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ORIGINAL PAPER

Genetic dissection of scent metabolic profiles in diploid rose populations

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Abstract The scent of flowers is a very important trait in ornamental roses in terms of both quantity and quality. In cut roses, scented varieties are a rare exception. Although metabolic profiling has identified more than 500 scent volatiles from rose flowers so far, nothing is known about the inheritance of scent in roses. Therefore, we analysed scent volatiles and molecular markers in diploid segregating populations. We resolved the patterns of inheritance of three volatiles (nerol, neryl acetate and geranyl acetate) into single Mendelian traits, and we mapped these as single or oligogenic traits in the rose genome. Three other volatiles (geraniol, β-citronellol and 2-phenylethanol) displayed quantitative variation in the progeny, and we mapped a total of six QTLs influencing the amounts of these volatiles onto the rose marker map. Because we included known scent related genes and newly generated ESTs for scent volatiles as markers, we were able to link scent related QTLs with putative candidate genes. Our results serve as a starting point for both more detailed analyses of complex scent biosynthetic pathways and the development of markers for marker-assisted breeding of scented rose varieties.

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Introduction

Roses are one of the most economically important ornamental plant crops worldwide (Heinrichs 2008). A major characteristic of ornamental roses, in addition to floral colour and morphology, is fragrance. Therefore, many biochemical investigations of the nature of rose scent volatiles have been conducted to date. More than 500 individual volatiles have been found to contribute to rose scent (Knudsen et al. 2006). Most of them can be classified into three main groups of metabolites: terpenoids, phenylpropanoids, and fatty acid derivatives. The major compounds of rose scent are 2-phenyl ethanol, geraniol, and alcohol acetates. However, some substances contribute significantly to typical rosy scent at low concentrations, such as β-ionone, β-damascenone, and the 'rose oxides'.

The production and emission of scent volatiles in rose flowers mainly occurs in petal epidermal cells (Bergougnoux et al. 2007). Scent emission follows a circadian rhythm and is dependent on the developmental stage of the flower (Helsper et al. 1998; Picone et al. 2004).

Studies of differences in gene expression between scented and non-scented roses have led to the identification of several genes coding for enzymes involved in the biosynthesis of scent volatiles. These include genes involved in the biosynthesis of 2-phenyl ethanol (phenyl acetaldehyde reductase and aromatic amino acid decarboxylase, Sakai et al. 2007); methylated aromatic compounds like 1,3,5-trimethoxybenzene (orcinol-*O*-methyltransferase 1, Guterman et al. 2002; Channeliere et al. 2002); methyleugenol ((iso)eugenol-*O*-methyltransferase, Lavid et al.

2002); sesquiterpenes (germacrene D synthase, Guterman et al. 2002); and alcohol acetates (*R. hybr.* alcohol acyltransferase1, Guterman et al. 2002). Most of these genes are specifically expressed in petals. Furthermore, the expression of some genes is restricted to certain taxonomic groups. For example, *RhOOMT*s are expressed only in Asian roses.

In contrast to the available enzymatic information, nothing is known about the inheritance of scent volatiles in roses. For other morphological and physiological traits, genetic and even genomic information is already available due to the utilisation of diploid segregating populations (Debener and Linde 2009). For example, genes for disease resistance (Kaufmann et al. 2003; Linde et al. 2004), double flowers (Debener and Mattiesch 1999), and recurrent flowering (Dugo et al. 2005) and QTLs for powdery mildew resistance (Linde et al. 2006), petal number (Hibrand-Saint Oyant et al. 2008), and growth rates (Yan et al. 2005b) have previously been located on rose linkage maps. Maps based on molecular markers are available for a number of diploid and tetraploid populations (Debener and Linde 2009). The most extensive map comprises more than 800 markers (Yan et al. 2005a and additional unpublished results from our lab). The goals of the present study were to analyse the inheritance of major scent metabolites by combining gas chromatographic (GC) analysis of floral volatiles in diploid populations with marker information from these maps and to map loci involved in the biosynthesis of scent volatiles.

Materials and methods

Marker analyses

The diploid rose populations 94/1 and 97/7 used in this study were described in Yan et al. (2005a) and Linde et al. (2006) and comprise introgressed hybrids of *R. multiflora* with garden roses. Population 94/1 resulted from a cross between half sibs (genotypes 93/1–117 and 93/1–119). Population 97/7 resulted from an intercross between unrelated genotypes.

DNA was extracted from 50 to 70 mg of leaf tissue as described by Linde et al. (2006). PCR primers were designed with Primer3 software (http://www.frodo.org) using default settings. Standard PCR reactions were performed with 50 ng of template DNA in a 25-µl PCR reaction containing 1× PCR buffer (Williams 1989), 5 pmol of each primer, 0.2 mM dNTPs, and 1 U Biotaq *Taq* polymerase (Bioline, Luckenwalde, Germany). PCR was conducted with a 5-min initial denaturation at 95°C, 30 cycles of 1-min denaturation at 94°C, 1-min annealing at a primer-specific annealing temperature, and 1 min/kb product elongation at 72°C, followed by a 10-min final elongation at 72°C.

CAPS markers were generated by digestion of PCR products in PCR buffer with 5 U of restriction enzyme for 2 h at temperatures specified by the manufacturers (for some enzymes, 0.1 M NaCl was added). For SSCP analysis, PCR products were diluted with an equal volume of loading buffer [95% (v/v) formamide, 0.01 M NaOH, 0.05% (w/v) xylene cyanol, and 0.05% (w/v) bromophenol blue], denatured for 3 min at 95°C, and immediately placed on ice. From this solution, 1.5–4.5 µl were loaded onto 0.5× MDE polyacrylamide gels and electrophoresed with 6 V/cm at 4°C for at least 16 h. DNA fragments were visualised by silver staining according to the protocol of Sanguinetti et al. (1994). The following known scent genes were investigated: *RhOOMT1* and *RhOOMT2* (Lavid et al. 2002), *RcOMT1*, *2*, and *3* (Wu et al. 2003), *POMT* (Wu et al. 2004), and *RhAAT1* and *GDS* (Guterman et al. 2002). Primer sequences are listed in Table 5 of the supplementary material. In addition, 40 different ESTs from internet sources and from a subtractive hybridisation experiment (data not shown) that showed similarity to scent related sequences from other species were tested for polymorphisms in the two mapping populations. For primer sequences of those ESTs that revealed polymorphisms, see Table 6 of the supplementary material.

