Progress Report

Project No.: 91-AA1

Project Title: Biochemical Markers for Bud-Failure Potential

Project Leaders: S. Arulsekar (916-752-1462)
Tom Gradziel (916-752-1575)
Pomology Department
University of California
Davis, CA 95616

Cooperating Personnel: D. E. Kester

Supporting Organization: Almond Board of California

The development of biochemical markers to monitor changes in bud-failure potential over time within and between sources of almond cultivars prior to the expression of symptoms has been one of the long term goals of BF research. In an earlier study, Fenton and Kester (1988) have shown that there are significant differences in protein synthesis levels between normal and bud failure derived cell suspension cultures. More recently, amino acid studies (Kester and Durzan. Annual Report 1990; Int. Hort. Cong. 1990) have also shown that there are significant differences in the amino acid patterns of normal and bud failure affected almond trees. Our primary objective of this research is to identify a specific protein and/or DNA marker that is closely correlated with the potential for BF symptom development in almond.

Research Results During 1991:

1. Protein Markers:

Vegetative bud samples were collected from Nonpareil clonal selections that showed no symptoms (normal) and from clonal selections that showed severe symptoms (bud failure). One set of these buds were incubated at 25°C for 8 hours and another set at 35°C for 8 hours. Total proteins were isolated using an acetone powder extraction procedure. Two-dimensional gel electrophoresis was done to compare the protein profiles between normal and bud failure trees. Significant differences, especially in the relative amount of expression of certain proteins, were observed between normal and bud failure trees at both 25°C and 35°C. Further research will be done to identify these proteins and their potential to be used as markers.

2. DNA Markers:

Bud failure in almond is known to be inherited to a large extent. Our objective is to identify specific DNA sequences that may be associated with bud failure in almond using the recent DNA amplification technology.

The procedure involves the following three steps:

1) Denaturation of DNA: A very small amount of genomic DNA (5 nanograms) extracted from the plant (i.e. leaves) is heated to 92°C to denature it and to separate the two strands of the DNA double helix.

2) Primer Annealing: The denatured genomic DNA is allowed to cool rapidly to 35°C in the presence of the primer DNA. The primer will anneal to regions of homology in the genomic DNA.

3) DNA Synthesis: The region between two annealed primers in the genomic DNA is synthesized at 72°C by an enzyme, TAQ DNA Polymerase.

The preceding three steps form one cycle of DNA amplification, a process which is repeated 25-45 times in a programmable thermal cycler. The amplified DNA sequences are then separated in a gel by electrophoresis and visualized by ethidium bromide staining (in the case of agarose gels) or silver staining (in the case of polyacrylamide gels). The primers that we have used are arbitrary nucleotide sequences 10 bases long. The amplified band pattern thus obtained is characteristic of the genomic DNA of the individual tree or clone.

We have isolated total genomic DNA from normal and BF affected selections of Nonpareil. A total of 40 different primers were used to amplify the genomic DNA of normal and BF affected clones. Preliminary results indicate that there may be differences between normal and bud failure affected trees (figure 1). Further work is needed to confirm these results. This work is currently in progress.

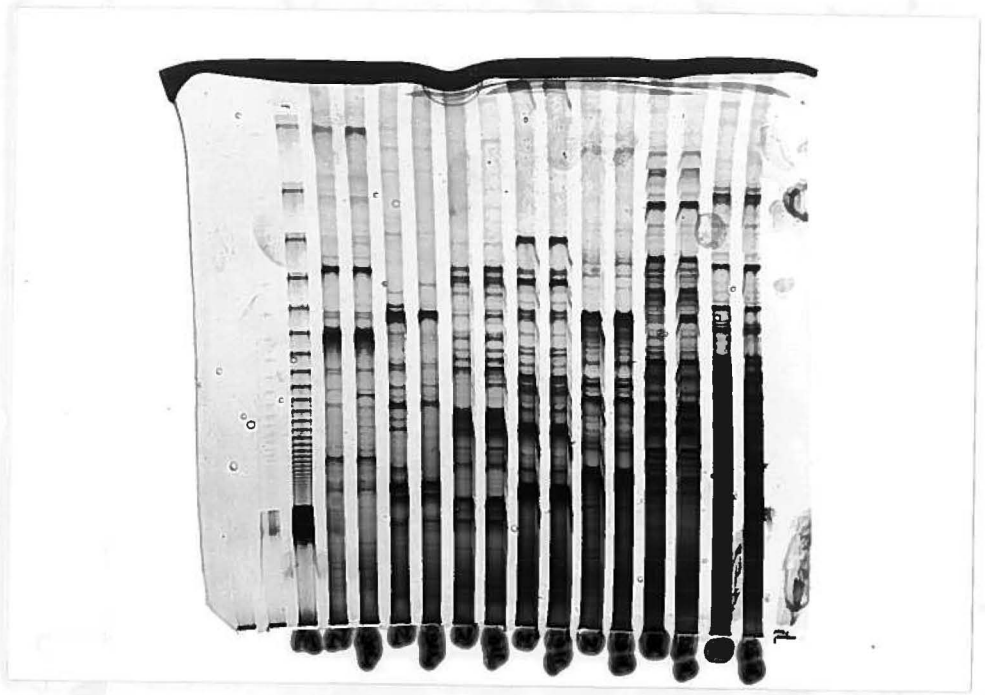

Figure 1. Normal and bud failure clones of Nonpareil almond showing differences in the DNA amplification patterns. the lane marked (M) is is the standard molecular size marker. Note band differences between normal (N) and bud failure (BF) clones in lanes 1 and 2; 3 and 4; 5 and 6.

AES/CE MAR 84 Workgroup/Department: Pomology

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year 1991-1992 Anticipated Duration of Project 1 year

Project Leader S.Arulsekar,T.Gradziel Location Davis

Cooperating Personnel D. E. Kester, F. Bliss

Project Title Biochemical Markers for Bud-failure Potential

Keywords Almond; bud-failure; markers; biotechnology

Commodity(s) Almond Relevant AES/CE Project No. _____

Problem and its Significance:

The Almond Board project on noninfectious bud failure (BF) has resulted in procedures for selection and management of sources of almond varieties with low BF potential. These procedures rely on long term orchard tests that require the actual expression of symptoms. The development of internal biochemical markers to monitor changes in BF potential over time within and between sources prior to the expression of symptoms has been one of the long term goals of BF research. The fact that BF probably results from the modification of expression of a normal physiological trait rather than from the infection of a foreign organism or the development of a distinct genetic mutation greatly complicates the program of developing markers. Recent amino acid studies (Kester and Durzan, Annual Report, 1990; Proc. Int. Hort. Cong., 1990) have uncovered information which will be useful in the development of biochemical markers. The first finding was the identification of a heat induced dormancy stage in midsummer as shown by correlated bud forcing and amino acid patterns in almond and its absence in the BF-affected plant. The second was evidence that aberrations in specific enzymes controlling pathways in amino acid synthesis (particularly the urea cycle) may lead to toxic conditions in BF symptoms.

The indication that there are amino acid and protein differences between normal (low potential) and bud-failure affected trees implies that there may be differences in the mRNA that corresponds to the observed protein differences. These mRNAs, when cloned as cDNAs, may serve as a molecular DNA marker for selection both in the breeding programs as well as in the nurseries.

This proposal is designed as a preliminary project to obtain evidence to support further outside research in this direction.

Objectives:

To identify DNA based molecular markers closely correlated with the potential for BF symptom development in almond.