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# Marker Assisted Background Selection for the Introgression of Black Spot Resistance into Cultivated Roses

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## Summary

In a model breeding program *Rdr1*, a gene conferring resistance to black spot (*Diplocarpon rosae* Wolf) was introduced from a wild rose species into the genetic background of cultivated tetraploid roses. The chromosomal set of the original diploid donor genotype, a *Rosa multiflora* hybrid, was doubled via colchicine treatment and crossed to a tetraploid hybrid tea variety. The resulting hybrid genotype, 91/100-5, was crossed to a Floribunda garden rose and the resulting progeny was tested for black spot resistance. AFLP markers were then used to screen a subset of the resistant progeny for the fraction of donor genome, which segregates from 32 to 84 % in this population. The genotype with the smallest number of donor-specific markers was further backcrossed to the Floribunda parent and the hybrid tea grandparent, and the analyses conducted in the previous generation were repeated. The $BC_2$ population segregated for the fraction of the original donor genome, ranging from 12.3 % to 32 %. A parallel screen for morphological markers in the $BC_1$ demonstrated the superiority of the molecular markers for the reduction of the genetic background of wild rose species in introgression programs allowing a more efficient utilization of wild rose germplasm.

## Zusammenfassung

**Markergestützte Hintergrundselektion zur Introgression von Sternrußtauresistenz in Kulturrosen.** In einem Modellzuchtversuch wurde das Resistenzgen *Rdr1*, welches Resistenz gegen den Sternrußtau an Rosen vermittelt, in den genetischen Hintergrund von Kulturrosen eingekreuzt. Der Chromosomensatz einer resistenten diploiden Donorlinie wurde durch eine Colchizinbehandlung verdoppelt und der daraus resultierende tetraploide Genotyp mit einer tetraploiden Teehybride gekreuzt. Die daraus hervorgehende resistente Hybride 91/100-5 wurde in der nächsten Generation mit einer Floribunda Gartenrose gekreuzt und die Nachkommenschaft auf Sternrußtauresistenz geprüft. Der resistente Anteil dieser Nachkommenschaft wurde dann mit AFLP-Markern untersucht um Individuen mit einem möglichst kleinen Anteil des Donorgenoms zu identifizieren. Dieser Genomanteil spaltet in der Nachkommenschaft von 32 % bis 84 % auf. Der Genotyp mit dem geringsten Anteil des Donorgenoms wurde zum Floribunda-Elter und zum Teehybriden-Großelter rückgekreuzt und die Analysen auf Sternrußtauresistenz wiederholt. Unter den resistenten Nachkommen spaltet der Anteil des Donorgenoms von 12,3 % bis 32 % auf. Paralleluntersuchungen morphologischer Marker in der $BC_1$ zeigen, dass molekulare Marker morphologischen Bonituren zur Bestimmung des Anteils des Donorgenoms überlegen sind.

**Key words.** *Rosa* – *Diplocarpon* – marker assisted selection – genetic background – black spot resistance – modified backcross method

## Introduction

Cultivated roses belong to the economically most important ornamental crops worldwide. Most varieties are tetraploid and have a complex history of hybridisation between different species (GUDIN 2000). Therefore, the genome of cultivated roses may be considered as a mosaic composed of fragments from the genomes of at least 10 different species.

Despite the large genetic diversity among cultivated roses, certain rose groups, like for example hybrid teas are lacking specific trait combinations. In particular, resistance genes to the most important fungal pathogens are underrepresented in the gene pools of some rose groups (for example genes for black spot resistance in hybrid tea garden roses and genes for powdery mildew resistance in cut roses).

This may be explained by the common practise of most rose breeders to develop new varieties from within relatively isolated gene pools, therefore creating genetic bottlenecks for specific traits.

