Final Report to The Viticulture Consortium and the American Vineyard Foundation

Project Title: Developing a Functional Genomics Approach to Berry Ripening and Defense

Principal Investigator: Douglas O. Adams
Department of Viticulture & Enology
University of California
Davis, CA 95616

Summary

Our work with fruit ripening and defense related genes is now complete with regard to collecting EST sequences. With the ones we have already collected and the many thousands that are now planned in efforts on this campus and internationally we feel that it will be possible to clone nearly any grape gene we are interested in by application of sequence information and PCR methods. This means that studies where information about gene expression in grape is needed can be undertaken with confidence that most of the genes of interest can be easily cloned. Nevertheless, the picture that has emerged from our work with the veraison stage library is very informative and is a major research accomplishment and result.

We divided the EST sequences we obtained into three classes. Class one are genes for which the role in fruit development or ripening is already known in other systems (e.g. tomato). An example of class-one genes would be polygalacturonase. The second class includes those genes whose function can be known with a high degree of certainty but where its role in fruit development or ripening is unknown. An example of class-two genes would be a tonoplast intrinsic protein. The third class contains those genes that are unknown (a match was found in the data base but no function has been assigned to the protein) hypothetical proteins (e.g. an open reading frame from Arabidopsis) or no match (i.e. no match at all was found in the data base). In our total EST collection 34% of the genes were assigned to class 1; 44% to class 2; and 22% to class 3.

In order to provide a functional classification for the genes in class 1 and class 2 above, we assigned each gene to a functional category. The major group in the functional category contained genes related to stress responses; either oxidative, osmotic or water stress. We found that 22% of the genes were stress related and 20% were related to protein synthesis, processing and degradation. We found that 18% of the genes to which we could assign a function are related to disease resistance, and 8% involved with signal transduction. There were 9% related to RNA processing or were known transcription factors. Other groups with about 5% each were cell wall chemistry, secondary metabolism and photosynthesis.

The view of grape berry ripening that has emerged from our work is much different than we expected when we began to sequence ESTs from veraison berries. The large number of stress induced genes and disease related genes we found was surprising and unexpected. Nevertheless, this result has drawn our attention to the function of stress responses and plant defense gene expression in fruit ripening and berry composition.

Objectives and Experiments Conducted to Meet Stated Objectives

The objectives for this proposal were

- Complete the EST sequencing effort from the veraison cDNA library.
- Continue to characterize individual ESTs and identify genes that may be fruit specific.
- Obtain the complete sequence of selected genes that will be most useful in physiological studies related to ripening.

Complete the EST sequencing effort from the veraison cDNA library.
The work to sequence an additional 400 ESTs was completed in February, 2002. We now feel that the sequencing effort from the veraison cDNA library is complete for our purposes. We have identified 538 new grape genes that are expressed in berries during ripening and this number is likely to increase as more refined BLAST searches are conducted on the sequences.

Continue to characterize individual ESTs and identify genes that may be fruit specific.
Work to characterize individual ESTs and determine which ones might be fruit specific is still underway but progress to date comparing expression in fruit with expression in leaves has revealed several disease and stress response genes that appear to be present in fruit but not in normal leaves. At this point we cannot say that the candidate genes we have found are fruit specific, just that they are expressed in normal fruit at veraison but not in normal leaves. Since some of the genes found in normal fruit at veraison are related to oxidative stress, water stress and pathogen attack, these same genes may be expressed in leaves when subjected to these stresses but are not present at detectable levels in normal leaves. Thus we have 23 candidate genes that may be fruit specific.