Scent phenotyping

Genotypes 93/1–117 and 93/1–119 and the hybrid population 94/1, with 88 plants, were used for scent phenotyping. Plants were cultivated in 3-l pots in the greenhouse until visible buds had formed. At this stage, the plants were transferred into a climate chamber for at least 1 week before the first sampling. Stage four to five flowers (fully opened flowers before the onset of anther senescence; Oka et al. 1999) were sampled at 11:30 pm and extracted with 2 ml hexane per gram petal fresh weight. Camphor (20 mg/l) was used as an internal standard. At least 500 mg of petals was extracted overnight in glass vials at −20°C. The extracts were dried with Na_2SO_4 overnight at −20°C. At least three different clones of each genotype were sampled. Flowers were pooled over several clones, and each genotype was sampled three to seven times.

Individual scent volatiles were identified by gas chromatography–mass spectrometry (GC–MS) and comparison with the Wiley, NBS54K (NIST), and LMC libraries. Separation of volatiles was achieved by injecting 1 µl of extract onto a high-resolution gas chromatograph (Fisons Instruments GC 8000 series) with cold-on-column injection (40°C) and equipped with a Phenomenex ZB-Wax column (30 m × 0.32 mm inner diameter, 0.25-µm film) and a Fisons Instruments MD 800 detector. The carrier gas was

He with prepressure of 50 kPa and flow rate of 1.8 ml/min. The temperature of the ion source was 200°C, that of the interface was 230°C, and that of the quadrupole was 100°C. The ionisation energy was 70 eV. Mass spectra were recorded from *m/z* 33 to 300.

Data were evaluated using Xcalibur Version 1.19 (Thermo). Peak identities were verified by coelution with standard substances (Fluka, Sigma-Aldrich).

Scent compounds were quantified using high-resolution gas chromatography (HRGC) with flame ionisation detection (FID) (HP 6890 series) with Gerstel KAS4 (cold application system) and Gerstel controller 505. The KAS parameters were splitless injection (0.5 min), initial temperature of 60°C, temperature increase of 12°C/s, and final temperature of 240°C (3 min). Separation was carried out on a Varian WCOT Fused Silica CP Wax 52CB column (30 m × 0.25 mm ID, 0.25-µm film). Detector parameters were FID at 250°C, H_2 carrier gas at 40 ml/min, prepressure of 58.4 kPa, and constant flow of 1.5 ml/min. Data were collected using a Hewlett Packard HP GC ChemStation, Version Rev. A. 0504, with the following temperature program: 40°C (3 min), 3°C/min up to 200°C, and 10°C/min up to 250°C (10 min). Repeated sampling was performed by a Hewlett Packard HP 6890 Series Injector. For peaks not detected in GC–MS but in quantitative analyses, Kovats indices were calculated. Relative peak areas were normalised to the internal standard and transformed to absolute concentrations.

Map construction

Linkage analyses and map construction were performed using JoinMap4 (Van Ooijen 2006). Linkage maps for both parents of population 94/1 were calculated independently. Grouping was performed using independence LOD. Groups were chosen from an LOD of 5–15. Mapping was performed using the mapping function of Kosambi, a recombination frequency of 0.3, a jump of 5, and LOD of 2.0. Only maps from the first round of calculation in JoinMap4 were used for QTL mapping in MapQTL6 (Van Ooijen 2009). QTL detection was performed using mean values of at least three biological replicates with peak areas normalised to absolute compound concentrations. The mean concentrations of geraniol, 2-phenyl ethanol, and β-citronellol could be used directly. For neryl acetate, log-transformed mean values were used (Yang et al. 2006). In the MapQTL6 software (Van Ooijen 2009), the non-parametric Kruskal–Wallis rank-sum test for single marker influence and the mixture model option for interval mapping (Lander and Botstein 1989) were used. Additionally, the significance threshold of LOD scores was estimated by the permutation test integrated in MapQTL6 (Churchill and Doerge 1994) with 1,000 permutations. QTLs were considered significant if they reached at least a chromosome-wide level of significance in interval mapping. Additionally, regions were considered to show significant influence on one of the traits if they contained several single markers for which the K values in the Kruskal–Wallis analysis were higher than the Bonferroni adjusted significance threshold ($df = 1 \rightarrow K \geq 7.24$; $df = 2 \rightarrow K \geq 9.88$; and $df = 3 \rightarrow K \geq 12.07$).

Results

Prior to the genetic analysis of rose scent, additional markers derived from published scent biosynthesis genes were added to the existing maps for populations 94/1 and 97/7. Furthermore, we mapped ten ESTs isolated from rose petal cDNA with significant similarity to volatile biosynthetic genes (unpublished results) and five markers for already known scent related genes of roses. PCR products were mapped as SCAR, CAPS, and SSCP markers in the two mapping populations (Table 1).

Genetic segregation of scent metabolites

Scent profiles of the parents

Scent volatiles were extracted with hexane from seven replicates of the parental genotypes 93/1–119 (mother) and 93/1–117 (father). The mean values of all replicates showed significant differences between the two genotypes (Table 2).

In total, about 250 GC peaks were detected in the different samples. Among these, several major and minor compounds were identified via GC–MS and coelution with standard substances. The six major compounds were neryl acetate, geranyl acetate, nerol, β-citronellol, geraniol, and 2-phenyl ethanol (Table 2). Scent extracts of the progeny also contained several minor compounds. These were substances with a very low odour threshold, such as β-ionone and β-damascenone. The concentrations of the minor compounds (neral, 3-*cis*-hexenol, 3-trans-hexenol, β-ionone, α-ionone, β-damascenone, 2-phenyl ethyl acetate, and benzyl alcohol) were below 0.1 mg/l, making reliable identification difficult with our experimental setup. Therefore, these substances were not considered in the subsequent analyses.

