-LRR class that was distributed on several of the *R. rugosa* clones (Kaufmann et al. 2003). The resulting 50 positive clones were characterised and assembled by hybridisation to probes from within the contig and by *Hin*dIII fingerprinting. In this way, we assembled about 30 overlapping BIBAC clones. Two steps of chromosome walking were necessary to connect one side of the contig to the centromeric end of the *R. rugosa* contig defined by clone 55O14. However, no overlap to the proximal clone

**Table 2** CAPS and SSCP markers derived from BAC clone end sequences

| Marker | Origin (BAC clone) | Primer sequences (5′→3′) | Expected product length (bp) | Ann. temp. (°C) | Restr. enzyme | Marker type | Recomb. freq.[a] |
|---|---|---|---|---|---|---|---|
| 72F | 72G18 | FW:TGAGCAGCCGAAAATGAAGC<br>BW:GCTGCAGGAGCTGCAAAACC | 760 | 60 | *Mbo*II | SSCP | 0.001 |
| 29F | 29O3 | FW:AGGATTTGGACGGTTCAGTG<br>BW:CCTGTTACTCGCCTTCTTCG | 2,679 | 55 | *Mbo*II | SSCP | 0 |
| 124F | 124F13 | FW:GACTGCTCCTGAAGTCCTGC<br>BW:GGGACTATATTCAGCTGCG | 671 | 56 | *Hin*fI | CAPS | 0.001 |
| 69F | 69E24 | FW:CCATGGCTAAGGGATGAAAG<br>BW:GATGTCATGAACCCTCGAC | 694 | 68 | *Mbo*II | SSCP | 0.002 |
| 53R | 53A15 | FW:TTGTGCAGGCTGAAAGTGCAA<br>BW:CCCGCCTCCGGTCATGATGT | 1,041 | 56 | *Mbo*I | CAPS | 0.003 |

[a] Recombination frequency calculated as number of recombinants among 974 plants

**Table 3** SSR markers derived from partial contig sequences

| Marker name | Derived from clone | SSR motive | Primer sequences (5′→3′) | Exp. length of product (bp) | Ann. temp. (°C) | Recomb. freq.[a] |
|---|---|---|---|---|---|---|
| 29Mic | 29O3 | $(CA)_7$ | F:ACAAAACATGCCCTCTTGCT | 162 | 58 | – |
| | | | R:GCTCTTGAGGCATGAATTGG | | | |
| 155SSR | 155F3 | $(GA)_{20}$ | F:GAAAAGAACGAGGGGTTTCC | 157 | 58 | 0 |
| | | | R:ACGGTCGGTAATCAAGATGC | | | |
| 69Mic | 69E24 | $(AG)_9$ | F:GGTTTGGGTTTTATTTGCTTTG | 249 | 60 | 0.001 |
| | | | R:ACGCAAGAAAATGAGGGGTA | | | |

[a] Recombination frequency calculated as number of recombinants among 974 plants

end with marker 55T was achieved. As furthermore *R. multiflora* BAC clones isolated with marker 20T from the telomeric end of the *R. rugosa* contig could not be immediately linked to the centromeric part of the contig, we increased the mapping population to almost 1,000 segregating individual plants.

We first analysed the additional plants with two molecular markers linked to each side of the gene at a distance of about 2 cM. The SSR marker RMS015, developed from *R. hybrida* (CON/CIPIO GmbH, Sangerhausen, Publication number WO/2003/097869) is located 2.26 cM telomeric to *Rdr1*. The CAPS marker Rd1, developed by Kaufmann et al. (2003), is located 2.1 cM from the opposite side of the gene, centromeric to *Rdr1*. Among these 441 plants of the five new populations, we identified 28 recombinants between the two flanking markers, in addition to the 14 recombinants detected in the original population of 538 plants. These were further analysed by inoculation with the black spot isolate 'Dort E4' (Debener et al. 1998) and with additional markers obtained from *R. multiflora* BAC clone ends (Table 2; Fig. 1).

Among the new markers, the SSCP marker 72F, obtained from one end of clone 72G18, defined the telomeric end, whereas the CAPS marker 124F, obtained from clone 124F13, defined the centromeric end of the contig. No recombinants were detected via the SSCP marker 29F, obtained from the inward end of clone 29O3. Markers 69F and 53R were mapped at 0.2 and 0.3 cM to the centromeric end, respectively.

