ABSTRACT

The toxic effects of the Fusarium graminearum trichothecene toxin deoxynivalenol were determined on ribosomes and leaf tissues of the fusarium head blight-resistant spring wheat cultivar Frontana and the susceptible spring wheat cultivar Casavant. The use of a poly-U-directed 14C-phenylalanine and deoxynivalenol ribosome-binding assays provided evidence of resistance to the protein-synthesis inhibition effects of deoxynivalenol in the head blight-resistant cultivar Frontana. This is probably due to the existence of a mutation in the peptidyl transferase. This cultivar also exhibited resistance to the membrane-damaging properties of this toxin compared to the other cultivar. This report summarizes the evidence for various kinds of "trichothecene tolerance" mechanisms in fusarium head blight-resistant wheat genotypes. Nat. Toxins 5:234–237, 1997.

Key Words: wheat; deoxynivalenol; Frontana; peptidyl transferase; fusarium head blight

INTRODUCTION

In temperate areas such as eastern North America and western Europe, fusarium head blight of wheat (Triticum aestivum L.) is caused by Fusarium graminearum Schwabe or F. culmorum (W.G. Smith) Sacc. [Miller, 1994]. Most strains of these fungi produce the trichothecene mycotoxin deoxynivalenol [Miller et al., 1991; Muller et al., 1997]. When present in feed, deoxynivalenol has a major impact on animal production [Prelusky et al., 1994]. In some parts of the world, deoxynivalenol is a public health concern. In the United States and Canada, there are government guidelines with respect to allowable concentrations in food [Kuiper-Goodman, 1994].

Many wheat-breeding programs have attempted to develop head blight-resistant cultivars as a solution to the mycotoxin problem, but this has proven difficult. A number of lines from South America and China have been shown to be resistant to fusarium head blight. These include the Chinese lines Su Mei 3 and the Brazilian line Frontana [Miller et al., 1984; Wang and Miller, 1987; Singh et al., 1995]. These lines share a parent descended from Italian, Dutch, and Japanese germplasm (Snijders, personal communication). Frontana was developed from the Italian cultivar Mentana and the Brazilian cultivar Frontiera in 1943 [Beckman, 1953; Singh et al., 1995]. We reported that our accession of Frontana was not only highly resistant to fusarium head blight, but also highly tolerant of the trichothecene mycotoxin deoxynivalenol [Wang and Miller, 1987]. Excepting mutant yeasts, no eukaryotic organism has been shown to display such resistance to a trichothecene.

Trichothecenes are potent inhibitors of peptidyl transferase and cause membrane damage [Feinberg and MacLaughlin, 1989; Khachatourians, 1990]. This report concerns evidence for a modified peptidyl transferase and resistance to electrolyte loss from membrane damage in Frontana, compared to a head blight-susceptible cultivar, Casavant.

MATERIALS AND METHODS

Isolation of Polysomes

Intact polysomes were isolated from seedlings after the method of Laroche and Hopkins [1987]. Seeds were surface sterilized, rinsed twice in sterile water, and planted in moist, sterile sand in trays. These were placed in a dark incubator at 25°C. After 3 days, approximately 20 g fresh weight coleoptile tissue were collected and placed in a mortar and ground with liquid nitrogen, taking care to keep the material covered in liquid nitrogen until powdered. The powdered tissue was then transferred to a smaller mortar in ice. Just as the plant tissue warmed up above 0°C, 60 ml of extraction buffer was added (3 ml/g tissue) and quickly stirred with a rubber spatula, avoiding the formation of ice. All subsequent operations were performed at about 4°C. The extraction buffer consisted of filter-sterilized 200 mM Tris-HCl, 0.6 mM sorbitol, 200 mM KCl, 35 mM MgCl₂, 12.5 mM EGTA, and 15 mM DDT (1,1-bis(p-chlorophenol)-2,2,2 trichloroeth-
and the pellet stored at pH 8.5. After centrifuging, the supernatant was discarded in a glass-teflon homogenizer and centrifuged again at 24,000 g for 10 minutes.

The supernatant was layered on 3 ml of sucrose buffer in Beckman polyallomer centrifuge tubes (14 × 95 mm, 12 ml) and centrifuged at 105,000 g for 4 hours. The sucrose buffer consisted of 1.5 M sucrose, 40 mM Tris HCl, 10 mM MgCl₂, 20 mM KCl, 5 mM EGTA, and 5 mM DTT adjusted to pH 8.5. After centrifuging, the supernatant was discarded and the pellet stored at −60°C. The suspension was diluted to 0.6 OD at 260 nm (Varian DMS 200, Varian Ltd., CA). One tube per run was used to assay purity by measuring absorbance of the suspension at 235, 260, and 280 nm. Values of 260/280 >1.4 and 260/235 >1.6 indicated acceptable purity. Polysomes prepared using this method from rye leaves have been shown to be capable of in vitro translation [Laroche and Hopkins, 1987]. Preparations were made from the Plant Research Centre, Agriculture Agri-Food Canada (Ottawa, Ontario) accessions of the fusarium head blight-resistant cultivar Frontana and the head blight-susceptible cultivar Casavant [Wang and Miller, 1987].

