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ORIGINAL PAPER

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Genetic mapping of QTLs controlling horticultural traits in diploid roses

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Abstract A segregating progeny set of 96 F_1 diploid hybrids ($2n = 2x = 14$) between "Blush Noisette" (D10), one of the first seedlings from the original "Champneys' Pink Cluster", and *Rosa wichurana* (E15), was used to construct a genetic linkage map of the rose genome following a "pseudo-testcross" mapping strategy. A total of 133 markers (130 RAPD, one morphological and two microsatellites) were located on the 14 linkage groups (LGs) of the D10 and E15 maps, covering total map lengths of 388 and 260 cM, respectively. Due to the presence of common biparental markers the homology of four LGs between parental maps (D10-1/E15-1 to D10-4/E15-4) could be inferred. Four horticulturally interesting quantitative traits, flower size (FS), days to flowering (DF), leaf size (LS), and resistance to powdery mildew (PM) were analysed in the progeny in order to map quantitative trait loci (QTLs) controlling these traits. A total of 13 putative QTLs (LOD > 3.0) were identified, four for FS, two for flowering time, five for LS, and two for resistance to PM. Possible homologies between QTLs detected in the D10 and E15 maps could be established between *Fs1* and *Fs3*, *Fs2* and *Fs4*, and *Ls1* and *Ls3*. Screening for pairwise epistatic interactions between loci revealed additional, epistatic QTLs (EQTLs) for DF and LS that were not detected in the original QTL analysis. The genetic maps developed in this study will be useful to add new markers and locate genes for important traits in the genus providing a practical resource for marker-assisted selection programs in roses.

Keywords *Rosa* · Molecular markers · Genetic map · Pseudo-testcross · QTLs

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Introduction

The genus *Rosa* includes approximately 100 wild species mainly distributed in the temperate and subtropical regions of the Northern Hemisphere. Roses are among the most valuable crops in ornamental horticulture. Despite their commercial significance little information is available about the inheritance of important agronomic characters (De Vries and Dubois 1978, 1984; Debener 1999; Gudin 2000; Debener et al. 2001; Crespel et al. 2002). Factors such as high heterozygosity, ploidy levels (most modern rose cultivars are tetraploids), and severe inbreeding depression after selfing make conventional genetic analysis difficult. Moreover, most diploid species have been reported as self-incompatible and non-recurrent for flowering. Therefore, breeding strategies in roses still rely on simple methods such as exploiting specific combining abilities to produce hybrids and subsequently selecting highly heterozygous, vegetatively produced new cultivars (Debener and Mattiesch 1999; Rajapakse et al. 2001).

Current demands in the rose industry require overcoming such difficulties by using new and more sophisticated breeding strategies (Gudin 2000). Inheritance of male sterility, moss character, recurrent flow-

ering, growth habit and absence of prickles on the petiole have been studied and some of them have already been mapped in different populations (Lewis and Basye 1961; De Vries and Dubois 1978, 1984; Debener et al. 2000; Rajapakse et al. 2001). Moreover, flower colour (pink versus white) and density of thorns were reported to show monogenic or oligogenic inheritance (Debener and Mattiesch 1999; Crespel et al. 2002) and recently, a QTL for petal number has been located (Debener et al. 2001). Apart from these ornamental characters, genes for resistance to the most important diseases blackspot and powdery mildew (PM) have been mapped as well (Malek and Debener 2000; Linde and Debener 2003). Increased knowledge of the genetics of additional important rose traits together with the use of tightly linked molecular markers could greatly accelerate the introgression of genes from wild rose species (mostly diploids) into the genetic background of modern roses.

Linkage maps covering the entire genome are required for some of the applications of markers in plant breeding. This is particularly true for the co-segregation analysis between markers and agronomic characters, often with polygenic inheritance, which allows the detection of quantitative trait loci (QTLs) responsible for the observed phenotypic variation. Up to now, three sets of molecular maps have been published that elaborate on diploid and tetraploid roses (Debener and Mattiesch 1999; Rajapakse et al. 2001; Crespel et al. 2002). Such maps contain molecular markers tightly linked to some of the genes controlling flower morphology, flower color, plant architecture or resistance to pathogens. Nevertheless, considering the narrow gene pool of modern roses, obtaining durable resistance in new rose varieties will require exploring the resistance responses in different genetic backgrounds and the introduction of several resistance genes simultaneously (Gudin 2000; Linde and Debener 2003).

Recently, comparative genome studies of key crop species in important families have also been reported. Researchers working on the Rosaceae family have initiated a comparative study with the goal of creating a unified map (Joobeur et al. 1998). With the aim of contributing to this effort, a number of microsatellites already tested for their utility in cross-species amplification (Guilford et al. 1997; Sosinski et al. 2000) was tested in our material.

The objectives of the present study were: (1) to develop genetic linkage maps from a cross between "Blush Noisette" (D10) and *Rosa wichurana* (E15) using morphological, isozyme, RAPD, and microsatellite markers, and (2) to identify QTLs controlling horticulturally interesting traits such as flower size (FS), days to flowering (DF), and leaf size (LS), as well as the resistance to PM. The final aim is to identify genetic markers linked to commercially valuable traits that could facilitate their introgression into the background of cultivated roses.

Materials and methods

Plant material

A progeny of 96 F_1 plants from an interspecific cross between diploid roses, "Blush Noisette" (D10), one of the first seedlings from the original "Champneys' Pink Cluster", and *R. wichurana* (E15), was used for map construction and QTL analysis according to a "pseudo-testcross" strategy (Grattapaglia and Sederoff 1994). The maternal parent (D10) is a pink double-flowered (> five petals), thorny and spreading rose with recurrent blooming and susceptibility to PM. The paternal parent (E15) has white flowers with five petals and is a ground-covering thornless bush rose with single seasonal blooming and resistance to PM. The progeny was maintained in the field in Córdoba, Spain, under standard culture conditions for phenotypic evaluation, disease assessment and collection of leaf tissue for isozyme and DNA extraction.

