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Mapping of quantitative trait loci for floral scent compounds in cowpea (*Vigna unguiculata* L.)

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Abstract

Floral scent is a very important trait in plant evolution. Currently, little is known about the inheritance of floral scent in cowpea (*Vigna unguiculata* L.) or changes that might have occurred during its domestication. Therefore, we analysed scent volatiles and molecular markers in a population of 159 F_7 recombinant inbred lines derived from a cross of a domesticated blackeye cowpea cultivar, '524B' and a wild accession '219-01'. Using gas chromatography-mass spectrometry (GC–MS) 23 volatile compounds were identified that fall into five general functional categories. Twenty-two of the compounds displayed quantitative variation in the progeny, and a total of 63 QTLs influencing the amounts of these volatiles were mapped onto the cowpea genetic marker map. Although QTLs for volatile compounds putatively involved in cowpea flower scent were found on 9 of the 11 cowpea chromosomes, they were not evenly distributed with QTLs mainly clustered on LGs 1, LGs 2 and LG 4. 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.

Key words: cowpea — quantitative trait locus — recombinant inbred lines — scent

Cowpea (*Vigna unguiculata* (L.) Walp) is an important food legume and an integral part of traditional cropping systems in the semi-arid regions of the tropics. It is cultivated in Africa, Asia, the Middle East, southern Europe, southern United States and central and south America. Other benefits derived from cowpea especially by the rural poor include animal feed, cash and spillover benefits to their farmlands through *in situ* decay of root residues and ground cover from cowpea's spreading and low growth habit. The potential of cowpea is limited by numerous factors, but field and storage insect pests are the most severe constraints, at such a scale that a couple of insecticide sprays usually multiply the yield 10-fold (Pasquet and Baudoin 2001, Timko et al. 2007a). However, most African farmers do not have access to them. Conventional breeding has made some progress towards developing and deploying insect-resistant cultivars, but the gene pool of cowpea lacks adequate sources of resistance for certain insect pests, including pod borers, weevils, pod bugs and thrips. Therefore, molecular biology seems to be the only way to introduce novel insect-resistance traits that will help solve this otherwise intractable problem (Popelka et al. 2006, Higgins et al. 2007).

However, environmental biosafety issues, such as transgenes escape from a genetic modified (GM) crop variety to its non-GM crop counterparts or wild relatives, are strongly debated worldwide, especially when these transgenes can bring evolutionary selective advantages or disadvantages to crop varieties or wild populations (Ellstrand et al. 1999, Ellstrand 2001, 2003, Snow 2002). Several ways of overcoming the gene escape problem were considered, with varied potential success: use of male-sterile plants or plastid genetic engineering (Stewart and Prakash 1998), cleistogamy (Fargue et al. 2006, Yoshida et al. 2007) or gene mitigation (Al-Hamad et al. 2005). A way that has not been considered yet is the manipulation of pollinator behaviour. Plant volatiles, together with some other compounds, are determinants in insect–plant interactions (Dobson 1994). Floral scent is an important component of reproductive biology of many flowering plants, advertising the presence of rewards (nectar or pollen) to foraging pollinators (Heinrich and Raven 1972, Kevan and Baker 1983, Robacker et al. 1988). Bees seem to detect the nectar level of a flower by its aroma and do not visit empty flowers (Cartar 2004). Therefore, changing flower aroma could be a way to prevent pollen flow.

A first step is to identify quantitative trait locus (QTL) that controls floral scent. This will improve our genetic understanding of these traits, enable us to analyse the possible associations between the different traits, clarify the relationships of QTLs to candidate genes and finally provide the basis for marker-assisted selection of these traits. With this in mind, this study was initiated to identify the chemical compounds in cowpea floral scent, map the QTLs controlling the amount of these scent compounds and identify closely linked molecular markers in the cowpea genome regions. A genetic linkage map was constructed for the F_7 RILs that are obtained from a cross of the two parents, '524B' and '219-01', and has been used for QTL analyses of data based on gas chromatographic separated and mass spectral identified volatiles.

Materials and Methods

Characteristics of the mapping population: One-hundred and fifty nine (159) F_7 recombinant inbred lines (RILs) were developed by single-seed descent from a cross between '524B', a California black-eyed type (developed from a cross between cultivars 'CB5' and 'CB3'), and '219-01', a unique wild perennial cowpea from subsp. *Vigna*

unguiculata var. *spontanea* (Schweinf.) Pasquet (formerly subsp. *dekindtiana* sensu Verdc.) collected from coastal Kenya (4°16'S 39°15′ E, elevation 210 m, soil from Mariakani sandstone geological formation) (Andargie et al. 2011). '524B' contains multiple disease and pest resistances and very few scent compounds, whereas '219-01' produces a rich floral scent and has outcrossing flower morphology (Lush 1979).

Floral scent collection and analysis: All floral scent samples were collected from newly opened flowers on rooted plants of both the parents ('524B' and '219-01') and from all the 159 recombinant inbred lines. Three seeds of each recombinant line were sown in August 2006 in the screen house at the Muhaka field station of the International Center of Insect Physiology and Ecology (4°19′S 39°31′E, 32 km south-southwest of Mombasa, Kenya), and all surviving seedlings were transferred when having 4–5 leaves into the field. In the field, the plants were positioned 3 m apart in rows separated by 3 m. Plants were watered on days with no rain. Floral scent was collected from all plants that flowered during the second half of October and until end of December 2006. Flower opening of individual plants varied from 30 min before until approxmiately 30 min after sunrise [in October–November, sunrise is around 05:30 (GMT + 2 h) local time]. Before bee visitation started, flowers were bagged individually in 170 × 210 mm polyacetate roasting bags (heat sealed from Toppits, Melitta Scandinavia AB, Box 504, SE-26423 Klippan, Sweden). Teflon tubes (5 cm long, 0.3 cm inner diameter) filled with either 20 or 40 mg of Tenax Gr (Scantech; Lab AB, Box 238, 433 24 Partille, Sweden), mesh size 60–80, were inserted into the bag and connected via silicon tubing to either a battery-driven membrane pump or an inverted aquarium pump running on net power. The air flow through the adsorbent plugs was between 90 and 110 ml per minute regulated for each individual sampling plug with a valve. Blank samples of either environmental air or plant parts were collected in parallel with all samples. Prior to use adsorbent plugs were cleaned sequentially with 1 ml of each of methanol, acetone and hexane and dried with pressurized carbon-filtered air. Floral scent was collected for approximately 2 h (individual collections ranged from 100 to 140 min). To avoid temperature rise in the bags because of strong sun during the last hour of floral scent collection, plants were shadowed with umbrellas, which as well was used as rain protection.