Several authors have already described the presence of resistances to fungal pathogens in wild rose species, which could serve as a valuable resource for resistance breeding (KNIGHT and WHEELER 1978; REDDY et al. 1992; WIGGERS et al. 1997; DEBENER et al. 1998; DEBENER 2000). However, as first generation hybrids between highly developed rose varieties, as e.g. hybrid teas

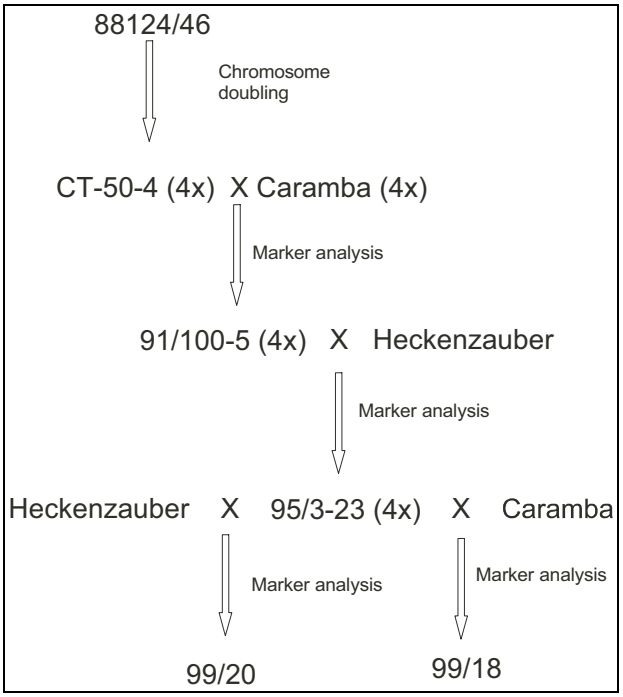



Fig. 1. Breeding scheme for the introgression of black spot resistance from a resistant diploid line to tetraploid rose varieties.

and wild roses, rarely comprise genotypes suitable for variety development, rose breeders restrained from using wild species for breeding these particular rose types.

A solution could be the elimination of unwanted genomic background of the donor species while retaining the resistance gene, by backcross breeding. As roses suffer from severe inbreeding depression, different varieties have to be selected as recurrent parents in each generation to retain a tolerable degree of vigour in the populations. This so called "modified backcross strategy" has already been practised over decades in apple breeding, where breeders introgressed scab resistance genes from wild species and subsequently crossed or "backcrossed" several generations to different high performing commercial varieties (CROSBY et al. 1992; GIANFRANCESCHI et al. 1994).

The advent of molecular marker techniques provided new tools for a more efficient design of introgression programs even for crops where introgression of genes from wild species has been common practise for several decades (PATERSON et al. 1991; GEBHARDT and SALAMINI 1992; RIBAUT et al. 1997). Apart from mapping single genes, the development of molecular markers suitable for MAS (marker assisted selection) and for the analysis of the inheritance and transmission of quantitative trait loci (QTL) has become a major focus of research on MAS (STUBER 1995).

In addition, the potential of molecular markers to select against the donor genome was evaluated theoretically and in practical introgression breeding programs (VISSCHER et al. 1996; FRISCH et al. 1999; BARONE et al. 2001; CHEN et al. 2001; HOSPITAL 2001). Especially for crops with less advanced breeding programmes, molecular markers could provide efficient tools for the introgression of important characters from wild species while keeping the fraction of donor genome cotransmitted with the trait of interest at a minimum.

In previous investigations of black spot resistant germplasm, the diploid *R. multiflora* hybrid 88/124-46 was identified as being resistant to five of the six races known at that time (DREWES-ALVAREZ 1992; VON MALEK and DEBENER 1998). This genotype displays many characters typical of the wild *R. multiflora* ancestor, such as small single white flowers, a large number of flowers per inflorescence, small leaflets etc (Table 2) which make it unsuitable for ornamental rose production. In order to introgress the black spot resistance from this line into the genomes of cultivated roses its chromosomal set was doubled via colchicine treatment, and the resulting tetraploid line CT 50-4 was crossed to the susceptible hybrid tea variety 'Caramba' (DREWES-ALVAREZ 1992, Fig. 1). The fully resistant hybrid 91/100-5 still displays many characters of the wild species ancestor *R. multiflora* (Table 2) and was therefore backcrossed to another garden rose variety the Floribunda variety 'Heckenzauber' (DEBENER et al. 1998, Fig. 1). The resulting population 95/3 comprises 119 individuals and segregates for resistance to black spot race 5 in a ratio of 5 resistant to 1 susceptible plants (VON MALEK and DEBENER 1998).