Isozyme analysis

Fourteen enzymatic systems were assayed: phosphogluconate dehydrogenase (PGD) [EC 1.1.1.44]; phosphoglucomutase (PGM) [EC 5.4.2.2]; malate dehydrogenase (MDH) [EC 1.1.1.37]; malic enzyme (ME) [EC 1.1.1.40]; esterase (EST) [EC 3.1.1]; aspartate aminotransferase (AAT) [EC 2.6.1.1]; triose phosphate isomerase (TPI) [EC 5.3.1.1]; shikimate dehydrogenase (SKD) [EC 1.1.1.25]; mannose phosphate isomerase (MPI) [EC 5.3.1.8]; aminopeptidase (AMP) [EC 3.4.11]; endopeptidase (ENP) [EC 3.4.–.–]; superoxide dismutase (SOD) [EC 1.15.1.1]; *N*-acetyl-glucosaminidase (NAG) [EC 3.2.1.30] and diaphorase (DIA) [EC 1.6.99.–]. Samples of young leaves were crushed on ice in a small volume of 0.1 M Tris-malate extraction buffer, pH 8 (Weeden and Emmo 1985). Horizontal starch gel electrophoresis was performed in 12% starch gels according to Gottlieb (1973) using three different systems depending on the isozyme in study: (1) Tris-citrate/lithium borate at pH 8 (Selander et al. 1971), (2) histidine at pH 6.5 (Cardy et al. 1980), and (3) citrate/*N*-(3-aminopropyl-morpholine), pH 6.1 (Clayton and Tretiak 1972). Electrophoresis was carried out at 50 mA (at maximum intensity) and 200–300 V for 3–4 h. All the assay solutions were adapted from those of Wendel and Weeden (1990).

DNA extraction and PCR reactions

Molecular analysis was carried out in young leaves from 96 F_1 plants. DNA was isolated according to Torres et al. (1993), replacing the extraction buffer by one adapted to woody species (Cheng et al. 1997). The RAPD analysis was as described by Torres et al. (1993).

A total of 55 primers from Operon Technologies (Alameda, Calif., USA) were surveyed in the parental lines. Thirty-nine primer pairs revealed intense and clearly scorable polymorphic bands and thus were screened for linkage analysis in the F_1 progeny. The amplified products were separated in 2% agarose gels. Electrophoresis was performed at 105 V and maximum amperage for 3 h. Gels were stained with ethidium bromide and photographed under UV light using the Kodak Digital Science 1D software versions 2.0 and 3.5.

To evaluate the potential of microsatellites for use in comparative genetic studies within the Rosaceae, 21 microsatellite primer pairs described in apple (Guilford et al. 1997), peach and cherry (Sosinski et al. 2000) were tested in the mapping progeny. Some of these markers have been reported to be linked to morphological traits and resistance genes to different pathogens. With the aim of obtaining a specific amplification in *Rosa*, several different amplification protocols for microsatellites (Guilford et al. 1997; Sosinski et al. 2000; Pozárková et al. 2002) were assayed. Amplified products were separated by electrophoresis at 90 V and maximum amperage in 2.5% Metaphor agarose gels that were stained and photographed as described above.

Phenotypic traits

Flower type (simple versus double corolla), found to be controlled by a single gene (designated as *Blfo*) (Debener and Mattiesch 1999), was recorded over 2 years after germination. Flowers with five petals were considered to be single, while flowers with more than five were considered to be double. Further morphological traits segregating in the progeny included the presence of prickles, growth habit (erect shrub versus prostrate), petal colour in the bud stage (pink, pale pink or white), and the number of flowers per inflorescence and these were also recorded for the entire progeny over 2 years.

Four quantitative traits (FS, DF, LS and resistance to PM) were scored over one season. The FS was measured at the widest diameter (in cm) when the flowers were completely opened and recorded as the average of five flowers per individual. Flowering date was recorded when 50% of flowers were fully opened. The parental line Blush Noisette (D10) was the earliest to flower (April 21). The number of days from April 21 to the date of flowering of each F_1 plant was calculated and used in the analysis. The LS (in mm^2) was measured as the mean value of five compound leaves from the middle part of the main shoot. The LS was estimated by scanning the leaves using the program Sigma Scan Pro 5.0. The maternal parent D10 was observed to be susceptible to PM, while the paternal parent E15 showed resistance. Assessment for PM resistance in the segregating progeny was carried out in the field after a natural infestation. Three consecutive surveys were performed during the infection period. Resistance to PM was scored using a scale of 0–2 (0 = no symptoms, 2 = heavy infection) and averaged over three surveys.

Map construction

Linkage analysis was carried out using JoinMap 3.0 (Van Ooijen and Voorrips 2002). The mapping progeny, termed the pseudo-testcross, resulted from a cross between two heterogeneously heterozygous and homozygous diploid parents (linkage phases unknown) and hence was coded as CP (for cross-pollination) in JoinMap. Only markers that fitted the expected ratios (1:1 for mono-parental markers; 3:1 for biparental markers) were included in the analysis (χ^2 test; $P > 0.01$). The map was constructed using a LOD of 5.0 for the grouping of the markers. In each linkage group, the order of the markers was inferred using the pairwise data of only those loci that showed a recombination frequency smaller than 0.35 and a LOD value larger than 2.0. The optimal marker order was determined by using the ripple value of 1.0 and the jump threshold of 5.0 and the final map was calculated using a weighted least squares procedure as described by Stam (1993). The Kosambi mapping function was used to convert recombination data to map distances. The linkage maps were drawn using MapChart 2.1 (Voorrips 2002).

QTL analysis

MapQTL Version 4.0 (Van Ooijen and Maliepaard 1996) was used to identify and locate QTLs linked to the molecular markers by performing the non-parametric test of Kruskal-Wallis as well as both interval mapping (Lander and Botstein 1989) and multiple-QTL mapping (MQM, Jansen and Stam 1994). In the regions of the putative QTLs, the markers with the highest LOD values were taken as co-factors. A backward elimination procedure was used to select cofactors significantly associated with each trait at $P < 0.02$ and used in the MQM. The LOD thresholds for QTL significance were confirmed by permutation tests (1,000 replications) in MapQTL with a genome-wide significance level of $\alpha_g = 0.05$ for significant linkages (Churchill and Doerge 1994). For each LOD peak, the 1-LOD support intervals were determined (Van Ooijen 1992).

