Construction of a Genetic Linkage Map to Locate QTLS Controlling Pathotype-Specific Powdery Mildew Resistance in Diploid Roses

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Abstract

Disease resistance is a sought-after trait in plant breeding programmes. One strategy to make resistance more durable is to increase the number of resistance genes, thereby increasing the number of pathotypes withstood. A diploid rose population (90 genotypes) derived from a cross between *R. wichurana* and *Rosa* 'Yesterday' was used to construct a genetic linkage map encompassing 20 AFLP primer combinations, 43 SSR, and 2 morphological markers. By applying the F1 pseudo test cross population strategy, 2 parental linkage maps were constructed (parent 'Yesterday' 536 cM; parent *R. wichurana* 526 cM). Both parental maps consisted of 7 linkage groups with an average length of 70 cM (Kosambi) corresponding to the 7 haploid rose chromosomes. These new maps were used to identify QTLs controlling disease resistance. The offspring population was screened for resistance to 2 powdery mildew pathotypes, R-E and R-P. QTLs for controlling pathotype-specific disease resistance were mapped by applying Kruskal-Wallis rank-sum tests and simple interval mapping. With 2 pathotypes analysed, 9 QTL loci were detected on linkage groups 2, 3, 5 and 6, explaining 15-73% of the phenotypic variance for pathotype-specific disease response. The genetic maps developed here will be useful for future rose breeding, pathotype-specific resistance research and development of a consensus map for roses.

***Keywords*: disease resistance, SSR, Molecular markers, AFLP, QTL mapping, *R. wichurana*, 'Yesterday'**

Introduction

Roses are the most economically important ornamental plants worldwide (Heinrichs 2008; Debener and Linde 2009). Molecular markers have been developed to enhance breeding efficiency through the identification and characterisation of genes controlling important traits (Hibrand-Saint Oyant et al. 2008; Spiller et al. 2011). Moreover, molecular markers are efficient for constructing genetic linkage maps (Agarwal et al. 2008). Genetic linkage maps have already been created for diploid roses (Debener and Mattiesch 1999; Crespel et al. 2002; Yan et al. 2005; Linde et al. 2006) and tetraploid roses (Rajapakse et al. 2001; Gar et al. 2011). Linkage maps are not only used to identify chromosomal regions controlling simple heritable monogenic traits, but also multiple loci controlling (parts of) polygenic quantitative traits by QTL analysis (Collard et al. 2005). In rose, powdery mildew (PM), caused by *Podosphaera pannosa* (Wallr.:Fr.) de Bary, is known to be one of the most important diseases in greenhouses and is also important for garden roses (Debener and Linde 2009). The objective of the present study is to develop genetic linkage maps from a cross between ´Yesterday´ and *R. wichurana* using morphological, SSR and AFLP markers to identify QTLs controlling resistance to powdery mildew. In addition, we have used race specific QTL mapping to learn more about disease resistance to powdery mildew, since many complete resistance genes (QTLs) exhibit pathotype-specificity (Talukder et al. 2004; Reddy et al. 2009). Moreover, QTLs are generally considered to be useful for wide-spectrum and durable resistance in breeding programmes (Roumen 1994). Finally, pathotype-specific QTLs can, by pyramiding resistance genes, enhance the efficiency of breeding plants with stable disease resistance.

Materials and methods

A diploid (2n = 2x = 14) rose population consisting of 90 individual genotypes derived from a cross between the cultivar 'Yesterday' and the species *R. wichurana* Crep. was used. The rose

population, all 3-year-old plants, were planted in the field. Experimental inoculations were made using 2 pathotypes of powdery mildew, R-P and R-E. The 2 monoconidial isolates were selected from the powdery mildew collection described by Leus et al. (2006). All experiments were performed with each pathotype separately for disease resistance segregation analysis. Linkage analysis and map construction were performed using the JoinMap software version 4.0 (Van Ooijen 2006) using the cross-pollination (CP) model. Parental maps were constructed separately using different sets of segregating markers: a set of markers present only in parent 'Yesterday' (seed parent), a set present only in *R. wichurana* (pollen parent) and a set of bi-parental markers that showed heterozygosity in both parents.