Three metabolites (geranyl acetate, β-citronellol, and 2-phenyl ethanol) were detectable in scent extracts of only one of the parents. Geraniol, neryl acetate, and nerol were detected in both parents, but differed in their respective amounts (Table 2).

Table 1 SCAR, CAPS, and SSCP markers developed in populations 94/1 and 97/7

Gene	Marker name	Marker type	Population	Linkage group
Alcohol acyltransferase1	RhAAT1-Intron, RhAAT1codingseq	SCAR	94/1, 97/7	2
Germacrene D-synthase	GDS-1, GDS-2	SSCP	94/1, 97/7	5
Orcinol-O-methyltransferase	RhOOMT1 and -2	CAPS	94/1	2
Eugenol-O-methyltransferase (RcOMT1)	EOMT_MboI	SSCP	97/7	4
Caffeic acid-O-methyltransferase3	RcOMT3-265 and -280	SCAR	94/1, 97/7	2, 4
Phloroglucinol-O-methyltransferase	POMT_MboI	CAPS	97/7	6
Carotenoid cleavage dioxygenase	CCD1_RsaI	CAPS		1
Terpene synthase-like *(Farnesyltransferase)*	T_1410_TPS-L	CAPS	94/1	4
Phenylacetaldehyde reductase	c3299-1, -2, -3_PAR-L	CAPS	94/1	1
BEAT-like	T_178_BEAT-L	SSCP	94/1	2
N-methyltransferase	G_189_NMT-L	CAPS	97/7	2
Terpene synthase-like	T_1994_TPS-L	SSCP	94/1	1
Alcohol acetyltransferase-like	T_45_AAT-L	SSCP	97/7	4
Benzyl alcohol acetyltransferase-like	T_81_BEAT-L	SSCP	97/7	6
(Di)Terpene synthase	c125_TPS_L	SSCP	94/1	5

First five markers are markers for already known scent related genes, markers indicated with T_, c, or G and marker CCD1_RsaI were developed from a SSH bank of EST sequences of rose petals

Table 2 Mean values for major scent compounds measured for the parental genotypes 93/1–117 and 93/1–119 and mean values for population 94/1

Scent compound	93/1–117	93/1–119	94/1
Neryl acetate	0.67 (0.47)	0.1 (0.24)	0.16 (0.13)
Geranyl acetate	5.09 (1.78)	0	1.58 (0.64)
β-Citronellol	0	20.25 (12.83)	4.15 (1.52)
Nerol	0.83 (0.14)	0.1 (0.23)	0.35 (0.25)
Geraniol	13.4 (4.01)	6.05 (2.81)	7.47 (2.92)
2-Phenyl ethanol	13.77 (3.46)	0	13.86 (3.82)

Values are given in mg/l extract. Standard deviations are shown in brackets

Scent profiles of the progeny

Two types of segregation for scent volatiles were detected among the progeny. Geranyl acetate, neryl acetate, and nerol were either present or absent, whereas β-citronellol, geraniol, and 2-phenyl ethanol were present in all progeny and showed quantitative inheritance (Table 3). However, the observed segregation profiles for nerol, neryl acetate, and geranyl acetate did not fit the pattern expected for single locus inheritance. Because nerol and neryl acetate are present in both parents, a segregation ratio of 3:1 (presence:absence) is expected if production of the volatile is caused by a single dominant locus that is heterozygous in both parents. The observed patterns of 1.75:1 for nerol and 1:1 for neryl acetate could be due either to distorted segregation of the responsible loci or to other genetic models (e.g., metabolite production only in heterozygotes). To distinguish between these possibilities, we analysed the segregation patterns of closely linked markers.

Mapping the nerol and neryl acetate traits in population 94/1 placed them close to molecular markers displaying the same type of segregation distortion. The nerol locus is located on LG3 (linkage group 3), closely linked proximally to P37M51-132 and distally to Rh50. For example, the dominant AFLP marker P37M51-136, which is present in both parents, segregates in a ratio of 1.4:1, instead of the expected ratio of 3:1. In addition, the most tightly linked SSR markers, Rh50, Rw12J12, and Rh65, show segregation distortion. This is caused by the presence of a self-incompatibility locus in the same region of LG3 (unpublished results).

The neryl acetate locus maps to a region on LG4 with a number of dominant AFLP markers that also show a shift from the expected ratio of 3:1 towards a 1:1 segregation ratio. In addition, the neryl acetate locus is tightly linked to the SSR marker Rw55E12, which also shows skewed segregation. These analyses indicate single locus segregation for both traits.

In contrast to the nerol and neryl acetate loci, geranyl acetate is present in the scent extract of only one parent. Therefore, if heterozygosity in this parent is assumed, it is expected to segregate in a ratio of 1:1 (presence:absence). The observed segregation pattern of 3:1 cannot be explained by segregation distortion. If treated as a single locusno marker in proximity to the geranyl acetate locus displayed skewed segregation in initial mapping experiments (data not shown). Alternatively, this segregation

Table 3 Segregation pattern of major scent compounds in population 94/1

Scent compound	Present in scent of parent		Expected presence to absence	Observed in 94/1 presence to absence	χ^2	*P* value
	93/1–117	93/1–119				
Nerol	+	+	3 to 1	54 to 34 (1.75 to 1)	3.02	0.15
Neryl acetate	+	+	3 to 1	47 to 41 (1 to 1)	21.88	≤0.001
Geranyl acetate	+	−	1 to 1	68 to 20 (3 to 1)	26.18	≤0.001
β-Citronellol	−	+	1 to 1	88 to 0 (quantitative)	–	–
Geraniol	+	+	3 to 1	88 to 0 (quantitative)	–	–
2-Phenyl ethanol	+	−	1 to 1	88 to 0 (quantitative)	–	–