In a pseudo-testcross progeny, a QTL can segregate for four different alleles ($Q_1Q_2 \times Q_3Q_4$). The means of the distribution of the quantitative trait associated with four different genotypes (Q_1Q_3, Q_1Q_4, Q_2Q_3 and Q_2Q_4) can be estimated. Following the method proposed by Knott et al. (1997), three allelic effects were calculated (Table 1): individual parental effects (difference in the effect of the alleles inherited from each parent), and interaction effects (i.e. deviation from additivity, where a value of zero indicates complete additivity). Finally, the percent of variance explained by each QTL were calculated.

Table 1 Model used to test the effect of QTL alleles

Parental cross	$Q_1Q_2 \times Q_3Q_4 \rightarrow Q_1Q_3, Q_1Q_4, Q_2Q_3, Q_2Q_4$
Maternal effect (D10)	$= (Q_1Q_3 + Q_1Q_4) - (Q_2Q_3 + Q_2Q_4)$
Paternal effect (E15)	$= (Q_1Q_3 + Q_2Q_3) - (Q_1Q_4 + Q_2Q_4)$
Interaction effect (I)	$= (Q_1Q_3 + Q_2Q_4) - (Q_1Q_4 + Q_2Q_3)$

Two-way interactions between QTLs were tested among all pairwise combinations of the markers using EPISTAT (Chase et al. 1997). A total of 10^6 trails were used in the Monte Carlo simulation to establish the statistical significance of the log-likelihood ratios of the interactions detected. The *P*-values found by the Monte Carlo simulation were transformed into $1-(1-P)^n$, thus, adjusting for the number of loci (n) searched (Lark et al. 1995).

Results and discussion

Isoenzymatic analysis

From the 14 enzymatic systems analysed, 7 (PGD, PGM, MDH, DIA, SOD, α-EST and AAT) were clearly resolved, but none of the loci displayed polymorphism in the rose progeny. For PGM and DIA, both parents were homozygous for different alleles and consequently their F_1 progeny was heterozygous, displaying a monomorphic pattern. In the cases of PGD, MDH, α-EST, SOD and AAT, both parents exhibited the same alleles for the different loci and therefore their progeny displayed an invariable banding pattern. Consequently no isozyme loci could be included in the map.

RAPD analysis

Of the 55 primers assayed in the parents, 39 were used to screen the mapping population. The selection was made based on the clearest and most reproducible patterns for unambiguous scoring. The average number of polymorphic bands per primer was four with molecular weights ranging between 700 and 1,800 bp. Of the 169 polymorphic markers, 104 were inherited from the female parent D10, 53 from parent E15, and 12 from both parents. Segregation ratios were tested using the chi-square test and 25 markers that did not fit the expected Mendelian ratios were omitted from further analysis. Fifteen of these discarded markers were inherited from the female parent, six from the male parent and four from both parents.

The proportion of markers with skewed segregation (14.8%) was similar to the level of segregation distortion, previously reported in roses by Debener and Mattiesch (1999) (16%) and lower than that obtained by Crespel et al. (2002) (38.8%). As stated by these authors, segregation distortion was more frequently observed in markers originated in the female than in the male parent, suggesting some form of selection at the female gamete level. Distorted segregation is known to be a regular outcome in interspecific crosses, where anomalous meioses are likely to occur. The fact that the percentage of markers showing Mendelian segregation in our population was relatively high (85%) suggest that the parental lines selected for the study are genetically close (Kianian and Quiros 1992). The particularly complex and largely unknown breeding history of most rose species and varieties makes it difficult to estimate genetic relationships and genetic diversity on the basis of pedigree data (Debener et al. 1996).

Microsatellite analysis

From the three previously reported amplification protocols tested (Guilford et al. 1997; Sosinski et al. 2000; Pozárková et al. 2002), we selected the latter one since it provided the clearest and most reproducible banding patterns. Of the 21 microsatellite primer pairs analyzed, 10 successfully amplified rose DNA. Out of these, only two (*Pchcms2* and *01a6*), produced polymorphic bands comparable in size with the corresponding PCR products of the homologous species (peach and apple, respectively). Both microsatellites were scored as dominant markers and mapped in the mapping population.

Although the microsatellite primer pairs used in this study were designed and optimized for cherry, peach and apple, about half of them (50%) provided amplification products in rose. All of these species belong to the family Rosaceae and the primer recognition sites seem to be well conserved across subfamilies (cherry and peach in Prunoidae, apple in Maloidae and rose in Rosoidae). However, the amplified DNA regions may be too highly conserved to allow for detection of significant polymorphism within rose, as seen with the low rate of polymorphism. A low rate of polymorphisms has been also described by Rajapakse et al. (2001) in an analysis of heterologous microsatellite markers in a tetraploid rose progeny.

Map construction

Of the 148 markers showing Mendelian segregation (144 RAPDs, 2 SSRs and 2 morphological markers), 133 (130 RAPDs, one morphological and two microsatellite markers) were successfully mapped (89.9%) on the seven LGs of each of the two maps. Due to the presence of the common biparental markers, the homology of four LGs (D10-1/E15-1 to D10-4/E15-4) could be inferred (Fig. 1). Correspondence among the remaining LGs from the two parents could not be determined due to the absence of common biparental markers. For this reason, these LGs were labelled differently (D10-5 to D10-7 for the female map and E15-8 to E15-10 for the male map).