On some individuals, scent samples were collected from more than one flower: either because the peduncle carried 2–3 flowers or because more than one sample was collected from one individual. This was corrected for in calculations of the total amount of scent produced per flower by calculating the average when two or more samples were collected from the same plant or by dividing the results with the number of flowers when more flowers were collected simultaneously. From the parental generation, flowers on 14 individually growing stems, vegetatively propagated from the paternal parent, and flowers on 43 individuals each originating from seed of the maternal line were sampled.

Individual samples were collected and wrapped in aluminium foil and placed in a freezer in double roasting bags. Samples were brought to Sweden where they were extracted immediately with 250 µl (20 mg plugs) or 400 µl (40 mg plugs) of either redistilled hexane or LiChrosolv hexane (98% purity, Merck). After extraction, 500 ng of methyl stearate was added as internal standard to all samples. Prior to analyses, samples were concentrated about 10-fold at room temperature.

The samples were analysed by coupled gas chromatography-mass spectrometry (GC–MS) on a HP 6890 connected to a HP 5973 mass selective detector (Agilent Technologies, Palo Alto, California, USA). A 30-m fused silica column with an inner diameter of 0.25 µm, coated with HP-Innowax (Agilent J&W GC columns, Santa Clara, California, US) at a film density of 0.25 µm as stationary phase was used. The injector temperature was 225°C, and the GC was programmed for 3 min at 40°C, increased by 8°C/min to 225°C, and then held steady for 5 min. Helium was used as carrier gas. Two microlitre of each sample was injected into the GC by an automated injector (Hp 7683 autosampler). Identifications were made by comparing obtained mass spectra and retention times with those of authentic reference samples.

Table 1: Floral volatiles ordered reflecting their biosynthetic pathways in F_7 recombinant inbred lines and in the parental lines 524B and 219-01 of cowpea

	Parents		RIL population	
Volatile compounds	524B (mean ± SD)	219-01 (mean ± SD)	(mean ± SD)	Range
Fatty acid derivatives				
3-octanone	0.000 ± 0.003	0.003 ± 0.005	0.002 ± 0.006	0.0–0.064
3-octanol	0.001 ± 0.002	0.001 ± 0.001	0.001 ± 0.001	0.0–0.007
1-octen-3-ol	0.030 ± 0.060	0.020 ± 0.050	0.025 ± 0.070	0.0–0.498
C6-C1 benzenoids				
Benzaldehyde	0.632 ± 0.087	0.027 ± 0.020	0.342 ± 0.660	0.0–5.690
Benzyl alcohol	0.001 ± 0.001	0.002 ± 0.001	0.009 ± 0.014	0.0–0.083
Methyl benzoate	0.008 ± 0.013	0.0002 ± 0.007	0.032 ± 0.080	0.0–0.724
Methyl salicylate	0.001 ± 0.002	0.0003 ± 0.001	0.0005 ± 0.001	0.0–0.067
C6-C2 benzenoids				
Acetophenone	0.0084 ± 0.030	0.702 ± 0.390	0.119 ± 0.190	0.0–1.9610
Methyl phenylacetate	0.000 ± 0.000	0.041 ± 0.030	0.006 ± 0.010	0.0–0.0039
1-phenylethanol	0.0012 ± 0.0012	0.046 ± 0.023	0.007 ± 0.007	0.0–0.0533
2-phenylethanol	0.003 ± 0.003	0.324 ± 0.179	0.059 ± 0.090	0.0–0.6919
Phenylacetonitrile	0.000 ± 0.000	0.002 ± 0.002	0.000 ± 0.0001	0.0–0.0014
Phenylacetaldehyde	0.0015 ± 0.002	0.041 ± 0.030	0.006 ± 0.010	0.0–0.0810
C6-C3 phenylpropanoids				
3-phenylpropanal	0.002 ± 0.004	0.000 ± 0.000	0.019 ± 0.037	0.0–0.2692
3-phenylpropanol	0.003 ± 0.006	0.001 ± 0.001	0.017 ± 0.038	0.0–0.3083
(Z)-cinnamic aldehyde	0.0672 ± 0.0372	0.000 ± 0.000	0.282 ± 0.497	0.0–2.8857
(E)-cinnamic aldehyde	0.1235 ± 0.1192	0.0023 ± 0.0061	1.019 ± 1.906	0.0–12.770
Methyl (Z)-cinnamate	0.0002 ± 0.0013	0.057 ± 0.072	0.035 ± 0.056	0.0–0.3193
Methyl (E)-cinnamate	0.007 ± 0.0048	0.260 ± 0.249	0.112 ± 0.182	0.0–1.1782
(E)-cinnamic alcohol	0.0001 ± 0.0001	0.000 ± 0.000	0.0002 ± 0.0006	0.0–0.0042
Nitrogen compounds				
Methyl anthranilate	0.001 ± 0.002	0.004 ± 0.005	0.031 ± 0.090	0.0–0.7620
2-Aminobenzaldehyde	0.0002 ± 0.001	0.004 ± 0.005	0.014 ± 0.060	0.0–0.7620
Indole	0.0001 ± 0.0002	0.009 ± 0.004	0.007 ± 0.013	0.0–0.1066

Quantifications: To produce calibration (dose–response) curves, a dilution series for each constituting compound was prepared in redistilled hexane ranging from 10^{-4} to 10^{-7} µg/ml. To each dilution, 500 ng of methyl stearate was added as an internal standard and the samples were analysed by GC–MS along with the floral scent samples. Ideally, a unique ion to quantify against should be selected for each compound. This was possible except for 1-octen-3-ol, where instead the largest ion (i.e. 57), was chosen and, therefore, the amount reported of this compound may be a slight overestimated because ion 57 often is present in small amounts in background contaminations. All calibration curves and later on quantification of floral scent samples were performed manually.

