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Specificity of *Ocimum basilicum* geraniol synthase modified by its expression in different heterologous systems

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ABSTRACT

Numerous aromatic plant species produce high levels of monoterpenols, using geranyl diphosphate (GPP) as a precursor. Sweet basil (*Ocimum basilicum*) geraniol synthase (GES) was used to evaluate the monoterpenol profiles arising from heterologous expressions in various plant models. Grapevine (*Vitis vinifera*) calli were transformed using *Agrobacterium tumefasciens* and the plants were regenerated. Thale cress (*Arabidopsis thaliana*) was transformed using the floral dip method. Tobacco (*Nicotiana benthamiana*) leaves were agro-infiltrated for transient expression. Although, as expected, geraniol was the main product detected in the leaves, different minor products were observed in these plants (*V. vinifera*: citronellol and nerol; *N. benthamiana*: linalool and nerol; *A. thaliana*: none). *O. basilicum* GES expression was also carried out with microbial system yeasts (*Saccharomyces cerevisiae*) and *Escherichia coli*. These results suggest that the functional properties of a monoterpenol synthase depend not only on the enzyme's amino-acidic sequence, but also on the cellular background. They also suggest that some plant species or microbial expression systems could induce the simultaneous formation of several carbocations, and could thus have a natural tendency to produce a wider spectrum of monoterpenols.

1. Introduction

Monoterpenols such as geraniol and linalool are important aromatic compounds belonging to the very large isoprenoid family, which includes more than 40,000 described molecules (Withers and Keasling, 2007). Isoprenoids are classified according to the number of carbon atoms in their skeletal structure: hemi (C5), mono- (C10), sesqui- (C15), di- (C20) and tri- (C30) terpenoids are the most basic representatives, and latex terpenoids (C > 1000) are the largest. Since prehistoric times, aromatic plants have been harvested or cultivated for their unusually high concentrations of fragrant volatiles. Each of these plants can contain several hundred different aromatic compounds, with in most cases very few of them representing a large majority of the total content. Interestingly, the composition of aromatic compounds can vary radically among cultivars of the same plant species, and such variations are in general highly dependent on the environmental conditions. It has been demonstrated that the sweet basil (*Ocimum basilicum*) fragrance varies between different cultivars, and differences in compounds such as geranial, neral, methylchavicol, and eugenol have been described (Morales and Simon, 1997; Iijima et al., 2004a). Furthermore, it has been shown that the molecular basis for this divergent composition can depend on point mutations in (*R*)-linalool synthase, or on the modification of geraniol synthase expression levels (Iijima et al., 2004b). Grapevine (*Vitis vinifera*) is also a plant for which cultivar-specific differences in aroma profiles have been described. The free monoterpenol contents of aromatic grapevine cultivars such as muscat and gewürztraminer range from 0.3 to 1.7 mg/L, whereas the non-aromatic cultivar free monoterpenol contents range from 0 to 0.06 mg/L (Günata et al., 1985). The specific aroma of muscat is determined mainly by (*L*)-linalool and geraniol, whereas that of gewürztraminer is determined mainly by geraniol. The total number of monoterpenols and their relative quantities in each grapevine cultivar may also be affected by other factors such as plant development (cultivation, yield) and environmental variations (pedology, water availability, sunlight), which are referred to as the 'terroir expression' (Styger et al., 2011).

Some plant isoprenoids are of great economical importance and are used as perfumes, food flavors, or for their pharmaceutical or industrial properties. Recent analyses of plant genomes have shown that the size of terpenoid synthase (TPS) families usually ranges from 30 to 50 members, with the exception of