Obtain the complete sequence of selected genes that will be most useful in physiological studies related to ripening.
We identified 38 genes that are of particular interest in fruit ripening studies. These represent such genes as adenosylhomocysteinase which is involved in secondary metabolism, several aquaporins that are associated with fruit water relations, ascorbate peroxidase that is involved in oxidative stress and fruit ripening, caffeic acid O-methyltransferase that is involved in phenolic synthesis. Our plan is to use several of these in a PCR approach to look at expression in conjunction with other projects and to make these sequences available to other workers investigating ripening phenomena in grape. In order to make them most useful we needed to obtain the entire sequence of the cDNA represented by the EST. For 31 of those we were able to complete the sequence by sequencing the vector insert from the T7 primer. Our first round of sequencing was from the T3 vector primer, which gave us 5' sequence. For seven of the genes the insert appears too long and the sequence from the T3 and T7 sequencing did not overlap in

the middle. In order to complete this objective we will design gene specific internal sequencing primers to complete the sequence.

Summary of Major Research Accomplishments and Results

The major accomplishment during the 2001 season is the number of new grape ESTs that we have characterized and the insight this has provided into the physiological processes that occur in fruit during veraison. This result would be important if it only applied to fruit, but we believe that many of the genes will be useful probes in other tissues. We have identified grape genes that can serve as standards and controls for expression studies and we are still screening many of the ESTs to identify fruit specific genes.

Our work with fruit ripening and defense related genes that has continued over the last three years is now complete with regard to collecting EST sequences. With the ones we have already collected and the many thousands that are now planned in efforts on this campus and internationally we feel that it will be possible to clone any grape gene we are interested in by application of sequence information and PCR methods. Nevertheless, the picture that has emerged from our work with the veraison stage library is very interesting and is a major research accomplishment and result.

We divided the EST sequences into three classes. Class one is where the role of the gene in fruit development or ripening is already known. The second class is where the identity of the gene can be known with a high degree of certainty but where its role on fruit development or ripening is unknown. The third class contains those genes that are unknown (a match was found in the data base but no function has been assigned to the protein) hypothetical proteins (e.g. an open reading frame from Arabidopsis) or no match (i.e. no match was found in the data base). In our total EST collection 34% of the genes were assigned to class 1, 44% to class 2 and 22% to class 3.

In order to provide a functional classification for the genes in class 1 and class 2 above, we assigned each gene to a functional category. The major group in the functional category contained genes related to stress responses; either oxidative, osmotic or water stress. We found that 22% of the genes were stress related and 20% were related to protein synthesis, processing and degradation and 9% were related to RNA processing or transcription factors. We found that 18% of the genes are related to disease resistance, and 8% involved with signal transduction. Other groups with about 5% each were cell wall chemistry, secondary metabolism and photosynthesis.

The view of grape berry ripening that has emerged from our work is much different than we expected when we began to sequence ESTs. The large number of stress related genes and disease related genes was surprising, but this result has drawn our attention to the role that these stress response genes may play in ripening.

Outside Presentations of Research

A poster presentation of this work was given at the Plant, Animal & Microbe Genomes X meeting held in San Diego California in January 2002.

Research Success Statements

Wine quality is largely determined by fruit composition at harvest. Grape variety, environment, soils, weather and cultural practices should be viewed as a biological continuum where the environment influences gene expression, which in turn determines the characteristics of the biosynthetic processes that ultimately determine fruit composition. Therefore, if we have any hope of understanding how the environment influences fruit composition we must determine what genes are expressed at a given time in berry development. We must also know what tissues those genes are expressed in. This project has already provided some of the tools needed to begin to address these biochemical and physiological issues.

The timing and level of expression of grape genes determine the quantity and nature of grape berry components. This research has continued to provide new tools to study fruit development and ripening. This project has identified and isolated several genes associated with disease resistance that can be used immediately in the search for an early molecular marker of the vine's response to Pierces Disease. We have identified several genes that are candidates for fruit specific expression under normal conditions. This work will have general applicability in the study of grapevine biology, and with the tools available from projects such as this, the response time required to address new problems can be shortened perhaps by years.

Funds Status

The funds remaining will be used to support the personnel for the remaining two months of the granting period and pay for additional DNA sequencing of the remaining seven plasmids.