GENE EXPRESSION ANALYSIS OF *VVAG3* IN GRAPEVINE REPRODUCTIVE TISSUES BY *IN SITU* HYBRIDIZATION

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Stenospermocarpy is the mechanism whereby certain genotypes of *Vitis vinifera* L., as 'Sultanina' (Thompson Seedless), produce berries with reduced seed size. In stenospermocarpy, fruit development begins normally after fertilization and embryo abortion occurs 2-4 weeks after fruit-set due to endosperm degeneration. Previous findings identified *VvAG3* as a candidate gene to control seed development in grape. This gene is a MADS-box transcription factor whose ortholog in *Arabidopsis* is involved in ovule identity control. According to *VvAG3* transcription profiles, higher *VvAG3* transcript accumulation occurred in developing seed rudiments throughout the berry development in seeded cultivars, while no transcript accumulation was observed in seedless ones. These results indicated that *VvAG3* is possibly related to grapevine seed morphogenesis, as its reduced transcription generated fruits with only seed traces. Therefore, the aim of this work was to characterize the spatial and temporal expression patterns of *VvAG3* in grapevine reproductive tissues by *in situ* hybridization. We used 'Chardonnay' (seeded) and 'Sultanina' (seedless) samples harvested at pre-anthesis flower, fruit-set as well as fruits at 2 and 4 weeks after fruit-set. The tissues were fixed in paraphormaldehyde, dehydrated in ethanolyc series and embedded in paraffin. Longitudinal and transversal sections (8-10µm) were prepared with a rotary microtome and mounted on silanized microscope slides. Gene-specific sense and antisense probes corresponding to 185 nucleotides of the *VvAG3* 3'-UTR segment were generated by digoxigenin (DIG)-labeling using T7 or SP6 RNA polymerase. After the detection of the hybridization signals by immunostaining, the slides were washed, dehydrated, and mounted using Entellan®. The results were documented using a binocular optical microscope. In 'Chardonnay' pre-anthesis and in fruit-set stages, VvAG3 was expressed in ovules at the chalazal and micropylar-end regions and in in the inner and outer integuments of fruits after 2 or 4 weeks of fruit-set. In 'Sultanina', no VvAG3 expression was observed nor in tissues nor in any stages evaluated. These results provide additional support to suggest the involvement of VvAG3 in grapevine seed morphogenesis.

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