Phytochemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Expression of terpene synthase genes associated with the formation of volatiles in different organs of *Vitis vinifera*

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ARTICLE INFO

Article history:
Received 27 January 2014
Received in revised form 11 June 2014
Available online xxxx

Keywords:
Vitis vinifera
Grapevine
Gene expression
VOCs
Monoterpenes
Sesquiterpenes
Terpene synthase genes

ABSTRACT

Plants produce a plethora of volatile organic compounds (VOCs) which are important in determining the quality and nutraceutical properties of horticultural food products, including the taste and aroma of wine. Given that some of the most prevalent grape aroma constituents are terpenoids, we investigated the possible variations in the relative expression of terpene synthase (TPS) genes that depend on the organ. We thus analysed mature leaves, young leaves, stems, young stems, roots, rachis, tendrils, peduncles, bud flowers, flowers and berries of cv Moscato bianco in terms of their VOC content and the expression of 23 *TPS* genes.

In terms of the volatile characterization of the organs by SPME/GC–MS analysis, flower buds and open flowers appeared to be clearly distinct from all the other organs analysed in terms of their high VOC concentration. Qualitatively detected VOCs clearly separated all the vegetative organs from flowers and berries, then the roots and rachis from other vegetative organs and flowers from berries, which confirms the specialization in volatile production among different organs.

Our real-time RT-PCR results revealed that the majority of *TPS* genes analysed exhibited detectable transcripts in all the organs investigated, while only some were found to be expressed specifically in one or just a few organs. In most cases, we found that the known products of the *in vitro* assay of *VvTPS* enzymes corresponded well to the terpenes found in the organs in which the encoding gene was expressed, as in the case of (*E*)-β-caryophyllene synthases, α-terpineol synthase and α-farnesene synthase. In addition, we found groups of homologous *TPS* genes, such as (*E*)-β-caryophyllene and β-ocimene synthases, expressed distinctively in the various tissues. This thus confirmed the subfunctionalization events and a specialization on the basis of the organs in which they are mostly expressed.

1. Introduction

Plants produce a plethora of volatile organic compounds (VOCs) which serve multiple roles in plants. For example they provide protection against herbivores not only by acting as toxins and feeding deterrents, but also by serving as signals for herbivore enemies (Degenhardt and Gershenzon, 2000). Both floral and vegetative parts of many species emit volatile substances with distinctive smells which act as an interface with the surrounding environment. VOCs are low molecular weight metabolites (less than 300 Da) with diverse chemical structures (hydrocarbons, alcohols, aldehydes, ketones, ethers, and esters) which can be divided in four major classes according to their metabolic origin: terpenoids, fatty acid derivatives including lipoxygenase pathway products, benzenoids/phenylpropanoids and amino acid derivatives (Dudareva et al., 2004). Nearly all of these classes are emitted from vegetative parts as well as from flowers (Knudsen et al., 1993) and roots (Steeghs et al., 2004) into the atmosphere or soil, allowing the plant to interact with other organisms while remaining anchored to the ground (Negre-Zakharov et al., 2009; Fineschi and Loreto, 2012). Leaf- or-root emitted volatiles can be involved in the plant's defence by directly repelling herbivores or pathogens, or recruiting enemies of their aggressors to limit or eliminate further damage (Kessler and Baldwin, 2001; Rasmann et al., 2005). VOCs emitted from flowers provide chemical cues to pollinators, thereby ensuring plant reproductive and evolutionary success (Reinhard et al., 2004).

Abbreviations: E–L, Eichhorn–Lorenz; FLcDNA, full length cDNA; MEP, methylerythritol phosphate; MVA, mevalonic acid; SPME, solid phase microextraction; TPS, terpene synthase; VOCs, volatile organic compounds; *VvTPS*, *Vitis vinifera* terpene synthase.

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http://dx.doi.org/10.1016/j.phytochem.2014.06.007
0031-9422/

VOCs are also important in determining the quality and nutraceutical properties of horticultural food products, including the taste and aroma of wine (Styger et al., 2011). Grapevine compounds contributing to the flavour of grapes and wine include terpenoids, shikimate/phenylalanine pathway derivatives (phenols and benzenic compounds), aliphatic compounds (from the lipoxygenase pathway), organo-sulfur compounds (volatile thiols) and methoxypirazines (amino acid derivatives). The most abundant class of VOCs derives from the terpenoid pathways, which give rise to mono-, sesqui- and diterpenes, apocarotenoids, and other irregular volatile terpenes. In the form of free volatiles and as glycoside conjugates, terpenoids are amongst the most important aroma compounds of grape berries and wine bouquet components (Lund and Bohlmann, 2006). They also have various pharmacological properties including antifungal, antibacterial, antioxidant, anticancer, anti-spasmodic, hypotensive and vasorelaxant anticancer activities (Santos et al., 2011; Lima et al., 2012; Bhalla et al., 2013). Terpenoids are synthesized by cytosolic mevalonic acid (MVA) and plastidial methylerythritol phosphate (MEP) pathways, the former giving rise to sesquiterpenes, irregular terpenes and geranyllinalool, and the latter to monoterpenes, hemiterpenes, diterpenes and volatile carotenoid derivatives (Dudareva et al., 2013).

