Generation of phenylpropanoid pathway-derived volatiles in transgenic plants: rose alcohol acetyltransferase produces phenylethyl acetate and benzyl acetate in petunia flowers

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Abstract

Esters are important contributors to the aroma of numerous flowers and fruits. Acetate esters such as geranyl acetate, phenylethyl acetate and benzyl acetate are generated as a result of the action of alcohol acetyltransferases (AATs). Numerous homologous AATs from various plants have been characterized using *in-vitro* assays. To study the function of rose alcohol acetyltransferase (RhAAT) *in planta*, we generated transgenic petunia plants expressing the rose gene under the control of a CaMV-35S promoter. Although the preferred substrate of RhAAT *in vitro* is geraniol, in transgenic petunia flowers, it used phenylethyl alcohol and benzyl alcohol to produce the corresponding acetate esters, not generated by control flowers. The level of benzyl alcohol emitted by the flowers of different transgenic lines was ca. three times higher than that of phenylethyl alcohol, which corresponded to the ratio between the respective products, i.e. ca. three times more benzyl acetate than phenylethyl acetate. Feeding of transgenic petunia tissues with geraniol or octanol led to the production of their respective acetates, suggesting the dependence of volatile production on substrate availability.

Introduction

Flower fragrance is a composite character that is determined by a complex mixture of volatile molecules. For many years, research into flower fragrance focused on its chemical elucidation (Knudsen et al., 1993), and as a result, hundreds of compounds were identified. Recently it has been shown that these compounds are biosynthesized through a relatively small number of often interconnected metabolic pathways (Dudareva et al., 2004).

Esters constitute a major class of floral volatiles, as well as contributing to the aromas of fruits (Croteau and Karp, 1991; Knudsen and Tollsten, 1993; Gang, 2005). Volatile acetate esters such as geranyl acetate, phenylethyl acetate and benzyl acetate are generated as a result of the action of alcohol acetyltransferases (AATs) belonging to a plant-specific AAT family designated BAHD (Dudareva et al., 1998; D'Auria et al., 2002). This family was named for the first four characterized enzymes: *Clarkia breweri* benzyl alcohol *O*-acetyltransferase (BEAT), *Gentiana triflora* anthocyanin

O-hydroxycinnamoyltransferase (AHCT), *Dianthus caryophyllus* anthranilate *N*-hydroxycinnamoyl/benzoyltransferase (HCBT), and *Catharanthus roseus* deacetylvindoline 4-*O*-acetyl-transferase (DAT) (St-Pierre and De Luca, 2000) and to date, includes several genes involved in scent and aroma formation (Gang, 2005). *In-vitro* characterization of these AAT homologues from various plant species revealed their broad substrate specificity towards alcohols and acyl donors (Dudareva *et al.*, 1998; Aharoni *et al.*, 2000; D'Auria *et al.*, 2002; Yahyaoui *et al.*, 2002; Shalit *et al.*, 2003; Beekwilder *et al.*, 2004; Boatright *et al.*, 2004). Beekwilder *et al.* (2004) generated transgenic petunia constitutively expressing the strawberry fruit *AAT* gene to analyze its function *in planta*. However, the transgenic flowers were not able to generate new esters or higher levels of native volatiles.

"Scent" enzymes have been identified by either first characterizing their enzymatic activity *in vitro* with substrates predicted to be the precursors of known products in the tissue from which they are derived (for review, see Dudareva *et al.*, 2004), or, as is more commonly practiced today, identifying homologous sequences in genomic or EST databases, expressing a protein in a heterologous system, and then biochemically testing the enzymatic activity with a variety of substrates (Aharoni *et al.*, 2000; Lange *et al.*, 2000; Gang *et al.*, 2001; Guterman *et al.*, 2002; Chen *et al.*, 2003; Dudareva *et al.*, 2003; Fridman and Pichersky, 2005). In both cases, the biochemical characterization of enzymes has shown that they are often capable of reacting with several substrates. Thus, the assignment of the "correct" *in-vivo* substrate (or substrates) depends on the kinetic parameters of the enzyme as well as on the information about the *in-vivo* product(s), and is generally tentative in the absence of additional lines of evidence derived from the analysis of mutants (Fridman and Pichersky, 2005; Tholl *et al.*, 2005). It is becoming clear that the substrate that is preferred *in vitro* is not necessarily the one reacted on *in vivo* (Negre *et al.*, 2003; Beekwilder *et al.*, 2004; Underwood *et al.*, 2005).

