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Protein extraction for *Cocos nucifera* with and without lethal yellowing phytoplasma: gel-based proteomics

Celso REYES-MARTÍNEZ[1], Danae MORALES-ANGELES[2], Maria NARVAEZ-CAB[3], Juan Alberto OSUNA-CASTRO[2], Daniel ZIZUMBO-VILLARREAL[3], Carlos Mariano OROPEZA-SALÍN[3], Elpidio PEÑA-BELTRÁN[2]
[1]*Universidad de Colima, Facultad de Ciencias Químicas, Colima, México*
[2]*Universidad de Colima, Facultad de Ciencias Biológicas y Agropecuarias, Colima, México*
[3]*Centro de Investigación Científica de Yucatán, Unidad de Biotecnología, Mérida, México*

Abstract

Two-dimensional gel electrophoresis (2-DE) is one of the two methodologies to separate and identify gene expression at the proteome level. However, the separation of proteins from plant tissue extract is often complicate, because it is necessary to modify the protocols depending upon the type of tissue and upon the presence of different interfering compounds. In this work it is established a routine procedure for the application of proteomic analysis to study interaction between palm coconut and lethal yellowing (LY) phytoplasma. The characteristics of protocol include: (i) cleanup of pigments and contaminants of the trunk palm coconut tissue, using a solution of acetone with TCA and 2-mercaptoethanol. (ii) Phenol extraction of proteins in presence of methanol with ammonium acetate. 2-DE analysis revealed differences in palm coconut with and without LY agent and similitude between palm ecotypes.

Key words: phytoplasma, lethal yellowing, *Cocos nucifera*, proteomic, 2-DE.

Introduction

The palm coconut (*Cocos nucifera* L.) is economically one of the species most important in the tropics, because it is food source, drink, coat and work; it is grown extensively in the humid tropics worldwide, including Mexico (Parrota, 1993). Lethal yellowing (LY) is a pandemic disease that affect coconut palm, and its agent is a phytoplasma. However, some palm coconut types are tolerant or resistant to phytoplasmal infections, being thus asymptomatic (Zizumbo *et al*., 2001). Knowledge of coconut phytoplasma biology is limited because they are noncultivable and experimentally inaccessible in their host, this was the main problem for the investigation of the plant-phytoplasma interaction (Lee *et al*., 1998; Christensen *et al*., 2005).

Two-dimensional gel electrophoresis (2-DE) approach could be applied to differentiate between palm coconut with and without LY phytoplasma. 2-DE was established by O'Farrell in 1975 and at this moment is quantitatively the strongest technique to separate and identify protein; it is cost-effective, and it is the most suitable proteomic approach for species in which the genome is not (fully) sequenced (Carpentier *et al*., 2005). The goal of this work is to establish a routine procedure for the application of proteomic analysis to study interaction between palm coconut and phytoplasma of lethal yellowing.

Materials and methods

The present work reports a study carried out in the national collection of germoplasm of Mexican palm coconut, located in the north coast of the peninsula of Yucatan, where LY was present from several years already.

Tissue samples consisting of wood shaving removed from interior basal trunk of palms with and without symptoms of LY; samples were extracted perforating around 10 cm in trunk of each palm using a portable electrical drill. Extraction of total protein was performed using two different protocols, first based on the procedure of Shewry *et al*. (1995) and Wang *et al*. (2003) (method 1), and the second developed by Wang *et al*. (2003) and Cho *et al*. (2006) (method 2), with some modifications. Protein was quantified by the Bio-Rad protein assay (Bradford, 1976) with bovine serum albumin as standard. For mini 2-DE, protein samples were applied in 125 µL of rehydration buffer by reswelling 7 cm Immobiline DryStrip (pH 3-10, Bio-Rad) for 16 h. IEF was performed in Protein IEF Cell (Bio-Rad) at 20ºC, applying 250 V for 20 min, 250 to 4,000 V for 2 h, and then 4,000 V until reaching 10,000 V/h. Focused strips were equilibrated using dithiothreitol and iodoacetamide solutions, and then positioned on a 12.5% minigel (1 mm thick). Secondary SDS-PAGE was carried at 80 V for 2.5 h. After electrophoresis, proteins were visualized with colloidal CBB G. Finally, the spots were quantified with the Delta 2D software.