Graphs of the $\log_{10}$ (response factor (calibration ion/internal standard ion)) as a function of $\log_{10}$ (amount of calibration compound) were made for each reference compound. The equation for the best-fitted line and its intercept with the *y*-axis for each reference compound was used to quantify most compounds in the samples. All calibration curves showed a linear relationship with correlation coefficient (r^2) between 0.98 and 0.999. (*Z*)- and (*E*)-cinnamic aldehyde and methyl (*Z*)- and (*E*)-cinnamate were quantified using their respective (*E*)-isomer, only.

Linkage mapping and QTL analysis: A genetic linkage map consisting of 202 simple sequence repeat (SSR) markers and four polymorphic morphological trait markers (flower colour, pod colour, pod position and root architecture) was constructed using 159 F_7 RILs derived from a cross of '524B' and '219-01', as described by Andargie et al. (2011).

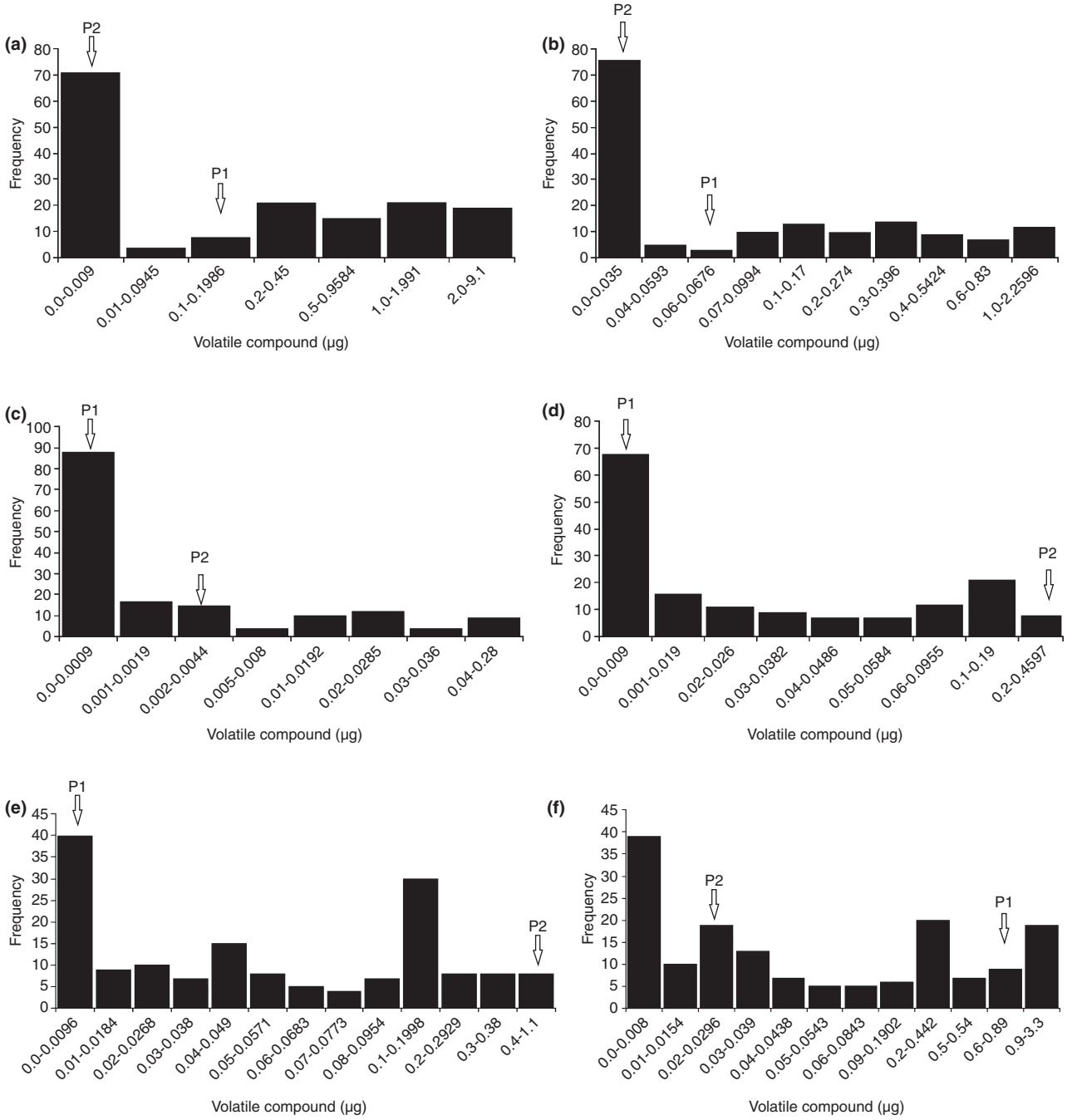

Fig. 1: Frequency distributions of key volatile compounds calculated as absolute amounts in micrograms produced per flower per hour in 159 F_7 RILs; (a) (E)-Cinnamic aldehyde, (b) (Z) - Cinnamic aldehyde, (c) 2-Aminobenzaldehyde, (d) 2-Phenylethanol, (e) Acetophenone, (f) Benzaldehyde, (g) Methyl anthranilate, (h) Methyl (E)-cinnamate and (i) Methyl benzoate. The mean concentration of volatiles in the two parental lines are indicated by the arrows (P1: '524B'; P2: '219-01')