We recently isolated a rose alcohol acetyltransferase (*RhAAT*) responsible for the formation of geranyl acetate from geraniol and acetyl-CoA in rose flowers (Shalit *et al.*, 2003). Biochemical characterization of the RhAAT enzyme *in vitro* revealed that in addition to geraniol, it exhibits activity towards a large number of alcohols, including the aromatic alcohols 2-phenylethyl alcohol and benzyl alcohol, which have been reported in petal tissue of petunia flower (Verdonk *et al.*, 2003; Boatright *et al.*, 2004). Here, we report the successful metabolic engineering of the benzenoid/phenylpropanoid pathway in *Petunia hybrida* cv. Mitchell by introducing *RhAAT* under a CaMV-35S promoter. Transgenic petunia flowers expressing *RhAAT* emitted a higher level of benzyl acetate and phenylethyl acetate, indicating that the *in planta* availability of substrates and biochemical properties of the enzyme determine the spectrum of emitted volatiles.

Materials and methods

Plant material

Petunia W115 (cv. Mitchell) was used for the generation of transgenic plants. All plants were grown under controlled temperature (27 °C/20 °C day/night) and natural photoperiod in the greenhouse.

RNA gel-blot analysis

Total RNA was extracted according to van Tunen *et al.* (1988). Subsequently, 5–10 μg of total RNA were fractionated in a 1% agarose gel containing formaldehyde and blotted onto Hybond N+ membranes (Amersham-Pharmacia Biotech, Buckinghamshire, UK). The blots were hybridized in 0.263 M Na_2HPO_4, 7% (w/v) SDS, 1 mM EDTA and 1% (w/v) BSA at 60 °C with ^{32}P-labeled cDNA probes (Rediprime; Amersham-Pharmacia Biotech). The membranes were washed twice in $2 \times$ SSC and 0.1% SDS at 60 °C for 20 min each and exposed to X-ray film (Fuji, Tokyo, Japan) with two intensifying screens at –70 °C.

DNA isolation and PCR analysis of transgenic plants

Total DNA from petunia leaves was isolated according to Izhaki *et al.* (2001). PCR amplification was performed using kanamycin primers

(Fw 5′-GAGGCTATTCGGCTATGACT-3′, Rev 5′-AATCTCGTGATGGCAGGTTG-3′) or 35S forward and *RhAAT* reverse primers (5′-TCTTTA CCCTTCAAAATCTCCAA-3′). The expected products of the PCRs were 0.8 and 0.5 kb, respectively.

Construction of a chimeric gene and plant transformation

The pBK-CMV phagemid carrying *RhAAT* (GenBank accession no. AY850287) was used to amplify the gene's coding region (1374 bp). The amplified fragment was inserted into a pCd shuttle vector between the CaMV-35S promoter and OCS terminator and then the entire construct was inserted into binary vector pCGN1559 (Zuker *et al.*, 2002). The construct was transferred via *Agrobacterium tumefaciens* to petunia variety W115 (cv. Mitchell) using the standard leaf-disk transformation method (Horsch *et al.*, 1985). Transformants were regenerated as described by van Tunen *et al.* (1989). To confirm stable transformation, kanamycin-resistant T_0 plants were analyzed by PCR.