In Vitro Protein Synthesis

Analysis of in vitro protein synthesis was modified according to Laroche and Hopkins [1987] and Wei and MacLaughlin [1974]. The reaction mixture contained 50 mM Tris HCl, 100 mM KCl, 18 mM Mg(C₂H₃CO₂)₂·4H₂O, 0.66 mM spermidine, and 5 mM DTT adjusted to pH 7.6. To 5 ml of the above, 2.5 mg ATP, 0.3 mg GTP, 1.3 creatine phosphokinase, 2.5 creatine phosphate, 2.5 mg Poly-U, and 2.5 mg wheat tRNAs were added. S-100 fraction from wheat germ was prepared according to Laroche and Hopkins [1987]. An amino acid mixture consisting of 19 amino acids minus phenylalanine was prepared to give a final concentration of 0.2 mM [Wei and MacLaughlin, 1974]. Each run involved freshly made ribosomes of the same batch. Typically, 5 tubes were prepared, each containing 100 µg ribosomes, 550 µl energy mix/buffer, 100 µl S-100 fraction, 100 µl amino acid mixture, and 50 µl 14C phenylalanine (ICN, 495 mCi/Mmole⁻¹; final volume 1 ml). Deoxynivalenol was added to the tubes as an ethanol solution which was evaporated under a nitrogen stream under aseptic conditions. Two tubes were used as controls, 3 with the desired concentrations of deoxynivalenol plus a further medium control.

The tubes were incubated in a water bath at 30°C for 3 hours. The reaction was stopped by adding 1 ml 10% trichloroacetic acid (TCA). The material was filtered on a glass microfibre filter (Whatman 2.4 cm GF/C) and washed with five 3-ml portions of 5% TCA. The filter was macerated in 10 ml of scintillation fluid (DuPont Aquasol 2) and refiltered. This was repeated twice to achieve a constant activity of the TCA-precipitated material counted with a Beckman LS-8000 instrument. Data were recorded as the net picomoles of phenylalanine as precipitated protein per 3 hours.

14C Deoxynivalenol Binding

Ribosomes were suspended in a buffer consisting of 200 mM Tris-HCl, 0.6 mM sorbitol, 200 mM KCl, 35 mM MgCl₂, 12.5 mM EGTA, and 15 mM DDT adjusted to pH 8.5. The concentration of ribosomes was adjusted to A₂₆₀ (A₂₆₀ = 1 mM). Concentrations of 14C deoxynivalenol (1.2 µCi/mg⁻¹; [Miller and Arnison, 1986]) of 1, 2, 4, and 6 µM were added as an ethanol solution, which was then taken to dryness. Two ml of ribosome suspension were added to Beckman quick seal polyallomer bell-top centrifuge tubes (13 × 25 mm). 100 µl were removed and radioactivity determined (correcting for quenching) and 100 µl buffer added. The materials were centrifuged with a Beckman Ti 80 vertical rotor at 354,000 g for 10 minutes. The supernatant was layered on 3 ml of sucrose buffer in a vertical rotor at 354,000 g for 4 hours. The resulting slurry was poured into Beckman polyallomer centrifuge tubes (14 × 95 mm, 12 ml) and centrifuged at 105,000 g for 4 hours. The sucrose buffer consisted of 1.5 M sucrose, 40 mM Tris HCl, 10 mM MgCl₂, 20 mM KCl, 5 mM EGTA, and 5 mM DTT adjusted to pH 8.5. After centrifuging, the supernatant was discarded and the pellet stored at −60°C. The suspension was diluted to 0.6 OD at 260 nm (Varian DMS 200, Varian Ltd., CA). One tube per run was used to assay purity by measuring absorbance of the suspension at 235, 260, and 280 nm. Values of 260/280 >1.4 and 260/235 >1.6 indicated acceptable purity. Polysomes prepared using this method from rye leaves have been shown to be capable of in vitro translation [Laroche and Hopkins, 1987]. Preparations were made from the Plant Research Centre, Agriculture Agri-Food Canada (Ottawa, Ontario) accessions of the fusarium head blight-resistant cultivar Frontana and the head blight-susceptible cultivar Casavant [Wang and Miller, 1987].

