Specific Mapping of Disease Resistance Genes in Tetraploid Cut Roses

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Abstract

Control of fungal diseases is a major constraint of cut-rose cultivation in greenhouses and in transportation around the world. Therefore, development of resistant cultivars is a promising way to reduce the use of chemicals required for controlling the diseases. Genetic analyses and breeding for resistance, however, are hampered by the high degree of heterozygosity and the polyploid nature of cultivated roses. Nucleotide-binding site (NBS) profiling of Van der Linden et al. (2004) was used as a tool enabling a more directed way of studying the genetics of resistance to pathogens responsible for diseases such as powdery mildew. NBS profiling is a multiplex screening technique, producing amplified resistance gene (R-gene) and resistance gene analogue (RGA) fragments by using degenerated primers based on the conserved motifs present in the NBS domain of resistance genes. Since RGAs are abundantly distributed and highly polymorphic within the plant genome, NBS profiling generates multiple markers of putative resistance genes. Twelve NBS degenerated primer/ restriction enzyme combinations were used to genotype the whole rose tetraploid K5 population (Yan, 2005) and its parents. To generate RGA profiles, the restriction enzymes: *Alu*I, *Hae*III, *Mse*I, and *Rsa*I were used in combination with degenerated primers NBS1, NBS3, and NBS5a6. The profiles were dominantly scored resulting in 106 polymorphic RGA markers which segregated in a 1:1 or 3:1 ratio. Uni-and bi-parental simplex markers will be mapped on the two available AFLP/SSR K5 maps (Yan, 2005) with Joinmap 4.0. The resulting parental tetraploid maps will be used to dissect the genetic variation for resistance to powdery mildew resistance. Additional Rosaceae SSRs mentioned in the literature are currently tested on the K5 population to obtain allelic bridges between the tetraploid and diploid genetic maps in rose and related species in order to align them. These bridges will improve cross-ploidy comparisons in roses in order to strengthen cut rose breeding.

INTRODUCTION

Roses are economically the most important ornamental crop world-wide. As an example, in 2008, for the Netherlands, over two billions flowers were exported (Centraal Bureau voor de Statistiek, 2009). Growers as well as end-users require a product free of diseases. Breeders need a reliable tool to pyramid resistance genes, and to get insight in association between resistance and desired aesthetical or other traits relevant for breeding cut roses Genetic analyses and breeding for resistance, however, are hampered by a high degree of heterozygosity and the polyploid nature of cultivated roses. Furthermore, despite the small size of the rose genome (600 Mb) and its relatively small number of chromosomes (x=7), no consensus genetic linkage map has been constructed yet. Since most of the commercial rose cultivars are tetraploid ($2n=4x$=28), genetic studies of traits directly relevant to breeders, such as quality traits and disease resistance also have to be done at that ploidy level. Up to now, two tetraploid maps have been constructed on two different rose populations, but they can not be linked yet because of a lack of sufficient markers (Rajapakse et al., 2001; Yan, 2005).

Besides, roses grown in greenhouses for the production of cut flowers are easily infected by pathogenic fungi. *Podosphaera pannosa* (Wallr.: Fr.) de Bary, responsible for

powdery mildew, can cause severe damage to leaves, stems, and flowers resulting in loss of quality and yield. This species is an obligate biotrophic ascomycete which can produce secondary spores within 4 to 10 days (Linde and Debener, 2003). Therefore the pathogen can quickly spread over all the rose plants present in a greenhouse. Breeders for this reason consider spraying of fungicides as a necessity to maintain healthy plants. Obviously, development of resistant cultivars will reduce the use of chemicals required for controlling the disease.

Linde and Debener (2003) found in a diploid rose population (97/9), segregating for powdery mildew resistance, a major dominant gene (*Rpp1*) controlling resistance to this disease. In 2004, they identified several molecular markers closely linked to *Rpp1*. They also located a SCAR marker at 2 cM from *Rpp1* but it turned out that this SCAR marker was not polymorphic in the mapping population (94/1) they used to construct their genetic linkage map. Another issue is that resistance to powdery mildew conferred by a single resistance gene is often rapidly overcome by new races of the pathogen. Therefore discovery of new molecular markers and development of markers linked to these genes could help to speed up breeding programs by marker-assisted selection and develop resistant cultivars.

Most of resistance genes identified are members of the Nucleotide Binding Site-Leucine Rich Repeat (NBS-LRR) containing resistance gene family (Young, 2000). NBS-containing resistance genes are widely distributed and highly polymorphic in plant genomes.