Collection of headspace volatiles and GC–MS analysis

The headspace volatiles of detached petunia flowers or leaves were trapped, eluted and concentrated as previously described (Lavid *et al.*, 2002). The volatile compounds collected from the headspace were analyzed by GC–MS composed of a Pal autosampler (CTC Analytic, Zwingen, Switzerland), a TRACE GC 2000 equipped with an Rtx-5SIL MS (Restek, Waldbronn, Germany; i.d. 0.25 μm, 30 m×0.25 mm) fused-silica capillary column and a TRACE DSQ quadrupole mass spectrometer (ThermoFinnigan, Hemel, UK). Helium was used as the carrier gas at a flow rate of 0.9 ml min^{-1}. The injection temperature was set at 250 °C (splitless mode), the interface at 280 °C, and the ion source adjusted to 200 °C. The analysis was performed under the following temperature program: 5 min of isothermal heating at 50 °C, followed by a 5 °C min^{-1} oven temperature ramp to 260 °C, and finally increasing from 250 to 280 °C at 10 °C min^{-1}. The detector temperature was 280 °C. The system was equilibrated for 1 min at 70 °C before injection of the next sample. Mass spectra were recorded at 2 scan s^{-1} with a scanning range of 40–450 mass-to-charge ratio, and an electron energy of 70 eV. Compounds were tentatively identified ($>95\%$ match) based on the NIST/EPA/NIH Mass Spectral Library (Data Version: NIST 05, Software Version 2.0d) using the XCALIBUR v1.3 program (ThermoFinnigan) library. Further identification of major compounds was based on a comparison of mass spectra and retention times with those of authentic compounds analyzed under similar conditions. For quantitative analyses, 10 μg ml^{-1} ethyl myristate was used as an internal standard (Lavid *et al.*, 2002; Shalit *et al.*, 2003).

Feeding experiments

Detached petunia stem sections with three to four leaves and flowers were placed in 20 ml of feeding solution for 16 h at 25 °C. After incubation, flowers or leaves were detached from the stems and used for volatile collection. The feeding solutions contained either 100 ppm (0.56 mM) geraniol (Sigma-Aldrich, St. Louis, MO) or 100 ppm (0.63 mM) 1-octanol (Sigma-Aldrich).

Partial purification of RhAAT recombinant enzyme and determination of kinetic properties

Recombinant *E. coli* BL21 (DE3) bacteria were obtained and grown as described by Shalit *et al.* (2003). After centrifugation, cells were resuspended in buffer A [3:1 (v/w) buffer:cells] containing 50 mM Bis–Tris–HCl pH 6.9, 10% glycerol and 10 mM β-mercaptoethanol. Resuspended cells were broken by French press (three passes at 1200 psi), and the cell debris was removed by centrifugation (30 min at 24,000×*g*). The supernatant was incubated with 1% (w/v) protamine sulfate for 30 min at 4 °C. After centrifugation (30 min at 24,000×*g*), the supernatant was dialyzed against 2 l of buffer A overnight at 4 °C. RhAAT protein was purified using a weak anion-exchange DEAE-cellulose column followed by Mono-Q anion-exchange and size-exclusion chromatographies.

RhAAT protein was loaded onto a DEAE-cellulose column (10 ml of DE53, Whatman, NJ) preequilibrated with buffer A at a flow rate of about 1 ml min^{-1}. After washing off unabsorbed material from the column with 60 ml of buffer A,

RhAAT was eluted with a linear gradient (60 ml) from 0 to 400 mM KCl in buffer A. Fractions (3 ml) were collected and assayed for AAT activity with geraniol and acetyl-CoA as substrates as described previously (Shalit et al., 2003). Fractions with the highest AAT activity in the 260–310 mM KCl range were pooled (total of 9 ml), dialyzed against buffer A, and subjected to ion-exchange chromatography on a Mono-Q column (gel bed volume 1 ml) (Amersham Biosciences, Piscataway, NJ) using the FPLC system. RhAAT protein was eluted from the column at 360–400 mM KCl using a 10 ml linear (0–400 mM) gradient of KCl in buffer A at flow rate of 0.5 ml min^{-1}. Fractions with the highest AAT activity (total of 2 ml) were pooled and subjected to size-exclusion chromatography (Superdex 200, 16 mm×60 cm). Fractions of 2 ml were collected at a flow rate of 1 ml min^{-1} and analyzed for AAT activity. Fractions exhibiting the highest activity were examined by SDS-PAGE followed by Coomassie Brilliant Blue staining of the gel.

For kinetics analysis, an appropriate enzyme concentration was chosen so that the reaction velocity was proportional to the enzyme concentration and was linear with respect to incubation time for at least 30 min. Protein concentrations were determined by the Bradford method (Bradford, 1976) using the Bio-Rad protein reagent and bovine serum albumin as a standard. Enzyme assays were performed as described previously (Shalit et al., 2003). Apparent K_m values for one substrate (geraniol, benzyl alcohol, phenyl ethanol or acetyl-CoA) were determined at saturating concentrations of the co-substrate. Kinetic data were evaluated using Lineweaver-Burk plots.