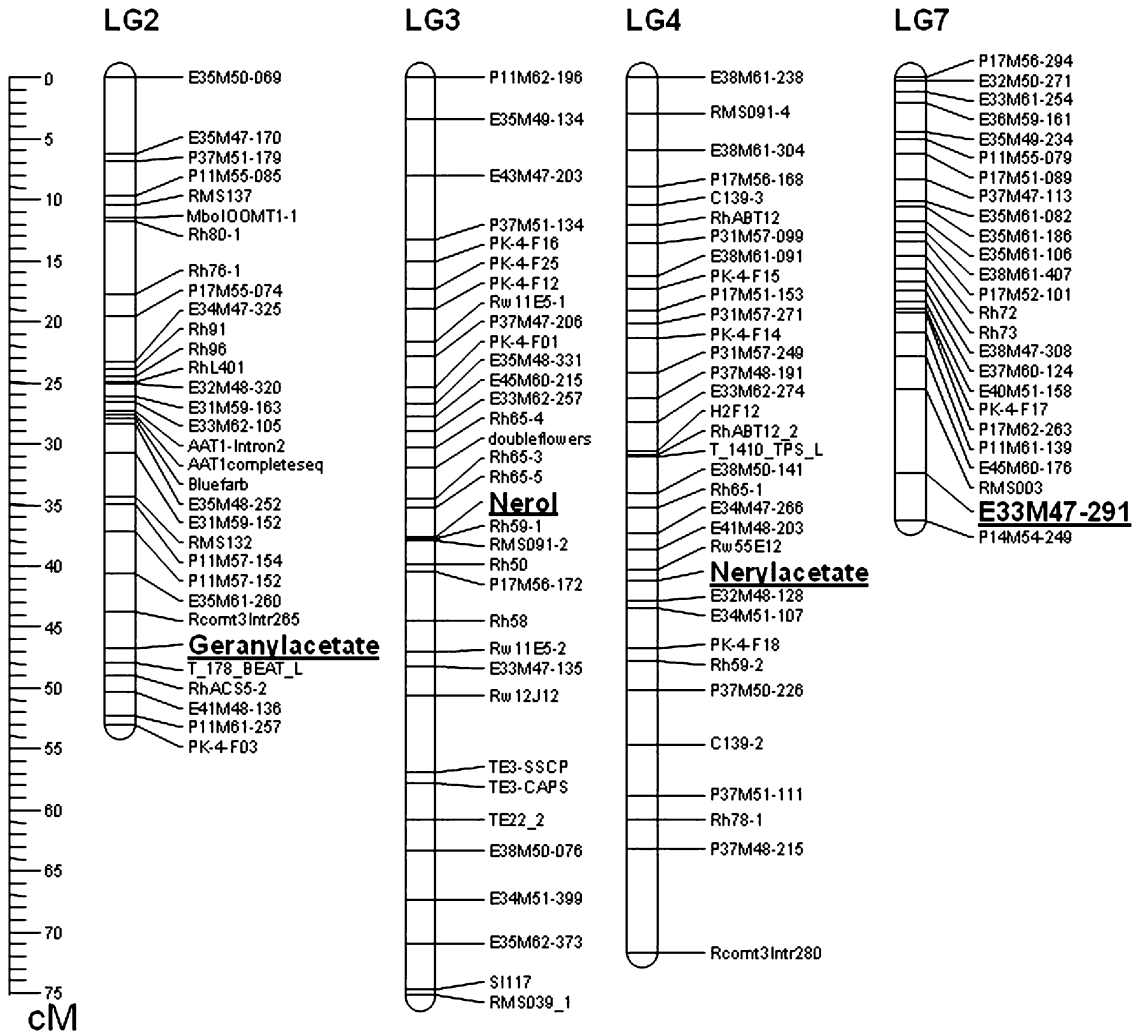

Fig. 1 Integrated linkage groups 2, 3, 4 and 7 of population 94/1. Loci for the production of individual scent volatiles following monogenic or oligogenic inheritance are indicated by *bold* and *underlined* locus names

pattern could be explained by the action of two unlinked dominant loci in a heterozygous condition. Because the JoinMap 4 software (Van Ooijen 2009) does not allow mapping traits based on two genes, we searched for linked markers manually. Such analysis of our set of markers using Excel spreadsheets revealed two loci that follow exactly the expected pattern. One locus cosegregates with one of the paralogues of the *RhAAT1* gene, which codes for an alcohol acyl transferase. The second locus is closely linked to marker E33M47-291 on LG7 (Fig. 1).

Table 4 Summary of QTLs for scent compounds detected in population 94/1

QTL	Linkage group	LOD score	Level of significance	2-LOD confidence interval (cM)
Geraniol	A1, B1	4.84, 5.6	Genome-wide	42 to 69, 43 to 84
2-Phenyl ethanol	A2, B2	4, 3.8	Chromosome-wide	21 to 55, 10 to 31
2-Phenyl ethanol	A5, B5	10, 6.55	Genome-wide	36 to 44, 10 to 33
β-Citronellol*	B1	$P \leq 0.0005$		42 to 72
β-Citronellol	B3	3, 3.1	Genome-wide	22 to 28, 21 to 27
β-Citronellol	A7, B7	3.22, 3.28	Chromosome-wide	45 to 59, 43 to 58

* Supported only by single markers

Mapping of QTLs

In contrast to the scent compounds that followed simple inheritance patterns, geraniol, *β*-citronellol, and 2-phenyl ethanol were present in the scent extracts of all progeny and showed quantitative inheritance (Table 3). The combination of QTL interval mapping and single marker analysis based on the non-parametric Kruskal–Wallis test, both implemented in MapQTL6 (Van Ooijen 2009), identified several major QTLs for each trait (Table 4).

Geraniol

Among the individuals of population 94/1, the quantity of geraniol was normally distributed (Kolmogorov–Smirnov $P = 0.15$) with values between 0.09 and 33 mg/l. One QTL region was detected located on LG1 with LOD scores of 4.8 (paternal map) and 5.6 (maternal map). Several highly significant markers detected by the Kruskal–Wallis test explained 23% of the observed variation (Fig. 2).