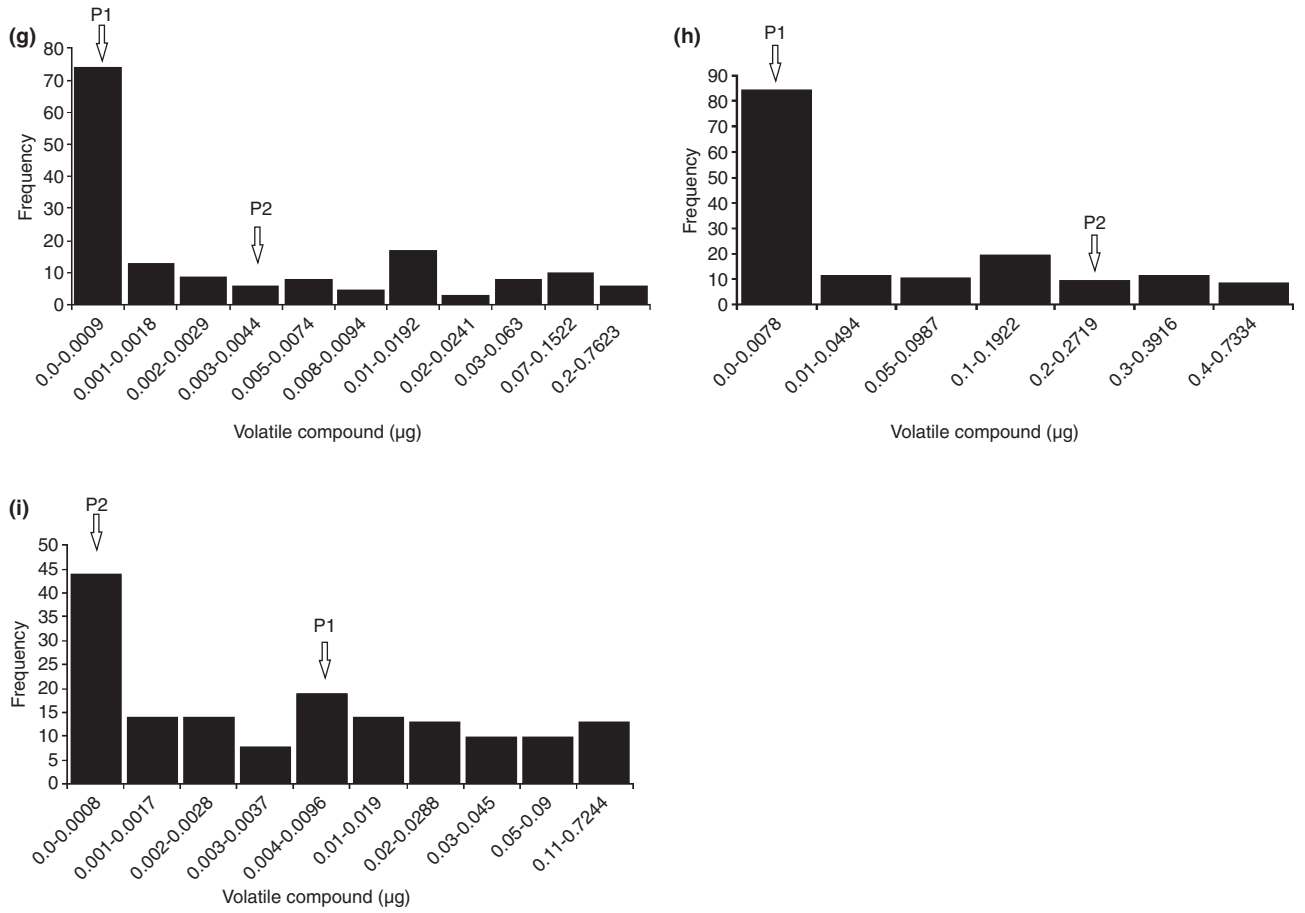

Fig. 1: (continued)

QTL detection was performed using QGene software (Nelson 2005). The LOD curves were created by scanning at 2-cM intervals, while a permutation test (1000 resamplings) was performed to determine the critical LOD score appropriate to empirically identify a putative QTL with a genome-wide error at a 0.05 confidence level (Churchill and Doerge 1994). QTL positions with highest LOD peaks were estimated by composite interval mapping (CIM). A LOD threshold of >2.5 was employed for declaring the significance of a putative QTL. A probability level of 0.001 was used as the threshold for the detection of a QTL.

Results

Identification and variability of scent compounds in cowpea

The floral scent of cowpea consists mainly of aromatic compounds and three fatty acid-derived compounds (Table 1) all of which have been reported in the floral scents of other plant species (Knudsen et al. 2006). In total, 23 compounds occurred consistently in varying quantities in one or both of the parental lines, '524B' and '219-01', and in most of the 159 F_7 RILs. The amounts of scent produced varied substantially between RILs and parental lines (Table 1). In addition, examples of frequency distributions of three esters (methyl (*E*)-cinnamate, methyl benzoate and methyl anthranilate), four aldehydes ((*E*)-cinnamic aldehyde, (*Z*)-cinnamic aldehyde, 2-aminobenzaldehyde and benzaldehyde), one alcohol (2-phenylethanol) and one ketone (acetophenone) are given in Fig. 1. The frequency distribution of the volatile compounds in the F_7 RILs was not normal, and in most cases, it was highly to moderately skewed towards the lower value (Fig. 1). All compounds exhibited a continuous variation in the progeny, which is typical for a polygenic inheritance and indicating that these traits are controlled by several QTLs. The phenotypic distributions of a few traits (e.g., the concentration of Acetophenone and Benzaldehyde) approached a bimodal frequency of distribution (Fig. 1). As described below, QTLs were estimated from these data.