Results

Emission of volatiles by transgenic petunia flowers expressing the RhAAT gene

After initial selection of transgenic petunia carrying the CaMV-35S promoter-RhAAT construct on kanamycin and screening the plants by PCR for the presence of the foreign DNA (data not shown), the expression of RhAAT in flowers of independent transformants (Figure 1) was examined by RNA gel-blot analysis. Transgenic plants expressed RhAAT at different levels, while no signal was
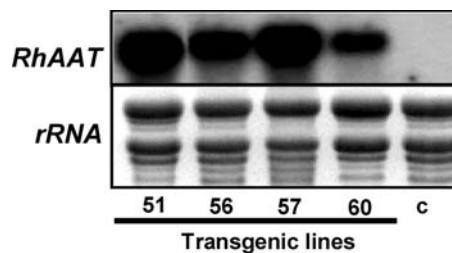

Figure 1. Molecular analyses of transgenic petunia plants expressing RhAAT. Expression of RhAAT driven by a CaMV-35S promoter in flowers of independent transgenic lines (51, 56, 57, 60) and control (C) flowers was analyzed by RNA blot. Ethidium–bromide staining of rRNA is shown.

detected in control, non-transgenic plants. The transgenic plants did not appear to be different from control plants with respect to overall morphology, time to flowering, growth rate, or flower architecture, development, or pigmentation.

Headspace analysis of non-transgenic petunia flowers showed the emission of benzaldehyde, benzyl alcohol and 2-phenylethyl alcohol, as well as of the esters benzyl acetate and methyl benzoate, similar to the previously published volatile profile of petunia flowers (Verdonk et al., 2003). Initial headspace analyses of flowers from four independent transgenic petunia lines revealed that they emit much higher levels of benzyl acetate and phenylethyl acetate, as compared to control flowers (Figure 2). Quantitative headspace analyses confirmed that all of these lines emit 5 (in line T51) to 10 (in line T60) times higher levels of these two acetate esters compared to control flowers (Figure 3). The level of emitted phenylethyl alcohol, the alcohol precursor of phenylethyl acetate, was elevated in transgenic plants (Figure 3B), whereas the level of emitted benzyl alcohol, the alcohol precursor of benzyl acetate, was lower (line T51) or not significantly different (other lines) from that measured in control, non-transgenic plants (Figure 3A).

Volatile production by RhAAT-transgenic petunia flowers and leaves fed with precursors

Kinetics parameters of recombinant RhAAT in vitro with geraniol, phenylethyl and benzyl alcohols, and acetyl-CoA were determined (Table 1) since in the previous study, K_m values for phenylethyl alcohol and benzyl alcohol were not obtained (Shalit et al., 2003). RhAAT K_m values for benzyl alcohol and phenyl ethanol using
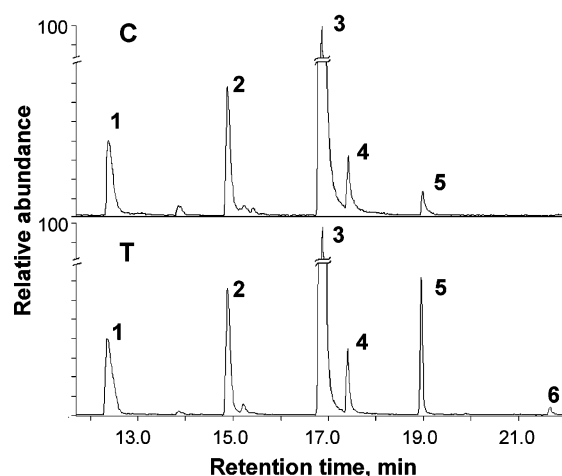
Figure 2. GC–MS headspace analysis of volatile compounds, emitted from detached flowers of *RhAAT*-transgenic (T, line 56) and control, non-transgenic (C) petunia plants. A representative GC chromatogram is presented. 1 – benzaldehyde, 2 – benzyl alcohol, 3 – methyl benzoate, 4 – phenylethyl alcohol, 5 – benzyl acetate, 6 – phenylethyl acetate. *In-vivo* production of novel acetates was assayed in detached flowers for 24 h.