Electrolyte Loss

Seeds of the wheat cultivars Frontana and Casavant were surface-sterilized with 1% sodium hypochlorite solution and planted in sterilized potting mixture and grown in a controlled environmental chamber at 21°C during the day and 18°C at night, with 16-hour fluorescent illumination (10,000 to 15,000 lux) for 21 days. Leaves were cut into 2 parts (350 mg), rinsed in distilled water, then immersed in 20 ml of either distilled/deionized water or 3 concentrations of deoxynivalenol in 25 ml culture tubes. Tubes were placed on a reciprocal shaker at 150 rpm at 25°C for 24 hours. Aliquots were then tested for electrolytic conductivity, (µ ohms) with a conductivity bridge YSI model 32 (Yellow Springs Instrument Co., OH) with a dip type electrode cell (K = 1.0 ohms/cm; conductance of water approximately 1.0–1.3). The effect of ion leakage from leaf tissues was measured by changes of the conductivity of the solution in which tissues were bathed. Electrolyte leakage (T1) from leaf tissue was determined by subtracting the electrolytic conductivity of DON stock solutions (EC1) from that of the treatment solution (EC2). Leaf segments were then removed and boiled in deionized water for 15 minutes to obtain total remaining electrolyte content (T2). Ten leaves were used at 3 deoxynivalenol concentrations, and the experiment was performed twice. The injury due to the deoxynivalenol
treatment solution (T1) was divided by the total electrolyte content (T1 + T2) to determine the percentage membrane damage [Cossette and Miller, 1995].

RESULTS AND DISCUSSION

Data on the effect of deoxynivalenol on in vitro protein synthesis are present in Figure 1. Mean control activity for the cultivar Casavant was approximately 5 ± 2.1 pmoles 14C phenylalanine per 100 µg polysomes/3h (mean ± standard deviation, n = 9). Protein synthesis at 10 µM deoxynivalenol was 32% of control activity, significantly different from controls (P < 0.05). Mean control activity for Frontana was about 1.1 ± 0.4 pmoles 14C phenylalanine per 100 µg polysomes/3h (n = 18). Protein synthesis did not differ from control values until 120 µM deoxynivalenol (P < 0.001).

The curves from these experiments showed a very steep drop around the effective concentration in accord with similar experiments on mammalian cells [Tate and Caskey, 1973]. This kinetic is found because protein synthesis proceeds until a sufficient number of ribosomes are inactivated by the trichothecene–ribosome binding.

14C-deoxynivalenol-binding experiments resulted in a maximum binding ratio on Casavant polysomes of 0.36 ± 0.13 (n = 4) and for Frontana, 0.17 ± 0.01 (n = 4). Using a paired t-test, these values are significantly different (P < 0.02). The calculated number of binding sites (Bmax) using the direct linear plot method in the 4 Casavant experiments was 70% of the actual value based on the moles of ribosome and deoxynivalenol present. For the Frontana experiments, the calculated number was 84% of the actual value. These 2 measurements provide biochemical evidence for a peptidyl transferase mutation in the ribosomal populations of the wheat cultivar Frontana. Spontaneous mutations of ribosomes affecting tolerance to trichodermin in Saccharomyces cerevisiae were reported by Schindl et al. [1974]. Evidently this category of mutation can occur in wheat.

Electrolyte losses from exposure to deoxynivalenol were lower in leaf tissue of the fusarium head blight resistant cultivar Frontana than the susceptible cultivar Casavant (Fig. 2). Snijders and Schepers [in press] showed that cell injury due to deoxynivalenol was lower in the head blight-resistant genotypes from the Netherlands Department of Agriculture breeding program, compared to susceptible types. Cossette and Miller [1995] demonstrated a similar phenomenon in corn genotypes resistant and susceptible to F. graminearum.

Comparisons of the resistant cultivar Frontana and the susceptible cultivar Casavant have revealed that: (1) germinating tissue of Frontana can tolerate high concentrations of deoxynivalenol [Wang and Miller, 1987]; (2) cell cultures of Frontana in suspension have the ability to conjugate and degrade deoxynivalenol [Miller and Arnison, 1986], (3) Frontana ribosomes have greater deoxynivalenol-tolerance, and (4) Frontana leaf tissue has cell membranes that resist damage by deoxynivalenol. Resistance to the phytotoxic actions of deoxynivalenol is a crucial component of fusarium head blight, explaining 70% of the variation of data from field inoculations of the disease [Snijders and Schepers, in press]. Conversely, the production of deoxynivalenol by the fungus is crucial to its virulence [Desjardins et al., 1996]. Without trichothecene tolerance, invasion by F. graminearum takes place in the absence of plant responses to fungal
invasion requiring the synthesis of new proteins, such as those required to synthesize lignin [Miller, 1989].

ACKNOWLEDGMENT

The method for determining the binding of deoxynivalenol to the wheat ribosomes was demonstrated to me by Dr. Calvin S. MacLaughlin, University of California–Irvine, during a short stay in his laboratory in 1989.

REFERENCES

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