The work presented here had two goals:

1. To introgress the resistance gene *Rdr1* into the genetic background of cultivated roses and therefore provide plant material suitable for variety breeding in various rose groups.
2. To assess the utility of AFLP markers for an efficient reduction of the donor genome in introgres-

Table 1. AFLP primer combinations used in the present study.

| | Primer combination | | Primer combination |
|---|---|---|---|
| 1 | Hind-ACG/Mse-AAG | 10 | Hind-ACC/Mse-AAT |
| 2 | Hind-ACC/Mse-ATG | 11 | Hind-AGA/Mse-AGG |
| 3 | Hind-ACC/Mse-AGA | 12 | Hind-ATA/Mse-AAA |
| 4 | Hind-ACC/Mse-ACG | 13 | Hind-AGA/Mse-AGT |
| 5 | Hind-ACC/Mse-ATT | 14 | Hind-ACC/Mse-AGG |
| 6 | Hind-ACA/Mse-AGA | 15 | Hind-ATA/Mse-AGA |
| 7 | Hind-ATA/Mse-AAG | 16 | Hind-ATA/Mse-ACG |
| 8 | Hind-ATA/Mse-AAC | 17 | Hind-ATA/Mse-ATG |
| 9 | Hind-AGT/Mse-ACG | 18 | Hind-ATA/Mse-AGT |

Table 2. Morphological characters and fraction of markers from the donor line in parental lines, the $F_1$-hybrid (91/100-5) and the selected $BC_1$ line 95/3-23. Standard deviations for mean values of petal numbers and flowers per inflorescence are given in brackets.

| Genotype | Petal number | Flowers per inflorescence | Flower colour | Fraction of markers from original donor |
|---|---|---|---|---|
| 88/124-46 | 5 (0.3) | 14,9 (4.3) | white 155b | |
| CT 50-4 | 5 (0.3) | 14,9 (5.3) | white 155b | 100 % |
| 'Caramba' | 53 (3.2) | 1,2 (0.4) | red 57a | 0 % |
| 'Heckenzauber' | 38 (4.4) | 1,6 (0.2) | red 55c | 0 % |
| 91/100-5 | 13 (2.1) | 4,1 (2.3) | red 61b | 91 % |
| 95/3-23 | 12 (4.7) | 1,7 (1.1) | orange 25b | 32 % |

sion programs for tetraploid roses using only few cycles of backcrossing.

## Materials and Methods

*Plant material*

The rose genotypes 88/124-46, 91/100-5, both resistant to black spot races 1-5, and the varieties 'Heckenzauber' and 'Caramba' have already been described earlier (CAIRNS 1993; VON MALEK and DEBENER 1998). Their position in the breeding scheme presented here is outlined in Fig. 1. The plants were cultivated under semi-controlled conditions in the greenhouse and crosses were performed as described in VON MALEK and DEBENER (1998) and DEBENER (1999). The terms $BC_1$ for the first backcross generation and $BC_2$ for the second backcross generation are used according to the definition of the modified backcross strategy of CROSBY et al. (1992). After this definition recurrent parents in the backcross cycles are not identical to parents of the $F_1$ generation.

*Evaluation of ornamental characters*

Average petal numbers were computed from 20 flowers per genotype. The average numbers of flowers per shoot were computed from up to ten shoots. The evaluation of flower colours was conducted visually on ten flowers directly after anthesis with the colour chart of the Royal Horticultural Society (RHS) as a reference. RHS colour codes are given along with the flower colours. The estimation of the fraction of wild species genome based on morphological traits was conducted by two breeders from rose breeding companies (Kordes Söhne, Klein-Offenseth Sparrieshoop, Germany and Rosen Tantau, Uetersen, Germany) and from one breeder of the Institute for Ornamental Plant Breeding, Germany.