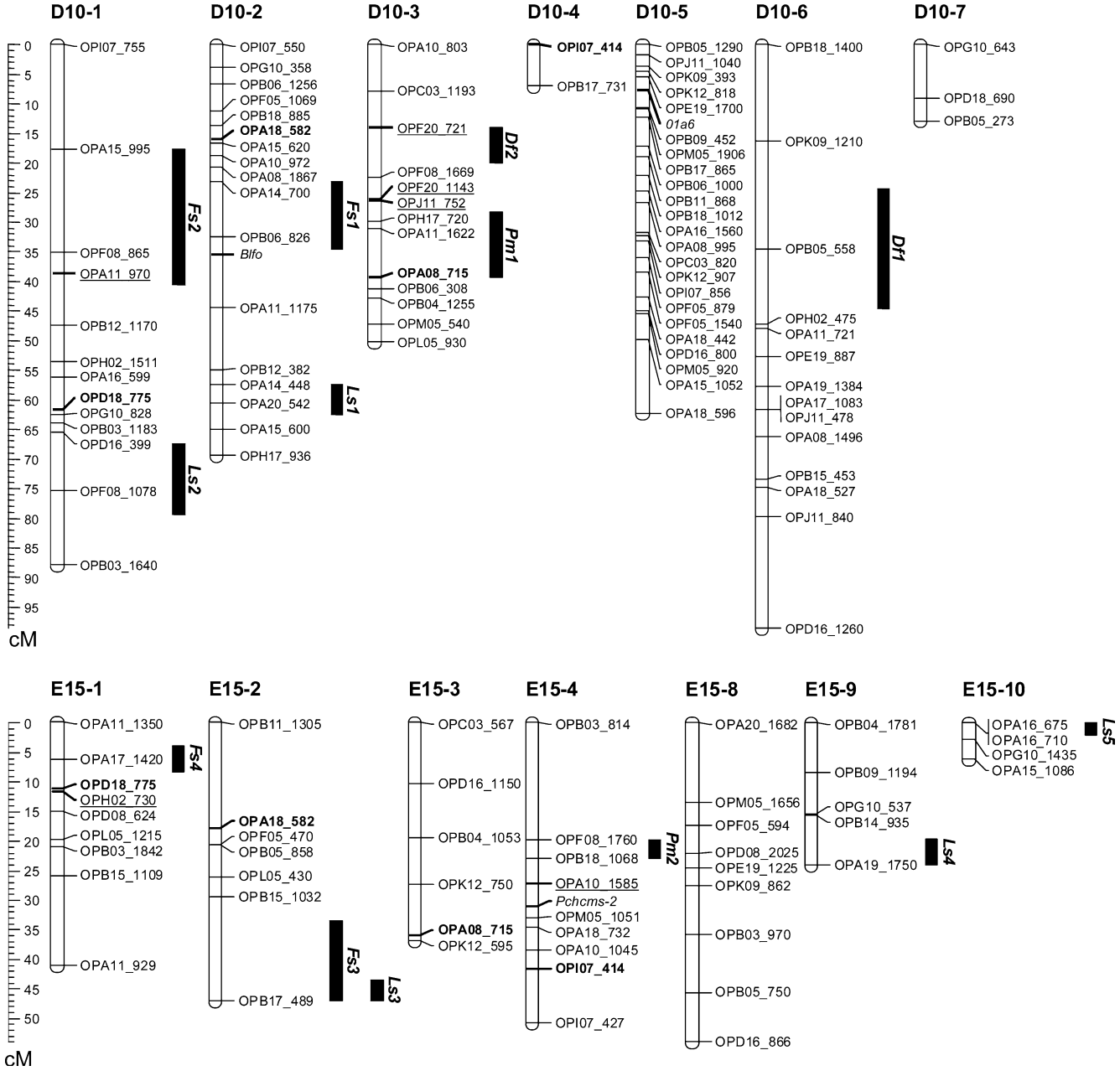

Fig. 1 Linkage maps of female parent (*Rosa chinensis* var. Blush Noisette: D10) and male parent (*R. wichurana*: E15) developed using their F_1 progeny. Map positions are given in cM using the Kosambi mapping function. LGs 1–4 are homologous in both maps. Common biparental markers are shown in *bold*. Microsatellites (*Pchcms2* and *01a6*) and a morphological marker *Blfo* are shown in *italics*. *Bars* indicate the QTL locations (i.e. 1-LOD support intervals) for each trait as detected using D10 and E15 linkage maps. Epistatic quantitative trait loci (EQTLs) for DF ($OPJ11_{752}$, $OPF20_{1143}$, $OPF20_{721}$ and $OPA11_{970}$) and for LS ($OPH02_{730}$ and $OPA10_{1585}$) are *underlined*

The map of Blush Noisette (D10) consisted of 87 (85 RAPDs, double corolla (*Blfo*, and apple microsatellite *01a6*) markers assigned to seven LGs. Among these LGs, five (D10-1, D10-2, D10-3, D10-5 and D10-6) are major ones that include 13 to 24 markers. The remaining two (D10-4 and D10-7), consist of two and three markers, respectively. The average size of the seven LGs was 55.47 cM, ranging from 6.97 (D10-4) to 98.64 (D10-6) cM. The female parental map covered 388.31 cM of the genome with an average intermarker distance of 5.62 cM. In the male parental map (E15), 50 markers (49 RAPDs and peach microsatellite *Pchcms2*) were assembled into seven LGs consisting of four (E15-10) to ten (E15-4) markers. The sizes of these LGs ranged from 6.05 (E15-10) to 53.99 (E15-8) cM. The map of *R. wichurana* thus covers 260.02 cM and the mean distance between markers is 5.83 cM. The length of the present maps is comparable to those reported by Debener and Mattiesch (1999); Rajapakse et al. (2001) and Crespel et al. (2002).

The map of the female parent Blush Noisette contains almost twice as many polymorphic markers as that of the male parent. This is an expected outcome when using

Table 2 Phenotypic values of quantitative traits for parental lines and the F_1 progeny. *FS* flower size (cm), *DF* days to flowering (days from April 21), *LS* leaf size (mm^2), *PM* resistance to powdery mildew

Traits	Parental lines			F_1 progeny		
	D10	E15	Mean	Range	Skewedness	Kurtosis
FS	4.84	4.02	4.06	2.05–5.72	−0.3574	−0.1446
DF	0.00	18.00	12.74	0.00–22.00	−0.0481	−0.4777
LS	8,644.50	1,353.00	2,200.09	593.49–5,157.25	0.3258	−0.0011
PM	1.00	0.00	0.85	0.00–2.00	0.3376	−0.5458

the pseudo-testcross strategy in progenies derived from interspecific crosses, as a consequence of the lower level of heterozygosity in one of the parents (Yin et al. 2001). In our cross, the hybrid origin of the female parent derived from Champneys' Pink Cluster, which was in turn obtained from a cross between a pink China rose (traditionally supposed to be Parsons' Pink) and *Rosa moschata*, might have contributed to the higher genetic variability.

Phenotypic data

A gene controlling flower type (simple versus double corolla), and designated as *Blfo* by Debener and Mattiesch (1999) or *d6* by Crespel et al. (2002) was located in the middle of LG D10-2. The closest marker ($OPB06_{826}$) was 2.9 cM away from the gene. The transformation of this marker into a standard marker such as a sequence characterised amplified region (SCAR) would facilitate the manipulation of this trait in future breeding programs. Moreover, it would provide an anchor point between LG D10-2, derived from the present study, and LGs B3 and A6 in the maps of Debener and Mattiesch (1999) and Crespel et al. (2002), respectively.