In conclusion, we could delimit the *R. multiflora* contig spanning *Rdr1* to five BIBAC clones with the outermost ends defined by end-derived markers detecting one recombinant each among 974 plants, therefore restricting the genetic interval to 0.2 cM.

A few *R. rugosa* BAC end markers failed to identify corresponding sequences in the *R. multiflora* BAC contig. Sequence analyses revealed that those *R. rugosa* BAC ends not present on the *R.multiflora* contig shared high similarity to retrotransposons. Interestingly, hybridization experiments revealed that the positions of some *R. rugosa*-BAC end sequences were inverted on the *R. multiflora* contig (data not shown).

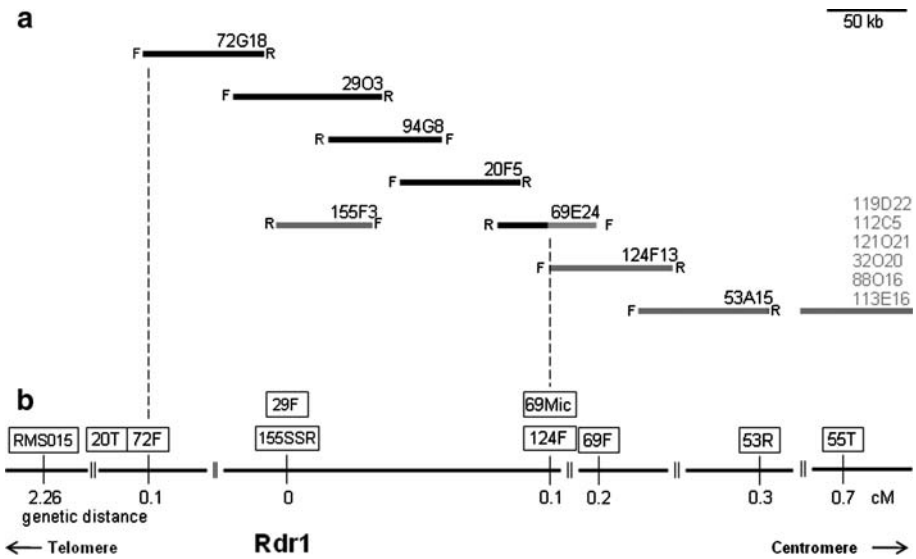


**Fig. 1** Genetic and physical map of the *Rdr1* genomic region in *R. multiflora*. Genetic distances were calculated as recombination frequencies and were directly converted to map units. **a** Overlapping *R. multiflora* BIBAC clones forming the contig of about 300 kb. The core sets of clones spanning the Rdr1 region are illustrated as *black bars*, others are coloured *grey*; redundant clones are not shown. *F* forward end of clone, *R* reverse end of clone. **b** High resolution mapping of the *Rdr1* genomic region in the eight rose mapping populations combining SSR and clone end-derived markers

## Development of new SSR markers based on sequence analysis

Within the scope of a running project aimed at sequencing the complete *Rdr1* contigs from *R. multiflora* and *R. rugosa*, partial sequence information is available for the contig clones (Terefe et al. in preparation). This allowed us to identify some microsatellite motifs on the contig, three of which were used for a conversion into SSR markers (Table 3).

Marker 29Mic, derived from BAC 29O3, is monomorphic between the parents in our mapping populations and is therefore not suitable for mapping in this particular population.

Marker 155SSR cosegregates without recombination to *Rdr1* among the 974 plants. The primers bind to two sites on BAC 29O3 that are not separated by any recombination. This results in the amplification of more than two SSR fragments in diploids and more than four in tetraploids (Table 4; Fig. 2). Furthermore, the heterozygous parents of the mapping population 97/7 share one null-allele for 155SSR, resulting in 25% double null progeny (Fig. 2).

Marker 69Mic detects the same recombinant as CAPS marker 124F. As this marker is derived from within the clone and is easier to analyse (Fig. 2), it was used to define the new centromeric border of the contig.