*Black spot inoculation and disease evaluation*

Assays for black spot resistance were done as described in DEBENER et al. (1998). For the inoculation experiments the single conidial isolate Dort E4 representing black spot race 5 was used at a concentration of $10^5$ conidia/ml.

*Marker analyses*

The isolation of genomic DNA and AFLP reactions are as described in DEBENER and MATTIESCH (1999). The AFLP primer combinations used in the present study are listed in Table 1. Marker fragments were recorded as present (1) or absent (0), and data storage and processing was performed with Microsoft Excel 97. A t-test in Excel 97 was performed to compare the marker fractions of population 99/18 and 99/20.

## Results

The breeding scheme for the populations on which the current work was based on has been described earlier (Drewes-Alvarez 1992; DEBENER et al. 1998; VON MALEK and DEBENER 1998). It comprised the following steps (Fig. 1):

– The identification of the resistant diploid donor line 88/124-46
– Doubling of the genome via colchicine treatment to obtain line CT 50-4
– Crossing the resulting tetraploid line to a hybrid tea garden rose to obtain the hybrid 91/100-5 (arbitrarily chosen among ten hybrids which are all resistant)
– Crossing the resulting hybrid to another garden rose variety
– Analysing the segregating progeny 95/3 for the segregation of disease resistance and molecular markers

To further reduce the genetic background of the resistance donor 88/124-46 in the next generations the following steps were conducted:

– Among the resistant fraction of population 95/3 the individual with the smallest fraction of donor genome was selected with molecular markers
– This individual was backcrossed to two garden rose varieties
– The resulting progeny was again screened for resistance and the resistant progeny was analysed with molecular markers to identify those individuals with the smallest fraction of donor genome.

In order to analyse the segregation of donor genome in the population 95/3 we investigated an arbitrary selection of 55 resistant individuals, the donor line 88/124-46, the varieties 'Caramba' and 'Heckenzauber' and the hybrid 91/100-5 with 18 AFLP primer combinations (Table 1).

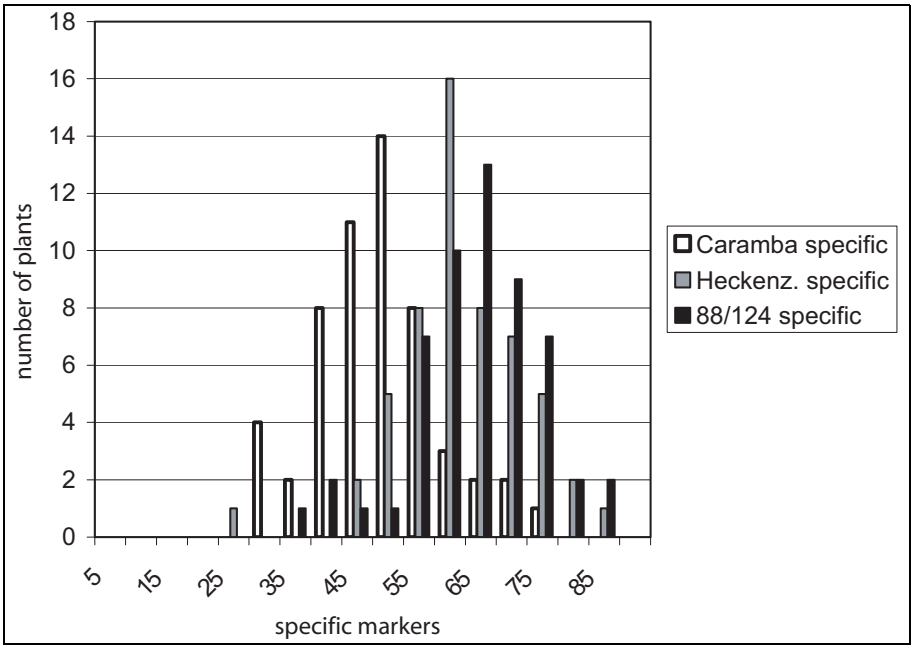



Fig. 2. Fraction of markers from the genome of different parents in the $BC_1$ population 95/3. The fraction of the donor line 88/124 and the varieties 'Caramba' (grandparent) and 'Heckenzauber' (female parent) are shown. The marker fractions are given as the percentage of the markers specific for each particular line.