The wide diversity of volatile terpenoids in plants is generated through the action of terpene synthases (TPSs), many of which can synthesize multiple products from a single prenyl diphosphate substrate (Degenhardt et al., 2009). This gene family has been divided into seven subfamilies (designated *TPS-a* through *TPS-g*) based on sequence relatedness, functional assessment, and gene architecture (Chen et al., 2011). Among these subfamilies, *TPS-a* contains mostly sesquiterpene and diterpene synthases, whereas the *TPS-b* and *TPS-g* clades consist mostly of monoterpene synthases. In *Vitis vinifera*, a recent analysis of the 12-fold coverage genome sequence identified 69 putatively functional *VvTPS* genes, 20 partial *VvTPS*, and 63 *VvTPS* probable pseudogenes (Martin et al., 2011). In total, 43 *VvTPS* FLcDNAs have been functionally characterized, representing mostly monoterpene and sesquiterpene synthases (Lücker et al., 2004; Martin and Bohlmann, 2004; Martin et al., 2011).

In a previous study (Matarese et al., 2013), regarding the transcript profiles of the genes of terpene synthases in grapes correlated with the accumulation patterns of volatiles, we found a linalool synthase gene that appeared to be specifically for vegetative organs. In addition, the relative expression patterns of the seven linalool synthases were similar in some tissues, and slightly or considerably different in others, thus suggesting a diverse regulation mechanism according to the type of tissue.

The aim of work was thus to increase our understanding of terpene metabolism in various organs of *Vitis vinifera*. Mature leaves, young leaves, stems, young stems, roots, rachis, tendrils, peduncles, bud flowers, flowers and berries of cv Moscato bianco were analysed for their volatile content and the expression of 23 *TPS* genes. Thus, the possible variations in the relative expression of terpene synthases in accordance with the specific organ were investigated.

2. Results

2.1. Tissue water content and dry weight

The water content in the different tissues varied from 69% in mature leaves to 90% in green and lag phase berries, while all other tissues showed around 80% of water except young leaves with 74% (Table 1). Since no significant differences ($P \geqslant 0.05$) were found by expressing the results of VOC proportions among organs per g of fresh tissue instead of dry tissue, we reported all the concentrations per g of fresh tissue for a better correlation with the gene expression analysis.

2.2. Free volatile compounds in different organs

Quantification results of the main volatiles from frozen ground tissues obtained by SPME/GC–MS analysis in the different organs of grapevine cv Moscato bianco are shown in Table 1. A total of 88 compounds were identified.

The composition of volatiles varied strongly according to the plant organ. Flower buds had the highest concentration of VOCs (667.12 $\mu g\ g^{-1}$) whilst berries at veraisón (E–L stage 35) were the least rich in VOCs (5.69 $\mu g\ g^{-1}$).

The dendrogram obtained from the hierarchical cluster analysis revealed that the quantity of volatiles (Fig. 1a) prevailed of over the presence/absence of the VOC profiles. In fact in this dendrogram, flower buds and open flowers, which had the highest concentration of VOCs, were separated from other organs which were clustered near to each other. We thus performed a hierarchical cluster analysis on transformed binary data (presence/absence of each compound in all organs). The resulting dendrogram (Fig. 1b) clearly revealed the presence of two clusters, one for vegetative tissues and another for flowers and berries. Of the vegetative tissues, the roots appeared to be the most different, young stems and peduncles were the closest to each other and young leaves were nearer to tendrils. In the second group, open flowers and bud flowers were very far from berries, which clustered together.

Monoterpenes were the main class of volatiles in peduncles, young stems, stems, tendrils and rachis. In the other organs, aliphatic compounds were prevalent (Table 2).

Aliphatic compounds ranged from 43% to 93% in all tissues and achieved their maximum value in berries at veraisón (E–L stage 35). The highest concentration was in flower buds while the lowest was in peduncles. Hexanal and 2-hexenal were the predominant compounds in all tissues except roots where 1-octen-3-ol prevailed.

Monoterpenes made up about 76% of the total volatile mass in rachis and peduncles and over 50% in young stems, stems and tendrils. In roots, young leaves, flower buds and open flowers, ranged from 22.36% to 36.72% and accounted for only 7.95% of the total quantity of VOCs in mature leaves. In berries the fraction of monoterpenes ranged from 6.56% to 13.55%, with the minimum in berries at veraisón (E–L stage 35) (Table 2).

Regarding monoterpenes, geraniol and geranic acid were the major components of all tissues except ripe berries (at stage E–L 37), where linalool prevailed (Table 1). The geraniol concentration was greatest in tendrils, where it was twice that in flowers and almost three-hundred times more than in berries. Other plentiful monoterpenes included linalool (especially in tendrils, rachis and young leaves), linalool oxides, nerol and β-citronellol (especially in tendrils, bud flowers and open flowers). Otherwise, roots were the richest in myrtenol, which represented 17% of total monoterpenes, and showed the highest amount of borneol among vegetative organs. Hydrocarbon monoterpenes represented an important class in flower buds and open flowers (13% and 12% of total monoterpenes, respectively), unlike the other tissues where they constituted only 1% of total monoterpenes.

The fraction of sesquiterpenes in all organs was very scarce (generally less than 1% of total VOCs; Table 2). They reached the highest concentrations in flower buds and open flowers (in flower buds the sesquiterpene concentration was about six times more than in open flowers) where α and β-farnesene were the major constituents (over 60% of total sesquiterpenes). They were found in higher amounts in tendrils with α and β caryophyllene, δ-cadinene and nerolidol, and young leaves with α-farnesene, α-bergamotene and nerolidol as major compounds.

Please cite this article in press as: Matarese, F., et al. Expression of terpene synthase genes associated with the formation of volatiles in different organs of *Vitis vinifera*. Phytochemistry (2014), http://dx.doi.org/10.1016/j.phytochem.2014.06.007