Is fusaproliferin associated with disease symptoms in maize plants?

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SUMMARY

The possible role of fusariotoxin-fusaproliferin in Fusarium disease was investigated with respect to ultrastructure responses in the cells of maize leaves. The seedlings of resistant (Lucia) and susceptible (Pavla) maize cultivars were grown on two fusaproliferin concentrations (5 and 35 $\mu g/mL^{-1}$). Only the higher concentration caused appearance of visible symptoms on the leaves. Structural changes of chloroplasts such as dilatation of grana thylakoids in the mesophyll chloroplasts, thylakoid disorganization, and an increased number of osmiophilic globules (plastoglobuli) in the stroma were observed in mesophyll and bundle sheath chloroplasts of both cultivars. The higher toxin concentration sporadically induced severe damage to the outer chloroplast membrane. The extent of ultrastructure disturbances depended on toxin concentration and it was greater in the susceptible cultivar Pavla. Fusaproliferin may be involved in Fusarium pathogenesis as a virulence factor or, by enhancing activity of some other toxins that might be concomitantly present in the diseased plants .

Key words: leaves, mesophyll, bundle sheath, chloroplast, thylakoids, osmiophilic globules

INTRODUCTION

Members of the genus *Fusarium* produce a range of chemically different phytotoxic compounds, such as fusaric acid, fumonisins, beauvericin, enniatin, moniliformin and trichothecenes. These possess a variety of biological activities and metabolic effects including wilting, chlorosis, necrosis, growth inhibition, inhibition of seed germination, and effects on calli (Desjardins and Hohn 1997). The possible use of fungal toxins as bioherbicides has been extensively reviewed (Duke and Lydon 1993), and some *Fusarium* toxins, such as enniatin and fumonisin, were evaluated for their herbicidal properties(Abbas *et al.* 1991).
A relatively "new" toxin fusaproliferin (FUP) was isolated and purified by Ritieni *et al.* (1997) and can induce teratogenic effects, e.g., cephalic dichotomy,
macrocephaly, and limb asymmetry, in chicken embryos. Later its toxicity to *Artemia salina*, SF-9 insect cells, and IARC/LCL 171 human B-lymphocytes was proved (Logrieco *et al.* 1996). In maize kernels the coproduction of fusaproliferin (FUP) with fumonisin (FUM), moniliformin (MON) known for their phytotoxicity was recorded in different concentrations according to susceptibility (Pascale *et al.* 2002). This led us to investigate the effects of FUP on maize seedlings, since from the phytopathological point of view a relationship between toxins in seed from infected ear and seedling development may exist.

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MATERIAL AND METHODS

Two maize cultivars provided by Zeainvent, Trnava, Slovakia, the resistant (cv. Lucia), and susceptible (cv. Pavla) to *Fusarium* infection (Pastirčák *et al.* 2002) were used. Seeds (20 of each cultivar) were surface-sterilized with 1% sodium hypochlorite (commercial bleach) for 2 min and rinsed three times in sterile distilled water for 2 min. The seeds germinated on moistened filter paper in Petri dishes in the dark at 21 °C for 3 days. The seedlings were selected for uniformity and the filter paper was cut into small pieces (1 cm^2), each containing one germinated seed. The pieces of filter paper with the seedlings were placed on a larger moistened filter paper in jar, on the surface of the solid potato-dextrose–agar (PDA) containing three levels of fusaproliferin concentrations: 0 (control), 5, and 35 $\mu g/mL^{-1}$. Under such conditions the seedlings (five per concentration) grew at the temperature of 21/15°C (day/night), and photoperiod 16/8 h. After 5 days of cultivation the leaves were removed for sampling.

The high toxin concentration in the agar (35 $\mu g/mL^{-1}$) had been effective in our previous experiments on chlorophyll and structure responses in maize seedlings (Šrobárová *et al.* 2004), and the low level ($5\mu g/mL^{-1}$) was the lowest concentration in embryotoxicity bioassay (Ritieni *et al.* 1997).