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V. vinifera, which exhibits a much larger number of TPS genes (Jaillon et al., 2007; Velasco et al., 2007; Martin et al., 2010). Mono, sesqui, di or tri terpenoid synthases share common basic structures, enabling the number of isoprene units to be predicted from the precursor used in the synthesis activity. However, it has until now been impossible to predict which terpenoid will be produced. The tremendous range of possible variations in the carbocationic reactions (cyclizations, hydride shifts, rearrangements, and termination steps) catalyzed by the terpenoid synthases explains the wide range of possible products. It is also interesting to note that the expression of a monoterpene synthase *in planta* does not automatically imply emission of a free volatile compound. Monoterpenes are often sequestered in the form of glycosylated or further processed non-volatile versions of the primary TPS products. The ratio of glycosylated/free terpenes depends on the plant species. Ratios of 60/1 have been described in *Arabidopsis thaliana* (Aharoni et al., 2003). In the grapevine, ratios between 1/1 and 1/2 have been described for muscat, and 1/15 for gewurztraminer cultivars (Günata et al., 1985), whereas 10 less aromatic cultivars have ratios ranging between 1/5 and 1/20.

The functional characterization of monoterpene synthases is usually performed using a combination of heterologous truncated cDNA expression in *Escherichia coli* (since the native plant enzyme targets the chloroplast, and the bacteria are unable to process the introns), together with an enzymatic TPS reaction performed on crude bacterial protein extracts. A large proportion of the characterized enzymes catalyzes the synthesis of several final products (Trapp and Croteau, 2001; Martin et al., 2010). Functional characterization of monoterpene synthases in the yeast *Saccharomyces cerevisiae* is not straightforward, as this species is not capable of spontaneously producing large quantities of monoterpenes. In a previous study, we engineered *S. cerevisiae* to produce a suitable heterologous expression system for a truncated *O. basilicum* geraniol synthase cDNA fragment, and observed the production of both geraniol and citronellol arising from the expression of GES (Fischer et al., 2011). The presence of citronellol was thought to be due to an endogenous yeast reductase transforming geraniol into citronellol. These results were therefore in agreement with those of Iijima et al. (2004a), indicating that geraniol was the only product synthesized after heterologous expression in *E. coli* crude protein extracts. In the present study, our aim was to determine whether expressing a full length *O. basilicum* GES DNA in several plant systems, which could be considered as suitable models for the functional characterization of terpene synthases, would also result in different monoterpenol profiles. We thus constructed transgenic *A. thaliana* and *V. vinifera* plants, constitutively expressing *O. basilicum* GES, and also assessed a faster method based on tobacco (*Nicotiana benthamiana*), transiently expressing *O. basilicum* GES. It was also decided to assess monoterpene production in *E. coli*, by constructing an improved strain enabling the detection of monoterpenes in the culture medium.

2. Materials and methods

2.1. Cloning the GES cDNA in E. coli and Agrobacterium tumefasciens

The *O. basilicum* GES cDNA was inserted between the pMDC83 *Pac*I and *Asc*I sites in frame with the green fluorescent protein (GFP) (Curtis and Grossniklaus, 2003). A control plasmid with the *O. basilicum* GES cDNA followed by two stop codons was inserted between the pMDC83 *Pac*I and *Asc*I to disrupt the frame with the green fluorescent protein (GFP). The resulting constructs were checked by sequencing. *A. tumefasciens* C58C1 were grown at 28 °C in a YEP medium containing rifampicin (50 mg/mL) and gentamycin (20 mg/mL), until an OD600 of 0.3 was reached. The cells were washed twice with Hepes 1 mM pH 7.0, and then resuspended in glycerol 10%. The binary vector was introduced into the desired *Agrobacterium* strain by means of electroporation (Bio Rad gene pulser II) in 0.2 cm cuvettes, with a field strength of 2.4 kV, using a 25 μF capacitance and 200 ohm resistor in parallel, to achieve a time constant of 3.8 ms. After a recovery period of 4 h, the bacteria were plated on a solid YEB medium and selected for rifampicin (50 mg/mL), kanamycin (50 mg/mL) and gentamycin (20 mg/mL) at 28 °C for 4 days. The colonies were checked by PCR to ensure the presence of the desired plasmids.

