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PROJECT TITLE: Biochemical Markers for Bud Failure

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## RESEARCH OBJECTIVE

The biochemical and genetic mechanism of bud failure development in alomond is not well understood. The limited results obtained thus far indicates that there are distinct differences in amino acid pool,protein composition as well as DNA between almond clones with bud failure symptoms and the ones without. Our objective in this research project is to study the differences in the DNA and to identify reliable diagnostic DNA marker which can be used to screen and select for almond clones/cultivars with no or low potential to develop bud failure disorder.

## RESEARCH RESULTS DURING 1992

DNA from different clonal selections of Nonpareil almond (from Wolfskill Experimental Orchard) showing different degrees of bud failure symptoms i.e., severe, mild and no symptoms were isolated and purified. The DNAs were then analysed using Southern blot analysis for differences in their methylation pattern of ribosomal genes.The genomic DNAs were digested with two restriction enzymes, MspI and HpaII, both recognizing the same sequence 5'CCGG 3'. MspI will not cleave the DNA if the 5'C is methylated and HpaII will not cleave if the 3'C is methylated. The restricted DNA fragments were separated in an agarose gel and transferred on to a nylon membrane. A wheat ribosomal gene clone pTA71 was labelled with radioactive P32 and hybridized with the almond genomic DNA fragments in the nylon membranes. The ribosomal genes are known to be involved in protein synthesis in living organisms and the methylated genes are known to be inactive. The results of this experiment clearly showed that the ribosomal DNA sequences in trees showing severe symptoms were partially methylated (Figure.1) However, the trees with mild symptoms as well as the ones with no symptoms did not show any differences in the methylation patterns (Figure.1) The same restricted DNA samples that were used in the methylation experiment were also used in a DNA amplification study using

random ten base primers. The DNA from the severely affected trees differentially amplified specific bands when compared with trees with no or mild symptoms confirming that there are qualitative differences in the DNA of the severely affected clones versus the clones with no or mild symptoms.The ability of these methods to be used as a diagnostic tool is still needs to be tested and that will be focus of the future experiments.

FIGURE.1

An autoradiogram showing the hybridization of ribosomalgene probe to the MspI digested almond genomic DNA. The lanes are as follows: 1.Severe symptom, 2.No symptom, 3.Mild symptom, 4.No symptom, 5.Severe symptom, 6.Mild symptom, 7.Mild symptom, 8.Severe symptom, 9.No symptom. The arrow indicates the methylated sequences.