## Benefits of new SSR markers

### *Application to diversity analysis*

To evaluate the utility of the SSR markers 155SSR, 29Mic and 69Mic in a broader range of rose taxa, we tested these markers with DNA from 28 rose genotypes of different species and varieties belonging to different sections of the subgenus *Rosa* as well as different subgenera (Table 4). This was done in comparison to marker RMS015 (CON/CIPIO GmbH, Sangerhausen, Publication number WO/2003/097869). The four markers detected between 8 and 17 different alleles, with 1–6 SSR fragments per genotype,

**Table 4** Marker data of SSRs 155SSR, RMS015, 69Mic and 29Mic in various rose species and varieties

| No. | Species/variety/genotype | Ploidy | RMS015 | 29Mic | 155SSR | 69Mic |
|---|---|---|---|---|---|---|
| 1. | *R. multiflora* hybrid 88/124-46 | 2x | f | – | i | h |
| 2. | *R. multiflora* hybrid 95/13-39 | 2x | fi | d | il | dh |
| 3. | *R. multiflora* hybrid 82/78-1 | 2x | hi | d | ln | d |
| 4. | *R. multiflora* hybrid 93/1-117 | 2x | f | f | hi | h |
| 5. | *R. multiflora* hybrid 93/1-119 | 2x | bh | df | ln | ad |
| 6. | *R. majalis* D054001 | 4x | n | cfg | lm | 0 |
| 7. | *R. majalis* 93/09-01 | 4x | aek | – | eiloqr | 0 |
| 8. | *R. majalis* 93/09-02 | 4x | aek | cfg | eiloqr | 0 |
| 9. | *R. omeiensis* 93/39-01 | – | aek | cfg | eiloqr | l |
| 10. | *R. multiflora* 93/27-02 | 2x | – | – | ln | ef |
| 11. | *R. multiflora* hybrid 91/100-5 | 2x | fo | – | – | bh |
| 12. | *R. rugosa* | 2x | m | g | l | 0 |
| 13. | *R. wichuraiana* Ahrensburg | 2x | ch | e | il | ei |
| 14. | *R. majalis* D052001 | 4x | bknp | eh | lqr | fk |
| 15. | *R. majalis* D051001 | 4x | mn | g | lr | h |
| 16. | *R. foetida* var. *persiana* 93/14-02 | 2x | k | adf | l | – |
| 17. | *R. pisocarpa* 93/33-01 | 2x | fo | ef | il | bh |
| 18. | *R. wichuraiana* England | 2x | de | f | hlpr | eh |
| 19. | *R. caudata* 94/103-1 | 4x | kn | ef | cdklq | 0 |
| 20. | *R. pimpinellifolia* | 4x | kn | afg | lr | 0 |
| 21. | *R. mollis* | 4x | bkn | b | r | gk |
| 22. | *R. carolina* | – | – | ceg | r | hk |
| 23. | *R. multiflora* 93/27-04 | 2x | fg | e | ln | ch |
| 24. | *R. multiflora* hybrid CT4 | 4x | – | e | – | h |
| 25. | 'Heckenzauber' | 4x | – | f | fgilmr | 0 |
| 26. | 'Caramba' | 4x | eio | – | abimr | bk |
| 27. | 'Mirato' | 4x | – | f | abilmr | k |

Lower-case letters represent different marker fragments whereas a '0' indicates a null allele in this genotype. Missing data are marked with '–'