Results

The 2 parents, 'Yesterday' and *R. wichurana*, and their segregating progeny were screened for polymorphisms using 20 AFLP and 43 SSR primer combinations. Each parental map consisted of 7 LGs corresponding to the 7 haploid rose chromosomes. For the numbering of the LGs, correspondence with the previously published maps was sought by checking for common SSR loci. Our study had 16 other SSRs in common with the study of Yan et al. (2005), and in our study, 10 of these were attributed to the same LGs for both rose genotypes. Four SSR markers attributed to different LGs as compared to the results of Yan et al. (2005) and Spiller et al. (2011). For the 3 SSR markers from other studies (Dugo et al. 2005; Rajapakse et al. 2001) only 1 was attributed to the same linkage group in our study.

MapQTL version 5.0 (MapQTL5, Van Ooijen 2004) was used to sketch the presence and locations of QTLs using the non-parametric Kruskal-Wallis test. Next, we used simple interval mapping (SIM) analysis (MapQTL5, Van Ooijen 2004) to more precisely locate the position of the QTLs on each LG on each parental map. The LOD threshold values for QTL significance were obtained by permutation tests (100 replicates) in MapQTL by an individual threshold (selected) per linkage group with a significance level of $\alpha_g = 0.05$ for significant linkages. Last, the percentage of variance explained by each QTL was calculated. Although QTLs were located on both parental maps, we found that most resistant alleles originated from parent *R. wichurana.*

Discussion

The analysis for significant QTLs for PM resistance for both pathotypes showed that QTLs exist in both parents. In most cases allele "c" originating from parent *R. wichurana* conferred resistance in the progeny. Nevertheless, the second-best allele was often contributed by parent 'Yesterday'. Moreover, QTLs for pathotype-specific resistance were found. We found 9 major QTLs for resistance to powdery mildew in total.

For resistance to pathotype R-P we found 2 QTLs located on LG2 and LG3 of 'Yesterday', while on parent *R. wichurana*, 2 QTLs were found on LG2 and 1 on LG5. One QTL on LG2 was found at the same position for both rose genotypes. For pathotype R-E, 2 QTLs for resistance were found on 'Yesterday'. One was located on LG3 on the same position as the QTL found for R-P; the other was located on LG6. Two QTLs were found for resistance towards pathotype R-E on *R. wichurana*: 1 QTL locus on LG5 and 1 on LG6. As both rose genotypes share 1 QTL on LG2 for resistance to pathotype R-P, this race-specific QTL is probably common in other rose genotypes.

The QTL found in common for both pathotypes on LG3 of parent 'Yesterday' is not specific for the pathotypes tested. Linde et al. (2006) found a cluster of QTLs on the same position on LG3 as well as 1 race-specific QTL locus for race 9. The 5 other QTL loci found in our study seem to be specific QTLs for the pathotypes tested. Three QTLs on LG6, one of which is race-specific to race 9, were reported by Linde et al. (2006), whereas two different QTLs were present on this linkage group in our study. Linde et al. (2006) did not report any QTLs on LG5, but we found QTLs on *R. wichurana* (1 QTL for each pathotype). Linde et al. (2006) tested PM resistance in different environments in different years and found a total of 28 QTLs. As they describe, there could have been a change in the population structure of *P. pannosa* from year to year, which could change the pathogenic strains that interact with the detection of particular QTL alleles.

We used 2 specific isolates under protected conditions and detected only 9 QTLs. Therefore we estimate that the use of additional pathotypes will lead to the detection of other pathotype-specific QTLs. Linde et al. (2006) reported that the susceptible parent contributes an average of 31% of the

explained variability for all resistance related QTLs. Keller et al. (1999) reported the same results for resistance to *Erysiphe graminis* in wheat, where 10 QTLs showed effects from the alleles of the resistant parent and 8 resistance QTLs originated from the medium-susceptible parent. Our study on powdery mildew resistance in the rose population can provide further insight into the nature of resistance in roses. QTLs for resistance against specific pathogen races used in the experiment could be identified.

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