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Glycoproteins from sugarcane plants regulate cell polarity of *Ustilago scitaminea* teliospores

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Summary

Saccharum officinarum, cv. Mayarí, is a variety of sugarcane resistant to smut disease caused by *Ustilago scitaminea*. Sugarcane naturally produces glycoproteins that accumulate in the parenchymatous cells of stalks. These glycoproteins contain a heterofructan as polysaccharide moiety. The concentration of these glycoproteins clearly increases after inoculation of sugarcane plants with smut teliospores, although major symptoms of disease are not observed. These glycoproteins induce homotypic adhesion and inhibit teliospore germination. When glycoproteins from healthy, non-inoculated plants are fractionated, they inhibit actin capping, which occurs before teliospore germination. However, inoculation of smut teliospores induce glycoprotein fractions that promote teliospore polarity and are different from those obtained from healthy plants. These fractions exhibit arginase activity, which is strongly enhanced in inoculated plants. Arginase from healthy plants binds to cell wall teliospores and it is completely desorpted by sucrose, but only 50% of arginase activity from inoculated plants is desorpted by the disaccharide. The data presented herein are consistent with a model of excess arginase entry into teliospores. Arginase synthesized by sugarcane plants as a response to the experimental infection would increase the synthesis of putrescine, which impedes polarization at concentration values higher than 0.05 mM. However, smut teliospores seem to be able to change the pattern of glycoprotein production by sugarcane, thereby promoting the synthesis of different glycoproteins that activate polarization after binding to their cell wall ligand.

Abbreviations: DFMO, α-difluoromethylornithine; HMMG, high molecular mass glycoproteins; I.d., internal diameter; IEC, intestinal epitelial cells; MMMG, mid-molecular mass glycoproteins; Tris-HCl, tris (hydroxymethyl) aminomethane hydrochloride

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Introduction

Smut is a major disease of sugarcane caused by *Ustilago scitaminea*. Spore germination occurs on the internode surface and it is followed by the formation of appressoria mainly on the inner scale of young buds and on the bases of emerging leaves (Waller, 1970). Entry into the meristem in the bud occurs between 6 and 36 h after the teliospores are deposited on the surface (Alexander and Ramakrishnan, 1980). Hyphal growth occurs throughout the infected plant, but mostly in the parenchyma cells of the lower internodes. In the upper internodes, hyphal growth concludes with the formation of the whip (sori with teliospores). Hyphae do not penetrate into the cells of the scale leaves (Singh and Budhraja, 1964), therefore buds tightly enclosed within scale leaves can escape infection. It has been proposed that varietal resistance is determined by morphological features of buds (Waller, 1970). However, other authors have suggested that resistance is based on chemical properties rather than on bud morphology. Lloyd and Naidoo (1983) isolated glycosidic substances from fresh bud scales and established a linear correlation between resistance to smut and glycoside concentration. These substances were previously identified as flavonoids, which inhibited teliospore germination (Lloyd and Pillay, 1980).

Resistance to disease seems to be a multifactorial process. The response phase includes accumulation of different compounds such as: phytoalexins (i.e. low molecular mass antimicrobial compounds that accumulate at sites of infection); systemic enzymes that degrade pathogens (e.g. chitinases, β-1,3-glucanases and proteases); systemic enzymes that generate antimicrobial compounds and protective biopolymers (e.g. peroxidases and phenoloxidases); biopolymers that restrict the spread of pathogens (e.g. hydroxyproline-rich glycoproteins, lignin, callose); and regulators of the induction and/or activity of defensive compounds (e.g. elicitors of plant and microbial origin, immune signals from primed plants and compounds, which release immune signals) (Kuc, 1990). Other glycoproteins are involved in resistance responses. Three varieties of sugarcane, defined by their relative resistance to smut, have been previously used to study the production of glycoproteins after smut infection. Barbados variety is extremely susceptible; Jaronu shows moderate resistance, while Mayari is extremely resistant to smut. Vegetative buds of sugarcane were inoculated with teliospores and cultured under field conditions for 12 months. Soluble polysaccharides and glycoproteins of juices increased after infection in Jaronu and Mayari plants, but decreased in Barbados specimens (Martínez et al., 2000). The primary response of smut-resistant sugarcane plants to infection is the production of several glycoproteins, defined as mid-molecular mass (MMMG) or high molecular mass (HMMG) macromolecules. Teliospore germination in the presence of both MMMG and HMMG decreased about 50% following 5 h of teliospore contact with glycoproteins. This may be related to the ability of glycoproteins to produce cytoagglutination. Binding of fluorescein-labelled glycoproteins was studied by fluorescence microscopy, showing that staining of cells was not even, but mainly restricted to the contact zone between two individual teliospores when aggregated (Fontaniella et al., 2002). Ungerminated fungal spores generally lack specific localization of their organelles and most are apparently able to germinate from any point. Hence, one of the major changes that must occur during germination is the establishment of cytoplasmic polarity (Fontaniella et al., 2002). Bachewich and Heath (1998) have shown that F-actin participates in initiating hyphal tip formation through the recruitment and stabilization of membrane-bound and cytosolic factors required to build the new tip in *Saprolegnia ferax*.

In this paper, we have examined the role of sugarcane glycoproteins in the resistance of sugarcane to smut. We found that they impede cell polarization by inhibiting the protrusion of the germinative tube and spore germination, which allows us to propose that inhibition of teliospore germination constitutes a defence mechanism involved in resistance of sugarcane to smut.

Material and methods

Plant material

Field-grown *Saccharum officinarum* L., cv. Mayari 55-14, was used throughout this work. Teliospores of *U. scitaminea* Syd. were collected from whips of infected plants in experimental crops of the National Institute for Sugarcane Investigation (INICA) in Matanzas, Cuba.

Preparation of soluble glycoproteins

Stalks from inoculated and healthy 22 month-old plants were mechanically crushed immediately after cutting and the raw juice was centrifuged at 5000*g* for 15 min at 2 °C. The pellet was discarded and the supernatant was filtered through filter paper. Centrifuged juice was chromatographed