Our study focused on the discovery of resistance markers in a tetraploid rose population. NBS profiling (Van der Linden et al., 2004) was used as a tool enabling a more directed way of studying the genetics of resistance to pathogens responsible for diseases such as powdery mildew. Furthermore, to meet the demands of the breeders, genetic linkage maps are being constructed on a tetraploid population where NBS profiling markers and SSRs are currently mapped this population, enabling linkage to existing diploid maps.

MATERIAL AND METHODS

The tetraploid population K5 from Yan (2005) investigated in this study consists of the offspring of a cross between two tetraploid genotypes P867 and P540 which are partially resistant to powdery mildew. The segregating progeny comprises 184 genotypes.

NBS profiling is a multiplex screening technique, producing amplified resistance gene analogue (RGA) fragments by using degenerated primers based on the conserved motifs present in the NBS domain of resistance genes. DNA was isolated as described by Esselink et al. (2003). NBS profiling was performed on 200 ng DNA as described in Van den Linden et al. (2004). Twelve NBS primer-restriction enzyme combinations were used to generate the NBS profiles: *Alu*I, *Hae*III, *Mse*I, and *Rsa*I combined with the degenerated primers NBS1, NBS3, and NBS5a6 (Fig. 1) from van den Linden et al. (2004). Amplified fragments of each primer-restriction enzyme combination were radioactively labeled ([γ-^{33}P] ATP), separated on polyacrylamide gels, and visualized by autoradiography. The resulting polymorphic markers were dominantly scored.

The generated NBS profiling markers will be added to the tetraploid parental linkage maps of Yan (2005). To this purpose, uni- and bi-parental simplex markers will be mapped by using Joinmap 4.0 (van Ooijen and Voorrips, 2001). Moreover, SSR markers (Süss and Schultze, 2003; Esselink, 2003; Hibrand Saint Oyant et al., 2008) from the literature are currently added to get sufficient anchoring points for proper alignment of rose maps.

Association study between NBS profiling markers and the powdery mildew resistance data that Yan (2005) and Yan et al. (2006) obtained by testing the K5 population against two monospore isolates of *Podosphaera pannosa* (2 and F1) was performed in a similar way as described by Yan (2005). The analyses comprises at first running single-marker ANOVAs to identify markers probably associated with powdery mildew resistance. A multiple regression analysis was subsequently performed using the

powdery mildew resistance trait as response variate and the pre-selected markers as regressors to determine the variance of resistance.

RESULTS AND DISCUSSION

NBS Profiling

A total of 181 K5 genotypes and their parents were genotyped by NBS profiling. NBS profiles showed polymorphic multilocus patterns with up to 24 polymorphic markers for primer-restriction enzyme combination NBS5a6-*Rsa*1 (Fig. 2). A total of 158 polymorphic markers were dominantly scored. Table 1 shows the classification of markers from NBS profiling based on segregation ratios with the assumption of tetrasomic inheritance. Uni-parental markers segregate with ratios 1 to 1 and 5 to 1. Bi-parental markers segregate with ratios 3 to 1, 11 to 1, and 35 to 1. Such segregation ratios are typical for an autotetraploid population of an autotetraploid species.

NBS Marker-Powdery Mildew Resistance Association

A preliminary association study between data on powdery mildew resistance from Yan et al. (2006), the markers they generated, and NBS profiling markers was performed, starting with single-marker ANOVAs. This was done to select markers possibly associated with resistance to isolate F1. ANOVAs showed that thirty-two markers were significantly associated to powdery mildew resistance. A multiple regression analysis was subsequently performed using those 32 pre-selected markers as regressors and the powdery mildew resistance trait as response variate. Seven markers, including 4 NBS profiling markers, two AFLP markers, and one SSR, explained together 21% of the variance of the resistance (R^2_{adj}=20.87). Therefore those 7 markers play a role in 1/3 of the heritability for powdery mildew resistance (h^2=62%; Yan, 2005). Yan (2005) found several isolate-specific markers with multiple regression analyses. One of them was the AFLP marker (E31M61-228) whose association to powdery mildew resistance is confirmed in this study as well.

Mapping the powdery mildew resistance trait will be the next logical step, but this will be complicated as Yan (2005) already indicated, since the two parental tetraploid maps needed will contain 56 linkage groups (4 homologous groups of 7 chromosomes for each parent) and because of the tetrasomic inheritance characteristic for autotetraploid species. Nevertheless, it will be interesting to compare location on the genetic maps of the K5 population between the powdery mildew resistance associated markers of Yan (2005) and the powdery mildew resistance associated NBS markers of this study.

