

Integrative Functional Genomic Resource Development in *Vitis vinifera*: Abiotic Stress and Wine Quality.

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Abstract

Water deficit affects important aroma, flavor and color constituents of grapes (*Vitis vinifera*). These changes are associated with improved wine quality and human health benefits. Regulated-deficit irrigation has been used successfully to grow grapes with less water, an important feature in arid regions throughout the world. We have taken an integrated functional genomics approach towards understanding how growth is affected and wine quality improvements might arise following abiotic stress exposure. To date, we have sequenced over 100,000 ESTs from cDNA libraries made from abiotically-stressed vines. A Unigene set was assembled at UNR from approximately 132,000 ESTs in GenBank in October 2003. Our group in collaboration with the Affymetrix GeneChip® Consortia Program, designed the first *Vitis* GeneChip® microarray, which became available publicly in June 2004. The GeneChip® contains probe sets for more than 14,000 *V. vinifera* transcripts and 1,700 transcripts from other *Vitis* species and represents approximately 30% of the grape transcriptome. We have found that these arrays deliver the high quality typical of Affymetrix; on average, gene expression exhibited a coefficient of variation of 16% across technical replicates. *Vitis* transcripts, proteins and metabolite profiles from the same organs were analyzed in parallel from a series of stress experiments. Microarray analysis of shoot tips of vines exposed to gradual and chronic water deficit or an equivalent salinity stress over a 16 day period revealed significant changes in transcript abundance. As water deficit progressed, a greater number of affected transcripts were involved in metabolism, transport and the biogenesis of cellular components than did salinity. Salinity affected a higher percentage of transcripts involved in transcription, protein synthesis and protein fate than did water deficit. In an acute shock experiment, sudden exposure of vines to chilling (5°C), water deficit (PEG) and an isoosmotic salinity (120 mM NaCl and 12 mM CaCl₂) for 1, 4, 8 and 24 hours showed no significant differences in transcript abundance between salinity and PEG prior to 24 hours. Forty-three percent of transcripts affected by stress versus control for 1 through 8 hours were affected only by chilling. Comparison of short- and long-term stress regimes indicated that a gradual long-term stress resulted in a larger, more complex stress response than acute stress. The functional categories most affected by stress were metabolism, protein metabolism, transcription, cellular transport and signal transduction. Microarray analysis of the three major tissue types of grape berries revealed that more than 28% of genes showed pronounced (≥ 2 fold) tissue-specific expression with the largest difference being observed between the seed and pulp/skin. Water-deficit affected the transcript abundance of 13% of the berry genes with differential expression patterns occurring mainly in the pulp and skin. Transcripts encoding genes for the general phenylpropanoid and ethylene metabolism, pathogenesis-related responses, energy, and interaction with the environment were significantly over-represented in the skin of berries from water-deficit-stressed vines. Over the course of berry development approximately 60% of transcripts exhibited significant differential expression between at least two out of seven stages of berry development with more than 28% of transcripts showing pronounced (≥ 2 fold) differences in

mRNA expression illustrating the dynamic nature of the developmental process. Ultimately, we wish to build a reliable prediction model for wine characteristics and plant stress tolerance that integrates transcriptomic, proteomic, and metabolomic data sets. Such a model will greatly facilitate our understanding of gene function and enable improved production efficiency and wine quality under environmentally adverse growing conditions.