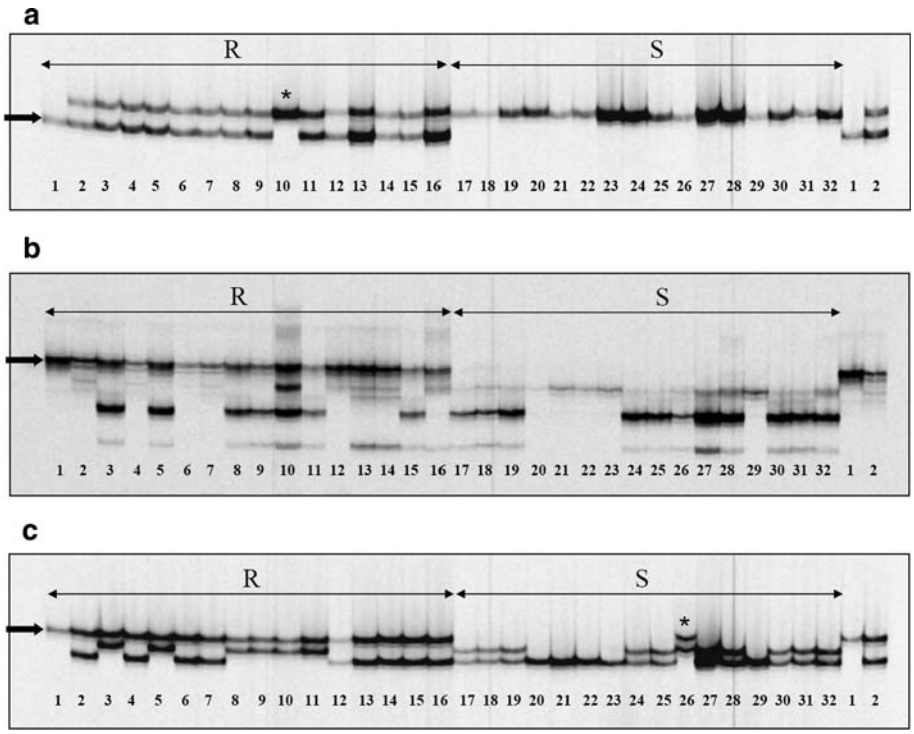


**Fig. 2** Marker pattern for SSR markers 69Mic (**a**), 155SSR (**b**) and RMS015 (**c**) linked to the resistance locus *Rdr1* in a subset of the diploid mapping population. *1* Homozygous resistant grandparent 88/124-46, *2* heterozygous resistant parent 95/13-39, *3–16* resistant plants (R), *17* homozygous susceptible parent 82/78-1, *18–32* susceptible plants (S); the following two recombinants are marked with an *asterisk '*':10* genotype 06/3-31 recombinant for marker 69Mic, *26* genotype 97/7-252 recombinant for RMS015

**Table 5** SSR marker characteristics in various rose species and varieties

| | RMS015 | 29Mic | 155SSR | 69Mic |
|---|---|---|---|---|
| No. of plants analysed | 24 | 23 | 28 | 14 |
| No. of different alleles | 15 | 8 | 17 | 10 |
| Total no. of alleles | 49 | 40 | 78 | 24 |
| Polymorphic information content | 0.9 | 0.8 | 0.86 | 0.84 |

and had PIC values between 0.78 and 0.9 (Table 5). Even marker 29Mic, which is not polymorphic in our mapping population 97/7, detected several polymorphic bands between the genotypes. Among these are the parents of another mapping population (93/1-117 and 93/1-119) that has been used to construct the first rose linkage map (Debener and Mattiesch 1999).

*Application of integration of genetic rose maps*

The SSR markers 155SSR and RMS015 (Fig. 2) were integrated in two different genetic maps for rose populations 97/7 and 94/1 (Linde et al. 2006; Yan et al. 2005). In both maps, they are located in the telomeric region of linkage group 1, closely linked to *Rdr1* (Fig. 3). The application of these microsatellites confirms the estimated position of the *Rdr1* allele in the population 94/1, which is not segregating for the resistance to the isolate DortE4.

## Discussion

We constructed a contig around the *Rdr1* gene for black spot resistance in roses and developed three new SSR markers from the locus that can now be used in genetic analyses of black spot resistance and linkage mapping.

As a prerequisite for cloning the *Rdr1* gene, we developed a BAC library from the *R. multiflora* hybrid 88/124-46 comprising about 60,000 clones with an average insert size of 48 kb. This is the third published BAC library for roses (Kaufmann et al. 2003; Hess et al. 2007). In contrast to the first two libraries, it is made in a transformation–competent vector that allows complementation with whole BAC clones via particle bombardment (Ercolano et al. 2004). In order to facilitate future complementation experiments, we cloned fragments with average insert sizes smaller than those generally used in BAC cloning projects (Cai et al. 1995; Marek and Shoemaker 1997; Woo et al. 1994).

For contig assembly, we made use of previously developed molecular markers derived from a *R. rugosa* BAC contig (Kaufmann et al. 2003). As the *R. rugosa* genotype used for constructing the first library did not carry the *Rdr1* allele conferring resistance and segregating in our populations, we could not use the original clones for complementation.