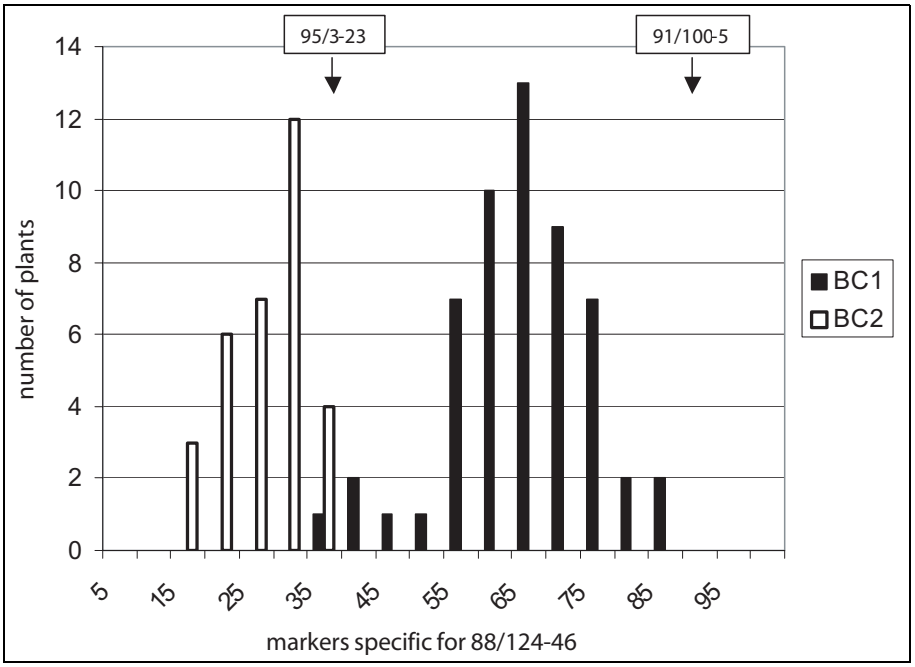



Fig. 3. Marker fractions specific for the donor line 88/124-46 in the $BC_1$ (95/3) and $BC_2$ (99/18 and 99/20) populations. Marker fractions are given as the percentage of 110 markers originally identified as specific for 88/124-46. The values for the $F_1$-hybrid 91/100-5 and the genotype selected in the $BC_1$ for further breeding (95/3-23) are indicated by arrows.

A total of 1660 AFLP fragments could be clearly distinguished, 491 of which were polymorphic between the two parents and segregated in the progeny. Among these, 110 were specific for the donor line 88/124-46 (e.g. they were absent in both 'Caramba' and 'Heckenzauber'), 114 were specific for 'Caramba' and 103 fragments were specific for 'Heckenzauber' (Fig. 2).

The fraction of markers specific for the donor line varied between 31.8 % (genotype 95/3-23) and 83.6 % (95/3-121) with a mean value of 62.1 %. The fractions of the markers specific for 'Heckenzauber' varied between 22.5 and 80.6 % with a mean of 59.7 %, whereas those for the variety 'Caramba' varied between 27.2 and 71.7 % with a mean of 46.4 %.

The population also segregates for a range of morphological and physiological characters. For example, the average number of petals per genotype varies between 5 and 125, the petal colours of individual genotypes in the population comprise white and different shades of red, orange and yellow, and scent segregates from absent to medium scented (data not shown).

Based on the smallest number of donor fragments (31.8 %) the genotype 95/3-23 (harbouring 62.8 % and 60.2 % of markers specific for 'Caramba' and 'Heckenzauber' respectively) was selected as a parent for the next breeding cycle. It was crossed as the male parent to the varieties 'Caramba' and 'Heckenzauber' (Fig. 1). The resulting population 99/18 ('Caramba' x 95/3-23)

Table 3. Morphological characters and fraction of markers from the donor line for selected genotypes of the $BC_2$ populations. Standard deviations for mean values of petal numbers and flowers per inflorescence are given in brackets.

| Genotype | Petal number | Flowers per inflorescence | Flower colour | Fraction of markers from original donor |
|---|---|---|---|---|
| 99/18-23 | 46.3 (3.2) | 1.2 (0.4) | yellow 16b | 13.7 % |
| 99/18-27 | 24.1 (2.1) | 1.0 (0.0) | red 58c | 14.1 % |
| 99/20-24 | 37.4 (4.0) | 2.1 (1.4) | red 55b | 14.2 % |

comprises 40 plants, whereas 99/20 ('Heckenzauber' x 95/3-23) comprises 64 plants.

