

## A MOLECULAR MARKER MAP FOR ROSES

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Keywords- molecular markers, AFLP, RAPD, microsatellites, mapping

### Abstract

In addition to an existing core map for diploid roses which comprised 305 molecular markers 60 additional markers were mapped to extend the map. As a first application of the information contained in the map, the map position of a resistance gene from roses, Rdr1, was determined by identifying closely linked markers on linkage group 1. Furthermore 21 microsatellite markers could be assigned to six of the seven linkage groups, therefore allowing their more efficient use in variety fingerprinting and general mapping in the near future. Apart from being a useful tool for genetic studies and map based cloning, the map could also be used in projects on the chromosome evolution within the rosaceae family.

### 1. Introduction

The paucity of information on the inheritance of major characters in roses and the difficult genetic system are obstacles in the development of sophisticated breeding strategies which are already applied to some of the major agricultural crops as for example maize, tomato and wheat. Genetic linkage maps would facilitate the implementation of these strategies in rose breeding and basic genetic studies. Furthermore, high density linkage maps would help to tag genes of interest for marker assisted breeding, map based cloning and the molecular characterisation of these genes. The construction of linkage maps has greatly profited from the invention of molecular markers like for example the RFLPs, RAPDs and AFLPs (Gebhardt *et al.* 1992, Paterson *et al.* 1991. Especially the makers based on the PCR reaction allowed the rapid map construction even in species with difficult genetic systems (Staub *et al.* 1996). Over the last years several maps have been generated for a number of woody species including the major fruit tree species (Davis *et al.* 1997, Baird *et al.* 1996, Hemmat *et al.* 1994). As most of these species like roses are highly heterozygous outcrossers and segregating populations can only be created by intercrossing two non related genotypes, the double-pseudo-test-cross strategy had to be employed (Grattapaglia *et al.* 1994). With these strategy the segregation of up to four alleles is expected in diploid populations and maps have to be computed for each parent separately if dominant markers like RAPD and AFLP markers are used.

A core map for diploid roses had been constructed utilising this mapping strategy (Debener and Mattiesch 1999). The segregating population for which the map was built resulted from a cross between *R. multiflora* hybrids displaying several characters of cultivated roses (Debener 1999, Debener and Mattiesch 1999) and selected for a maximum of marker polymorphism in an earlier study (Debener and Mattiesch 1996). In the present paper the extension of the core map as well as first applications for the localisation of markers and traits analysed in other rose crosses will be presented.

### 2. Materials and methods

For the construction of the chromosome map 60 plants from the diploid

segregating population 94/1 were used (Debener 1999). The parents of this population 93/1-119 and 93/1-117 were half sibs originating from open pollinated progeny of the *R. multiflora* hybrid 81/42-15 (Debener 1999).

The generation of the segregating rose population and all procedures for the generation and mapping of molecular markers have been describe previously (Debener 1999, Debener and Mattiesch 1999). The isolation of microsatellite markers (to be published elsewhere) was performed essentially as described by Wiel *et al.* (1999).

### 3. Results and discussion

Based on the core map for diploid roses (Debener and Mattiesch 1999) we analysed 60 additional markers including 20 AFLP markers, 27 microsatellites, 8 RFLP fragments (scored as dominant markers) and 5 SCAR markers. A total of 365 markers were analysed in the extended data set (Table 1). After the computation of separate maps for both parents and the subsequent alignment of the maps by the markers segregating from both parents, 321 could be placed on seven linkage groups (data not shown).

The female parent 93/1-117 contributed 129, the male parent 93/1-119 contributed 155 markers to the data set (Table 1). A relatively large number of 81 markers (22%) segregate from both parents (Table 1). This is most probably the result from the close genetic relationship of the half sib parents of the mapping population. Although dominant RAPD and AFLP markers of this segregation type provide only limited information, they are useful for the alignment of the parental maps. Most useful markers for a double-pseudo-test-cross mapping strategy would be markers that can differentiate different alleles. In the current mapping set only one RFLP marker and 9 microsatellite markers allowed the differentiation of alleles between the parents and could be scored in a codominant way. The overall effectiveness of markers for map construction is not only dependant on the number of detectable alleles but also on the total number of useful markers that can be obtained in a single marker analysis. If RAPD and AFLP markers are compared for the total number of scorable, segregating markers per single marker reaction (which means per PCR reaction), the advantages of AFLP markers become obvious because they yield the sevenfold number of scorable markers compared to RAPDs (Table 2). As AFLPs can be generated on automated sequencers which allow multiplex PCR reactions (e.g. processing multiple primer combination in a single PCR reaction) this difference in efficiency will be even larger.

A first application of the core map was the localisation of Rdr1, a resistance gene to blackspot, in the genome of roses. Rdr1 was characterised both genetically and by linkage to molecular markers in different diploid and tetraploid segregating populations. As no general marker data were available in these populations and Rdr1 is not segregating in the mapping population 94/1, a marker (BMA) linked to Rdr1 at 0,75 cM was used as an RFLP marker in 94/1 to locate the gene on the map (Figure 1 ). This lead to an additional marker from the map (P 479\_491\_2) which was mapped conversely in the populations segregating for Rdr1 close to the gene (data not shown). In the near future the map may be used not only to map genes which have been scored in other population but it may also provide additional markers which might be linked to the target genes in the other population at a distance much closer than the original markers.

A more efficient strategy however would be a grid of highly polymorphic markers which cover every linkage group, which can be scored in a codominant manner and which can be generated by PCR reactions. Therefore we mapped 27 microsatellite marker fragments either codominantly or where more than one locus was detected, as dominant markers. So far 21 different microsatellites could be located on six of the seven linkage groups (Figure 1). As microsatellite are ideal markers for variety fingerprinting for the protection of breeders rights the mapping information helps to locate markers evenly distributed over the genome. This information is important to avoid conclusions on genetic relatedness of rose varieties solely based on markers from one or a few chromosomal regions.

Future applications of the rose marker maps may also be the analysis of syntenic relationships with other rosaceous species as for example apple, pear and strawberry. In all of these species maps have been generated (Davis *et al.* 1997, Baird *et al.* 1996, Hemmat *et al.* 1994) and mapping of common markers on these different maps would not only provide insight into the genome evolution within the *rosaceae* family but could also provide additional resources for gene mapping and isolation from these other species.

### Acknowledgements

The authors wish to thank Danny Esselink for excellent technical assistance. The microsatellite primers discussed in this publication are available for academic research upon request to the last author.

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## Tables

1. Analysis of marker segregation for three different marker types in the mapping population 94/1.

	from 93/1-117	from 93/1-119	from both parents	total
markers analysed in the segregating population	129	155	81	365
expected segregation ratios	1:1	1:1	3:1	
markers with deviation from the expected ratio at $p=0,005$	28(22%)	20 (13%)	15 (19%)	63 (17%)

2. Effectiveness of RAPD and AFLP primer combination to produce segregating markers

	RAPD	AFLP
Number of primer combinations used in the segregating population	114	17
Number of useful polymorphic markers	161	164
Number of polymorphic markers per primer combination/PCR reaction	1.4	9.7

## Figures