

Isolation of the three grape sub-lineages of B-class MADS-box *TM6*, *PISTILLATA* and *APETALA3* genes which are differentially expressed during flower and fruit development

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Abstract

The B class of MADS-box floral homeotic genes specifies petal and stamen identity in angiosperms. While this group is one of the most studied in herbaceous plant species, it has remained largely uncharacterized in woody species such as grapevine. Although the B class *PI/GLO* and *AP3/DEF* clades have been extensively characterized in model species, the role of the *TM6* subgroup within the *AP3* clade is not completely understood, since it is absent in *Arabidopsis thaliana*. In this study, the coding regions of *VvTM6* and *VvAP3* and the genomic sequence of *VvPI*, were cloned. *VvPI* and *AtPI* were confirmed to be functional homologues by means of complementation of the *pi Arabidopsis* mutant. Expression analysis revealed that *VvPI* and *VvAP3* transcripts are restricted almost exclusively to inflorescences, although *VvPI* was detected at low levels in leaves and roots. *VvTM6* expresses throughout the plant, with higher levels in flowers and berries. A detailed chronological study of grape flower progression by light microscopy and temporal expression analysis throughout early and late developmental stages, revealed that *VvPI* expression increases during pollen maturation and decreases between the events of pollination and fertilization, before the cap fall. On the other hand, *VvTM6* is expressed in the last stage of anther development. Specific expression of *VvAP3* and *VvPI* was detected in petals and stamens within the flower, while *VvTM6* was also expressed in carpels. Moreover, this work provides the first evidence for expression of a *TM6*-like gene throughout fruit growth and ripening. Even if these genes belong to the same genetic class they could act in different periods and/or tissues during reproductive organ development.

Abbreviations

AP3, APETALA 3; AtPI, *Arabidopsis thaliana* PISTILLATA; DEFDEFICIENS; GLO, GLOBOSA; GOI, gene of interest; I domain, Intervening domain; K domain, Keratin like domain; M domain, MADS domain; PI, PISTILLATA; TM6, Tomato MADS-box gene 6; VvPI, *Vitis vinifera* PISTILLATA; VvAP3, *Vitis vinifera* APETALA 3; VvTM6, *Vitis vinifera* TM6; WPBB, weeks post bud break.

Keywords

ABC model; *Vitis vinifera*; Flowering; Berry; Quantitative RT-PCR; Arabidopsis mutant complementation; AP3; PI; Grape Genome Sequence