acetyl-CoA as an acyl donor were 5.5 and 12 times higher (15.4 and 34.2 mM), respectively, than with geraniol. The apparent catalytic efficiency of the recombinant RhAAT, *in vitro*, for alcohol substrates with acetyl-CoA was 155 s^{-1} M^{-1} for geraniol, 21 s^{-1} M^{-1} for phenyl ethanol and 8 s^{-1} M^{-1} for benzyl alcohol (Table 1), indicating that, *in vitro*, geraniol is the preferred substrate. The K_m obtained for acetyl-CoA was comparable for the three different alcohols (47.38–134.33 μM); however, catalytic efficiency was higher when geraniol was used as the alcohol substrate (Table 1).

To test whether RhAAT in transgenic plants could use geraniol, the preferred substrate in the *in vitro* assay, we analyzed the headspace of

detached flowers following feeding with geraniol. Flowers with ca. 10 cm stems were incubated in a 10 mM solution of geraniol for 2 h at 22 °C. Flowers were then detached from the stems and headspace analyses of the emitted volatiles were performed. Transgenic petunia flowers, fed with geraniol, produced and emitted geranyl acetate. The flowers of control petunia plants did not generate this acetate ester following feeding with geraniol (Figure 4B). The production of geranylacetate was also observed in detached leaves of transgenic but not control petunias fed with geraniol (Figure 4A).

We also tested the ability of recombinant RhAAT to acetylate 1-octanol, an additional

Table 1. Kinetic parameters of RhAAT protein.

	K_m (μM)	V_{max} (pkat/mg)	K_{cat}^e (s^{-1})	K_{cat}/K_m^f (s^{-1} M^{-1})
Acetyl-CoA[a]	47.38 $\pm$ 7.22	5475 $\pm$ 327	0.28 $\pm$ 0.02	6148 $\pm$ 523
Acetyl-CoA[b]	86.38 $\pm$ 7.85	2473 $\pm$ 77	0.128 $\pm$ 0.004	1502 $\pm$ 99
Acetyl-CoA[c]	134.33 $\pm$ 10.46	10414 $\pm$ 314	0.54 $\pm$ 0.02	4051 $\pm$ 225
Geraniol[d]	2783 $\pm$ 460	8222 $\pm$ 1039	0.43 $\pm$ 0.05	155 $\pm$ 6
Benzyl alcohol[d]	15403 $\pm$ 774	2294 $\pm$ 76	0.119 $\pm$ 0.004	8 $\pm$ 0.2
Phenyl ethanol[d]	34213 $\pm$ 3714	13988 $\pm$ 1171	0.72 $\pm$ 0.06	21 $\pm$ 0.5