For technical reasons a subset of both populations (31 individuals from 99/18 and 54 individuals from 99/20) was screened for resistance to black spot race 5 with the isolate DortE4. The segregation of 15 resistant to 16 susceptible genotypes in population 99/18 ($\chi^2$=0.03, P=0.86) and 24 resistant to 30 susceptible genotypes in 99/20 ($\chi^2$=0.67, P=0.41) fits a 1:1 segregation and indicates that line 95/3-23 carries the resistance gene *Rdr1* in the simplex configuration.

In order to analyse the segregation of the original donor genome in the $BC_2$, both populations were analysed with the same 18 AFLP primer combinations that were used for population 95/3. For technical reasons only a selection of 15 and 17 resistant plants were analysed from population 99/18 and population 99/20 respectively and only the 110 markers specific for 88/124-46 were considered this time. With an average of 22.1 % of donor fragments present in 99/18 and 25.1 % in 99/20, both populations do not show significant differences (t=0.115, $\alpha$=0.05). Therefore, both datasets were merged and the distribution of the donor fragments was computed for the joint data (Fig. 3, Table 3). Genotypes with the lowest fraction of donor genome are candidates for further breeding cycles or commercial variety breeding. The most promising candidates are 99/18-23 (13.7 %), 99/18-27 (14.1 %) and 99/20-24 (14.2 %). For these three genotypes, some of the key morphological traits for ornamental roses as, e.g., petal number, petal colour and the number of flowers per inflorescence are already more similar to the parental cultivars than to the donor line and the parental hybrids 91/100-5 and 95/3-23 (Table 2 and 3).

In parallel to the molecular analyses, the $BC_1$ population 95/3 was evaluated based on morphological traits by three breeders. Apart from the number of flowers per inflorescence, flower and leaf size and petal numbers the overall plant habit was used to classify every genotype into a scale between 1 (corresponding to the hybrid tea 'Caramba') and 5 (corresponding to the donor line 88/124-46). All three breeders scored the genotype 95/3-23 as being intermediate between 'Caramba' and the donor line with a score of three. For the line 95/3-121 two of the scores were three, whereas one was four. The means of these scores are shown in Fig. 4.

## Discussion

Wild rose species are an invaluable resource of resistance genes against the most important rose diseases (KRÜSSMANN 1974; HORST 1983). Though several species contribute to the genome of present day rose varieties (GUDIN 2000) introgression breeding for the in-

