AROMA ACCUMULATION TRENDS DURING BERRY DEVELOPMENT AND SELECTION OF GRAPE AROMA CANDIDATE GENES SUITABLE FOR FUNCTIONAL CHARACTERIZATION[•]

Claudio D'ONOFRIO
Department of Fruit Science and Plant Protection of Woody Species 'G. Scaramuzzi', Fruit Science Section, University of Pisa, Via del Borghetto 80, 56124 Pisa, I.
E-mail: c.donofrio@agr.unipi.it

Key words: sesquiterpenes, monoterpenes, *Vitis vinifera*

1. INTRODUCTION

The technological potential of a grape cultivar depends on some compounds derived from secondary metabolism, such as flavonoids and flavour compounds, which in turn may undergo modification during grape must fermentation and wine ageing, determining the organolectic parameters used to define wine quality. Grape flavour management in the vineyard requires knowledge of the derivation of individual flavour and aroma characteristics and the effects that different concentrations and interactions between these compounds have on flavour potential. But, although hundreds of secondary metabolites that potentially contribute to wine flavour have been detected in grapes, knowledge of the biosynthesis of these compounds and the effects of growth conditions on their accumulation is not well understood.

The aim of this research is the discovery of candidate genes thought to contribute to the volatile profiles of grapes by an analysis of correlations between flavour compound concentration and gene expression levels in flowers and during berry development of 'Aleatico', 'Moscato bianco' and 'Sangiovese' grapevine cultivars cultivated in Tuscany.

2. MATERIALS AND METHODS

In Tuscany, during the years 2007-2009, samples of closed flowers, open flowers and berries sampled at regular intervals of 10-11 days covering the developmental stages from fruit set to technological ripening or withering, of 'Aleatico', 'Moscato bianco' and 'Sangiovese' have been analysed for their aroma content and the expression of genes that are predicted to be involved in grape sesquiterpene and monoterpene biosynthesis.

Volatile and glycoconjugated monoterpenes were extracted from fresh berries by solid phase extraction (SPE) using packed cartridges and aglycones of the glycoconjugated forms were liberated using glycosidase enzymes. Sesquiterpenes were extracted from samples stored at -80 °C by a solid phase micro-extraction (SPME) procedure with carbowax/divinylbenzene fibers. Both monoterpenes and sesquiterpenes were identified and quantified using a Thermo GC-MS.

[•] QUAD. VITIC. ENOL. UNIV. TORINO, 31, 2009-2010

Gene expression was determined by real-time RT-PCR using a MJ Opticon thermocycler (Bio-Rad) and ubiquitin was used as the 'housekeeping' gene to normalise the relative gene expression of the candidate genes. It was evaluated the expression of a large group of candidate genes involved in terpenoid pathways identified by analysis of sequence homology with known sequences. The most interesting putative terpene biosynthesis genes were selected by an analysis of correlations between flavour compound concentration and gene expression levels.

3. RESULTS AND DISCUSSION

The aroma analysis reveals that in all the three cultivars analysed, monoterpenes are mostly synthesised in flowers and in ripening berries but also in green berries; C13-norisoprenoids are mainly synthesised during ripening, while sesquiterpenes are largely synthesised in flowers (D'Onofrio *et al.*, 2009a, b; 2010). Among the analysed genes, some showed only a peak of expression in flowers, while others are expressed also during berry ripening. By analysing the correlations among the concentrations of single flavour compounds and gene expression patterns during berry development, about 30 putative genes involved in terpene biosynthesis were selected for further study. Among these genes there are some putative monoterpene and sesquiterpene synthases.

With regards to the sesquiterpenes, all the cultivars analysed had a peak of accumulation in open flowers and α-farnesene represented almost all of the total sesquiterpenes present (fig. 1). Most of the selected sesquiterpene synthase genes showed a peak in expression in closed flowers (fig. 1A). However, we also identified a putative sesquiterpene synthase (VvTSseq_2) that had a peak in expression in open flowers in all three cultivars studied and thus had an expression pattern that highly correlated with α-farnesene production (fig. 1B).