Several rose phenotypic characteristics were evaluated to infer their genetic basis of inheritance. The flower type was recorded during the 2 years following germination. Blush Noisette is characterized by a double corolla (> five petals) and recurrent blooming, whereas *R. wichurana* is a single flowered type (five petals) with single seasonal blooming. In the first year of scoring, double versus single corolla segregated in a 3:1 ratio (84 double:25 single; $\chi^2 = 0.25$; $P = 0.62$) suggesting that the trait was controlled by two complementary genes. This outcome can, however, also result from a possible association between single flowering and non-recurrent blooming since the second year of growth, number of

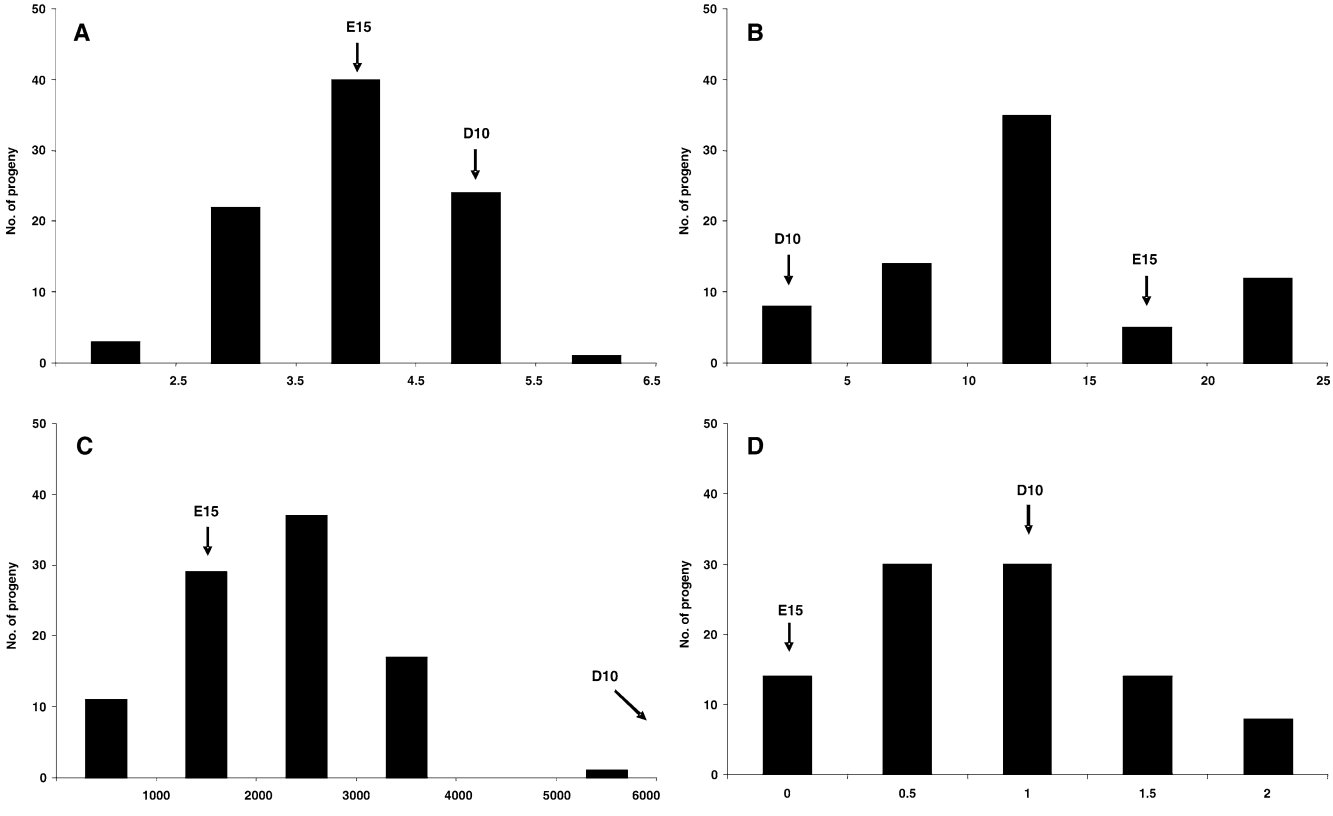

Fig. 2 Frequency distribution in 96 F_1 plants for **a** Flower size (cm), **b** Days to flowering (*days* from April 21), **c** Leaf size (mm^2) and **d** Resistance to PM (evaluated on a scale of 0–2. 0 = no infection, 2 = heavy infection). Parental trait values are indicated by *arrows*

flowering plants was higher and most of the new individuals displayed a single flowered type (97 double:78 single). In accordance with previous studies (Debener 1999; Debener et al., 2001; Crespel et al. 2002), the trait fits a 1:1 ratio ($\chi^2 = 2.06$; $P = 0.15$) consistent with the monogenic inheritance of this character. Assuming that single flowered *R. wichurana* is homozygous recessive, the observed segregation suggests that Blush Noisette is heterozygous.

Presence of prickles, growth habit, flower colour in the bud stage and number of flowers per inflorescence were recorded as well in the entire population during the 2 year study period. In all cases it was very difficult to unambiguously classify the progeny into different phenotypic groups, and data recorded for individual plants often differed between the two observation years. Since the correlations were low between the two scorings, the corresponding data were disregarded for further analysis.

Mean phenotypic values and basic statistical parameters for FS, DF, LS and resistance to PM are presented in Table 2. Frequency distributions for these traits are shown in Fig. 2. The values of FS and LS were normally distributed as checked by Shapiro-Wilk normality test ($W = 0.98$, $P = 0.48$; $W = 0.97$, $P = 0.23$, respectively). Although the parental lines had similar values for FS, the F_1 plants showed a great variability for the trait with clear transgression in both directions. In case of LS the frequency distributions displayed a clear bias toward the parent with less foliar area (E15). Moreover, 19 F_1 plants had, on average, a smaller LS than E15 suggesting negative dominance and transgressive segregation for this trait. The progeny distribution of DF values showed a platykurtic distribution (kurtosis = −0.48) and transgression toward the higher values, while the distribution of PM values was both platykurtic and skewed toward the lower values. A significant ($P < 0.01$) positive correlation was observed between FS and LS ($r = 0.45$) but no other comparisons among the analysed traits yielded significant correlations (Table 3).