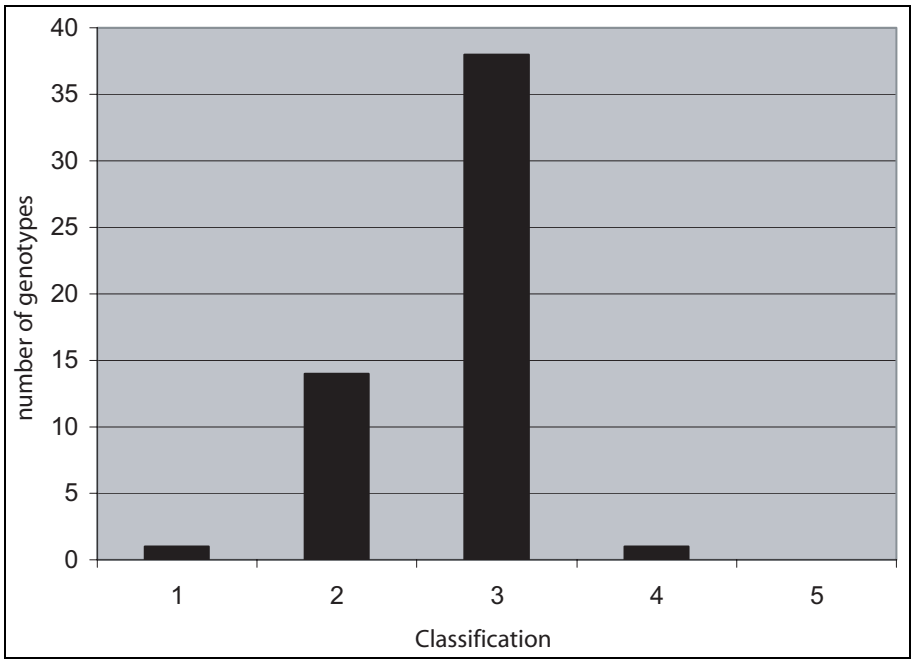



Fig. 4. Frequency distribution of scores for the morphological screening of the $BC_1$-population. For each genotype the mean value from the individual score of three breeders were used.

corporation of new resistance specificities is complicated by the tetraploid nature of most varieties and relatively simple breeding strategies in commercial companies which mainly select varieties from within $F_1$ populations. A major obstacle for introgression breeding in rose is the genome of the wild donor species which affects important trait complexes over the first generations. Another obstacle is the difference between the ploidy levels of many wild diploid species and tetraploid rose varieties, leading to sterile triploid hybrids in conventional crosses.

The project presented here aims at the development of strategies for the introgression of black spot resistance from diploid donor genotypes and the efficient reduction of the background of undesired donor genome. Therefore, we initiated a model breeding program starting with a chromosome doubling of resistant diploid genotypes and the creation of $F_1$ hybrids between wild and cultivated roses. Backcross populations were then analysed with molecular markers to identify individuals with a minimum amount of donor genome to be used in further backcross cycles. The segregation of random dominant markers specific for the donor line was used as an indicator for the amount of donor genome retained in the introgression lines. In a previous project on the construction of a rose molecular marker map, AFLP marker fragments from 10 primer combinations were found to be distributed over all 7 rose chromosomes (DEBENER and MATTIESCH 1999). Although the 110 markers considered in the present study will most probably not cover the whole rose genome, they may be taken as a rough indicator for the fraction of the donor genome under the assumption that most of the marker fragments represent independent loci. Markers with known positions and an even distribution over the rose genome would be more effective than random markers. However, only few microsatellite, RFLP- and CAPS markers with known map positions are available for roses yet (DEBENER et al. 2001). The distribution of markers in the $F_1$-hybrid and the, $BC_1$- and $BC_2$-populations is in accordance with the present knowledge about rose genetics: The $F_1$-hybrid 91/100-5 carries 91 % of the fragments specific for the donor. As all markers heterozygous in the diploid donor will be present in the duplex configuration in line CT50-4, the majority of the heterozygote loci will be transmitted to the $F_1$-progeny due to a 5:1 segregation (LINDSTROM 1936).

The distribution of parent specific markers in the $BC_1$-population 95/3 reflects the structure of the pedigree. The mean percentage of fragments specific for the parents 'Heckenzauber' and CT 88/124-46 are 59.7 % and 62.1 % respectively while the fraction of 'Caramba' as one of the grandparents has already decreased to an average of 46.4 % (Fig. 2). In the second backcross generation the fraction of donor markers was already reduced to an average of 23.7 %. The marker analysis in the $BC_1$ identified the genotype 95/3-23 with only 31.8 % of the markers from the donor line. This line would not have been identified by scoring morphological characters alone, as three independent evaluations by rose breeders placed it with a value of 3 in the medium range of scores from 1 (hybrid tea like) to 5 (donor like). In conventional breeding programs more than 95 % of the seedlings are generally discarded within the first four months of selection before selected lines are clonally propagated for further tests (NOACK 2003). Therefore, the selected line 95/3-23 would not have been considered for further breeding cycles. This already demonstrates the superiority of marker assisted background selection in introgression programs in roses over the use of morphological characters.

