A MOLECULAR MARKER SYSTEM TO DETERMINE MANGO LEAF AGE

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Application of potassium nitrate to mango leaves has been shown to stimulate flowering. However, the developmental-physiological state of leaves receptive to this stimulation has not been critically defined. The use of this practice to manipulate mango production is thus uncertain and not cost effective at the present time.

We are interested in developing a molecular system to mark the developmental/physiological stages of mango leaves. Once established, this marker system can be used to link the developmental-physiological stage(s) of mango leaves with their responsiveness to potassium nitrate stimulation for flowering. The ultimate goal is to develop a simple yet reliable diagnostic test for effective application of this stimulator to manipulate mango production.

The protein profile of mango leaves, as generated by the technique of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), was selected for evaluation as a marker system. The objective is to identify developmental-physiological stage-specific protein(s) and use them as molecular markers or indicators to time the application of potassium nitrate for flower induction.

Pilot experiments have been carried out to find if the protein profiles of mango leaves at different developmental stages show distinction in their polypeptide species. A procedure was established to extract total protein from mango leaves using a buffer containing 0.05M Na phosphate buffer, pH 7.5, 0.1M NaCl, 2 percent 2-mercaptoethanol and 1 percent SDS. The total proteins from leaves of three developmental stages were then extracted and analyzed by SDS-PAGE. Results indicate that distinct protein species can be detected in leaves at different developmental stages.

To improve the resolution of proteins, a two-dimensional gel electrophoresis procedure was established for mango leaf proteins. Total leaf protein was prepared by extracting leaf acetone powder with 50mM Tris buffer, pH 6.8, containing 2% SDS and 2mM EDTA. The leaf proteins were first separated by isoelectric focusing (IEF) (pH range 4.5-6.5) and then by SDS-PAGE. Results reveal that many proteins differing in charge as well as size can be detected by this technique. Use of silver instead of Coomassie staining can further enhance the number of detectable proteins, but the background of the gel is higher. This 2-D gel system was applied to analyze the proteins of 2-, 8-, and 13-week-old leaves. Results reveal that although most of the proteins are common to these leaves, there are proteins specific to each of the developmental stages.

Finally, we explored the use of an in vivo labeling technique to further enhance the sensitivity of protein detection. Freshly harvested leaves were incubated with 35[S] methionine for one hour before their proteins were extracted. The radioactive proteins were then separated by 2-D gel electrophoresis and detected by autoradiography. Figure 1 shows the labeled protein profile of a 10-week-old mango leaf; many proteins can be clearly detected and identified in the autoradiogram. When this technique was used to analyze the leaf protein profiles of 3-, 13-, and 20-week-old leaves, distinct proteins, qualitatively and/or quantitatively, specific or characteristic for each of the leaf ages, can be identified (Figure 2).

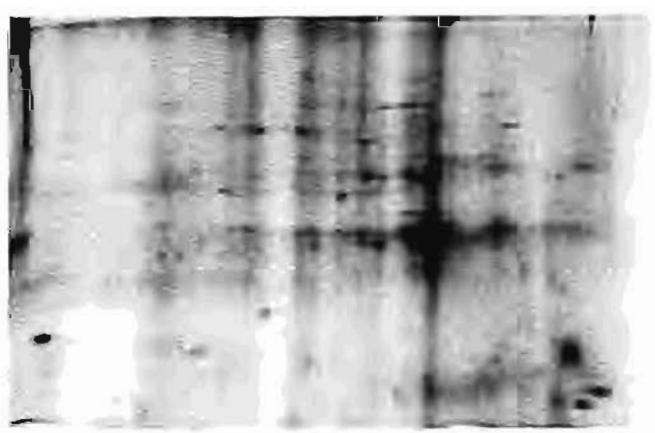

Figure 1. Two-dimensional gel electrophoresis of in vivo labeled mango leaf proteins (horizontal separation by IEF, vertical separation by SDS-PAGE).

In summary, we have developed a molecular marking system, which involves 2-D gel electrophoresis and in vivo protein labeling techniques, for mango leaf proteins. It has high resolution and sensitivity in detecting mango leaf proteins. Using this system, proteins specific to leaf ages can be identified. Further development and extension of this system to establish a diagnostic test for effective application of potassium nitrate to manipulate mango production appears promising.

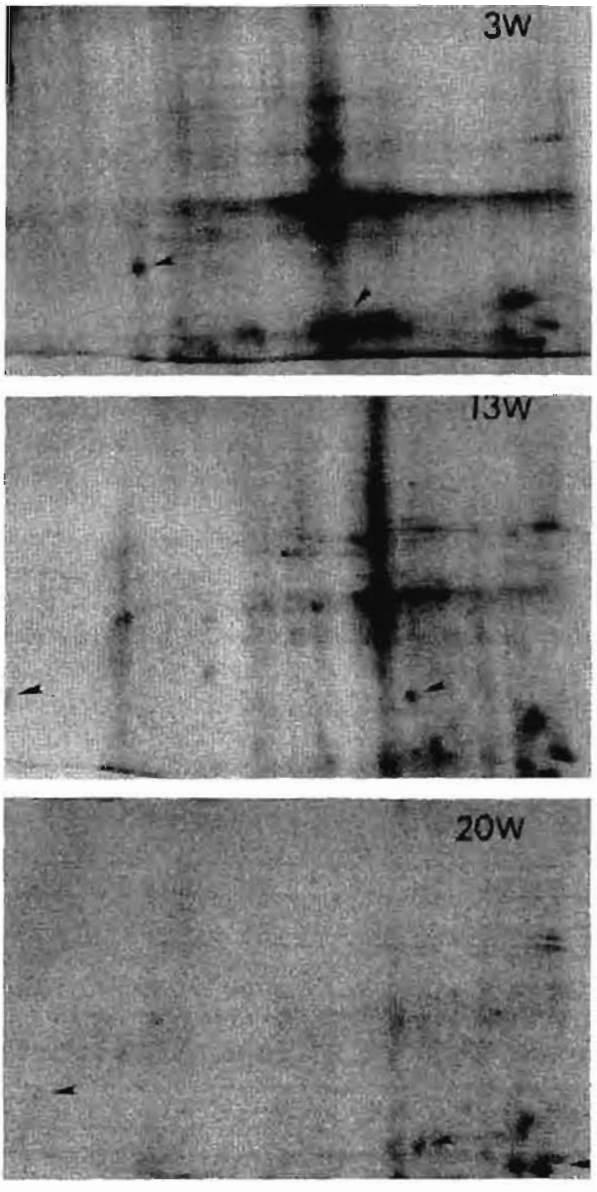

Figure 2. Two-dimensional gel electrophoresis of in vivo labeled proteins of 3-, 13-, and 20-week-old mango leaves (horizontal separation by IEF, vertical separation by SDF-PAGE).