2.2. Transformation of the GES cDNA in various plant species: V. vinifera, A. thaliana, N. benthamiana

For the grapevine transformation, embryogenic calli (EC) were obtained from the somatic cells of the anther filaments of the 41B grapevine rootstock (*V. vinifera* Muscat × *V. berlandieri*) (Perrin et al., 2004). The EC were transferred onto a fresh MPM1 medium prior to the transformation. A transformed *Agrobacterium* strain was grown in a YEB medium containing $MgSO_4$ (667 μM), rifampicin (50 mg/mL), kanamycin (50 mg/mL) and gentamycin (20 mg/mL). The transformation was performed using the procedure described by Krastanova et al. (1995). However, all of the media were replaced by a MPM1 basis for selection of the transgenic cells resistant to Hygromycin (50 mg/L), and establishment of transgenic calli until plant regeneration (Perrin et al., 2001). Three independent lines were obtained. The transformed plants were confirmed by PCR and RT-PCR.

The Columbia-0 *A. thaliana* ecotype was transformed using the floral dip method (Clough and Bent, 1998). Five independent lines were obtained. The transformed plants, obtained after selection with kanamycin, were confirmed by semi quantitative RT-PCR.

The transient expression of GES in *N. benthamiana* was performed using the agro-infiltration method (Voinnet et al., 2003). The agrobacteria were resuspended in 10 mM of $MgCl_2$ containing 100 μM of acetosynringone (OD 0.3), and were infiltrated into leaves of 2–4-week-old wild type *N. benthamiana*. The GES products were analyzed 3 days after agro-infiltration.

2.3. Extraction and quantification of monoterpenes

Leaves from *V. vinifera* (0.5–1.4 g), *A. thaliana* (8.0 g) and *N. bentamiana* (0.2–0.6 g) were ground in 2–10 mL UHQ water, and the supernatant obtained after centrifugation of the crude extract (5000 × *g* for 5 min) was adjusted with NaOH to a pH of 8.0, prior to the addition of 50 mg PVP. 3-octanol (40 μg, >99%, Fluka) was added in order to evaluate the extraction efficiency. Control extracts were performed with geraniol (120 μg/g leaves, 98% pure Aldrich) to test whether added geraniol can be converted to any of the minor products that were later found. We found no detectable geraniol conversion. The mixture (2–3 mL) was loaded on a Bond Elut C18 (5 g) SPE cartridge (Varian), preconditioned with methanol (3 mL × 5 mL) and water (3 mL × 5 mL). Glycosylated and free monoterpenols were eluted from the cartridge with ethanol (2 mL). A 20 mL citrate buffer at pH 4.5 was added to the eluate, and the glycosylated monoterpenols were hydrolyzed by means of 12 h of incubation at 37 °C with an AR2000 enzyme (70 mg/mL, DSM). Following hydrolysis, the samples were loaded on a Bond Elut C18 (5 g) SPE cartridge (Varian) conditioned with methanol (3 mL × 5 mL) and water (3 mL × 5 mL). After washing with water (3 mL × 5 mL), the monoterpenols were eluted with dichloromethane (6 mL). The sample was dehydrated with anhydrous Na_2SO_4, concentrated to 500 μL under a nitrogen stream, following which *m*-cresol (20 μg) was added as an internal standard, and the samples were stored at −20 °C prior to GC–MS analysis.

The cells from an *E. coli* stationary phase culture were harvested by centrifugation (5000 × *g* for 5 min). Octanol (4 μg) and ethylheptanoate (4 μg) were added as an internal standard to 20 mL of the culture medium supernatant. The monoterpenoids were extracted using stir bar (Twister; Gerstel, Mülheim a/d Ruhr, Germany) sorptive extraction liquid desorption gas chromatography mass spectrometry (SBSE-LD–GC–MS) (Coelho et al., 2008) with the following modifications: the solvent used was acetonitrile, with a 1 μL injection volume. Control extracts were performed with geraniol added in a culture supernatant from an *E coli* control strain not producing monoterpenols (0.6 μg/mL, 98% pure Aldrich) to test whether added geraniol can be converted to any of the minor products that were later found. We found no detectable geraniol conversion. Similar control extracts were performed with geraniol added in a culture supernatant from an *S. cerevisiae* A9K control strain not producing monoterpenols (6 μg/mL, 98% pure Aldrich). In both cases we found no detectable geraniol conversion.