QTL analysis of floral scent compounds

We recently reported the construction of a genetic linkage map consisting of 202 simple sequence repeat (SSR) markers and four polymorphic morphological trait markers using 159 F_7 RILs derived from a cross of '524B' and '219-01'. The map consists of 11 linkage groups (LG1–LG11) and spans a total genetic distance of 677 cM. Using composite interval mapping (CIM), 63 significant QTL were discovered that govern floral scent composition and these QTLs have been marked on the cowpea genetic map shown in Fig 2. The QTLs account for 22 of the 23 chemical compounds investigated in this population (Table 2), with only phenylacetaldehyde showing a lack of variability among parents and RILs. Generally, one QTL was identified for a specific volatile under high stringent conditions for significance. For most of the volatiles, more than one QTL was found, with 3-octanone having five QTLs, two of which occurred on the same linkage group (LG2), 3-octanol with five QTLs, two of which are on LG4, benzyl alcohol with eight QTLs, two of which are on LG4 and acetophenone, (E)-cinnamic aldehyde and methyl (Z)-cinnamate each with four QTLs (Table 2).

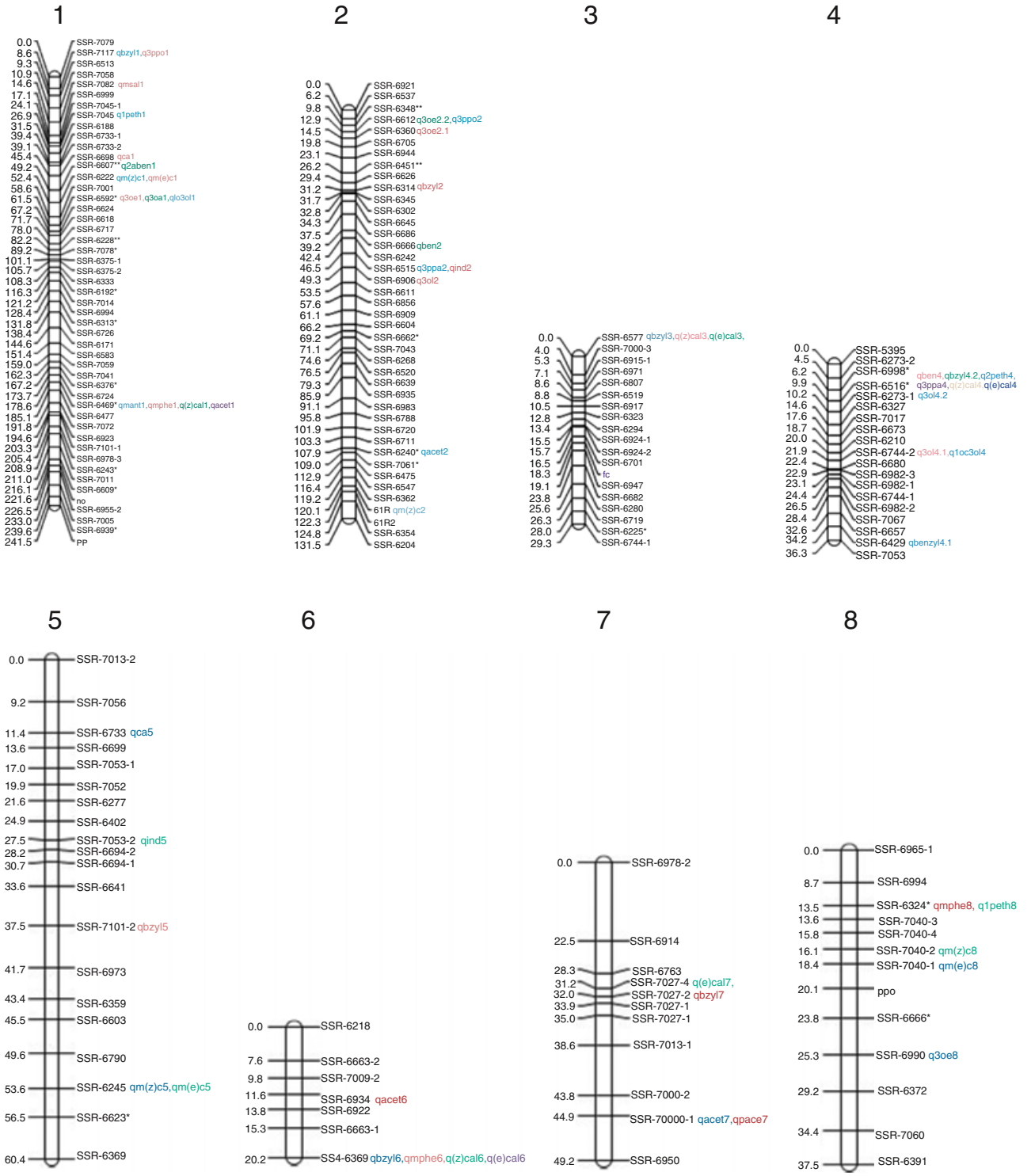

Fig. 2: Complete genetic linkage map of cowpea showing the location of QTL associated with floral scent. Detected QTLs are represented by the name of the chemical compound followed by numbers showing the respective LGs. The asterisks show the x2 levels of significance (* P = 0.05; ** P = 0.01)

Individual QTLs explained 5.6–6.7% of the phenotypic variation of 3-octanone, 6.0–12.9% of the phenotypic variation of 3-octanol, 5.6–12.6% of the phenotypic variation of benzyl alcohol, 5.9–9.4% of the phenotypic variation of acetophenone, 6.2–13.1% of the phenotypic variation of (E)-cinnamic aldehyde, 5.6–9.9% of the phenotypic variation of methyl (Z)-cinnamate (Table 2). All together, the QTLs detected for the above compounds in these regions of the chromosome explained 30.5%, 47.4%, 60.2%, 37.4%, 47.2% and 33.7%, respectively, of the variation. The amount of variation in all these traits explained by these regions is quite substantial in view of the quantitative nature of the traits.