Fusaproliferin (FUP) isolated and purified by Ritieni *et al.* (1997) was dissolved in methanol >99% Mikrochem Bratislava to make a stock solution (concentration 0.5 $mg.mL^{-1}$). To obtain the final concentrations, the stock solution was diluted with PDA. For electron microscopy, segments from central part of the 3rd leaf were fixed with 3% glutaraldehyde and 1% OsO_4, buffered with Na-cacodylate to pH 7.2, dehydrated in ethanol and embedded in Spurr's medium. Ultrathin sections from five embedded specimens of each treatment were stained with uranyl acetate and Pb-citrate, and investigated with the EM Tesla BS 500.

RESULTS AND DISCUSSION

Under control conditions, there were no substantial differences in mesophyll or bundle sheath chloroplasts between the resistant (Lucia) and susceptible (Pavla) cultivars. Mesophyll cells in control plants of both cultivars had large central vacuoles with thin peripheral layer of cytoplasm. In the cytoplasm, organelles like endoplasmic reticulum (ER), mitochondria, nucleus, and plastids were present. A well-developed inner membrane of the chloroplasts was built up from high grana tied up to well developed stroma thylakoids occupying almost the entire chloroplast volume (Fig. 1A). Plastoglobuli were seen sporadically in the mesophyll chloroplasts of the resistant cultivar, and in a higher amount in the susceptible one. The chloroplasts were typically arranged along the cell walls. The cells of bundle sheath were rich in numerous large chloroplasts. The plastid apparatus possessed all structural peculiarities of the C_4 chloroplasts in the studied maize plants: they were typically agranal, with well-developed parallel stroma thylakoids (Fig. 1B). No starch grains were observed in the cells of control samples. No macroscopic symptoms were observed on leaves and roots of the plants grown on the lower $5\mu g/mL^{-1}$ concentration of fusaproliferin. Root growth of these plants was inhibited (data not shown). In these samples, the mesophyll chloroplasts revealed changes in their structure (Fig. 2). Loosening of the tight arrangement of grana thylakoids and disordered orientation of the stroma thylakoids were most common. In the grana, dilatation of thylakoids

was observed (Fig. 2A). In mesophyll cells of the susceptible cultivar, vacuolation of cytoplasm in addition to plasmolysis were observed. The observed structural responses were more pronounced in the susceptible cultivar. The above-mentioned structural modifications of chloroplast thylakoids may be associated with decreased content of chlorophyll after FUP treatment on maize seedlings in *vivo* and *in vitro* (Nadubinská *et al.* 2003).

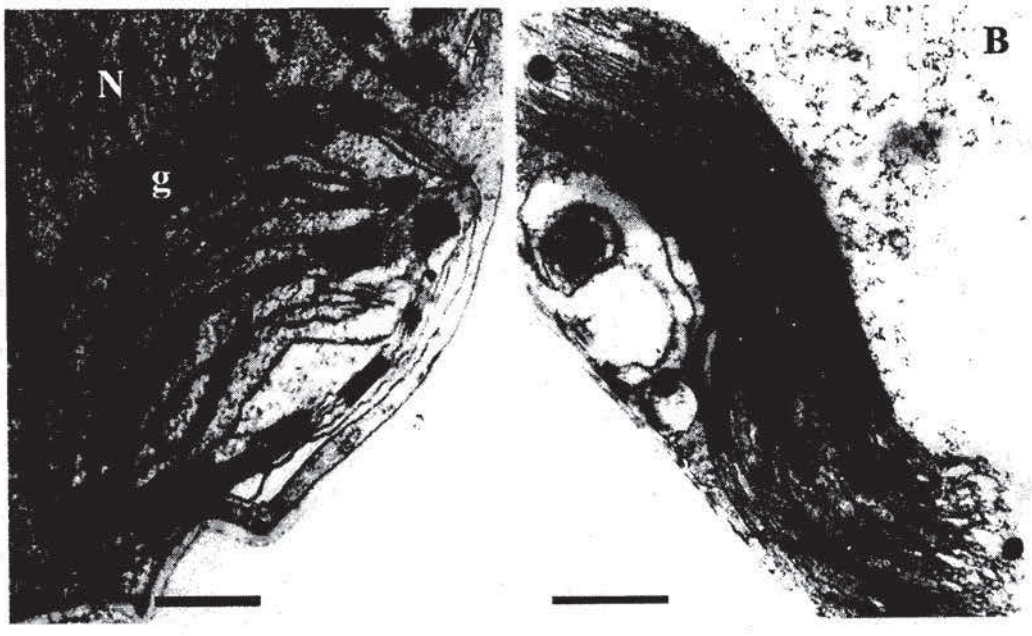

Fig. 1.Chloroplasts in control maize plants. A. Mesophyll, tolerant cultivar Lucia. B. Bundle sheeth, susceptible cultivar Pavla. N = nucleus, g = granum, arrows indicate plastoglobuli. Bars represent 1 μm.