To narrow down the contig to a small number of clones, we increased the resolution of the genetic map around *Rdr1* by increasing the size of the original mapping population to 974 plants. This large number of individual plants

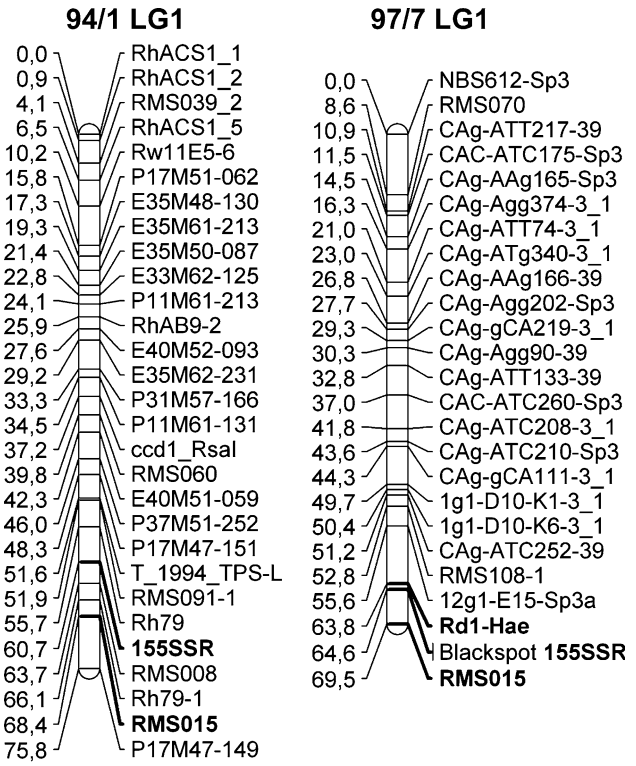


**Fig. 3** Genetic maps for linkage groups 1 of the populations 94/1 and 97/7. The markers 155SSR, RMS015 and Rd1-Hae closely linked to *Rdr1* are shown in *bold*

improved the genetic resolution to 0.1 cM on each side of the contig. Thereby, the contig was restricted to five clones, for a final size of 368 kb. We estimate that these BACs span a region of about 300 kb, based on an estimation of overlaps from *Hin*dIII fingerprints (data not shown). This is a clear improvement as compared to the previous *R. rugosa* contig spanning a physical distance of 400 kb, corresponding to a genetic region of 0.36 cM (Kaufmann et al. 2003).

Not all of the *R. rugosa* BAC end sequences detected corresponding clones in the *R. multiflora* contig. However, as these BAC ends contained retroposon sequences, the missing corresponding clones might indicate a different sequence organisation of this region in *R. rugosa* and *R. multiflora.*

The locations of some *R. rugosa*-BAC end sequences were inverted on the *R. multiflora* contig, which is also an indication of rearrangements in the *Rdr1* region between *R. rugosa* and *R. multiflora*. Such intergenic rearrangements have often been observed, especially in complex genomic regions containing resistance loci. Ballvora et al. (2007) found gene-by-gene co-linearity disrupted by non-allelic insertions of retrotransposon elements, stretches of diverged intergenic sequences and differences in gene content and even gene order between two homologous chromosomes of one *Solanum demissum* genotype. Wicker et al. (2005) reported a multitude of deletions and insertions at the barley Hv-elF4E locus, as well as the insertion of numerous transposable elements.

The complete sequence of one *R. multiflora* BIBAC clone is available, and sequence analysis revealed the presence of various copia-like retrotransposons on it (Terefe et al. in preparation). These repetitive elements are also dispersed over the whole *R. rugosa* contig and severely impede correct contig assembly (Kaufmann et al. 2003).

From the partially available contig sequence, we developed two SSR markers, one of which is cosegregating among 974 plants with *Rdr1* (155SSR). Marker 69Mic detects one recombinant on the centromeric side of the gene, thereby representing the right border of the contig.

The third microsatellite marker, 29Mic, could not be located in relation to the *Rdr1* gene, as it does not segregate in our mapping populations. However, as it originates from BAC 29O3 also harbouring marker 155SSR, which is one of the three central clones of the contig, it is both physically and genetically close to *Rdr1* and is therefore useful in mapping this locus in populations with different genetic backgrounds. As 29Mic differs in size from the other two SSRs, multiplexing of all three markers would be feasible in automatic detection systems using fluorescently labelled primers.

The new SSR markers represent a valuable tool for the high-throughput analysis of the *Rdr1* locus in roses. With this marker set, it is possible to analyse additional segregating rose populations in a short time, allowing for the determination any new black spot resistance specificities that map to the *Rdr1* interval. A recent example for the application of these two markers is that of Whitaker et al. (2009). Here, one resistance locus from the *R. hybrida* variety 'Love and Peace' was found to cosegregate with both markers whereas a new locus from the *R. hybrida* variety 'George Vancouver' displayed independent assortment from both indicating that a new resistance locus named *Rdr3* was found.

