Transcriptomic and Proteomic Response of Fruit Trees to Abiotic Stress

M. Wisniewski, C. Bassett, D. Macarisin, S. Korban
J. Norelli and T. Artlip
Department of Natural Resources and Environmental Sciences
Appalachian Fruit Research Station
Kearneysville, WV 25430
Illinois 61801
USA

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Abstract
Together, temperature and water availability are the primary determinants of the global distribution of major vegetation biomes and as such have a major impact on the cultivation of temperate fruit trees. The regulation of both low temperature and water deficit stress has been widely studied in herbaceous plants using transcriptomics, proteomics, and transformation technologies. These studies have revealed stress signaling pathways, specific stress-tolerance genes, and transcriptional regulators. Using direct data or empirical approaches, biotechnology has been utilized to produce transgenic plants that have greater stress tolerance. For example, plants overexpressing the transcription factor CBF (under the control of a low-temperature-inducible promoter) have increased freezing tolerance. However, only recently, have these same approaches been used to study stress tolerance in woody plants and more specifically fruit trees. Evidence suggests that although there is a high level of conservation in mechanisms of stress tolerance between annual herbaceous plants and perennial woody plants, the perennial habit has also resulted in additional mechanisms that are specific to perennial plants. We have utilized several different global approaches to study stress tolerance in apple and peach. These include subtractive hybridization (SSH), bioinformatics analysis of ESTs derived from stress-induced cDNA libraries, and 2D Difference in-Gel Electrophoresis (DiGE) for proteomic analyses. These approaches are beginning to reveal the complexity of stress response in fruit trees and helping us develop a comprehensive understanding of stress tolerance in fruit trees. An overview of the results of these studies is presented and discussed.

INTRODUCTION
Extremes of both temperature and water availability are primary factors that determine the worldwide distribution of plants (Graham et al., 2006). Woody plants, including those species that have been domesticated for fruit and nut production, have evolved mechanisms of resistance and adaptation to these environmental stresses. Cold hardness is a complex trait that is influenced by a number of factors (Welling and Palva, 2006; Wisniewski et al., 2003; Wisniewski and Arora, 2000) and likewise, drought tolerance is determined by complex interactions between plant anatomy, physiology, and biochemistry, all of which are directly under genetic control (Street et al., 2006; Verslues et al., 2006). While the genetic regulation of low temperature and drought tolerance has been extensively studied in Arabidopsis and other herbaceous plants (Sreenvivasulu, 2007), limited information is available on how temporal patterns of gene expression and types of genes differ between Arabidopsis and woody plants during cold acclimation and exposure to dehydration (Bassett et al., 2006; Renaut et al., 2004). Due to the complexity of environmental stress resistance, we propose that a systems approach is needed in order to develop a comprehensive knowledge of this trait. This approach includes the use of “omic” technologies, such as transcriptomics, metabolomics, and proteomics, the use of transgenics to conduct functional genomic studies, as well as disciplines such as ecophysiology, and anatomy and morphology. Over the past several years, we have
developed a comprehensive approach to studying abiotic stress resistance in fruit crops that includes expressed sequence tag (EST) analysis, subtractive-suppressive hybridization (SSH), proteomic difference in gel electrophoresis (DiGE), and functional genomic studies. In this article, we would like to draw attention to some of the highlights of these studies and discuss their relevance to abiotic stress resistance in fruit crops.

**TRANSCRIPTOMIC STUDIES**

We conducted an extensive EST analysis of the response of apple to low temperature and water deficit (Wisniewski et al., 2008). More than 22,600 clones from nine libraries were sequenced. Libraries were derived from leaf, bark, xylem, and root tissues exposed to 24h of a cold-acclimating temperature (5°C) or water deficit (45% of saturated pot mass for 2 weeks). The analysis indicated a distinct down-regulation of genes involved in general metabolism and photosynthesis, while a significant increase in defense/stress-related genes, protein metabolism and energy was observed (Fig. 1). The number of stress-related genes in response to low temperature was lower than that in the water-deficit libraries, perhaps reflecting the shorter (24h) exposure to stress. Specific lists of genes in each library were also provided as supplemental material. Genes of particular note that were over represented in the low temperature libraries included dehydrin and metallothionein-like proteins, ubiquitin proteins, a dormancy-associated protein, a plasma membrane intrinsic protein and an RNA binding protein. Genes highly represented in the water-deficit libraries included dehydrins, heat shock proteins, and photosynthesis-related proteins. While these results were similar to other global studies of plant response to abiotic stress, a more comprehensive analysis of specific tissue responses was provided that indicates the complexity of the whole plant response (Wisniewski et al., 2008).