2-Phenyl ethanol

With concentrations of mean values for single individuals ranging from 0.1 to 56 mg/l, the quantity of 2-phenyl ethanol was nearly normally distributed among the progeny (Kolmogorov–Smirnov $P = 0.05$). The most significant QTL detected in this study, which explained 60% of the phenotypic variation, was found on LG5, with a LOD of 10.0 (Fig. 3).

A second QTL that explained 28% of the variation was found on LG2 (LOD 4.0).

β-Citronellol

As for geraniol, all scent extracts showed a normal distribution for the quantity of *β*-citronellol (Kolmogorov–Smirnov $P = 0.37$). Concentrations of this compound ranged from 0.12 to 23 mg/l. Three QTL regions were detected. A marker interval toward one end of LG7 (LOD 3.2) supported by two markers found to be significant in the Kruskal–Wallis test explained 15% of the phenotypic variation (Fig. 4). A second locus on LG1 within the QTL interval for geraniol content explained 20% of the observed variation in *β*-citronellol content. However, this is based only on markers highly significant in the Kruskal–Wallis test (P values ≤ 0.001). A third region on LG3 with a LOD of 3.0 was linked to markers Rh50 and *double flowers* and explained 15% of the phenotypic variation.

Discussion

Long an important trait for practical plant breeding, floral scent has received increasing attention in basic research due to its ecological function as well as the biosynthesis of its individual compounds (Dudareva et al. 2004; Pichersky and Dudareva 2007; Schwab et al. 2008). Here, we present the first genetic dissection of scent production in roses and the localisation of some of the responsible genes in the rose genome.

Many enzymes involved in the biosynthesis of scent metabolites have already been identified and functionally characterised (Pichersky et al. 2006). Some of the structural genes and biosynthetic pathways are well studied, primarily in model systems, such as *Clarkia breweri* (Dudareva et al. 1996a, b, 1998a, b), *Petunia* (Boatright et al. 2004), and *Antirrhinum* (Tholl et al. 2004). One of the best-studied examples is volatile synthesis in *Clarkia* sp., for which six genes have been characterised. The biochemical functions of the enzymes encoded by these genes have been analysed in detail. Several genes involved in the production of volatiles have been identified in non-floral tissues of herbs and fruits. As an example, geraniol synthase was isolated and characterised from peltate glands of *Ocimum basilicum* (Iijima et al. 2004). Even in roses, several genes involved in the biosynthesis of scent compounds have already been identified. Several genes for *O*-methyl transferases involved in the production of methylated aromatic compounds are known (Channeliere et al. 2002; Guterman et al. 2002; Wu et al. 2003, 2004). In addition, two genes coding for enzymes that