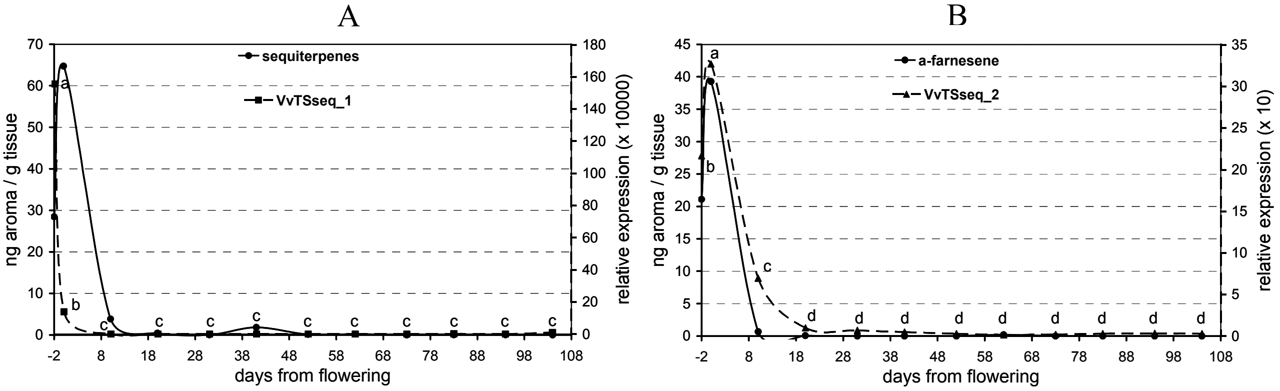

Fig. 1 - Sequiterpene concentration and the expression of putative *Vitis vinifera* sesquiterpene synthase gene VvTSseq_1 (A) in closed flowers, open flowers and berries of 'Moscato bianco'; α-farnesene levels and expression of the putative *Vitis vinifera* sesquiterpene synthase gene (VvTSseq_2) in closed flowers, open flowers and berries of 'Moscato bianco' (B). Different letters indicate statistically different values ($P<0.05$) according to Tukey's test for gene expression level.