Cumulatively, QTLs for volatile compounds involved in cowpea floral scent mapped to nine of the 11 cowpea linkage groups (Fig. 2). The largest number of QTLs mapped to LG1, with major QTLs associated with 15 of the 23 compounds

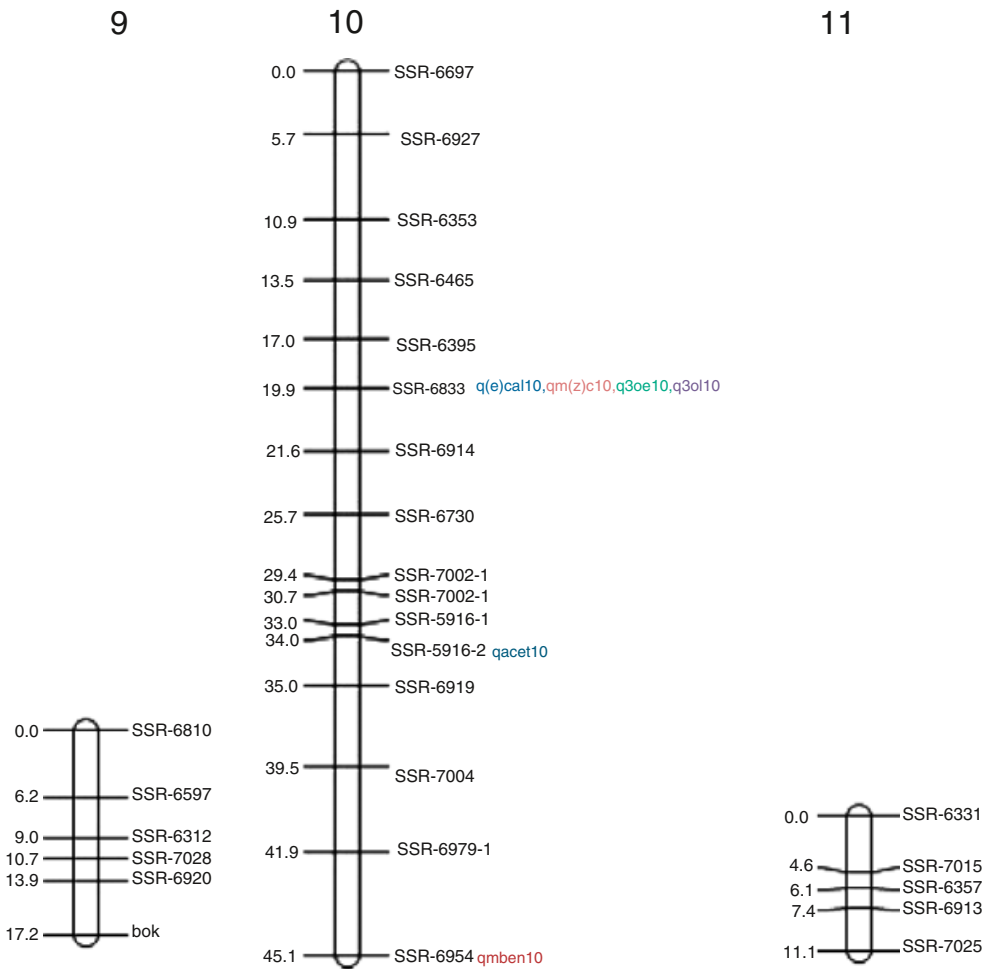

Fig. 2: (Continued)

included in the analysis, and this linkage group included representatives of all functional groups, that is, alcohols, esters, aldehydes and ketones found on cowpea floral scent. The LOD confidence interval showed that (a) the three fatty acid-derived compounds, (b) the two stereo-isomers of methyl cinnamate and 2-aminobenzaldehyde, (c) methyl phenylacetate, methyl anthranilate, acetophenone and (*Z*)-cinnamic aldehyde share a common part of this linkage group in their respective region (Fig. 2).

Discussion

Variability in floral scent composition in cowpea

Twenty-three volatile compounds were identified by gas chromatography-mass spectroscopy (GC-MS), and their quantitative variation in the parental lines '524B' and '219-01' as well as in the 159 F_7 recombinant inbred lines was determined under field conditions. The majority of the volatiles were aromatics including aldehydes, alcohols, esters, ketones, a nitrile as well as indole. In addition, three fatty acid-derived compounds: two alcohols and one ketone were identified and quantified.

Single floral scent compounds showed different frequency distributions in the parents as well as in the offspring. In addition, many of the frequency distributions of the compounds show ranges beyond the parent phenotypes indicating a polygenic nature of the traits. This is what to expect because the biosynthesis of the aromatic compounds found in cowpea most likely follow a common route until chorismic acid, where after the nitrogen containing compounds, except phenylacetonitrile, follow the tryptophan pathway and the remaining the phenylalanine pathway (Wakasa and Ishihara 2009). This transgressive segregation highlights hidden genetic variation in the parents that was masked by epistatic interactions of their genomes, but was freed from these restrictions in the RILs. Generally speaking, the occurrence of such transgressive segregants could be associated with the interaction of complimentary QTL alleles from two parents or overdominance of a gene. In addition, transgressive segregation occurred in both directions for the floral scents, suggesting that several genes may be involved in this trait in both parents. The diversity and plasticity of the final products derived from phenylpropanoids are catalysed by oxygenases, ligases, oxidoreductases and various transferases with differing substrate specificities (Boatright et al. 2004, Long et al. 2009, Vogt 2010). Although scent is a highly appreciated organoleptic attribute, little is known about the inheritance of scent production and the main compounds associated with it apart from the information available on the biochemistry of scent volatiles. A few studies have been carried out in tomatoes (Causse et al. 2001, Saliba-Colombani et al. 2001), strawberries (Carrasco et al. 2005, Olbricht et al. 2008), and the inheritance of Mendelian loci for terpenoid composition in *Mentha* sp. has been reported (Gershenzon et al. 2000) along with a study identifying QTLs responsible for terpene oil content in Eucalyptus (Shepherd et al. 1999). In the present study, we found that most of the cowpea scent compounds analysed showed