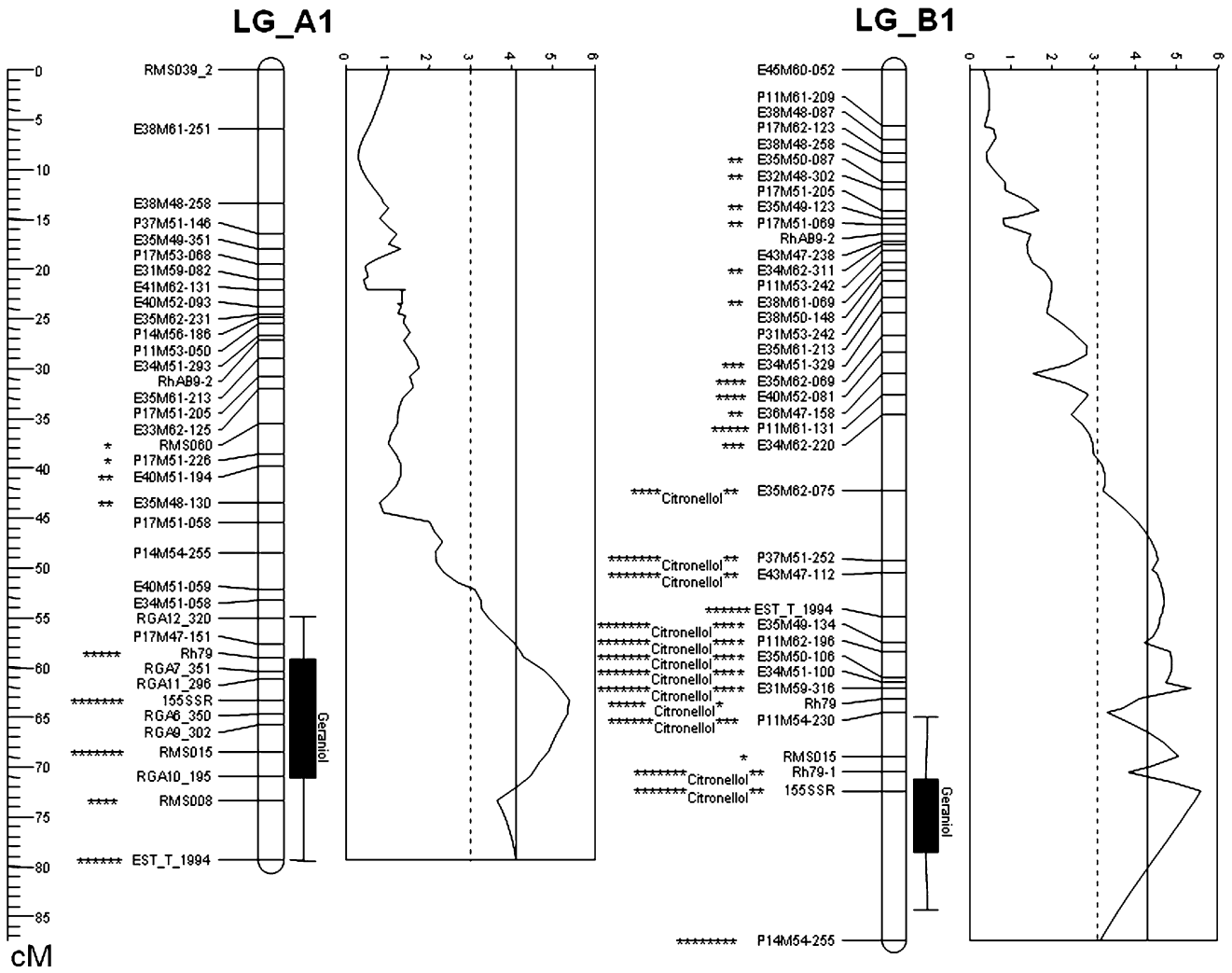

Fig. 2 QTLs for geraniol on linkage groups *A1* and *B1* of population 94/1. Loci that show significant *K* values in non-parametric QTL mapping are indicated with *asterisks*. *Graphs* show the corresponding LOD score curves from interval mapping. *Dashed lines* show chromosome-wide significance thresholds based on permutation tests, while *solid lines* show genome-wide thresholds. The 1- and 2-LOD confidence intervals are indicated with *filled bars* and *whiskers*

catalyse the production of 2-phenyl ethanol (Sakai et al. 2007), a sesquiterpene synthase (Guterman et al. 2002) and a short chain alcohol acyltransferase (Shalit et al. 2003), have been identified.

Despite the information available on the biochemistry of scent volatiles, little is known about the inheritance of scent volatiles in plants. Only a few studies have investigated the genetics of scent production. A few reports on the inheritance of Mendelian loci for terpenoid composition in *Mentha* sp. are available (Gershenzon et al. 2000), along with one study identifying QTLs responsible for terpene oil content in *Eucalyptus* (Shepherd et al. 1999) and a QTL mapping study for aromatic compounds in apple (Dunemann et al. 2009).

In the present study, we resolved the inheritance of some components of rose scent into single Mendelian loci. Single loci were mapped for nerol and neryl acetate, and two loci were detected for geranyl acetate. Although the quantities of geraniol, *β*-citronellol, and 2-phenyl ethanol displayed continuous variation, we were able to locate two QTLs for each of these volatiles. This is the first study reporting map locations of loci responsible for scent volatiles in roses. The only published analysis of scent in a segregating population was conducted in tetraploid roses and provided values for heritability of scent volatiles, but it did not link these to Mendelian factors (Cherri-Martin et al. 2007).

Our strategy to resolve the complex inheritance of scent into single factors was based on two prerequisites: genetic analysis in a diploid population polymorphic for scent volatiles, and the availability of a dense marker map to support genetic analyses. This approach is exemplified by our genetic analyses of the inheritance of nerol and neryl acetate. The availability of a dense marker map allowed us to distinguish between alternative genetic hypotheses that