Anchor points to other Rosaceae maps

Two microsatellites(*01a6* and *Pchcms2*) were mapped in LGs D10-5 and E15-4, respectively. *Pchcms2* was previously ascribed to LG G7 in the reference *Prunus* map (Aranzana et al. 2003), based on a F_2 progeny of the cross between "Texas" almond and "Earlygold" peach (Joobeur et al. 1998), thus allowing to establish the correspondence between the rose LG E15-5 in the present study and the *Prunus* LG G7. Although the number of anchoring markers in the present map to other Rosaceae maps is low, these markers serve as a starting point for genome comparison among the Rosaceae.

Table 3 Pearson correlations among traits. *FS* flower size (cm), *DF* days to flowering (days from April 21), *LS* leaf size (mm^2), *PM* resistance to powdery mildew

Traits	FS	DF	LS
DF	0.06^{ns}		
LS	0.45^{*}	-0.13^{ns}	
PM	0.19^{ns}	0.00^{ns}	0.05^{ns}

^{ns} $P > 0.05$, * $P < 0.01$

QTL mapping

Two separate QTL analyses were performed with the D10 map (LGs D10-1 to D10-7) and the E15 map (LGs E15-1 to E15-4 and E15-8 to E15-10). As mentioned previously, due to the low number of biparental markers, homology could be inferred for only four LGs (D10-1/E15-1 to D10-4/E15-4).

Table 4 lists major QTLs affecting FS, DF, LS, and resistance to PM detected on the D10 and E15 maps. Four putative QTLs for FS (*Fs1*, *Fs2*, *Fs3* and *Fs4*) were detected on LGs D10-2, D10-1, E15-2 and E15-1, respectively. The strongest signal (*Fs1*, LOD = 7.58) was detected on the linkage group D10-2 in the vicinity of the locus *Blfo* controlling flower type that might suggest a possible pleiotropic effect of this gene on FS. Although the homologies of these QTL regions are yet to be definitely established with adequate number of common markers, it is likely that the QTLs *Fs1* and *Fs3* and *Fs2* and *Fs4*, detected in the maternal and paternal maps and explaining 45.7/47.9 and 47.0/36.1% of the phenotypic variance of the trait respectively, are homologous. All the QTLs for FS have shown considerable parental effects (both maternal and paternal) as well as interaction effects, suggesting that the parents were heterozygous and heterogeneous for the QTLs in question.

Two QTLs, *Df1* and *Df2*, controlling flowering time were detected on D10-6 and D10-3, respectively, while no significant QTL was found using the E15 map. *Df1* and *Df2* explained 31.8 and 39.8% of the phenotypic variance, respectively. It appears that the maternal line D10 was heterozygous for *Df1*, carrying an allele for early flowering, while the E15 was homozygous for a *Df1* allele causing delayed flowering.

Five QTLs affecting LS were found on D10-2, D10-1, E15-2, E15-9, and E15-10, explaining from 10.6 to 35.1% of the phenotypic variance of the trait. Possible homology between putative QTLs could be established between *Ls1* on D10-2 and *Ls3* on E15-2. *Ls1* showed substantial maternal effect while the paternal effect was null suggesting that the male parent E15 was homozygous. The paternal effect was more pronounced in *Ls2* and *Ls4* while the maternal effect was null for *Ls5* suggesting that the female parent D10 was homozygous.

Finally, two QTLs for PM resistance, *Pm1* and *Pm2*, were detected on D10-3 and E15-4, explaining 45.2 and 24.9% of the phenotypic variance of the trait, respectively. Estimates of the paternal effects of these QTLs indicate that the resistant parent, E15, was heterozygous

Table 4 Quantitative trait loci for flower size (*Fs1-4*), days to flowering (*Df1-2*), leaf size (*Ls1-5*) and resistance to powdery mildew (*Pm1-2*) detected in F_1 progeny of *Rosa chinensis* var. Blush Noisette (D10) and *R. wichurana* (E15). *TLOD* represents the LOD significance threshold corresponding to genome-wide significance level $\alpha_g = 0.05$ as obtained by permutations

QTLs	Linkage group	Peak position (cM)	Nearest marker(s)	Maximum LOD score	Allelic effects (maternal, paternal and interaction) as described in Table 2			% Phenotypic variation	TLOD
					D10	E15	I		
Fs1	D10-2	29.1	$OPA14_{700}/OPB06_{826}$	7.58	−1.77	0.90	0.56	45.7	3.6
Fs2	D10-1	25.6	$OPA15_{995}/OPF08_{865}$	3.82	1.26	−1.57	0.47	47.0	3.6
Fs3	E15-2	39.5	$OPB15_{1032}/OPB17_{489}$	4.01	−0.60	0.83	1.82	47.9	3.5
Fs4	E15-1	6.3	$OPA17_{1420}$	3.44	1.26	−0.84	0.91	36.1	3.5
Df1	D10-6	38.5	$OPB05_{558}/OPH02_{475}$	4.92	−13.49	0.00	0.00	31.8	3.5
Df2	D10-3	14.0	$OPF20_{721}$	4.38	1.43	14.03	−7.18	39.8	3.5
Ls1	D10-2	59.4	$OPA14_{448}/OPA20_{542}$	5.30	−1,598.72	0.00	0.00	10.6	3.3
Ls2	D10-1	75.4	$OPF08_{1078}$	4.04	71.70	−1,831.12	−918.28	35.1	3.3
Ls3	E15-2	47.1	$OPB17_{489}$	3.47	−1,356.33	901.31	1,082.89	30.1	3.5
Ls4	E15-9	24.1	$OPA19_{1750}$	4.68	−631.11	1,591.01	−1,048.49	30.9	3.2
Ls5	E15-10	0.0	$OPA16_{675}$	4.11	0.00	−1,539.86	0.00	18.1	3.2
Pm1	D10-3	37.0	$OPA11_{1622}/OPA08_{715}$	4.62	−1.03	0.91	−0.90	45.2	3.7
Pm2	E15-4	23.0	$OPB18_{1068}$	3.63	0.26	−0.98	−0.74	24.9	3.7

for both QTLs conferring resistance to PM. Moreover, both QTLs exhibit a strong interaction effect, which suggests some degree of non-additive (i.e. dominant or epistatic) expression for alleles at these QTLs.