[a] With 5 mM geraniol.
[b] With 50 mM benzyl alcohol.
[c] With 40 mM phenyl ethanol.
[d] Alcohols were assayed using saturating concentrations of acetyl-CoA (180 μM for geraniol, 480 μM for benzyl alcohol and phenyl ethanol).
[e] K_{cat}, turnover number.
[f] K_{cat}/K_m, catalytic efficiency.
Results are an average of three independent experiments $\pm$ a 95% confidence interval of the mean.
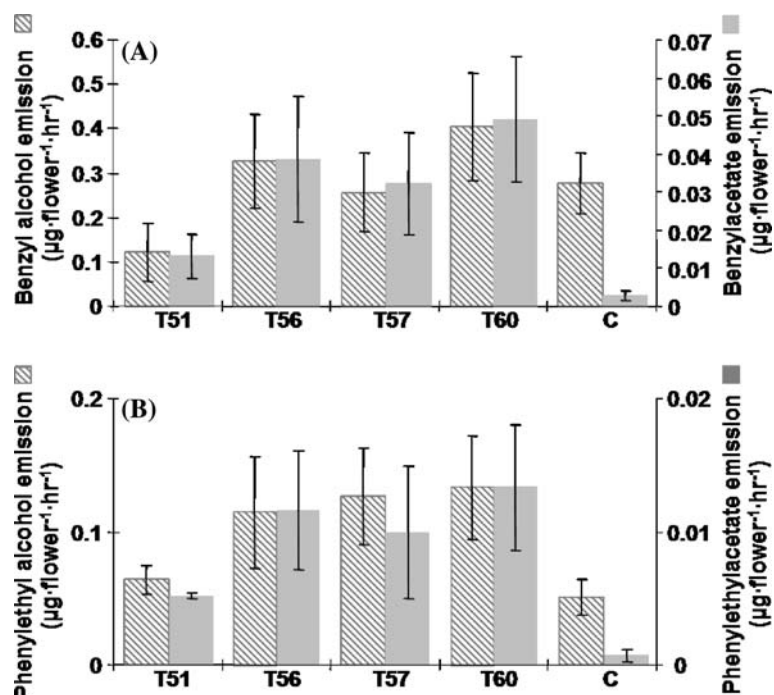
Figure 3. Emission of benzyl alcohol and benzyl acetate (A) and phenylethanol and phenylethyl acetate (B) from *RhAAT*-transgenic petunia flowers. Quantitative headspace GC–MS analyses of benzyl acetate and phenylethyl acetate in transgenic (T) petunia (independent lines 51, 56, 57, 60) and control (C) flowers were performed using ethyl myristate as the standard. Flowers one day after anthesis were used and means of five replicates $\pm$ SE are presented.

potential substrate (Shalit *et al.*, 2003). Similar to geraniol, following feeding, octanol was used as a substrate *in planta* by transgenic petunia flowers to produce octylacetate, but control flowers did not emit any octylacetate (Figure 5B). The production of octylacetate was also observed in detached leaves of transgenic but not control petunias fed with octanol (Figure 5A).

Discussion

Acetate esters represent one of the major groups of volatiles determining the scent of flowers and aroma of fruits (Knudsen and Tollsten, 1993). These volatile esters are synthesized from alcohols, generated via various metabolic pathways, by AATs belonging to a unique plant protein family of BAHD acyltransferases (Dudareva *et al.*, 1998; D'Auria *et al.*, 2002). Numerous genes encoding BAHD AATs have been isolated and biochemically characterized using *in-vitro* systems (Dudareva *et al.*, 1998; Aharoni *et al.*, 2000; D'Auria *et al.*, 2002; Yahyaoui *et al.*, 2002; Shalit *et al.*,

2003; Beekwilder *et al.*, 2004; Boatright *et al.*, 2004). It has been shown that each of these enzymes can convert various alcohols, and typically the prediction of their *in-vivo* activity is based on the highest substrate affinity *in vitro*. For example, the rose RhAAT was shown to be able to use geraniol, citronellol, nerol, octanol, hexanol, phenylethyl alcohol and benzyl alcohol *in vitro* (Shalit *et al.*, 2003). As the enzyme showed the highest preference to geraniol and citronellol, and rose flowers emit geranyl acetate, the protein was designated geraniol/citronellol acetyltransferase. Similarly, an enzyme found in the benzyl-acetate-emitting *Clarkia breweri* flowers that exhibited the highest activity with benzyl alcohol was designated benzyl alcohol acetyltransferase (BEAT), although it could also acetylate cinnamyl alcohol, 2-naphthalene ethanol and phenyl ethanol, albeit at lower rates (Dudareva *et al.*, 1998).

