

MOLECULAR ANALYSIS OF THE GRAPEVINE shortened berry DEVELOPMENT MUTATION (Vitis labrusca L. cv. Isabel Precoce) USING cDNA-AFLP

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Grape is a non-climacteric fruit and despite of the advances in grape genetics and genomics, little is known about developing-related expression genes. In an attempt to identify genes associated with the developmental process of the berry growth stage (green phase), cDNA-AFLP analyses was employed. Isabel Precoce (Vitis labrusca L.) is a grapevine mutant, with the berry growth phase reduced in 30-35 days when compared to the wild type and thus constitutes an informative model to investigate many aspects of fruit growth and development. We illustrate in this work the usefulness of cDNA-AFLP to identify development-related genes during the grape berry growth. Total RNA from berries at three stages of development (collected at 10, 40 and 83 days after fruit set) was used for synthesis of stage-specific cDNAs. Thirty combinations of primers MseI(+1, +3) and EcoRI(+1, +2) were used in the final amplification. Fourty-six transcript-derived fragments (TDF's) were excised from dried polyacrilamide gels and successfully re-amplified, cloned and sequenced. Blast analysis against the Grape Genome Browser (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/) resulted in 21 clones that did not showed significant matches and ten clones and one contig (from twelve redundant sequences) obtained a specific match. The clones sequenced showed homology with putative AT-hook DNA-binding proteins, putative mature anther specific proteins, serine-threonine protein kinases, abscisic acid-inducible protein kinases, among others. In polyacrylamide gels, these genes presented an antecipated temporal expression profile in Isabel Precoce when compared to Isabel. In order to confirm the observed expression profiles the selected candidate genes, along with two reference genes are being evaluated through quantitative real-time PCR. The results of this work in combination with the ongoing comparative analysis of gene expression profiles of candidate genes involved in the control of berry development will provide a better characterization of the shortened berry development mutation in the cv. Isabel Precoce.

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