Epistatic interactions

Using the program EPISTAT (Chase et al. 1997) we screened for pairwise epistatic (non-additive) interactions occurring between markers on different LGs (Table 5). Significant epistatic interactions were detected for DF andLS. The analysis for DF revealed the marker $OPA11_{970}$ on LG D10-1 that interacts with markers $OPJ11_{752}$, $OPF20_{1143}$, and $OPF20_{721}$ on D10-3. In the vicinity of these markers the primary QTL, *Df2*, was detected. Thus, an allele at the epistatic locus located near the marker $OPA11_{970}$ on LG 10-1 significantly shortens the number ofDF, but only when combined with an allele at the primary QTL *Df2*. One significant epistatic interaction for LS was detected involving the marker $OPA10_{1585}$ on E15-4 and the marker $OPH02_{730}$ on E15-1. It is worth mentioning that in the interval between the markers $OPH02_{730}$ and $OPD08_{624}$ on the LG E15-1 the putative QTL action was detected using multiple-QTL mapping, but the peak LOD value of 2.94 did not exceed the LOD significance threshold corresponding to a genome-wide significance level of $\alpha = 0.05$. Since the primary QTL, *Ls2*, is identified on the homologous D10-1 LG, it is possible that the homolo-

Table 5 Epistatic interactions between unlinked markers. The epistatic quantitative trait loci (EQTL1 and EQTL2) and the linkage groups are given. *LLR* represents the log-likelihood ratio if an epistatic model is compared with an additive model. *P*a represents the additive *P* values associated with the LLRs derived from the Monte Carlo simulation. *P*t represents the transformed *P* values adjusted for the number of loci searched

Trait EQTL1/LG		EQTL2/LG		LLR	*P*a	*P*t
DF		$OPA11_{970}$/D10-1				
		ab	aa			
$OPJ11_{752}$/D10-3	ab[a]	12.72	13.72			
	aa	7.20	17.27	7.93	0.0004	0.0330
DF		$OPA11_{970}$/D10-1				
		ab	aa			
$OPF20_{1143}$/D10-3	ab	7.20	17.27			
	aa	12.79	13.65	7.88	0.0004	0.0364
DF		$OPA11_{970}$/D10-1				
		ab	aa			
$OPF20_{721}$/D10-3	ab	6.43	16.29			
	aa	13.00	14.31	8.01	0.0004	0.0393
LS		$OPA10_{1585}$/E15-4				
		aa	ab			
$OPH02_{730}$/E15-1	aa	2,379.17	2,565.60			
	ab	2,181.11	1,315.80	7.44	0.0006	0.0304

[a] The mean phenotypic values for all four marker classes (see Results and discussion)

gous QTL is located in the vicinity of the marker $OPH02_{730}$ on the LG E15-1, having not only additive but also epistatic effect on this trait.

Conclusions

As in an earlier mapping study with roses (Debener and Mattiesch 1999), the pseudo-testcross mapping strategy together with the RAPD assay was used in this study. This approach allows gene mapping using a progeny derived from a cross between two heterogeneously heterozygous and homozygous diploid parents (Grattapaglia and Sederoff 1994). This strategy has been the method of choice for species in which inbred lines cannot be developed. However, if an integrated map for the two parents is to be developed, only those homologous maternal and paternal LGs sharing at least two biparental markers can be identified and fully integrated with the JoinMap 3.0 package (Van Ooijen and Voorrips 2001). Since our mapping population is derived from an interspecific cross between two diploid roses, Blush Noisette and *R. wichurana*, a low number of biparental markers (heterozygous for both parents) can be expected because the parents are not closely related. Indeed, only six out of 133 markers mapped (4.5%) were shared by the parents and were used to assign LG homologies between the two maps. In order to develop a fully integrated map, an offspring cross strategy (Atienza et al. 2002) is currently being followed after selecting and crossing contrasted individuals from the current offspring. This approach is likely to reveal a fair level of polymorphisms between the parents, but additionally the number of biparental markers is expected to be considerably higher with respect to the original pseudo-testcross mapping progeny because the parents are full-sibs. By comparing linkage maps obtained by the pseudo-testcross and offspring cross strategies as well as the results of QTL analyses we should be able to obtain an idea about the efficiency of the offspring cross strategy.

The SSRs are highly suitable markers for the purpose of integrating parental maps with the added advantage of providing the potential of anchoring loci between the maps of related species within the Rosaceae. To achieve this objective, 24 microsatellites from *R. hybrida* (Esselink et al. 2003) and 25 from peach, japanese plum and almond (kindly provided by Dr. Arús) are currently being tested in our mapping population.

The present map has allowed the localization of markers linked to valuable genes (flower type), and QTLs (FS, LS, DF and resistance to PM) in the species. Six of the 13 QTLs reported in Table 4 were consistently detected on homologous LGs of the D10 and E15 maps (*Fs1*, *Fs2*, *Fs3*, *Fs4*, *Ls1* and *Ls3*). Additional common markers are required to determine if QTLs in homologous LGs from the two parental maps are the same. Other QTLs were only found either on the D10 map (*Df1*, *Df2*, *Ls2* and *Pm1*) or on the E15 map (*Ls4*, *Ls5* and *Pm2*). This outcome might be due to the lack of coverage of the genome regions bearing these QTLs on the alternative parental map, or because the homologous groups have not been recognized yet. It will be necessary to increase the number of molecular markers and the size of the segregation population in order to saturate the linkage maps and eventually bridge homologous arrays. As mentioned before, multiallelic markers such as microsatellites will be powerful tools for this purpose. While mapping additional markers may fill some gaps, increasing the population size will improve the resolution of the QTL region. As a result, putative QTLs located in unmapped genome regions or QTLs with minor effects could be identified. A more focused search for the exact position of these QTLs can be done by applying the bulked segregant analysis technique (Michelmore et al. 1991) that may identify additional polymorphisms close to the region of interest.

