DIFFERENTIAL DISPLAY PROTEIN PROFILING OF SOME COMMON PRODUCTION AND POSTPRODUCTION PROBLEMS IN ROOTCROPS

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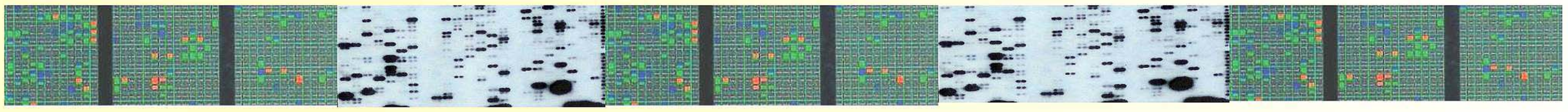


Abstract

Protein expression profiling is a powerful tool used to discover proteins associated with the biochemical state of a sample in response to some stimuli. To obtain fundamental knowledge on the biochemical events underlying the causes of existing production and postproduction problems in rootcrops, protein expression profiles were established on major disorders and organoleptic constraints. Five expression profiles were established to address the following concerns: acrid and non-acrid taro corms, anthracnose-infected and non-infected yam, spider mites and scale-insects infested and non-infested cassava and two profiles for different varieties of blight-infected taro. Differences in banding patterns of proteins extracted from different samples were compared and analyzed for overexpression and non-expression of specific proteins. Several differentially expressed specific proteins were identified which can be used as markers for diagnostic purposes and target biomolecules for designing appropriate control measures.

Introduction

The application of biotechnology in rootcrop agriculture offers tremendous potentials in solving production and postproduction problems of root crops. However, before such measures could be introduced, it is necessary to obtain fundamental knowledge on the biochemical events underlying the causes or existence of such problems. Protein expression profiling has provided researchers the overall pattern of gene activity in a sample and knowledge as to which particular genes turn on and off in response to some influence. The pattern serves as a shorthand signature reflecting the biochemical state of a sample under some specific condition. Expression profiling has proved invaluable to cell biologists because knowledge of the proteins that predominate after a tissue is exposed to different conditions can provide insights on how the tissue normally compensates for disruptions and what goes wrong when diseases develop. Such signature proteins are not only useful for diagnostic purposes but also in tailoring appropriate interventions through controlled expression in solving production and postproduction problems in root crops.

Objectives

1. To establish protein expression profiles of acrid and non-acrid taro corms, anthracnose-infected and non-infected yam, spider mites and scale-insects infested and non-infested cassava, and different varieties of blight-infested and non-infested taro.
2. To identify "signature" proteins (molecular mass only) as biochemical markers of the above phenotypic and organoleptic constraints.

Methodology

Wash and air dry

Cut into fine pieces and macerate using a mortar and pestle in the presence of sterilized broken pasteur pipette or glass

Homogenize with lysis buffer

Centrifuge for 5000 rpm, 30 min

precipitate

CLEAR EXTRACT

DISCARD

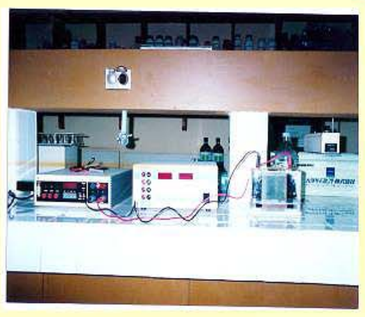
ELECTROPHORESIS

Preparation of Cellular Extracts for Protein Expression Analysis

SAMPLE PREPARATION
10 ug protein sample + 10 ul of SDS-loading buffer

Heat at 98°C for 3 min → Cool on Ice

Apply To a 12 % Electrophoresis Gel



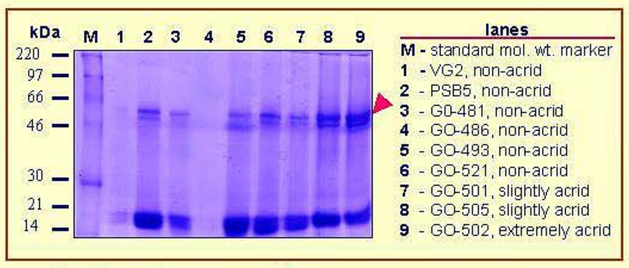
Run the Gel at 40 V for 3 Hours

Stain with COOMASSIE BRILLIANT BLUE for 1 H

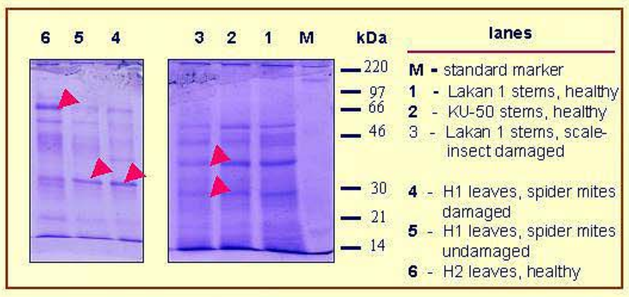
Destain w/ methanol-acetic acid solution until bands are clear