The monoterpenol extracts were analyzed by GC–MS using an Agilent 6890N gas chromatograph coupled to an Agilent 5975B inert MSD (Agilent Technologies), equipped with a Gerstel MPS2 sampler. The gas chromatograph was fitted with a DB-Wax capillary column (60 m × 0.32 mm i.d. × 0.50 μm film thickness, J&W Scientific) and helium was used as carrier gas (1 mL/min constant flow). The GC oven temperature was programmed to increase from 45 °C to 82 °C, at 20 °C/min, and then to increase to 235 °C at 2.7 °C/min (hold 15 min). The injector was set to 230 °C and used in the pulsed splitless mode (25 psi for 0.50 min). The temperatures of the interface, MS ion source and quadrupole were 270 °C, 230 °C and 150 °C, respectively. The mass spectrometer was operated in the electron impact ionization mode (EI, 70 eV) and the masses were scanned over a *m*/*z* range of 29–450 amu. Agilent MSD ChemStation software (G1701DA, Rev D.03.00) was used for the instrument control and data processing. The mass spectra were compared with the Wiley's library reference spectral bank. The total amounts of geraniol, linalool, citronellol, nerol and α-terpineol were determined using linear calibration curves, leading to a value of 0.98 for R^2, over the range of concentrations from 0 to 100 mg/mL.

3. Results

3.1. Characterization of GES transgenic plants

GES-expressing transgenic *A. thaliana* plants exhibited a viability, growth and phenotype similar to those of control *Col-0* plants. On the other hand, the viability of GES-expressing *V. vinifera* transgenic plants was severely impaired, when compared with the control plants. This was noticeable at all stages of the transformation procedure, with a higher proportion of embryogenic calli, somatic embryos at the torpedo stage, and somatic embryos developing into normal plants, exhibiting the typical brownish coloration which ultimately leads to the clone's death. These results are in agreement with the fact that monoterpenols are toxic to plants, and tend to be sequestered in glandular trichomes in plant species exhibiting high monoterpenol concentrations (Biswas et al., 2009). We were however able to obtain three plants that could be used for the extraction of monoterpenoids from their leaves.

3.2. Monoterpene analyses

For the purposes of statistical analysis, one way analyses of variance (ANOVA) (p value < 0.05) were performed. As was expected, geraniol was the main monoterpene detected in every GES expression system used (Table 1). However, it is noteworthy that other products were also detected, depending on the heterologous expression system under consideration. Monoterpene GC–MS analysis allowed minor monoterpenols to be detected, with a detection threshold corresponding to *ca* 0.1 μg/g leaves (S/N = 3), and a quantification threshold of *ca* 0.3 μg/g leaves (S/N = 10). The resulting expression in *V. vinifera* was 93% geraniol, together with 3% citronellol and 4% nerol as minor products, whereas the expression in *N. benthamiana* resulted in 95% geraniol, 3% linalool and 2% nerol as minor products. Conversely, the expression in *A. thaliana* resulted in 100% geraniol, with no detectable minor product. Control experiments performed with empty vectors did not lead to the detection of any of these monoterpenes in *V. vinifera*, *N. benthamiana* and *A. thaliana*. In the case of *S. cerevisiae*, the monoterpene production was strain-dependent. An unimproved *S. cerevisiae* (A9K cells transformed with pMO5 plasmid expressing *O. basilicum* GES, deleted for the first 28 amino acids) did not lead to a detectable monoterpene profile modification upon GES expression, whereas in engineered yeast (A9D transformed with the same plasmid), very significant quantities of monoterpenes, including 90% geraniol, 5% linalool, 5% citronellol and traces of nerol, were obtained.