Table 2: Quantitative trait loci for scent/aroma traits identified by composite interval mapping (CIM) method

Trait	QTL name	Linkage group	Nearest marker	Position (cM)	Highest LOD score	1-LOD Interval	Phenotypic variation	Additive effect
3-octanone	q3oe1	1	SSR-6592	200.5	2.807	178.6–217.2	5.6	−0.01
	q3oe2.1	2	SSR-6360	64.5	2.984	53.9–79.8	6.7	0.002
	q3oe2.2	2	SSR-6612	53.9	2.865	36.8–64.5	5.8	−0.002
	q3oe8	8	SSR-6990	133.3	2.758	117.8–150.2	6.1	−0.004
	q3oe10	10	SSR-6838	91.9	2.933	69.0–108.6	6.3	0.003
3-octanol	q3ol1	1	SSR-6592	200.5	4.752	178.6–217.2	12.9	−0.001
	q3ol2	2	SSR-6906	287.3	2.755	270.5–301.5	6.0	−0.01
	q3ol4.1	4	SSR-6744-2	143.9	3.491	127.0–159.4	9.6	−0.01
	q3ol4.2	4	SSR-6273-1	67.2	4.125	51.9–84.6	11.3	0.0015
	q3ol10	10	SSR-6838	91.9	2.728	69.0–108.6	7.6	0.0013
1-octen-3-ol	q1o3ol1	1	SSR-6592	200.5	3.219	178.6–217.2	6.8	−0.04
	q1o3ol4	4	SSR-6744-2	143.9	3.141	127.0–159.4	7.4	−0.017
Benzaldehyde	qben2	2	SSR-6666	232.2	2.957	215.5–252.4	5.8	0.296
	qben4	4	SSR-6516	51.9	2.793	34.2–67.2	6.4	−0.163
Benzyl alcohol	qbzyl1	1	SSR-7117	18.6	2.763	0.0–22.3	6.9	−0.009
	qbzyl2	2	SSR-6314	150.2	2.848	134.4–164.7	6.8	−0.003
	qbzyl3	3	SSR-6577	0.0	2.715	0.0–18.0	6.2	−0.02
	qbzyl4.1	4	SSR-6429	248.2	2.986	232.6–252.3	8.3	−0.004
	qbzyl4.2	4	SSR-6516	51.9	4.657	34.2–67.2	12.6	−0.009
	qbzyl5	5	SSR-7101-2	188.5	2.798	171.6–208.7	7.8	0.005
	qbzyl6	6	SSR-6369	86.2	2.841	72.3–86.2	6.0	−0.016
	qbzyl7	7	SSR-7027-2	87.0	2.704	77.2–97.9	5.6	−0.003
Methyl benzoate	qmben10	10	SSR-6964	236.1	3.226	221.9–236.1	8.9	−0.158
Methyl salicylate	qmsal1	1	SSR-7082	51.6	2.771	34.9–67.1	6.9	−0.001
2-amino-benzaldehyde	q2aben1	1	SSR-6607	159.2	2.683	145.4–172.4	7.5	−0.01
Methyl anthranilate	qmant1	1	SSR-6469	555.6	2.667	537.7–575.1	6.1	−0.06
Acetophenone	qacet1	1	SSR-6469	555.6	2.945	537.7–575.1	9.4	−0.127
	qacet2	2	SSR-6240	535.9	2.925	520.9–536.0	8.1	−0.076
	qacet6	6	SSR-6934	50.6	2.755	33.8–60.8	7.7	−0.064
	qacet7	7	SSR-7000-1	153.9	2.848	143.8–162.7	6.3	0.072
	qacet10	10	SSR-6916-2	171.0	2.997	165.0–186.0	5.9	0.041
Methyl phenylacetate	qmphe1	1	SSR-6469	555.6	3.098	537.7–575.1	5.9	0.01
	qmphe6	6	SSR-6369	86.2	3.891	72.3–86.2	6.7	0.0164
	qmphe8	8	SSR-6324	36.6	2.903	18.7–49.6	5.6	0.01
2-phenylethanol	q2peth4	4	SSR-6516	51.9	2.828	34.2–67.2	5.7	−0.031
1-phenylethanol	q1peth1	1	SSR-7045-2	96.9	2.785	84.1–111.5	5.9	−0.004
	q1peth8	8	SSR-6324	36.6	2.936	18.7–49.6	6.5	−0.006
Phenylacetonitrile	qpace7	7	SSR-7000-1	153.9	2.745	143.8–162.7	7.1	−0.01
3-Phenylpropanal	q3ppa2	2	SSR-6515	270.5	2.896	252.4–287.3	7.0	−0.032
	q3ppa4	4	SSR-6516	51.9	3.318	34.2–67.2	9.2	−0.012
3-phenylpropanol	q3ppo1	1	SSR-7117	18.6	3.105	0.0–22.3	8.6	−0.022
	q3ppo2	2	SSR-6612	53.9	3.415	36.8–64.5	6.5	−0.018
(*Z*)-cinnamic aldehyde	q(z)cal1	1	SSR-6469	555.6	2.866	537–575.1	5.8	0.264
	q(z)cal3	3	SSR-6577	0.0	4.368	0.0–34.3	11.9	−0.839
	q(z)cal4	4	SSR-6516	51.9	4.105	34.2–67.2	11.2	−0.157
	q(z)cal6	6	SSR-6369	86.2	3.211	72.3–86.2	6.2	−0.296
(*E*)-Cinnamic aldehyde	q(e)cal3	3	SSR-6577	0.0	4.864	0.0–18.0	13.1	−2.966
	q(e)cal4	4	SSR-6516	51.9	3.968	34.2–67.2	10.9	−0.756
	q(e)cal6	6	SSR-6369	86.2	3.046	72.3–86.2	5.8	−1.128
	q(e)cal7	7	SSR-7027-4	77.2	3.287	61.3–87.0	7.9	−0.602
	q(e)cal10	10	SSR-6838	91.9	3.446	69.0–108.6	9.5	0.865
Cinnamic alcohol	qca1	1	SSR-6698	145.4	2.798	138.1–159.2	5.9	0.012
	qca5	5	SSR-6996	40.4	2.885	22.2–57.6	5.6	0.0014
Methyl (*Z*)-cinnamate	qm (z)c1	1	SSR-6222	172.4	2.942	159.2–178.6	6.8	0.065
	qm (z)c2	2	61R	598.1	2.844	585.2–600.3	5.7	−0.016
	qm (z)c5	5	SSR-6245	265.6	2.713	251.6–289.5	5.7	0.034
	qm (z)c8	8	SSR-7040-2	75.1	2.903	62.8–88.4	5.6	−0.019
	qm (z)c10	10	SSR-6838	91.9	3.587	69.0–108.6	9.9	0.041
Methyl (*E*)-cinnamate	qm (e)c1	1	SSR-6222	172.4	4.701	159.2–178.6	12.7	0.211
	qm (e)c5	5	SSR-6245	265.6	2.912	251.6–289.5	8.1	0.124
	qm (e)c8	8	SSR-7040-1	88.4	2.623	75.1–103.1	5.7	−0.11
Indole	qind2	2	SSR-6515	270.5	2.772	252.4–287.3	6.6	−0.011
	qind5	5	SSR-7053-2	142.5	2.907	124.9–150.2	5.9	0.003