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) : Sample preparation and set-up

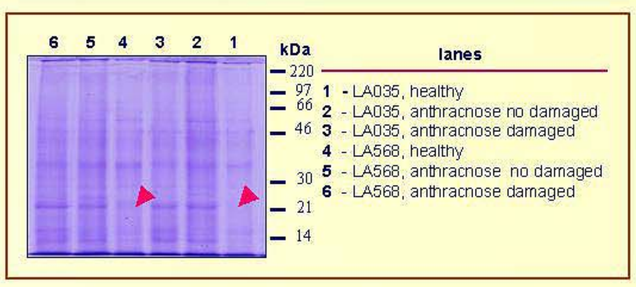
Results



Acrid and non-acrid taro corms

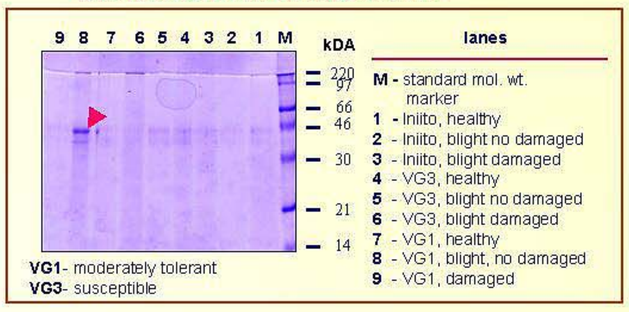


Spider mites and scale-insects infested cassava stems and leaves

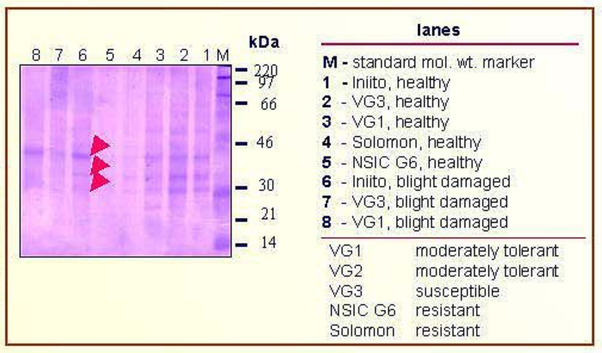


Healthy and anthracnose-infected yam leaves

Fresh and stored cassava roots



Healthy and blight-infested taro leaves



Healthy and blight-infested taro leaves

Potential biochemical markers

Condition	kDa protein
Acridity in taro	55 kDa overexpression
Blight infestation in taro	45 kDa overexpression
Scale insect infestation in cassava stems	40 kDa non-expression
	28 kDa non-expression
Spider mites infestation in cassava stems	70 kDa non-expression
	30 kDa overexpression
Anthracnose infection in yam	22 kDa expression
Stored cassava roots	35 kDa non-expression

Discussion

Five protein expression profiles were established. These were: profiles for acrid and non-acrid taro corms, anthracnose infected and non-infected yam, spider mites and scale-insects infested and non-infested cassava and two profiles for different varieties of blight-infected taro. Differences in protein bands of samples under comparison were observed. For instance in acrid and non-acrid taro corms, a 55 kDa protein is overexpressed in all acrid varieties than the non-acrid ones. In the case of anthracnose infection in yam, highly susceptible varieties were able to display a very distinct protein band of 22 kDa which is absent in the non-infected yam varieties.

Insect infestation in cassava such as spider mites and scale-insects also produced highly differentially-expressed proteins than the control non-infested ones. A 30 kDa protein is consistently expressed and upregulated in the spider mites-infested varieties indicating that this protein was produced by the host plant in response to this infestation.

The different varieties of susceptible varieties of taro to bacterial blight also produced an overexpressed 45 kDa protein band which was distinguishable from the resistant varieties.

Conclusion and Implication

Thus, protein expression profiling provides a tool for determining biochemical markers that can be used for diagnostic purposes. It can also be exploited to develop control measures for prevention of root crop diseases as well as for genetic improvement of crops for useful traits or desirable qualities.

References

1. The Integrated National Root Crops Industry Research and Development Agenda 2000. Prepared by the National RDE Network and Philippine Root Crops Research and Training Center.
2. Faylon, P. S. Status and Prospects of Biotechnology in Agriculture and Natural Resources. Paper presented during the Zonal Seminar-Workshop on Biotechnology, 6-7 December 2000, Visca, Baybay, Leyte.

Acknowledgment

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