Hattendorf and Debener (2007) cloned various RGAs from roses which contained the common motifs from the NBS-LRR genes. Some of them have been mapped onto a diploid rose map. They concluded to the polygenic nature of the resistance since, markers associated with resistance were found on different chromosomes. Linde et al. (2006) detected in a mapping study with a diploid population 4 QTLs in the vicinity of RGA markers. Two QTLs, however, were found in two map regions where no RGAs were detected.

Tetraploid Genetic Linkage Maps

Two parental maps are being constructed by using uni-parental simplex markers. Markers from several origins are used to construct the maps. SSRs from the literature which were developed for diploids did not amplify very well on the K5 population. 38% of the SSRs from Hibrand Saint Oyant et al. (2008) and 8% of the SSRs from Süss et al. (2003) were polymorphic, and used to construct the genetic linkage maps. SSRs from other Rosaceae (strawberry, peach and plum) did not amplify or showed no polymorphism for most of them. Therefore a good tool to study synteny for Rosaceae is not available yet.

Nevertheless a total of 250 markers could be used, and are currently being mapped. Due to the fact that JoinMap is not developed for autotetraploids, stringent

grouping parameters were set, and coupling phases were checked within every linkage group to be the same.

CONCLUSIONS

Many NBS markers were generated due to the high heterozygosity of the K5 population. By examining the segregation ratios of the markers, we can confirm that the K5 population behave as an autotetraploid as Yan (2005) concluded as well. The preliminary association studies indicated association between some NBS markers and resistance to powdery mildew resistance, suggesting that RGAs may play a role in the resistance. A complete coverage of the maps with markers will possibly enable the discovery of more candidate genes affecting resistance.

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Tables

Table 1. Classification of markers from NBS profiling based on the observed segregation ratios. S: simplex, D: duplex.

Marker origin	Uni-parental		Bi-parental			Total
	S	D	SxS	SxD	DxD	
	1:1	**5:1**	**3:1**	**11:1**	**35:1**	
P867	57	3				60
P540	49	1				50
P867 & P540			41	6	1	48
Total	106	4	41	6	1	158

Figures

Fig. 1. Scheme of the NBS domain of disease resistance genes indicating the position of the degenerated NBS primers used to generate NBS markers (adapted from Van der Linden et al., 2004).

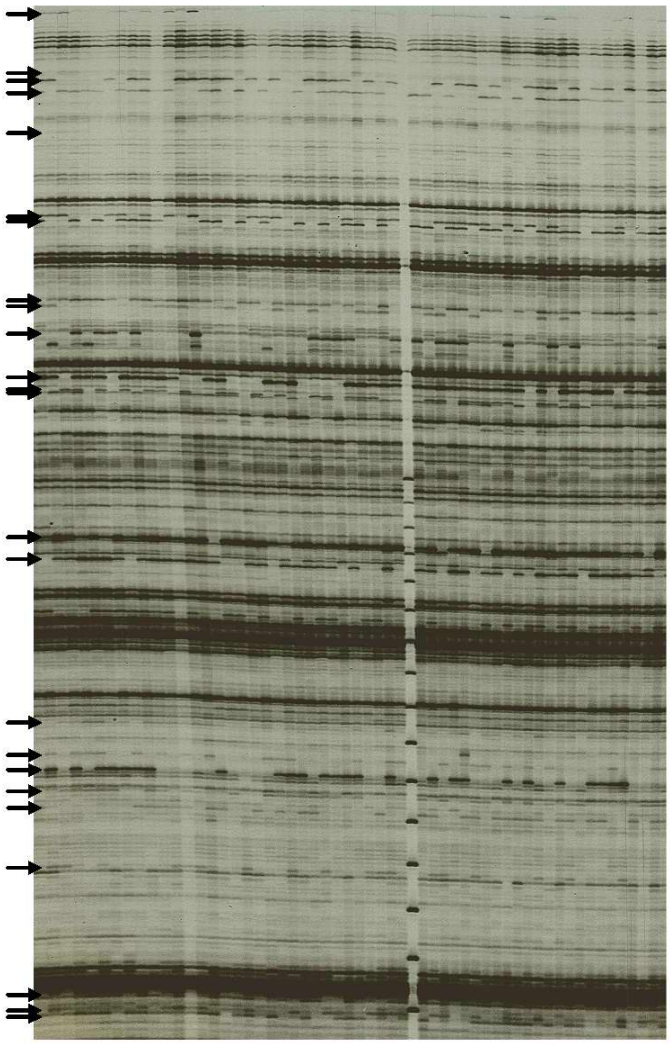

Fig. 2. Example of a NBS profile of tetraploid roses with primer NBS5a6 and restriction enzyme *Rsa*1. Arrows indicate the 24 polymorphic bands which were scored dominantly.