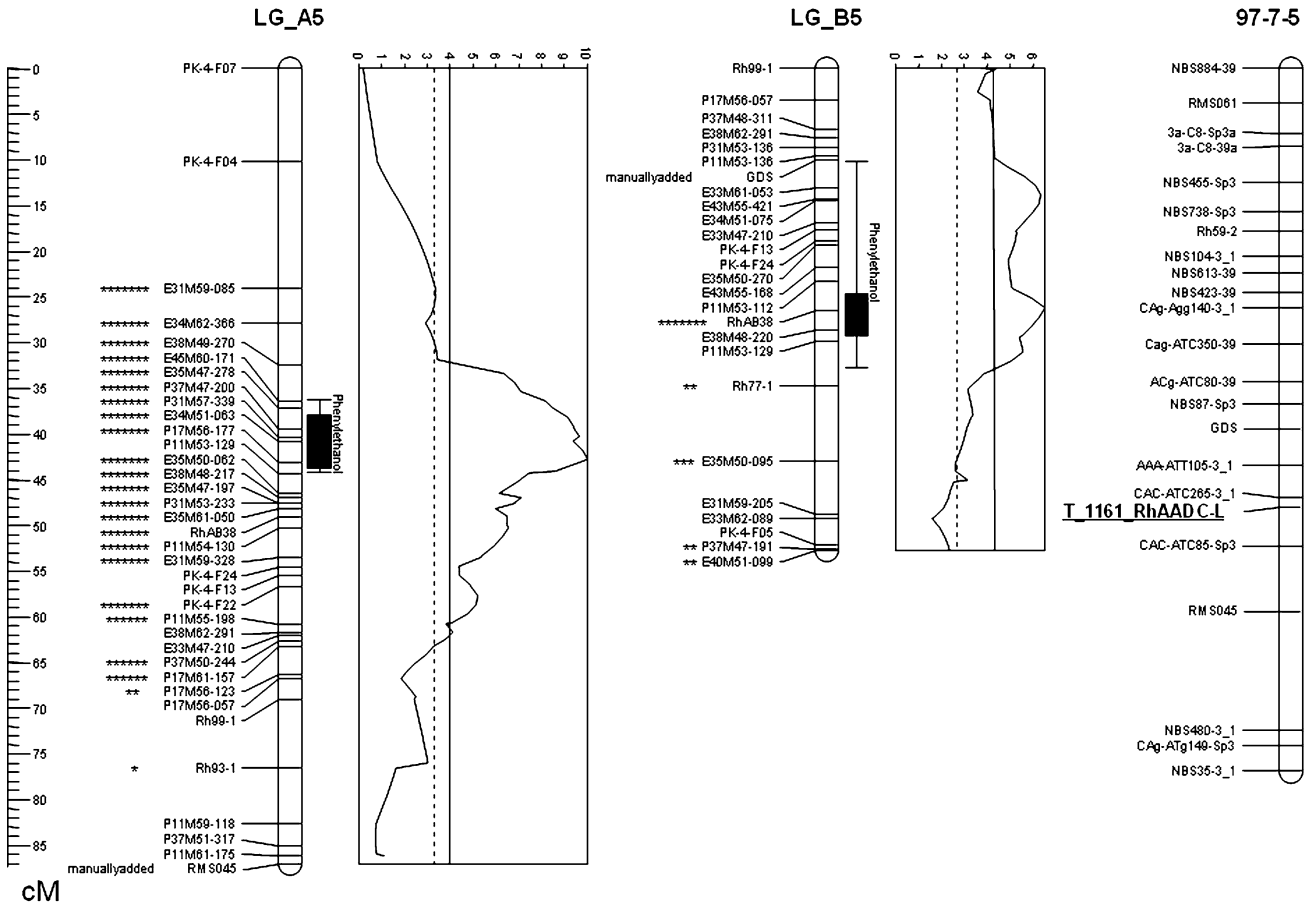

Fig. 3 QTLs for 2-phenyl ethanol on linkage groups *A5* and *B5* of population 94/1 and the homologous linkage group 5 from population 97/7. Markers connecting the linkage groups of the two populations are shown in *bold*. Marker T1161_RhAADC-like is indicated in *bold* and *underlined*. Loci that show significant *K* values in non-parametric QTL mapping are indicated with *asterisks*. *Graphs* show the corresponding LOD score curves from interval mapping. *Dashed lines* show chromosome-wide significance thresholds based on permutation tests, while *solid lines* show genome-wide thresholds. The 1- and 2-LOD confidence intervals are indicated with *filled bars* and *whiskers*. The highest LOD score for 2-phenyl ethanol (10.04) was found on LG A5

otherwise would have required time- and resource-consuming procedures (such as backcrosses) in several segregating populations. Furthermore, marker analyses immediately provide information on map positions for Mendelian factors. For geranyl acetate, we found an even more complex inheritance pattern with two unlinked dominant loci. In addition, the clear separation on our map of two loci encoding *RhAAT1* orthologues expands the *AAT* gene family in roses. The mapping results are supported by the observation of four distinct but closely related sequences of *AAT* PCR products from each of the diploid genotypes 93/1–117 and 93/1–119.

These results indicate that at least five loci are involved in the production of alcohol acetates (four for geranyl acetate and one for neryl acetate). Notably, the two paralogues of the *RhAAT1* gene do not cosegregate with any of the loci involved in alcohol acetate production that were characterised in this study. Apart from these two paralogues of the *RhAAT1* gene, the nature of the other genes is unknown. However, they are most likely either acetyltransferases or regulators of acetyltransferases, since no acetyltransferase cofactors are known to date (D'Auria 2006).

Geraniol

Within the QTL region for geraniol on LG1, several markers are also linked to the black spot resistance gene *Rdr1* in population 97/7, such as the SSR markers 155SSR and RMS015, which are linked to *Rdr1* at zero and three centimorgans, respectively (Biber et al. 2009). Since a BAC contig around *Rdr1* has already been sequenced (Terefe et al. unpublished data), several transcription factors have been identified. One example is a sequence displaying similarity to transcription factor B3 from *Medicago*. Several transcription factors (such as MyB)