The *O. basilicum* GES product accumulation levels were slightly different from one system to another. Greater quantities of geraniol were obtained in *V. vinifera* transgenic plants (50.7 ± 17.6 μg/g leaves) and transgenic *N. benthamiana* (93.0 ± 53.1 μg/g leaves) than in transgenic *A. thaliana* (18.7 ± 7.5 μg/g leaves). In *S. cerevisiae* and *E. coli*, the monoterpenols were extracted from the culture media. Taking the weight of the pelleted bacteria and yeast cells into account, we estimated the geraniol production of *E. coli* to be 7.85 ± 1.2 μg/g fresh weight, and that of *S. cerevisiae* to be 449 ± 188 μg/g fresh weight.

4. Discussion

Our observations show that the heterologous models we tested led to the production of differing quantities of monoterpenes. Some of these differences are obviously due to sub-cellular location differences, but our results also suggest putative variations in the endogenous ability of these systems to generate GPP, differences in the expression level of *O. basilicum* GES or in GES protein stability, or different abilities to sequester monoterpenols in leaf tissues or different water content of the plant tissues. In this study, we found that the *V. vinifera* and *N. benthamiana* plant background has a better performance, in terms of the accumulation of monoterpenols in leaves, than *A. thaliana*. It is also noteworthy that the production of volatile monoterpenols is drastically affected by light, with a maximum occurring during the day and a minimum at night (Aharoni et al., 2003). This is not surprising, since GPP is the basic molecule used to synthesize all monoterpenes. Its IPP precursor biosynthetic pathway was extensively studied in the 1950s, and the cytosolic mevalonate pathway (MVA) was originally described as being common to all living organisms (Spurgeon and Porter, 1981). The existence of a second IPP pathway, the MEP (2-*C*-methyl-D-erythritol 4-phosphate) pathway, was more recently detected in plant chloroplasts and some micro-organisms (Schwender et al., 1996; Lichtenthaler et al., 1997), and the different steps involved in this pathway have been described (Eisenreich et al., 2001; Kuzuyama and Seto, 2003). It is now admitted that the MVA pathway provides IPP for sterols, sesquiterpenes and the side chain of ubiquinone biosynthesis (Disch et al., 1998), whereas the MEP pathway is active in plastids for the production of monoterpenes, diterpenes, and carotenoids, and the side chains of chlorophyll and plastoquinones (Arigoni et al., 1997; Lichtenthaler et al., 1997; Hirai et al., 2000). Accordingly, in *V. vinifera*, the difference in GPP availability between aromatic and non-aromatic cultivars has recently been demonstrated to rely on variations in 1-deoxy-d-xylulose 5-phosphate synthase, one of the first enzymes in the chloroplastic GPP biosynthesis pathway (Battilana et al., 2009a,b).

Table 1
Monoterpenol content and relative % extracted from *Vitis vinifera*, *Arabidopsis thaliana*, *Nicotiana benthamiana* leaves, transformed or untransformed with a GES-GFP plasmid or a GES plasmid, or extracted from a minimal medium during the stationary growth phase for the AE9D and AE9K yeast strains, and *E. coli* transformed or untransformed with a GES plasmid.