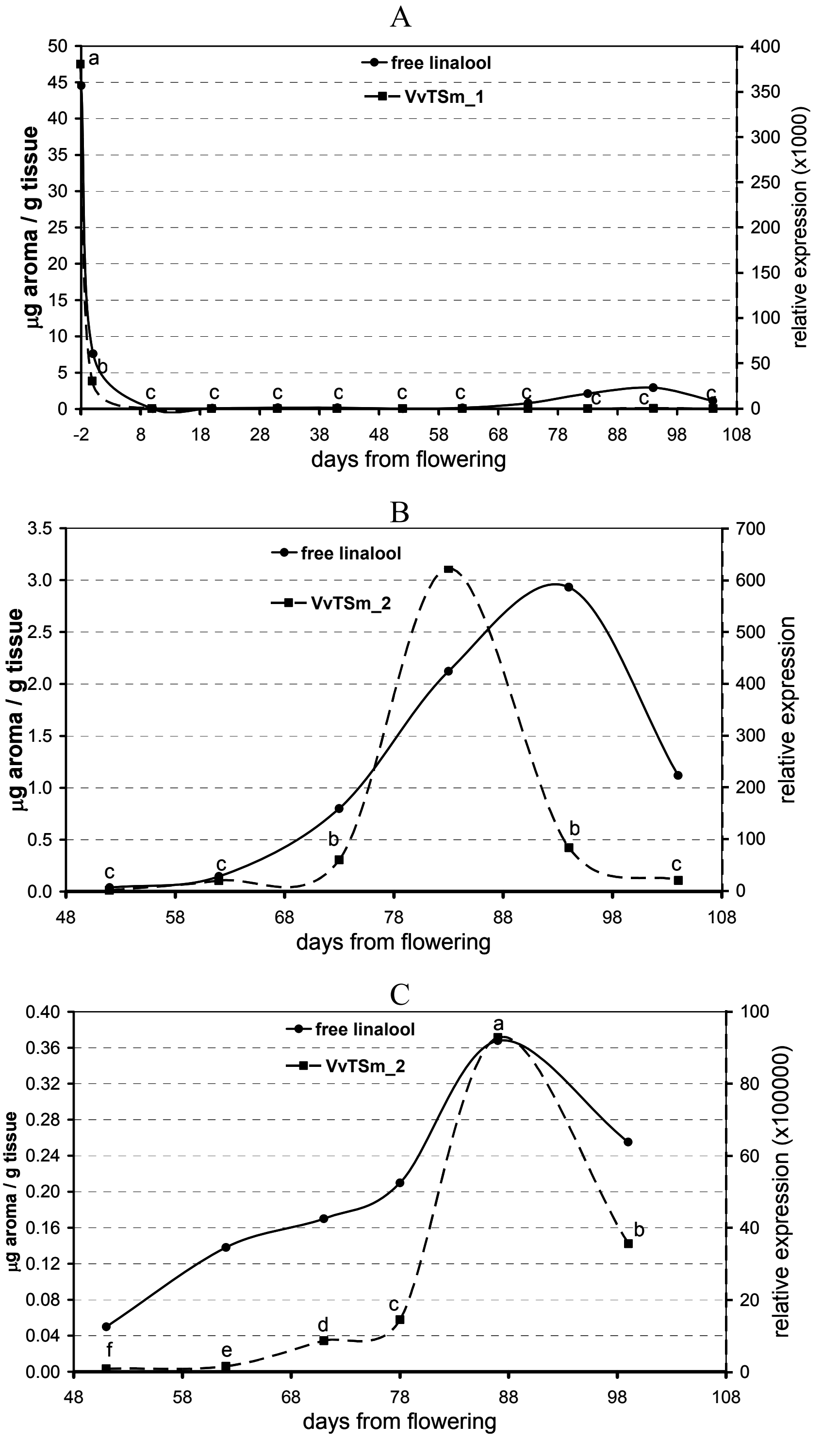

Fig. 2 - Free linalool concentration and expression of putative *Vitis vinifera* linalool synthase genes VvTSm_1 (A) and VvTSm_2 (B) in berries of 'Moscato bianco' and free linalool levels and expression of putative linalool synthase gene (VvTSm_2) in berries of 'Aleatico' (C).

For the candidate monoterpene synthase genes, we focused our attention on some putative linalool synthases selected in the initiation screen of genes. In all three cultivars there was a peak of free and glycoconjugated linalool in closed flowers but also a second minor peak of free linalool during berry ripening at 87-104 days after flowering in 'Aleatico' and 'Moscato bianco' (fig. 2) and a second peak of glycoconjugated linalool at 45 days after flowering in 'Sangiovese'. Among the putative linalool synthase genes, most had a single peak of expression in closed flowers in correspondence to the highest peak of linalool accumulation (fig. 2A). However, there was one candidate linalool synthase gene that had both the peak of expression in closed flowers and a secondary increase in expression during berry development in good correlation with the increased concentration of free linalool in 'Moscato bianco' (fig. 2B), 'Aleatico' (fig. 2C) and glycoconjugated linalool in 'Sangiovese'. Another putative monoterpene synthase is expressed during ripening of 'Moscato bianco' and 'Aleatico' when geraniol accumulates.

4. CONCLUSIONS

The analysis of correlation among flavour compounds and gene expression patterns in flower and during berry development in 'Aleatico', 'Moscato bianco' and 'Sangiovese' has allowed us to identify a putative α-farnesene synthase, a candidate linalool synthase gene expressed in flowers and during berry ripening and a putative geraniol synthase gene expressed during berry ripening. We are now working towards the functional characterization of these *Vitis vinifera* genes involved in terpene pathways.

Acknowledgements

Research supported by University of Pisa, Fondazione Monte dei Paschi di Siena, Fondazione socio-culturale Montecucco funds.

Abstract

Grape flavour management in the vineyard requires knowledge of the derivation of individual flavour and aroma characteristics and the effects that different concentrations and interactions between these compounds have on flavour potential. But, although hundreds of secondary metabolites that potentially contribute to wine flavour have been detected in grapes, knowledge of the biosynthesis of these compounds and the effects of growth conditions on their accumulation is not well understood. The aim of this research is the discovery of candidate genes thought to contribute to the volatile profiles of grapes by an analysis of correlations between flavour compound concentration and gene expression levels in flowers and during berry development of 'Aleatico', 'Moscato bianco' and 'Sangiovese' cultivated in Tuscany. The results demonstrate that the analysis of correlation among flavour compounds and gene expression patterns in flower and during berry development is a powerful tool for selection of most promising candidate genes involved in terpene biosynthesis pathways. The analysis of the above correlations in 'Aleatico', 'Moscato bianco' and 'Sangiovese' has allowed us to identify a putative α-farnesene synthase, a candidate linalool synthase gene expressed in flowers and during berry ripening and a putative geraniol synthase gene expressed during berry ripening. We are now working towards the functional characterization of these *Vitis vinifera* genes involved in terpene pathways.

Literature cited

D'Onofrio C., Boss P.K., Cox, A. - 2009a -. Flavour biosynthesis pathways in grape cell cultures: sesquiterpene biosynthesis. *9th Int. Conf. on Grapevine Genetics and Breeding. Acta Hort.* (ISHS), 827, 331-336.

D’Onofrio C., Cox A., Davies C., Boss P.K.- 2009b - Induction of secondary metabolism in grape cell cultures by jasmonates. *Functional Plant Biology*, 36, 323–338.

D’Onofrio C., De Lorenzis G., Boss P.K. – 2010 - Molecular characterization of aroma genes in *Vitis vinifera* var. ‘Moscato bianco’. *Selected papers and abstracts from the 8th Int. Symp. on Grapevine Physiology and Biotechnology. Australian Journal of Grape and Wine Research*, 16-S1: A21.