In woody plants, cold acclimation occurs in response to a combination of short photoperiod (SD) and low temperature (LT). In general, SD has only a minor effect on cold hardiness while LT has a much greater effect (Juntilla et al., 2002). To achieve the highest levels of cold acclimation, woody plants must go dormant. SSH has been used in an earlier global study of peach (*Prunus persica*) to identify genes regulated by low temperature and/or short photoperiod (Basset et al., 2006). This study provided lists of treatment-specific genes (LT vs. SD) that were either upregulated or downregulated. It also identified genes common to both environmental stimuli. A noteworthy finding of both the SSH and EST study was the upregulation of many biotic stress related genes (defense genes, pathogenesis-related genes) by both low temperature and water deficit. These include chitinases, thaumatin, glucanases, and metallothionein-like proteins. The upregulation and functional overlap of genes induced by both biotic and abiotic stress is often overlooked and should be further studied.

**FUNCTIONAL GENOMIC STUDIES**

Cold acclimation involves the induction of several mechanisms that collectively contribute to augment freezing tolerance (Guy, 2003). Such changes include both the accumulation of cryoprotective molecules (sugars, compatible solutes, and proteins) and alterations in membrane composition. In *Arabidopsis*, a family of transcription factors (*CBF1-3*) that regulate subsets of cold-responsive genes by binding to a C-repeat element in the promoter region of target genes has been identified (Jaglo-Ottosen et al., 1998, 2001). We have cloned three transcription factors from apple (Fig. 2) sharing high homology with CBFs from other species, and we are in the process of conducting functional genomic analyses of these genes by generating transgenic lines of apple (cv. M.26) that either overexpress or silence a specific CBF gene.

Overexpression of *AfrsMdcbf1* (Appalachian Fruit Research Station *Malus domestica* C-repeat binding Factor 1) resulted in non-acclimated and acclimated plants that were several degrees more cold hardy than non-transformed plants. The LT$_{50}$ (defined as the temperature at which 50% of electrolytes are leaked from the plant tissue) of line 126 (overexpressing *AfrsMdcbf1*) in the non-acclimated state was -6°C compared
to an LT$_{50}$ of -3°C in the untransformed control (WT). The LT$_{50}$ of the acclimated plants was -17°C and -15°C for line 126 and WT, respectively. Plant overexpressing AfrsMdcbf1 also had smaller stature, at least initially (Fig. 3). Phenotypic analyses of other AfrsMdcbf1 overexpressing lines, as well as lines where AfrsMdcbf1 has been silenced are in progress. We are also conducting RT-PCR studies to characterize the temporal expression patterns of all three apple MdCBF genes in response to a wide array of environmental conditions (low temperature, short photoperiod, drought, etc.). It is expected that these studies will lead to a comprehensive understanding of the regulatory role of CBF genes in apple.

PROTEOMIC STUDIES

Although a great deal of knowledge has been gained by transcriptomic studies, this information must be balanced by the fact that mRNA abundance and protein levels are often not correlated (Gygi et al., 1999). Furthermore, gene expression studies do not provide information about either the subcellular localization of gene products or post-translational protein modifications that may be essential for its function, transport, or activation (Rose et al., 2004). Proteomics, the study of global changes in proteins, can provide a critical link between the transcriptome and the metabolome, the latter of which is the end result of transcriptional and proteomic activity (Renaut et al., 2006).