The content of chlorophyll *a* was slightly decreased after *in vitro* treatment with FUP, while chlorophyll *b* increased. The authors report that *in vivo* chlorophylls *a, b* in the susceptible cv. Pavla were less reduced by FUP (9.4 %) than in the resistant one (18.2 %). Similarly, a reduction of photosynthetic capacity in maize and banana plants was induced by *F. verticillioides* living as endophyte (Pinto *et al.* 2000). These effects may reflect a reduction of chlorophyll contents and an impairment of electron transport in the thylakoid membranes leading to a decrease in the electron transport components. The authors suppose a possible reduction in the maximum yield of photosynthesis by toxins produced by the endophytic fungi. Nevertheless, the leaves were without symptoms similarly as in our experiment.

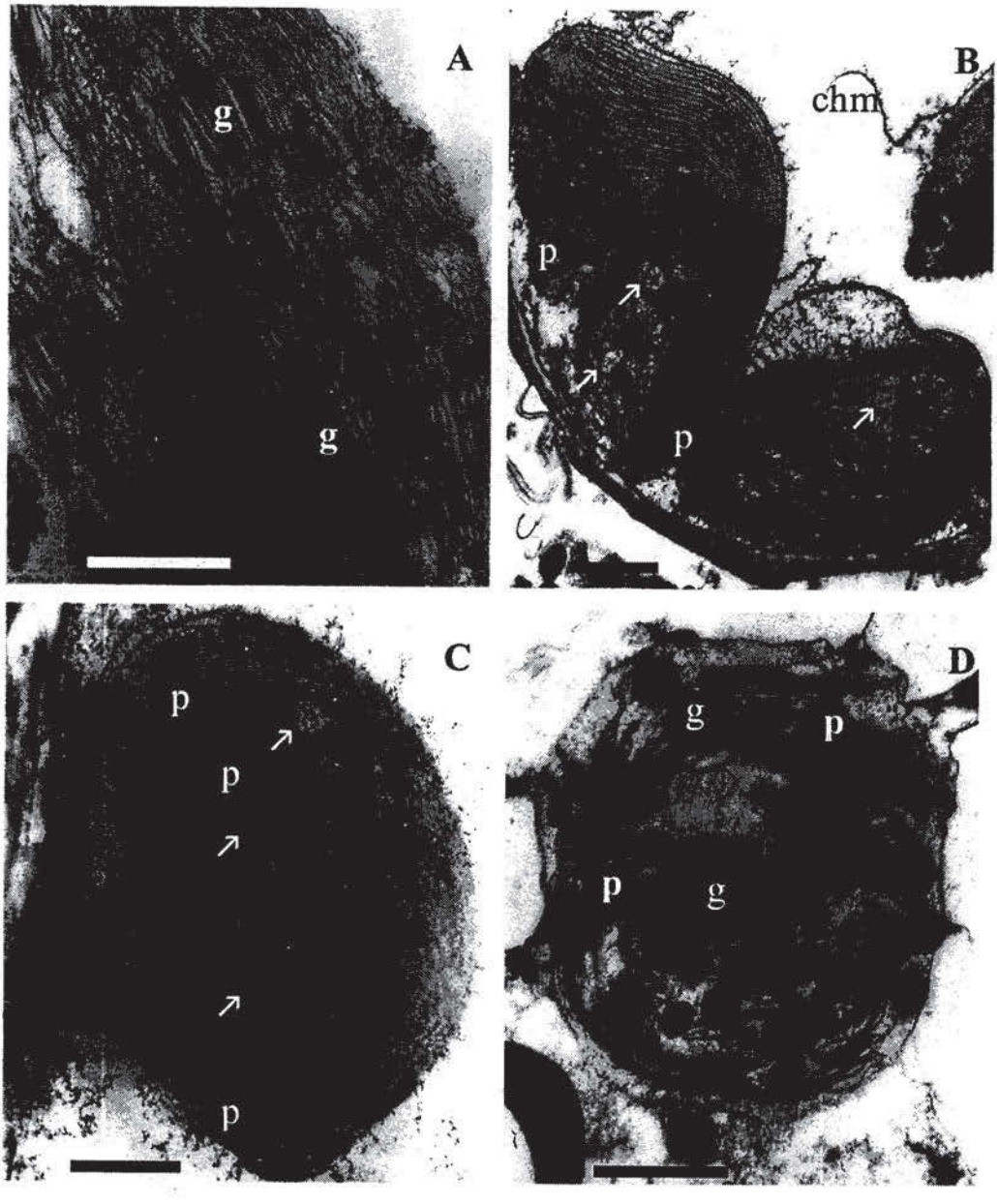

Fig. 2.Chloroplasts in plants of the susceptible (A,B,D), and resistant (C) maize cultivars treated with 5 μgL^{-1} FUP, leaves without visible symptoms (A) or 35 μgL^{-1} FUP, leaves with the symptoms (B-D). A. Loosening of grana (g) and thylakoid dilatation (arrows). B. Disorder in thylakoid arrangement (arrows) and damaged continuity of chloroplast outer membrane (chm). C. Disintegrated grana and disordered thylakoids (arrows). D. Grana (g) with dilated thylakoids (arrows) and an increased number of plastoglobuli (p). Bars represent 1 μm.

In addition to FUP, *F. verticillioides* can also produce moniliformin and fumonisin (Pascale *et al.* 2002), which are known by their phytotoxicity and inducing macroscopic effects on the diseased plants. Visual symptoms occurred in the apical and marginal part of the leaves of the plants cultivated on the higher fusaproliferin concentration (35 $\mu g/mL^{-1}$), such as chlorosis, a shrivelled appearance or water-soaking. The affected parts of the leaves contain the developmentally oldest cells, and their death was likely induced by FUP. This symptom is non-specific and can be induced by numerous pathogens, e.g. *Phytophthora* spp. (Farr *et al.*1989). However, more specific responses of infected plant tissues can be expected as, specific non-covalent interactions have been detected recently between both single- and double-stranded model oligonucleotides and FUP with 1:1 stoichiometry (Pocsfalvi *et al.* 2000).