a distribution in the F_7 recombinant inbred lines, which indicate that they are under genetic control.

The cohesion of the parental differences was markedly reduced in the RILs, suggesting that the genes, which determined the trait differences between the parents, underwent recombination after hybridization. However, significant associations were maintained in some of the floral scent traits, indicating that genes controlling the scent of the flowers were not randomly distributed on the chromosomes. QTLs for floral scent volatiles were clustered mainly on linkage groups 1, 2 and 4 suggesting the involvement of these regions in volatile metabolism. The regions characterized by the largest cluster of

QTLs were the upper portion of linkage groups 1, 2 and 4 (Fig. 2). Colocalizations of QTLs for the alcohols benzyl alcohol, 3-phenylpropanol, 1-phenylethanol, 3-octanol and 1-octen-3-ol, and the methyl esters, methyl salicylate, methyl (Z)- and methyl (E)-cinnamate at the upper portion of linkage group 1 could be due to biochemical relationships. For the alcohols, it most likely is a reductase that reduces the aldehyde to its corresponding alcohol, while the methyl esters are produced from their corresponding acids using methyl transferases. QTLs for benzyl alcohol, 2-phenylethanol, 3-phenylpropanol and 3-octanol and the two aldehyde QTLs on linkage group 4 may have a common last step physiological origin due to related reductases. Colocalization of QTLs for aroma volatiles derived from the same metabolic pathway was also found in tomato (on LG1-fatty acid metabolism derived two volatiles and on LG9 two phenolic compounds) (Saliba-Colombani et al. 2001). In another report by Tiemann et al. (2006), multiple QTL loci were also identified that affected sets of related volatiles. Clustering of QTLs can occur either due to the presence of a single locus with pleiotropic effects on several volatiles and/or as a result of tightly linked different loci or the physical linkage of multiple genes. Such loci may encode transcription factors that coordinately regulate genes, or they may encode enzymes that catalyse limiting steps in single pathways (Tiemann et al. 2006). Closely related methyl transferases or even a single methyl transferase may utilize a range of substrates although with different relative efficiency (Kapteyn et al. 2007, Tiemann et al. 2010). The absence of QTL for the volatile compound, phenyl acetaldehyde could be attributed to lack of variability for this trait in the parents.

Floral scent, which is considered as a very important trait for practical plant breeding, has received increasing attention in basic research due to the biosynthesis of its individual compounds as well as its ecological function (Dudareva et al. 2004, Pichersky and Dudareva 2007, Schwab et al. 2008). To the best of our knowledge, this is the first report on identification of QTLs for floral scent compounds in cowpea and for flowers in general. We were able to identify nine genomic regions that were found to be associated with 63 QTLs. Among the QTLs reported in this study, some may be useful for molecular breeding approaches. The potential for using molecular markers such as SSRs linked to QTLs in breeding programmes depends on the magnitude of the QTL effects. The percentage of the total phenotypic variance explained by the QTL was large in several cases and reached up to 60%, indicating genetic control by major as well as multiple minor effect genes. In addition, the robust QTL with high phenotypic variation can also be fine mapped for the identification of genes or it can be exploited in MAS programme. In general, identifying the linked molecular markers and knowing the genes that are responsible for controlling the levels of these different volatiles will be an important tool for blocking pollen flow from domesticated to wild cowpea, thus preventing the risk that insect-resistance transgene moves into wild cowpea gene pool and potentially turn wild cowpea plants into aggressive weeds.

Generally, although it needs a stringent effort for QTL validation, 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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