In the $BC_2$-generation the genotypes with the smallest fraction carried 13.7 % of the donor markers. Key morphological characters of these lines as e.g. petal number, petal colour and number of flowers per inflorescence are already in the range of garden rose varieties (Table 2 and 3). Therefore, these lines will probably segregate for the key characters like other cultivated roses and may be used as a starting point for breeding resistant rose cultivars. As for both generations only a relatively small number of plants (55 for the $BC_1$ and 32 for both populations of the $BC_2$) have been analysed, the efficiency with which the donor background can be reduced may be increased by increasing the size of the BC populations. Simulation studies for diploid crops indicated that with increasing population sizes not only the fraction of the donor genome could be reduced more efficiently but also the number of marker analysis may be reduced without loosing resolution (FRISCH et al. 1999, 2000).

Tools for marker assisted selection of important characters have been developed for a number of different crop species (PATERSON et al. 1991; GEBHARDT and SALAMINI 1992; STAUB et al. 1996) with the majority of the reports concentrating on markers linked to single loci or QTLs. Only relatively few reports are available on the application of markers for a broad selection in order to reduce a particular genetic background (BARONE et al. 2001; CHEN et al. 2001; HERRERA et al. 2002). In all of these reports at least part of the analyses were conducted by means of markers with known map positions. HERRERA et al. (2002) scored 11 RFLP and 13 microsatellite markers in two interspecific $BC_1$s of coffee species (*Coffea arabica* crossed to *C. canephora*) comprising 28 and 45 individuals respectively. They detected an average of 24.5 % of the alleles of *C. canephora* in both $BC_1$-populations. CHEN et al. (2001) analysed three backcross and one $F_2$-population of rice cultivars with SCAR markers linked to an introgressed resistance gene and 129 randomly chosen polymorphic AFLP markers. They were able to recover 98.8 % of the recurrent parent genome. BARONE et al. (2001) introgressed resistance to *Erwinia carotovora* from diploid *Solanum commersonii* to tetraploid potatoes utilising a similar breeding scheme as we used for roses in the present study. They used 34 RAPD- and 61 AFLP-fragments specific for *S. commersonii* and recovered genotypes with a minimum of 80 % and 82 % of these markers in the $BC_1$ and 48 % and 55 % of markers in the $BC_2$ for each marker type, respectively. The differences between marker segregation among the published studies including our work on roses are most probably due to the variability between the species used and to differences in their breeding systems. Furthermore, different marker types and different amounts of marker information were used. However, they all demonstrated the utility of molecular markers to monitor the amount of genetic background of donor genotypes

in introgression programs. In combination with markers linked to the traits to be introgressed, marker assisted selection can lead to a reduction of linkage drag within few generations (FRISCH et al. 2000). But even for crops like roses, for which only little information about markers linked to agronomically important traits is available, marker assisted background selection could be very useful. Several important traits like, e.g., resistances against major pathogens or stress tolerances can be found among the large number of wild rose species, many of which carry numerous traits detrimental to variety breeding (GUDIN 2000). Introgression of traits from these species could be performed by selecting $F_1$-hybrids from a cross with a cultivar for the traits of interest (e.g. a particular resistance gene). These hybrids would then be backcrossed to a different cultivar in order to reduce inbreeding depression. In this $BC_1$ population the donor genome would segregate and could be monitored with random molecular makers or, in the future with markers of known position. Parallel to the background analysis this information could be used to detect markers linked to the trait of interest and to detect recombination between these markers and the trait. For the next generation individuals which carry a minimum of donor genome and ideally show recombination between linked markers and the trait would be chosen as a parent for the $BC_2$ population to be crossed to a third cultivar. Background selection with markers as well as selection on the trait of interest will be repeated in the $BC_2$ and genotypes with a minimum of donor genome will be selected for further conventional breeding. Only from the $BC_3$ on, selection on ornamental characters along with selection for the trait of interest will be performed. This strategy would allow the exploitation of genetic resources currently unsuited for rose breeding. As the pressure to produce rose varieties with enhanced resistances to pests and pathogens is rising constantly, it would be a simple and efficient method to access resistance genes currently not represented in the gene pool of ancient and modern rose varieties.

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