To evaluate the function of AATs *in planta*, Beekwilder *et al.* (2004) recently generated transgenic *P. hybrida* cv. Mitchell expressing strawberry SAAT, which exhibited its highest activity *in vitro* with geraniol among the substrates tested, but
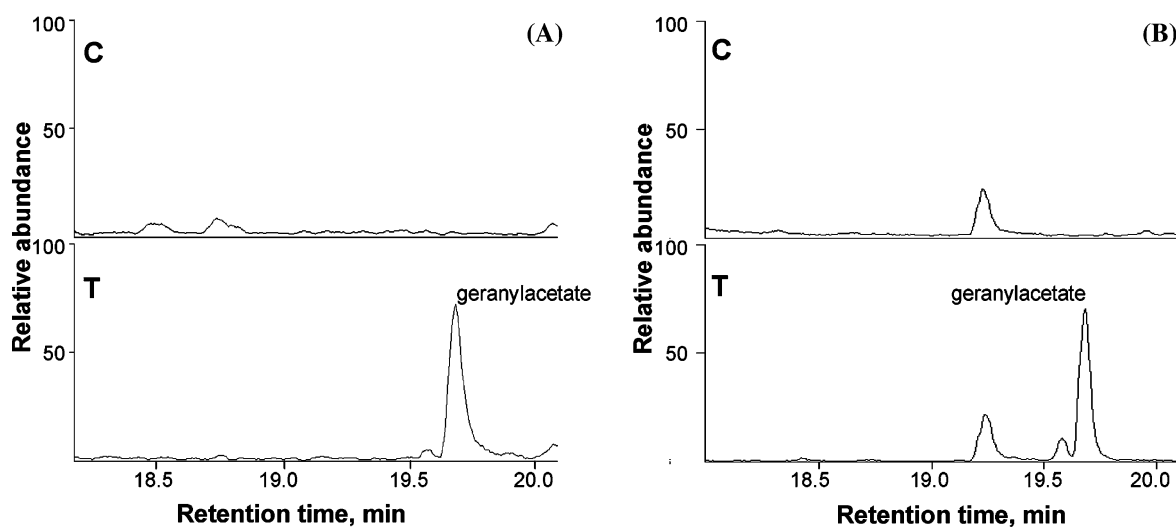
Figure 4. Production of geranyl acetate by *RhAAT*-transgenic petunia following feeding with geraniol. GC–MS headspace analysis of volatile compounds emitted from leaves (A) and flowers (B) of *RhAAT*-transgenic (T, line 56) and control, non-transgenic (C) petunia plants. Stems with leaves and flowers were placed in feeding solution containing geraniol for 16 h at 25 °C. After incubation, flowers or leaves were detached from the stems and used for collecting volatiles. The peak at 19.2 min represents eugenol.

whose substrate in the strawberry fruit was not known. Petunia flowers do not synthesize geraniol but produce 2-phenylethyl alcohol and benzyl alcohol, which are very poor substrates for SAAT *in vitro* (7% and 3% of SAAT activity with geraniol, respectively) (Beekwilder *et al.*, 2004). These transgenic petunia flowers were not able to generate any new esters. In contrast, the transgenic

P. hybrida cv. Mitchell, expressing the rose RhAAT generated in this study, emitted much higher levels of benzyl acetate as well as phenylethyl acetate, demonstrating the possibility of direct metabolic engineering of phenylpropanoid pathway-derived volatiles.

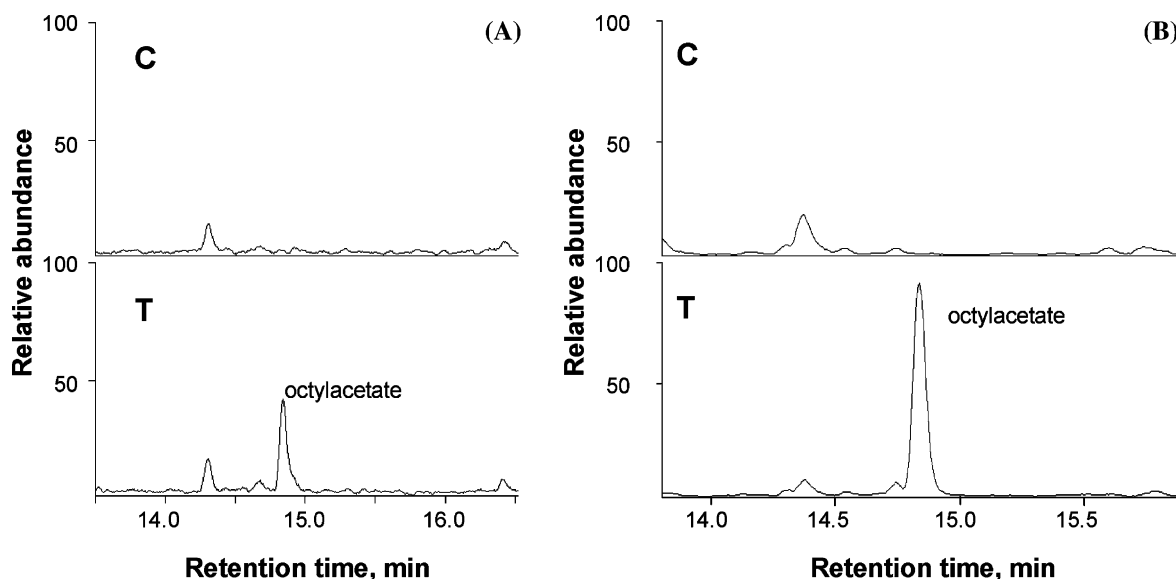RhAAT is the closest homologue of SAAT in the database and the substrate specificity of these