Following the treatment by 35 μgL^{-1}, several changes in chloroplast structure were observed in both cultivars. The outer membrane of chloroplasts was only sporadically disrupted (Fig. 2B). In bundle sheath (Fig. 2B) as well as in mesophyll (Fig. 2C) chloroplasts remarkable disturbation of thylakoid arrangement occurred and plastoglobuli were more frequent.

Particularly the mesophyll chloroplasts of the susceptible cultivar Pavla were filled with large osmiophilic globules (Fig. 2D). These may contain lipids resulting from thylakoid membrane disintegration, as occurs during senescence (Kaup *et al.* 2002) or as induced by numerous biotic and abiotic stresses. Disruption and swelling of thylakoids is the most universal structural response of the inner membrane system of cells to a stress, and can occur in bean plants under conditions of water deficit and temperature stress (Dekov *et al.* 2000), or barley plants given high concentrations of salicylic acid (Stoyanova and Uzunova 2001).

Up to now the possible role of toxins in *Fusarium* plant diseases has been supported by several studies only for DON and FUM (Lemmens *et al.* 1997, Munkvold 2003). The alteration of plasma membrane permeability and electrolyte leakage is a well known effect of some of fusariotoxins like zearalenone and fusaric acid (Vianello and Macri 1978, Pavlovkin *et al.* 2004), but only indirect experiment has been done to study the FUP activity in plants. According to the results of Zonno and Vurro (1999), out of 14 assayed toxins most active in a depressing germination capability of *Striga hermonthica*, 7 belonged to *Fusarium* toxins, and the FUP belonged into the third group of toxins with a low level of activity. These and our data can indirectly indicate that FUP may act as a virulence factor in the disease caused by *Fusarium* pathogens.

We suppose FUP could have an important role in disease development or in the expression of symptoms through interactions between FUP and nucleic acids during the first steps of cellular dividing in the meristem or during leaf development. FUP might have very important role in endophytic mode of life of its producers.

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