

Glucanases and quitinases detected in the sugarcane – *Sporizorium scitamineum* interaction.

Marcia Fajardo (1), Ricardo Acevedo (1), María La O (1), Rosemary López (2), Eida Rodríguez (1) y Ondina León (2). (1) Instituto Nacional de Investigaciones de la Caña de azúcar (INICA), MINAZ. Carretera al CAI Martínez Prieto Km 2 ½, Boyeros, Ciudad de La Habana, Cuba. E-mail: acevedo@inica.edu.cu. (2) Centro Nacional de Sanidad Agropecuaria (CENSA), MES, Cuba.

The defense of the plants against the attack of the pathogens is physiologically complex and being of structural and chemical nature (1). The answer of induced resistance is accompanied by such physiologic changes like hypersensitive answer, cellular wall alterations, production of new secondary metabolites and actively synthesized proteins well-known as proteins related with the defense (PRs). Among the “PR proteins” they stand out the glucanases and quitinases. In this work were used two immunochemical techniques to detect these proteins in sugarcane plants infected with *Sporizorium scitamineum* (Piepenbring, Stoll & Oberw.). 30 buds of Ja60-5 (susceptible) and M31/45 (resistant) were inoculated with the fungus and same number of buds were left without inoculation. The Western blot was carried out at 6, 24, 72 hours and 7 days post-inoculation (2). For immunoelectron microscopy, 1 x 4 mm of tissue samples were fixed in 4% paraformaldehyde in 0.025 M cacodylate buffer pH 7.2 for 1 h, postfixed in 1% osmium tetroxide in the same buffer, dehydrated in an ethanol series embedded in LRW resin (3). Immunogold labeling was performed on ultrathin sections mounted on nickel grids. Antiserum against glucanases and quitinases were used like the primary antibody and anti-IgG of rabbit with colloidal gold as secondary antibody. Controls were performed by omitting incubation with the primary antibody. Similar proteins of β -1,3-glucanases were detected as constitutive (PM 47.5 KDa approx.) and induced by the fungus (PM between 60 y 42 KDa) at 6 h post-inoculation in the resistant cultivar and at 24 h in the susceptible. Quitinases with PM 73 KDa are constitutive in both cultivars and the quitinases with 60 and 36 KDa were detected in M31/45 starting from the 6 h. The observation of the immunogold with the electronic microscope could be defined that the glucanases is disseminated by the protoplasm and the quitinases are inside of some vacuoles, lake reported by other investigators (4). The pattern of temporary and space expression of proteins β -1,3-glucanases and quitinases was determined. The two immunochemical methods used allowed to detect these proteins in the sugarcane - *S. scitamineum* sistem.

References

1. Collinge *et al.*, Modern Approaches and perspectives. (1993), 391-433, New York.
2. Lopez, R. Ph D Tesis (2002), CENSA, La Habana, 118 p.
3. Acevedo *et al.*, Chromosoma (2002), 110: 559-569.
4. McCollum *et al.*, Physiol. Plant (1997), 99: 486-494.



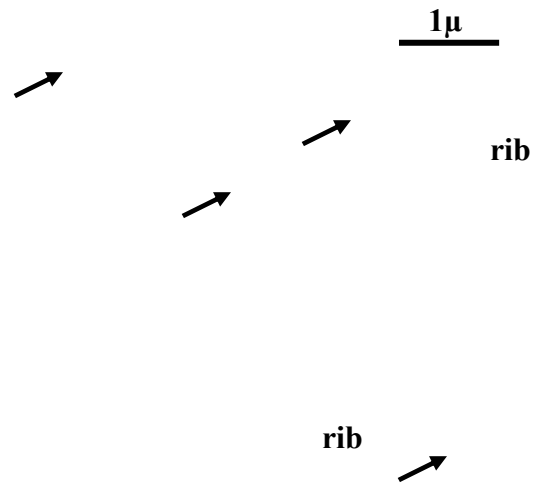


Fig. 1. Immunolabeling of the β -1,3-glucanases proteins (arrow), located in the cellular protoplasm of M31/45 buds tissues. Abundant ribosomes are present (rib).

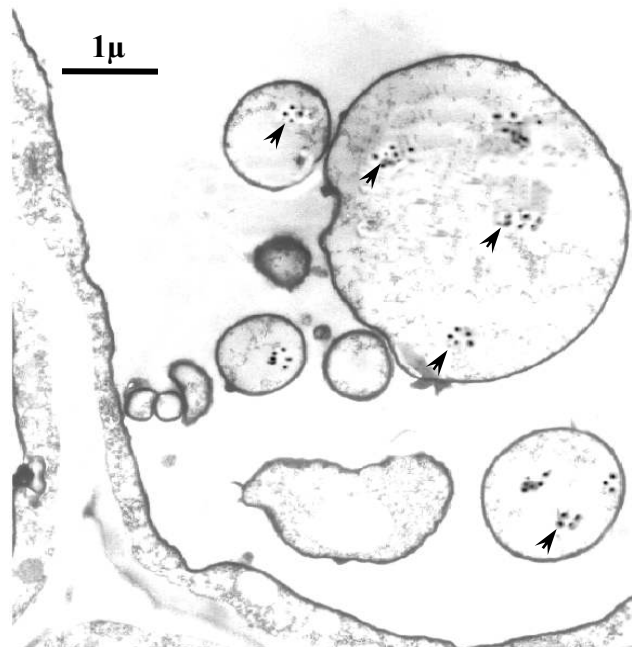


Fig. 2. Immunolabeling of the quitinases proteins (arrow head), located in vacuoles of the cellular cytoplasm of M31/45 buds tissues.