	Geraniol	Linalool	Citronellol	Nerol	α-Terpineol
V. vinifera GES-GFP[a]	50.7 ± 17.6[d] 93%	[c]	1.82 ± 2.37[d] 3%	2.11 ± 1.51[d] 4%	[c]
V. vinifera control[a]	[c]	[c]	[c]	[c]	[c]
A. thaliana GES-GFP[a]	18.7 ± 7.5[d] 100%	[c]	[c]	[c]	[c]
A. thaliana control[a]	[c]	[c]	[c]	[c]	[c]
N. benthamiana GES-GFP[a]	93.0 ± 53.1[d] 95%	3.27 ± 1.89[d] 3%	[c]	1.52 ± 1.20[d] 2%	[c]
N. benthamiana GES[a]	83.6 ± 37.9 96%	2.73 ± 0.79 3%	[c]	0.78 ± 0.67 1%	[c]
N. benthamiana control[a]	[c]	[c]	[c]	[c]	[c]
S. cerevisiae A9D GES[b]	3.05 ± 1.28[e] 90%	0.17 ± 0.11[e] 5%	0.15 ± 0.02[e] 4.5%	0.004 ± 0.006[e] <0.1%	[c]
S. cerevisiae A9D control[b]	0.14 ± 0.01[e]	0.13 ± 0.02[e]	0.003 ± 0.001[e]	[c]	0.006 ± 0.001[e]
S. cerevisiae A9K GES[b]	0.0030 ± 0.0012[e]	0.0021 ± 0.0016[e]	[c]	[c]	[c]
S. cerevisiae A9K control[b]	0.0010 ± 0.0003[e]	0.0010 ± 0.0009[e]	[c]	[c]	[c]
E. coli GES	0.185 ± 0.028[e] 49%	0.013 ± 0.001[e] 3.5%	0.139 ± 0.007[e] 36.5%	0.043 ± 0.002[e] 11%	[c]
E. coli control	[c]	[c]	[c]	[c]	[c]

[a] Results are the mean of 5 experiments ± standard deviation.
[b] From Fischer et al. (2011).
[c] No peak detected.
[d] μg/g leaves.
[e] μg/mL.

On the other hand, qualitative monoterpenoid profiling indicates that *O. basilicum* GES expression results not only in the production of monoterpenoids, which are different for plants and microbial systems, but also in the production of monoterpenoids which differ from one plant to another. Plants have a more diverse profile of possible products, since the expression in *V. vinifera*, *N. benthamiana*, or *A. thaliana* results in the formation (or not) of variable quantities of linalool, citronellol and nerol, in addition to the expected production of geraniol. The expression of GES in *E. coli* results in the production of linalool, citronellol and nerol, and its expression in *S. cerevisiae* resulted in the production of linalool and citronellol as the only minor products (Fischer et al., 2011). It should be noted that when the GES activity was previously assessed in a crude bacterial extract, no minor products were reported (Iijima et al., 2004a). Such differences between heterologous models could result from minor differences in the protein structure, related to different post-translational modifications or different enzyme environments.

Monoterpene synthesis requires a basic phosphorylated precursor (Fig. 1). For the case of monoterpenoids, GPP was the first precursor to be described, but neryl diphosphate (NPP) has since been demonstrated to be another possible precursor (Schilmiller et al., 2009). Interestingly, the conversion of GPP into geraniol is not obtained through a simple dephosphorylation event. It has been shown that it rather involves the formation of a carbocation intermediate, and that the formation of geraniol results from the hydroxylation of this carbocation (Iijima et al., 2004a). The formation of other simple acyclic monoterpenols such as linalool and nerol, or cyclic monoterpenols such as α-terpineol, has been proposed to follow a similar biosynthetic route, with different carbocation intermediates being hydroxylated to give linalool, nerol or α-terpineol (Davis and Croteau, 2000). The reaction can be stereospecific, generating for example either (*R*)- or (*S*)-linalool. Bergamot mint (*Mentha citrata* Ehrh.) *R*-linalool synthase uses GPP as a substrate, and specifically synthesizes (*R*)-linalool (Crowell et al., 2002), whereas *Clarkia breweri* (*S*)-linalool synthase specifically synthesizes (*S*)-linalool (Pichersky et al., 1995). We emphasize the fact that on some occasions, a monoterpene synthase can also use a phosphorylated molecule, very similar to the end product, as a substrate. Bergamot mint (*R*)-linalool synthase can also use (3*R*)-linalyl pyrophosphate as a precursor (Crowell et al., 2002). *Clarkia breweri S*-linalool synthase preferentially uses GPP as a substrate, but can also use (3*S*)-linalyl pyrophosphate as a substrate, at a rate which is 6 times slower, or even (3*R*)-linalyl pyrophosphate, at a rate which is 150 times slower (Pichersky et al., 1995). Subtle differences in protein structure and/or environment could impact the intermediate carbocation profiles, resulting in different products depending on the heterologous systems considered. Engineered *A. thaliana* would have a carbocation profile leading to the exclusive synthesis of geraniol, whereas engineered *E. coli*, *S. cerevisiae*, *V. vinifera* and *N. benthamiana* would generate a carbocation profile leading to the biosynthesis of a mixture of geraniol, linalool and nerol in leaves. This hypothesis means that a variation in a plant's aromatic composition may not only result on the presence of TPS genes and proteins in each plant, but may also be influenced by the cellular context. The cellular background can affect the enzyme for post-translational modifications, but can also affect the enzyme *via* differences in the cytoplasmic environment. A given TPS activity may thus also be influenced by tissue/cell type and by environmental factors affecting precursor availability and product conversion activity.

