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SHORT COMMUNICATIONS

Correlation of Antifungal Properties and β-1,3-Glucanases in Aqueous Extracts of Kernels from Several Varieties of Corn

Keywords: *Aflatoxins; Aspergillus flavus; enzyme inhibitors; hydrolases*

INTRODUCTION

The complex relationship between fungal infection (*Aspergillus flavus*) and aflatoxin formation in major crops including corn is an area of intense research worldwide. Currently, one of the objectives in solving the problem in corn is the identification of resistance genes and specific proteins that could be transferred within the species through classical breeding or genetic engineering. It is recognized in plant pathology that chitinases, glucanases, and other hydrolases are enzymes that have an antibiotic role in plants (Boller, 1985), that is, they do not attack endogenous carbohydrates but rather protect plants from fungal pathogens by digesting the invading fungal cell walls. It is known from earlier reports that fungal walls contain significant amounts of β-1,3-glucans and chitin that are readily attacked by glucanases and chitanases.

In this study, our objective was to develop a similarity profile of β-1,3-glucanase activity and antifungal properties in the kernels of several varieties of mature corn. Kernels from open-pollinated varieties with extremes in aflatoxin contamination due to *A. flavus* infection in the field were chosen (Zummo and Scott, 1989).

EXPERIMENTAL PROCEDURES

Protein Extraction. One hundred milligram samples of milled kernels from six varieties were extracted in 1.0 mL of water using a mortar and pestle at 25 °C. The homogenates were clarified by centrifugation at 5000*g* for 30 min at 25 °C. Protein contents of each solution were determined by the standard BCA procedure (Pierce Chemical Co.).

Analytical Procedures. Separation of native cationic proteins by cathodic polyacrylamide gel electrophoresis, PAGE (Bio-Rad), was conducted on vertical slabs at pH 8.5 according to the procedure of Mikola (1965) using a borate–potassium hydroxide buffer system (Figure 2). The stacking gels contained 4% (w/v) acrylamide, and the running gels contained 7.5% (w/v) acrylamide. Electrophoresis was conducted at a constant current of 50 mA/slab for approximately 2 h at 10 °C. The active β-1,3-glucanase enzyme bands were visualized on the gels as described by Pan et al. (1989). Each gel was incubated at 40 °C for 30 min in a mixture containing 75 mL of 0.05 M potassium acetate (pH 5.0) and 1 g of laminarin dissolved in 75 mL of water by heating in a boiling water bath. The gel was washed three times with distilled water after incubation. The PAGE gel was then put into a glass tray containing 0.3 g of 2,3,5-triphenyltetrazolium chloride in 200 mL of 1.0 M NaOH. The tray was kept in a boiling water bath until red bands appeared (about 10 min). The β-1,3-glucanase activities of the extracts in Table 1 were detected by the laminarin–dinitrosalicylate assay of Abeles and Forrence (1970). Bioassays of fungal growth inhibition were carried out in tissue cluster plates using potato dextrose agar as described in an earlier study (Neucere and Zeringue, 1987).

RESULTS AND DISCUSSION

A comparative activity profile of β-1,3-glucanases in three resistant and three susceptible genotypes of corn is shown in Table 1. Considerable differences were noted in extractable protein from the six genotypes (range of 0.50–0.93 mg/mL). On the basis of total protein content, all of the resistant genotypes contained slightly higher glucanase activity than the susceptible ones.

Assays for inhibition of *A. flavus* growth by the crude extracts are shown in Figure 1. The arrows point to rows of test wells that showed inhibition of spore germination and fungal growth. Only one of the resistant lines, MP 313 e (row 3), showed inhibition, whereas two of the three susceptible lines, T 216 (row 4) and SC 212 m (row 5), showed inhibition of fungal growth.

Assays for β-1,3-glucanase activity in crude extracts on polyacrylamide gels are shown in Figure 2. The cathodic system used here detected positively charged isozymes. Glucanase activity appeared as bright red zones on the gels. All of the bands were diffused and migrated between R_f 0 and 1.5. On the basis of the

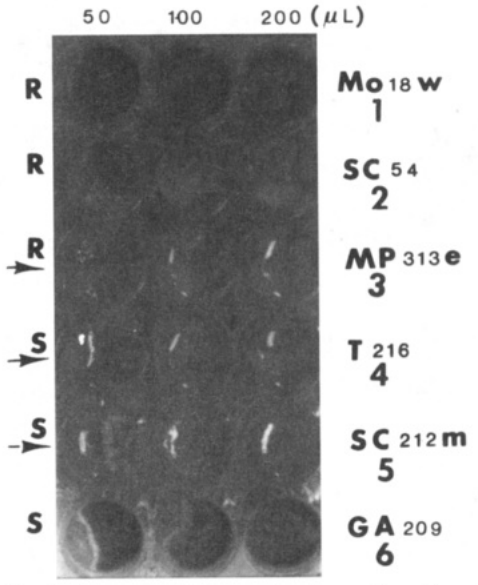

Figure 1. Antifungal effects on *A. flavus* inoculum in solid agar of several aliquots of extracts from resistant (R) and susceptible (S) varieties of corn. Dark areas denote massive mycelial growth after 7 days of incubation. Arrows point to inhibition of fungal growth.

Table 1. Measurement of β-1,3-Glucanases in Kernels of Field Corn That Are Resistant (R) and Susceptible (S) to *A. flavus* Infection

genotype	protein[a] (mg/mL)	β-1,3-glucanase activity[b] (units/mg of protein)
1, MO 18W (R)	0.49	0.55
2, SC 54 (R)	0.93	0.64
3, MP 313 e (R)	0.67	0.63
4, T 216 (S)	0.85	0.49
5, SC 212 m (S)	0.52	0.42
6, GA 209 (S)	0.52	0.37

[a] Data obtained from water extracts by the standard BCA procedure. [b] One unit of β-1,3-glucanase activity liberates 1 mg of glucose from laminarin for 2 h, pH 5.5, 50 °C.

intensity of staining, varieties 1, 3, and 6 showed the highest activity.

In summary, the extractable protein in the six genotypes of corn ranged from 0.5 to 0.93 mg/mL. Slightly higher specific glucanase activity occurred in the resistant genotypes compared to the susceptible ones. *In vitro* bioassays of fungal growth inhibition, however, did not correlate with observed contamination of *A. flavus* and aflatoxin production in field studies. These results established the presence and differences of glucanases in resistant and susceptible corn kernels, but their direct involvement as components of plant defense mechanisms remains for future analytical studies. The complex relationship between *A. flavus* infection and aflatoxin formation *in vivo* is difficult to assess because multiple parameters such as insect damage and agronomic conditions are integral factors for consideration.

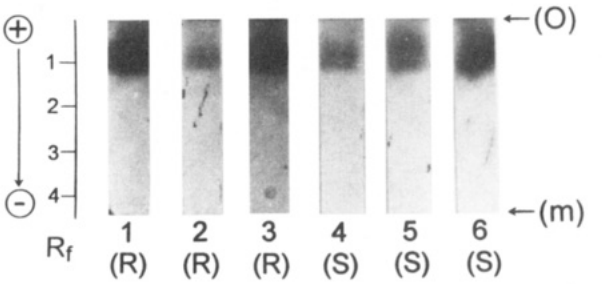

Figure 2. Cathodic PAGE of the native proteins from resistant and susceptible corn varieties 1–6 followed by colorimetric assay for glucanase activity. Semiquantitative differences in total activity are noted by the intensity of staining (Pan et al., 1989). O corresponds to the origin of electrophoresis, and m refers to the lead marker dye.

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