To evaluate the utility of our new SSR markers for general applications in genetic analysis of roses, we analysed these markers in a set of additional genotypes. Some of these belong to species from different sections of the subgenus *Rosa*, such as *R. majalis, R. rugosa, R. caudata, R. pisocarpa* (section *Cinnamomea*), *R. foetida* and *R. omeiensis* (section *Pimpinellifoliae*) as described in Wissemann (2003).

With a few exceptions due to PCR failures, all markers detected several polymorphic bands in the rose species and varieties analysed. PIC values were between 0.78 and 0.9, indicating that all markers are highly informative and well-suited for diversity analysis of roses.

Genetic maps have been previously constructed in diploid (Dugo et al. 2005; Crespel et al. 2002; Debener and Mattiesch 1999) and tetraploid (Zhang et al. 2006;

Rajapakse et al. 2001) roses. These maps were constructed with dominant multilocus markers. Hibrand-Saint Oyant et al. (2008) and Yan et al. (2005) constructed genetic maps including SSR markers. For the establishment of a consensus linkage map for roses, SSRs can be used as anchor points. Hibrand-Saint Oyant et al. (2008) demonstrated that some of their SSR markers provide anchor points between their maps and the tetraploid rose maps (Zhang et al. 2006). Both SSR marker RMS015 and 155SSR, developed in this study, represent anchor markers combining the genetic maps for population 97/7 (Linde et al. 2006) and population 94/1 (Yan et al. 2005). In both maps, the markers could be integrated in linkage group 1 in close vicinity to the black spot resistance gene *Rdr1* (Fig. 3). The new SSR markers may contribute to the construction of a rose consensus map and provide a valuable tool for identifying the location of *Rdr1* in other genetic maps. Markers 29Mic and 69Mic, which have not yet been mapped, are both polymorphic between the parents of mapping population 94/1 (93/1-117, 93/1-119). Additionally, the latter marker is polymorphic between the parents of population 97/7 (95/13-39, 82/78-1), demonstrating their potential for map integration.

## References


Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME (1993) Optimizing parental selection for genetic linkage maps. Genome 36:181–186

Ballvora A, Jöcker A, Viehöver P, Ishihara H, Paal J, Meksem K, Bruggmann R, Schoof H, Weisshaar B, Gebhardt C (2007) Comparative sequence analysis of *Solanum* and *Arabidopsis* in a hot spot for pathogen resistance on potato chromosome V reveals a patchwork of conserved and rapidly evolving genome segments. BMC Genomics 8:112–125

Cai L, Taylor JF, Wing RA, Gallagher DS, Woo SS, Davis SK (1995) Construction and characterization of a bovine bacterial artificial chromosome library. Genomics 29:413–425

Crespel L, Chirollet M, Durel CE, Zhang D, Meynet J, Gudin S (2002) Mapping of qualitative and quantitative phenotypic traits in *Rosa* using AFLP markers. Theor Appl Genet 105:1207–1214

Debener T, Mattiesch L (1999) Construction of a genetic linkage map for roses using RAPD and AFLP markers. Theor Appl Genet 99:891–899

Debener T, Drewes-Alvarez R, Rockstroh K (1998) Identification of five physiological races of black spot, *Diplocarpon rosae*, Wolf on roses. Plant Breed 117:267–270

Dickson EE, Arumuganathan K, Kresovich S, Doyle JJ (1992) Nuclear DNA content variation within the *Rosaceae*. Am J Bot 79:1081–1086

Dolezel J, Bartos J, Voglmayr H, Greilhuber J (2003) Nuclear DNA content and genome size of trout and human. Cytometry 51A:127–128

Dugo ML, Satovic Z, Millan T, Cubero JI, Rubiales D, Cabrera A, Torres AM (2005) Genetic mapping of QTLs controlling horticultural traits in diploid roses. Theor Appl Genet 111:511–520

Ercolano MR, Ballvora A, Paal J, Steinbiss HH, Salamini F, Gebhardt C (2004) Functional complementation analysis in potato via biolistic transformation with BAC large DNA fragments. Mol Breed 13:15–22