The presence of other monoterpenol modifying enzymes is one of the factors to be taken into account, in particular for monoterpenols that cannot be obtained from a phosphorylated precursor, when the synthesis takes place through the conversion of one monoterpenol into another monoterpenol. For example, it is not possible to obtain citronellol directly from GPP, since citronellol is obtained by reduction from a geraniol or nerol precursor. Evidence of such conversions was obtained long ago in the rose, since geraniol and nerol are reduced to citronellol by a solubilized enzyme preparation from the rose petals. The terpene reductase has NADPH as a cofactor, and was reported to be specific for primary terpene alcohols with a *cis* or *trans* allylic double bond (Dunphy and Allcock, 1972). Although the existence in plants of enzymes able to produce citronellol has been demonstrated, there is no currently available sequence for a reductase or a citronellol synthase (Luan et al., 2005). Other monoterpenoid reductase genes have been described in plants, and these belong to the SRD (short-chain

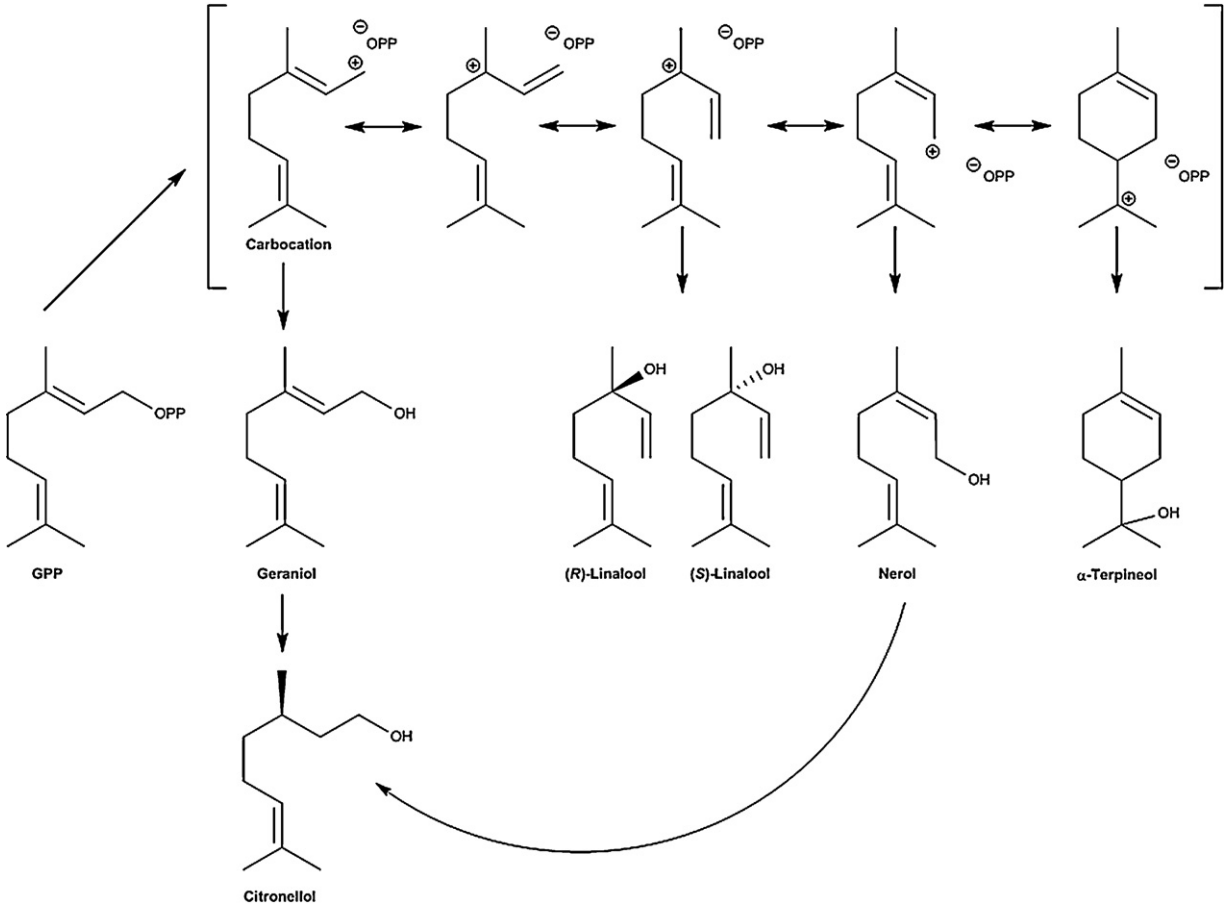

Fig. 1. Model of production of monoterpenols (geraniol, nerol, linalool, α-terpineol) by monoterpenol synthases *via* an intermediate carbocation. Citronellol is obtained *via* a reduction from geraniol or nerol conducted by another additional enzyme.

dehydrogenase/reductase) superfamily (Kallberg et al., 2002). In the experiments described in the present paper, *A. thaliana* and *N. benthamiana* do not express an enzymatic set enabling the conversion of geraniol or nerol into citronellol. On the other hand, in these experiments, *E. coli*, *S. cerevisiae* and *V. vinifera* may express an endogenous enzymatic set leading to the biosynthesis of citronellol.

