Project Number 82-JA1

BIOCHEMICAL Markers for Identifying Non-Infectious Bud Failure
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Objectives

The objectives of this research as stated in the project title is the development of biochemical techniques in the identification of bud failure.

Interpretive Summary

The latent syndrome of disease development in bud failure produces a particulary devastating disease since one can be misled by investing time, money, and effort into nurturing a time bomb. The basis for the disease has yet to be discovered but we are working to develop a method for its rapid identification and in the process have a chance at disclosing its origin.

First, we have characterized the cellular response to temperature in order to examine normal and bud failure cells under a condition where they are most likely to display their inherent differences and thereby enhance our chances of detecting useful biochemical markers. The results support the contention that bud failure cells "perceive" temperature differentially with 27°-30°C having the largest difference in response. The relationship of response velocity to temperature provides the biophysical framework that supports these contentions. The initial reaction akin to the energy of activation is identical but the reaction velocities are different. Bud failure cells have a greater reaction velocity and their components perceiving temperature breakdown faster with the transition occuring between 27°-30°C. Apparently, there is a real intrinsic difference between normal and bud failure cells in either the quantity or interaction of their cellular components.

The nature of the bud failure symptomology parallels that of a disease class produced by viroids (latency, temperature induction). All known viroids have been characterized as a unique fraction of RNA falling within a narrow molecular size range. If was of interest to determine if bud failure cells contained this type of RNA. A known viroid marker (potato spindle tuber viroid) was obtained to facilitate the interpretation of the electrophoretic pattern obtained from almond cells. An RNA position identical to the potato spindle tuber viroid has been discovered in almond cells. The nature of this RNA has yet to be determined. Experiments in progress have been designed to compare the RNA sequence between the known viroid and the almond RNA position, and to determine if it is infectious. The apparent contradiction between the noninfectious label given the disease and the possible involvement of an infectious agent must be resolved. If the involvement of a

viroid proves to be positive, nucleic acid hybridization probes will be made as a means for rapid identification of bud failure clones.

A method to serologically identify bud failure has been initiated using hybridoma technology. We have used this system to identify markers for the cling peach breeding program and are now applying it to bud failure. Antibodies have been induced against components of bud failure cells and these will be tested to determine if they differentiate between normal and bud failure clones. If this proves to be positive, it will also be the means for rapid identification.

Experimental Procedures

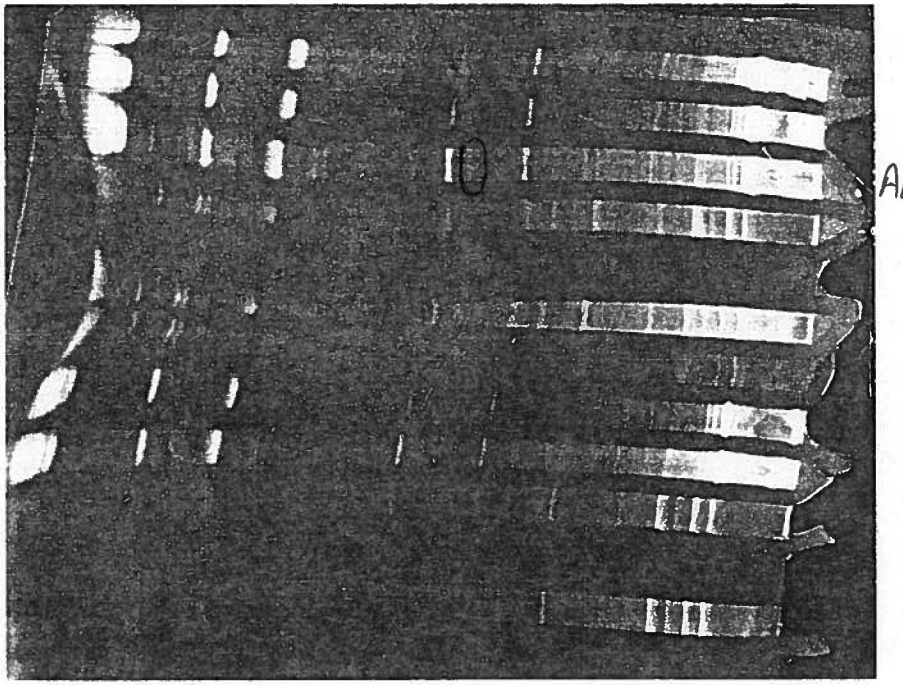
1. Cells of normal and bud failure nonpareil, harpareil, and jordanolo are being grown in tissue culture for a source of uniform material differing only in being initiated from normal or diseased tissue sources.
2. Potato spindle tuber viroid infected plants are being cultivated in the Pomology greenhouse.
3. RNA is extracted from these tissue sources by a variety of techniques and is subjected in polyacrylamide gel electrophoresis. The RNA is detected by ethidium bromide fluorescence, the RNA positions of interest are cut out of the gel and electrophoretically eluted.
4. The RNA positions of interest will be copied by reverse transcriptase to produce a radioactive probe as the initial means of comparing potato spindle tuber viroid and the almond RNA position. If this proves to be positive, the almond RNA will be cloned to prepare nucleic acid hybridization probes to detect the presence of this RNA in trees displaying varying degrees of bud failure symptoms.
5. The almond RNA will be infected into indicator plant cells to determine if it can be recovered and thereby ascertain its infectious nature.
6. Protein from nonpareil bud failure cells have been used to inoculate spleen cells from BALB/C mice. The antibody producing cells are being cultured to determine if they produce unique antibodies to bud failure cell components. The enzyme-linked immunosorbent assay (ELISA) will be used to determine the nature of the antibody produced. All cross reactive antibodies will be eliminated and only those that faithfully distinguish bud failure cells will be saved. This will not only determined the unique nature of bud failure cells but will also serve as a means of rapid identification of bud failure clones.

Results and Discussion

Based on internal molecular weight standards (ribosomal and transfer RNA) and the electrophoretic mobility of potato spindle tuber viroid, the almond RNA position has been calculated to be the equivalent of 390 bases long. This places it in the same size class as potato spindle tuber viroid as well as others. The RNA position has been detected in normal tissue culture cells but not from normal leaf tissue. Viroids are a minor class of

RNA in infected cells but are induced by stress. Since subjecting tissue to adapt to tissue culturing can be viewed as a form of stress, it is not suprising that a putative viroid can be amplified in culture. We must determine if the almond RNA is a result of differential genic activity of a normal almond gene that is activated upon tissue culturing since the RNA position has been seen in normal almond tissue culture cells. An alternative explanation would be that this almond clone harbors a viroid that is of very low titer until some induction event elicits its reproduction. The two approaches to distinguish between these alternatives is to compare the RNA sequence of a known viroid and the almond RNA, and to determine whether it is infectious, particularly in a good indicator host. Until these approaches are taken, we will not be certain about the nature of the RNA observed.

The application of the hybridoma technology in developing a marker system for the cling peach breeding program has proven to be a very powerful tool. Hybridoma clones have been produced which recognize distinguishable allelic domains of protein which segregate in a mendelian pattern. The same technology has been applied to bud failure protein from nonpareil tissue culture cells. Hybridoma cells have been derived and are being nurtured until their population is sufficiently large to allow for the detection of antibodies in their spent culture medium. This aspect brings in a powerful tool in the ability to recognize specific differences using a rapid assay technique. If it proves to be positive, it would serve the purpose of screening for bud failure clones.



ALMOND RNA EXTRACT