Up to now, few quantitative genetic studies have been conducted on horticultural traits in *Rosa*. Crespel et al. (2002) reported two QTLs for density of thorns and Debener et al. (2001) a QTL for petal number. In the present study putative QTLs for FS, flowering time, LS, and resistance to PM were identified. Although these QTL regions need to be validated in other environments and mapping populations, the information generated so far regarding the genetic complexity and map position of new quantitative traits will be valuable for further genetic analyses as well as for their manipulation in future rose breeding programs.

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References

Aranzana MJ, Pineda A, Cosson E, Dirlewanger E, Ascasibar J, Cipriani G, Ryder CD, Testolin R, Abbott A, King GJ, Iezzoni A, Arús P (2003) A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. Theor Appl Genet 106:819–825

Atienza SG, Satovic Z, Petersen KK, Dolstra O, Martín A (2002) Preliminary genetic linkage map of *Miscanthus sinensis* with RAPD markers. Theor Appl Genet 105:946–952

Cardy BJ, Stuber CW, Goodman MM (1980) Techniques for starch-gel electrophoresis of enzymes from maize (*Zea mays* L.). Mimeo Series, North Carolina State University Department of Statistics, Raleigh

Chase K, Adler FR, Lark KG (1997) Epistat: A computer program for identifying and testing interactions between pairs of quantitative trait loci. Theor Appl Genet 94:724–730

Cheng FS, Brown SK, Weeden NF (1997) A DNA extraction protocol from various tissues in woody species. Hortscience 32:921–922

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Clayton JW, Tretiak DN (1972) Amino-citrate buffers for pH control in starch-gel electrophoresis. J Fish Res Bd Can 29:1169–1172

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 (1999) Genetic analysis of horticulturally important morphological and physiological characters in diploid roses. Gartenbauwissenschaft 64:14–20

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, Bartels C, Mattiesch L (1996) RAPD analysis of genetic variation between a group of rose cultivars and selected wild rose species. Mol Breed 2:321–327

Debener T, Janakiram T, Mattiesch L (2000) Sports and seedlings of rose varieties analysed with molecular markers. Plant Breed 119:71–74

Debener T, von Malek B, Mattiesch L, Kaufmann H (2001) Genetic and molecular analysis of important characters in roses. Acta Hort 547:45–49

De Vries DP, Dubois LAM (1978) On the transmission of the yellow flower colour from *Rosa foetida* to recurrent flowering hybrid tea-roses. Euphytica 27:205–210

De Vries DP, Dubois LAM (1984) Inheritance of the recurrent flowering and Moss characters in F_1 and F_2 hybrid Tea × R. *centifolia muscosa* (Aiton) Seringe populations. Gartenbauwissenschaft 49:97–100

Esselink GD, Smulders MJM, Vosman B (2003) Identification of cut rose (*Rosa hybrida*) and rootstock varieties using sequence tagged microsatellite site markers. Theor Appl Genet 106:227–286

Gottlieb LD (1973) Enzyme differentiation and phylogeny in *Clarkia franciscana*, *C. rubicunda*and *C. amoena*. Evolution 27:205–214

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross mapping strategy and RAPD markers. Genetics 137:1121–1137

Gudin S (2000) Rose: genetics and breeding. Plant Breed Rev 17:159–189

Guilford P, Prakash S, Zhu JM, Gardiner S, Basset H, Forster R (1997) Microsatellites in *Malus* × *domestica* (apple): abundance polymorphisms and cultivar identification. Theor Appl Genet 94:249–254

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447–1455

Joobeur T, Viruel MA, De Vicente MC, Jauregui B, Ballester J, Dettori MT, Verde I, Truco MJ, Messeguer R, Batlle I, Quarta R, Dirlewanger E, Arús P (1998) Construction of a saturated linkage map for *Prunus* using an almond × peach F_2 progeny. Theor Appl Genet 97:1034–1041

Kianian SF, Quiros CF (1992) Generation of a *Brassica oleracea* composite RFLP map: Linkage arrangements among various populations and evolutionary implications. Theor Appl Genet 84:544–554

Knott SA, Neale DB, Sewell MH, Haley CS (1997) Multiple marker mapping of quantitative trait loci in an outbred pedigree of loblolly pine. Theor Appl Genet 94:810–820

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lark KG, Chase K, Adler F, Mansur LM, Orf JH (1995) Interactions between quantitative trait loci in soybean in which trait variation at one locus is conditional upon a specific allele at another. Proc Natl Acad Sci USA 92:4656–4660

Lewis WH, Basye RE (1961) Analysis of nine crosses between diploid *Rosa* species. Proc Am Soc Hort Sci 78:572–579

Linde M, Debener T (2003) Isolation and identification of eight races of powdery mildew of roses (*Podosphaera pannosa*) (Wallr:Fr) de Bary and the genetic analysis of the resistance gene *Rpp1*. Theor Appl Genet 107:256–262

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

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Pozárková D, Koblízková A, Román B, Torres AM, Lucretti S, Lysák M, Dolezel J, Macas J (2002) Development and characterization of microsatellite markers from chromosome l-specific DNA libraries of *Vicia faba*. Biologia Plantarum 45:337–345

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

Selander RK, Smith MH, Yang SY, Johnson WE, Gentry JB (1971) Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Univ Texas Publ 7103:49–90

Sosinski B, Gannavarapu M, Hager LD, Beck LE, King GJ, Ryder CD, Rajapakse S, Baird WV, Ballard RE, Abbott AG (2000) Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. Theor Appl Genet 101:421–428

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

Torres AM, Weeden NF, Martín A (1993) Linkage among isozyme, RFLP and RADP markers in *Vicia faba*. Theor Appl Genet 85:937–945

Van Ooijen JW (1992) Accuracy of mapping quantitative trait loci in autogamous species. Theor Appl Genet 84:803–811

Van Ooijen JW, Maliepaard C (1996) MapQTL, Version 3.0: software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen The Netherlands

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

Voorrips RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Weeden NF, Emmo AC (1985) Isozyme characterization of Kentucky bluegrass cultivars. Can J Plant Sci 65:985–994

Wendel JF, Weeden NF (1990) Visualization and interpretation of plant isozymes. In: Isozymes in plant biology Dioscorides Press, Portland, Oregon, pp 5–45

Yin T, Huang M, Wang M, Zhu L-H, Zeng Z-B, Wu R (2001) Preliminary interespecific genetic maps of the *Populus* genomes constructed from RAPD markers. Genome 44:602–609