Figure 5. Production of octylacetate by *RhAAT*-transgenic petunia following feeding with octanol. GC–MS headspace analysis of volatile compounds emitted from leaves (A) and flowers (B) of *RhAAT*-transgenic (T, line 56) and control, non-transgenic (C) petunia plants. Stems with leaves and flowers were placed in feeding solution containing octanol for 16 h at 25 °C. After incubation, flowers or leaves were detached from the stems and used for collecting volatiles. The peak at 14.4 min could not be identified.

two enzymes is also very similar *in vitro*. Furthermore, petunia with the same genetic background was used for the activity analyses of both rose and strawberry AATs *in planta*. The differences obtained *in planta* may be due to protein-sequence divergence, which may affect the *in planta* enzyme affinity and catalytic efficiency toward the substrates, i.e. phenylethyl alcohol and benzyl alcohol, despite the similar enzyme–substrate preferences *in vitro*. Within the flower tissue, these enzymes have to compete with the native benzoyl-CoA:benzyl alcohol/phenylethanol benzoyltransferase (BPBT) (Boatright *et al.*, 2004) for alcohol substrates. Moreover, BPBT can also potentially use acetyl-CoA. Thus, the observed levels of volatile-ester emission reflect the relative protein levels of introduced RhAAT and the endogenous BPBT, their maximal velocities, and substrate affinities for alcohol substrates and acyl donors.

Surprisingly, three out of the four transgenic petunia lines examined emitted higher levels of phenylethanol (Figure 3B), while the level of benzyl alcohol was lower (one transgenic line) or relatively unaffected (Figure 3A). Biosynthesis of these alcohols follows two different routes. The first step in the synthesis of benzyl alcohol is the conversion of L-phenylalanine to *trans*-cinnamic acid by phenylalanine ammonia lyase (PAL), whereas this step is not required for the formation of phenylethanol (Boatright *et al.*, 2004). The reason for the higher levels of phenylethanol in transgenic lines is currently unknown. However, these results reflect the complexity of the mechanisms involved in the regulation of biochemical pathways leading to secondary-metabolite formation.

Recent advances in the isolation of scent biosynthetic genes have provided the basis and created the opportunity for the biotechnological manipulation of floral scent. In the last few years, several attempts have been made to metabolically engineer floral scent using not only the genes responsible for the synthesis of volatile compounds, but also those involved in the secondary modification of scent compounds, as well as transcriptional regulators (Vainstein *et al.*, 2001; Dudareva *et al.*, 2004; Lucker *et al.*, 2004; Dudareva and Negre, 2005; Verdonk *et al.*, 2005). The information generated from these studies revealed additional, unexpected problems that can be encountered in the genetic engineering of flower fragrance: modification of the scent compound into a non-volatile form, e.g. by glycosylation, masking by other volatiles, or the emitted amount being insufficient for olfactory detection by humans (summarized in Dudareva and Negre, 2005). The results presented here demonstrate the possibility of engineering volatile phenylpropanoid esters using an AAT with broad substrate specificity. Our data clearly show that the *in-vivo* function of such an enzyme is not only determined by its *in-vitro* substrate specificity, but is also strongly dependent on the level of available substrate. Detailed analyses of levels, availability and types of potential substrates and detailed biochemical characterization of enzyme activity towards various substrates are hence a prerequisite for *in planta* modulation of metabolic flow for floral-scent modification.

Acknowledgements

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