Factors other than co-expressed enzymes may be involved in the generation of various monoterpenoid profiles. The intracellular pH could be a possible cause of the chemical transformation of monoterpenols. The pH in the chloroplast lumen is slightly acidic, with values ranging from 5.8 to 6.5. (Kramer et al., 1999). As monoterpenols are stable at a neutral pH, but tend to interconvert under acidic conditions (Fischer et al., 2011), one could speculate that minor differences in chloroplast lumen pH, related to the ambient luminous intensity or stress conditions, could also be a factor affecting the production of monoterpenes. Another chemical factor potentially affecting the reaction is the availability of GPP substrate in the cell. It is however noteworthy that experiments conducted in transgenic *A. thaliana*, expressing a dual linalool/nerolidol synthase, have demonstrated that diurnal variations in the amount of available GPP do not affect the enzyme's specificity (Aharoni et al., 2003).

The heterologous expression of GES from *O. basilicum* in various microbial and plant systems demonstrates that the selected system greatly influences the amount of product(s) isolated from leaf tissues or culture media, but more surprisingly also affects the qualitative profile of the GES products. As could be expected, the main product of *O. basilicum* GES is in all cases geraniol, and the observed differences in the present study concern only minor products. More drastic changes for monoterpenol synthase in other plants are, however, not excluded. Our results also highlight the fact that some plant species, with similar numbers of monoterpenoid synthase genes in their genomes, are likely to produce a broader range of monoterpenes than others.

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