We have conducted a quantitative proteomic analysis of short photoperiod and low-temperature responses in bark tissues of peach using DiGE technology (Renaut et al., 2008). DiGE technology (GE Healthcare, Buckinghamshire, UK) has addressed many of the problems encountered with existing 2DE-technology and greatly improved the reliability, quantitative analysis, and efficiency of proteomic research (Renaut et al., 2006). In our study of peach, we presented a general picture of the peach proteome in response to factors that result in the induction of cold acclimation and dormancy in woody plants. Out of 114 proteins that were observed to be differentially regulated, a total of 57 spots were identified with mass spectrometry. The identified proteins were associated with carbohydrate metabolism, defense or environmental stress, energy production, and cytoskeletal organization. For a discussion of the possible role of these proteins in cold acclimation and dormancy see Renaut et al., 2008).

We have also used a proteomic approach to observe the responses of crabapple (Malus pumila) to water deficit, and determine the ability of the non-protein amino acid, β-aminobutyric acid (BABA), to induce drought tolerance through a process of sensitizing the plant to water deficit rather than by inducing global changes in gene expression as occurs with the application of ABA. Details on the ability of BABA to induce resistance to both biotic and abiotic stresses in herbaceous plants has been previously reported (Ton and Mauch-Mani, 2004; Ton et al., 2005; Zimmerlie et al., 2000). Sensitization or potentiating of resistance mechanisms represents an alternative to directly inducing changes in gene expression (by chemicals or genetic transformation) that may increase resistance but compromise growth (Jakab et al., 2005).

In our crabapple study, plants were watered with 100 µM ABA, 500 µM BABA, or plain water on day 0 and then changes in relative leaf water content and in the leaf proteome were followed over a 12 day period as the plants experienced an increasing water deficit. Results indicated that BABA could induce drought resistance. After 8 days, water loss from leaves was approximately 2, 8, and 30% from the ABA, BABA, and water treatments, respectively. A typical 2D gel obtained during the course of this study is presented in Figure 4. Statistical analysis of the results revealed that by 10 days after the onset of the experiment, 138 proteins were differentially regulated by ≥1.5-fold in at least one of the treatments. Among these proteins, some showed almost identical patterns of up- or down-regulation in both BABA and ABA treated seedlings. This supports the concept that BABA-induced abiotic stress resistance is at least partially achieved by potentiating an ABA-regulated pathway rather than an immediate induction of ABA-induced proteins. More specifically, while similar proteins were induced by BABA and ABA, with ABA, the proteins are induced immediately after application, in the absence of
or prior to the water stress, while with BABA these proteins were expressed only after the onset of the stress. Suppression of lignin biosynthesis, activation of pectin methyl esterase, and proteins associated with the production of compatible solutes were associated with drought tolerance.

**SUMMARY**

Environmental stress tolerance is a complex trait in woody plants regulated by many genes. Both transcriptomic and proteomic analyses indicate that there is substantial overlap in the induction of both genes and proteins in response to biotic and abiotic stress. The functional role of the majority of genes/proteins associated with defense and stress resistance, however, is not known and needs to be further explored. There are at least three CBF-like transcription factors in apple. Overexpression of the native apple AfrsMdcbf1 increased cold hardiness and initially reduced growth. BABA increased drought tolerance in crabapple seedlings and is a useful tool to study both disease and environmental stress resistance in woody plants. Due to the complexity of abiotic stress tolerance, a systems biology approach will be needed to develop a comprehensive understanding of how this trait is genetically regulated.

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**Literature Cited**


Figures

Fig. 1. Increase or decrease in the percentage of apple ESTs belonging to various functional categories in response to water deficit or low temperature. Percent changes are relative to the control which was set at 100%.

Fig. 2. Amino acid comparison of three apple CBF transcription factors.
Fig. 3. Effect of overexpression of CBF1 on the stature of apple plants (cv. M.26). Plants of line 126 overexpressing CBF1 are numbered 1-5, wild-type plants are numbered 6-9.

Fig. 4. 2D gel of leaf proteins obtained from crabapple plants treated with ABA, BABA, or water (control) and subjected to water deficit.