Results

The extraction of total proteins in tissue of palm coconut was carried out by two different protocols, which differ only in the cleaning of the plant tissue, the precipitation of the proteins is similar in both protocols. The first used protocol (method 1) showed low resolution in the pattern of proteins in 1-DE (figure 1A). The method 2 was where the isolated proteins of tissue trunk of the coconut palms protocol showed major resolution in 1-DE and 2-DE (figures 1B and 1C). Besides, the pH 3-10 Immobiline DryStrip pattern reveals well-resolved spots throughout the gel in 2-DE analysis.

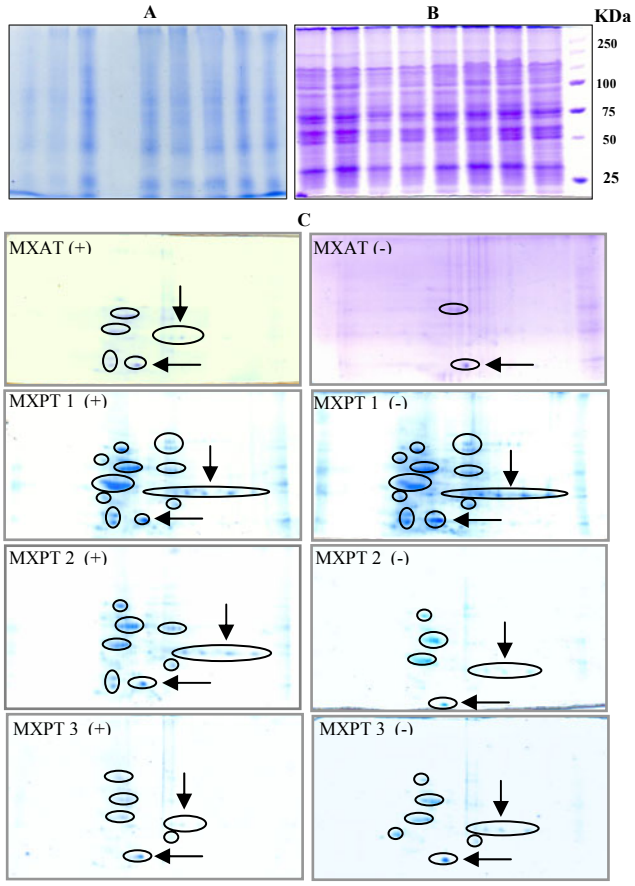

Figure 1. Representative 1-DE and 2-DE patterns of the proteins. (A) 1-DE developed by method 1. (B) 1-DE developed by method 2. (C) About de 50 µg of total protein were resolved by 2-DE for the different ecotypes. Arrows indicate the zone between the different ecotypes with and without LY agent.
(In colour at www.bulletinofinsectology.org).

In figure 1C, are shown the different ecotypes of coconut palm with and without presence of the phytoplasma, analyzed in gels 2-DE: they differ in quantity and in expression of proteins. The areas marked with circles in the gels 2-DE represent proteins that are common in the different analyzed ecotypes, the numbers of spots was estimated in the Delta 2D software, the greater amount from expressed proteins was in ecotype MXPT1.

Discussion

This is the first study of 2-DE in palm coconut establishing the extraction conditions for this plant species. Our study demonstrates as the use of a simple protocol that involves organic solvents, such as 10% TCA/2-mercaptoethanol in acetone, can remove water-soluble contaminants after, intensive washing with organic solvents (2-mercaptoethanol in acetone). The tissue can be extracted in homogenization buffer, followed by phenol extraction methanol/ammonium acetate precipitation. We picked several zones (arrow, figure 1C) where one or several proteins are present in the different ecotypes with and without the presence of the LY agent. A remarkable reduction in the expression in the ecotypes without phytoplasma, with the exception of MXPT1, was observed. The number of estimated spots in the Delta 2D software is present in the ecotype MXAT, where 90% of palm mortality was observed; the spot number was minor in comparison with spots observed in the ecotypes MXPT (70% of resistance). This result could have relationship with the fact that this ecotype is the more susceptible to the LY disease. It will be now necessary to determine if the proteins expressed in the different ecotypes, have or not relationship with tolerance or resistance to LY.

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Corresponding author: Elpidio PEÑA (e-mail: Elpidio.pb@prodigy.net.mx), University of Colima, Facultad de Ciencias Biológicas y Agropecuarias, Colima, CP 28100, México.