Differential Gene Expression During Chilling Requirement Fulfillment in *Vitis riparia* buds.

[1]Kathy L Mathiason, [1]Dong He, [2]Etti Or, [3]David Galbraith and [1]Anne Y Fennell

[1]Department of Horticulture, Forestry, Landscape, and Parks, South Dakota State University, Brookings, SD 57007, USA; [2]ARO, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel, [3] Department of Plant Science, University of Arizona, Tucson AZ 85721

Introduction

In temperate climates, grapevines cycle seasonally from active growth to endodormancy in order to survived winter conditions. Once endodormant, the vine requires a genotype specific amount of chilling during which the overwintering axillary buds become capable of budbreak and growth upon exposure to favorable temperatures (ecodormant). Control of dormancy release is very important for economic production of grapes and is of increasing concern with global warming trends as prolonged dormancy can result in delayed and weak growth, delayed and protracted flowering and decline in vine vigor. Improved cultural management of grapevine dormancy and ability to select grapevines for greater regional adaptation is dependent on establishing a comprehensive understanding of the gene activity involved in dormancy release. Bud dormancy cycling has been associated with changes in hormones, water, lipids, carbohydrates, proteins and gene expression; however, gene activity regulating the progression of chilling accumulation has not been thoroughly characterized. Therefore transcriptomic analysis of V*. riparia* buds was conducted at increasing chilling hour accumulation to identify the coordinated temporal changes in gene expression associated with the transition from endodormancy to ecodormancy.

Materials and Methods

Plant Materials. Short days at 25C were used to induce endodormancy in *Vitis riparia* Michx potted grapevines. The endodormant vines were than moved to cold storage (4.4/C) for chilling requirement fulfillment. Buds from basal nodes 3 to 12 were harvested from 60 vines at 0, 500, 1000, 1500, and 2000 hours of chilling, immediately frozen in liquid nitrogen, and stored at -80/C. Basal nodes 3 to 5 of ten canes were removed from cold storage at the indicated time points and placed at room temperature under
constant lights in test tubes filled with 1 inch of water. The amount and timing of bud break was monitored weekly for 28 days.

Transcriptomic analysis. RNA was extracted with the method of Chang et al (1993) and purified with the
RNeasy plant mini kit (Qiagen). RNA was amplified using the Amino Allyl MessageAmpTM II aRNA Amplification Kit (Ambion). Custom cDNA microarrays constructed from various *Vitis* bud dormancy induction and release cDNA libraries were hybridized with RNA from the five time points. Pairs of samples were labeled with Cy3 and Cy5 dyes, and a loop design was developed for microarray hybridizations. The loop design was replicated three times with buds from 3 different years of treatment. The microarray expression data were normalized and analyzed using the R software package LIMMA (Smyth, 2004). Differential expression analysis was based on the single channel intensities, and analysis of variance extracted genes at least one time point significantly differentially expressed compared to all other time points at p-value<0.05.

Real-time PCR. Primers were designed for selected candidate genes and optimized on cDNA synthesized from 500 hr RNA. Real-time PCR reactions were run in triplicate on 500, 1000, 1500, and 2000 hr RNA from 3 years of buds. The mathematical model presented by Pfaffl (2001) was used to calculate the relative expression ratio of the candidate gene transcripts in comparison to the eIF4A housekeeping gene transcript.

Results and Discussion

Chilling accumulation. Percentage of budbreak increased with increasing chilling hours. The rate of budbreak also increased with increasing chilling hours. There was no difference in budbreak at 0 and 500 hours of chilling. Although budbreak eventually reached 100% in 28 days with 1500 chilling hours, the

rate of budbreak was much more rapid after 2000 hours of chilling than 1500 hours, indicating complete fulfillment of chilling requirement.

Transcriptomic analysis.
A cohort of genes were consistently expressed throughout the chilling accumulation period, and an increasing number of genes were expressed with increased chilling time. A total of 456 genes were significantly expressed at p-value < 0.001 when 1000, 1500, and 2000 hr time points were compared to 500 hr of chilling (Fig. 1). Blastx (NCBI) and gene ontology (KEGG) analysis revealed that the majority of genes both up- and down-regulated were involved in cell defense and stress response, metabolism, genetic information processing, and cell structure. All differentially expressed genes between 500 and 1000 hours of chilling are also significant at the other time points. The majority of the genes differentially expressed in response to chilling accumulation were down-regulated and all 25 of the genes common to the three comparisons were down-regulated. Half of these genes are involved in either metabolism or cell defense/stress response. Real-time PCR analysis of seven genes verified the microarray results. The expression of dehydrin, glutathione S-transferase, pathogenesis related protein and xyloglucan endotransglycolase decreased while metallothionein, peroxidase and cyclase increase. In addition, changes in pathogenesis-related protein, dehydrin, peroxidase and cyclase showed differences in level of expression with increased chilling. The continued change in expression levels corresponds to changes in budbreak percentage and rate of budbreak with increased chilling.

Acknowledgements
Funding received from US-Israel Binational Agricultural Research and Development Fund (BARD) IS-3340-02 and South Dakota State University Agriculture Experiment Station. This material is based upon work conducted using the South Dakota State University Functional Genomics Core Facility supported in part by the National Science Foundation/EPSCoR Grant No. 0091948 and by the State of South Dakota.

References
1. Chang S, J Puryear, J Cairney. 1993. A simple and efficient method for isolating RNA from pine trees. Plant Mol Bio Rep 11:113-116.
2. Pfaffl MW. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research 29:2002-2007.
3. Smyth, GK. 2004. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 3(1)Article 3.

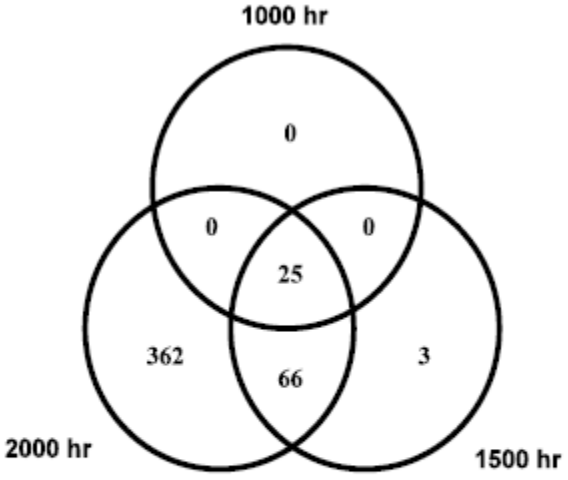

Figure 1. Distribution of differentially induced and repressed genes in axillary buds after chilling. A p-value <0.001 was used to determine transcripts differentially expressed at 1000, 1500 and 2000 chilling hours relative to 500 hours of chilling, n=3.