Hess G, Scheuring D, Byrne DH, Zhang D (2007) Towards positional cloning of the everblooming gene in plants: a BAC library of *Rosa chinensis* cv. Old Blush. Acta Hort 751:169–174

Hibrand-Saint Oyant L, Crespel L, Rajapakse S, Zhang L, Foucher F (2008) Genetic linkage maps of rose constructed with new microsatellite markers and locating QTL controlling flowering traits. Tree Genet Genomes 4:11–23

Horst RK (2007) Compendium of rose diseases, 2nd edn. The American Phytopathological Society, St. Paul

Jones JDG, Shlumukov L, Carland F, English J, Scofield SR, Bishop GJ, Harrison K (1992) Effective vectors for transformation, expression of heterologous genes, and assaying transposon excision in transgenic plants. Transgenic Res 1:285–297

Kaufmann H, Mattiesch L, Lörz H, Debener T (2003) Construction of a BAC library of *Rosa rugosa* Thunb. and assembly of a contig spanning *Rdr1*, a gene that confers resistance to blackspot. Mol Gen Genomics 268:666–674

Linde M, Hattendorf A, Kaufmann H, Debener T (2006) Powdery mildew resistance in roses: QTL mapping in different environments using selective genotyping. Theor Appl Genet 113:1081–1092

Marek LF, Shoemaker RC (1997) BAC contig development and fingerprint analysis in soybean. Genome 40:420–427

Matallana E, Bell CJ, Dunn PJ, Lu M, Ecker JR (1992) Genetic and physical linkage of the *Arabidopsis* genome: methods for anchoring yeast artificial chromosomes. In: Koncz C, Chua N-H, Schell J (eds) Methods in *Arabidopsis* research. World Scientific, Singapore, pp 145–169

Orita M, Suziki Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879

Rajapakse S, Byrne DH, Zhang L, Anderson N, Arumuganathan K, Ballard RE (2001) Two genetic linkage maps of tetraploid roses. Theor Appl Genet 103:575–583

Sambrook J, Russell DW (2001) Molecular cloning. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Stam P (1993) Construction of integrated genetic-linkage maps by means of a new computer package—JoinMap. Plant J 3:739–744

Tao Q, Zhang HB (1998) Cloning and stable maintenance of DNA fragments over 300 kB in *Escherichia coli* with conventional plasmid-based vectors. Nucl Acids Res 26:4901–4909

Van Ooijen JW, Voorrips RE (2001) JoinMap 3.0. Software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Von Malek B, Debener T (1998) Genetic analysis of resistance to black spot (*Diplocarpon rosae*) in tetraploid roses. Theor Appl Genet 96:228–231

Von Malek B, Weber WE, Debener T (2000) Identification of molecular markers linked to *Rdr1*, a gene conferring resistance to blackspot in roses. Theor Appl Genet 101:977–983

Whitaker VM, Bradeen JM, Debener T, Biber A, Hokanson SC (2009) *Rdr3*, a novel locus conferring black spot disease resistance in tetraploid rose: genetic analysis, LRR profiling and SCAR marker development. Theor Appl Genet. doi: 10.1007/s00122-009-1177-0

Wicker T, Zimmermann W, Perovic D, Paterson AH, Ganal M, Graner A, Stein N (2005) A detailed look at 7 million years of genome evolution in a 439 kb contiguous sequence at the barley Hv-elF4E locus: recombination, rearrangements and repeats. Plant J 41:184–194

Wissemann V (2003) Conventional taxonomy of wild roses. In: Roberts A, Debener T, Gudin S (eds) Encyclopedia of rose sciences. Academic Press, London, pp 111–117

Woo SS, Jiang J, Gell B, Paterson AH, Wing RA (1994) Construction and characterization of a bacterial artificial chromosome library of *Sorghum bicolor*. Nucl Acids Res 22:4922–4931

Yan Z, Denneboom C, Hattendorf A, Dolstra O, Debener T, Stam P, Visser PB (2005) Construction of an integrated map of rose with AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers. Theor Appl Genet 110:766–777

Yokoya K, Roberts AV, Mottley J, Lewis R, Brandham PE (2000) Nuclear DNA amounts in roses. Ann Bot 85:557–561

Zhang LH, Byrne DH, Ballard RE, Rajapakse S (2006) Microsatellite marker development in rose and its application in tetraploid mapping. J Am Soc Hort Sci 131:380–387