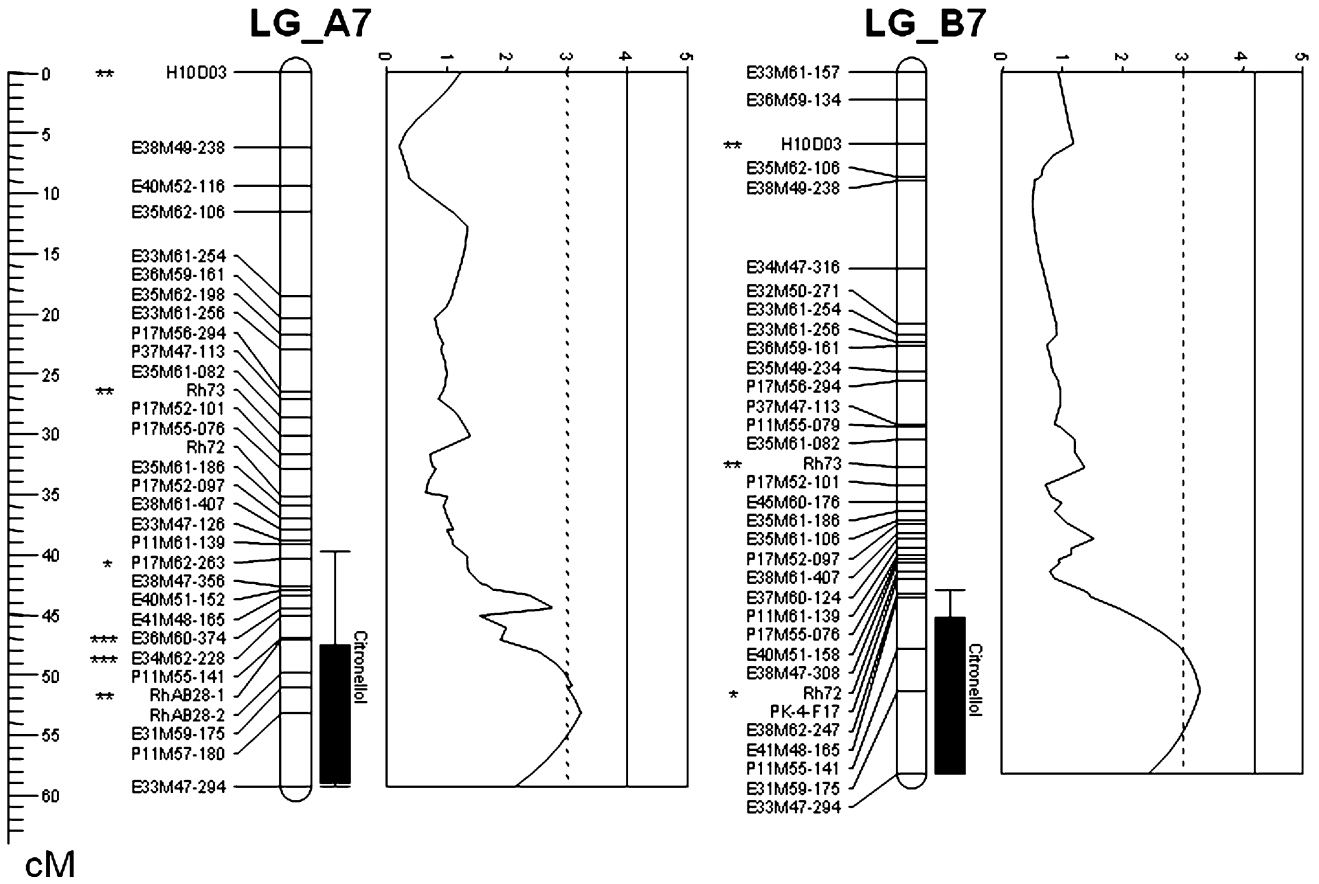

Fig. 4 QTLs for *β*-citronellol on linkage groups *A7* and *B7* of population 94/1. Loci that show significant *K* values in non-parametric QTL mapping are indicated with *asterisks*. *Graphs* show the corresponding LOD score curves from interval mapping. *Dashed lines* show chromosome-wide significance thresholds based on permutation tests, while *solid lines* show genome-wide thresholds. The 1- and 2-LOD confidence intervals are indicated with *filled bars* and *whiskers*

have already been shown to play key roles in phenylpropanoid production (van Schie et al. 2006; Ben Zvi et al. 2008). Regulation of terpenoid biosynthesis by transcription factors is likely.

β-Citronellol

One of the three QTLs for *β*-citronellol is located on LG7. However, this region shows only chromosome-wide significance, and only two AFLP markers within it show a significant influence on *β*-citronellol concentration. Therefore, stronger statistical support could be obtained by the analysis of additional progeny.

A second locus on LG1 comprises several markers that influence both geraniol and *β*-citronellol concentration. *β*-citronellol is synthesised by the reduction of geraniol or nerol (Banthorpe et al. 1972). A QTL influencing both metabolites may contain either a gene involved in the biosynthesis of a common precursor or a regulatory factor responsible for the biosynthesis of both monoterpenoids. Together with the third QTL, which is linked to the dominant allele of the double flower gene (Debener and Mattiesch 1999) on LG3, a total of 50% of the phenotypic variation can be explained.

2-Phenyl ethanol

Two QTLs, a highly significant locus on LG5 (LOD 10.0) and a second locus on LG2, influence the amount of 2-phenyl ethanol. A putative candidate gene for the QTL on LG5 is EST_T1161, which maps to the homologous region in population 97/7 between markers GDS and RMS045, indicated in Fig. 3. This EST shares significant similarity to an aromatic amino acid decarboxylase gene (*RhAADC*; Sakai et al. 2007) that catalyses one of the final steps in the biosynthesis of 2-phenyl ethanol. Polymorphism in this gene may be responsible for the observed variation. However, EST_T1161 can be linked only indirectly to the QTL on LG5, since mapping was only possible in population 97/7. Verification of its functional relationship to the QTL could be obtained by scent profiling of population 97/7 in future experiments. A marker (c_3299) which represents the sequence homologue of the enzyme which catalyses the last step in the biosynthesis of 2-phenyl

ethanol (PAR) could be developed also and placed on LG1. This locus shows no influence on the 2-phenyl ethanol content. The regulation of the 2-phenyl ethanol production therefore takes place on the RhAADC step.

The second QTL region, located on LG2, comprises paralogues of *RhAAT1* (*R. hybrida* alcohol acyltransferase 1; Guterman et al. 2002). Since RhAAT1 has been shown to acylate 2-phenyl ethanol in heterologous expression studies, one of the paralogues may be a candidate gene for the QTL due to the reduction of 2-phenyl ethanol through its acylation process. However, the concentrations of 2-phenyl ethyl acetate were too low to verify any correlation in the present study. This could be improved by increasing the sensitivity of the scent profiling through the analysis of concentrated scent extracts.

The six scent metabolites for which genetic loci were analysed in this study represent only a small fraction of the approximately 570 metabolites identified to date in various rose genotypes (Knudsen et al. 2006). This is due to the fact that our model population emits lower amounts of volatiles compared to heavily scented rose varieties such as 'Fragrance Cloud' (Guterman et al. 2002), as identified by previous headspace analyses (data not shown) and human perception. However, the substances analysed in our study are important components of the rose scent. Geraniol and 2-phenyl ethanol contribute the basic rose scent, while nerol and β-citronellol smell like fresh lemon and the acetates contribute fruity notes.

Our results will serve as a starting point for further analysis of the structure and regulation of the biochemical pathways for scent metabolites. This could include correlating expression and proteomic data with the segregation of scent metabolites in progeny in order to test hypotheses on the involvement of single genes or enzymes in the production of particular metabolites. It could also include analysis of the genomic structure of some of the structural genes such as *RhAAT1*. Our preliminary analyses have already shown that a second gene with high similarity to *RhAAT1* exists. Data on SSCP polymorphisms in amplicons from this gene family indicate the existence of additional copies, but this requires further confirmation (data not shown). Furthermore, genes at the additional loci for alcohol acetyltransferases mentioned above may be isolated either by candidate gene approaches or by positional cloning. A larger segregating population consisting of an extension of population 94/1 with about 1,000 individuals is currently being generated.

Because the concentrations of the analysed metabolites varied to some extent between samples of the same genotype, we tried to reduce the effect of the environment by strictly controlling it in a climate chamber. The application of our results to commercial rose breeding will therefore require confirmation of the QTLs under greenhouse or field conditions and in additional populations. We expect that variability in the number and locations of QTLs will be observed in different environments, similarly to the results obtained by Dunemann et al. (2009). Effects of the environment on the production of volatiles have been monitored previously (Mathieu et al. 2009).

A future application would be the selection of genotypes with enhanced concentrations of scent volatiles and with particular metabolite combinations based on tightly linked molecular markers. However, a frequent observation in cut rose cultivation is that the quantity of scent volatiles emitted is negatively correlated to the vase life of cut flowers. Before our results on the genetics and biochemistry of scent metabolites can be useful in commercial cut rose breeding, this putative correlation must be investigated in more detail in tetraploid varieties.

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