INTRODUCTION

Pyrenophora teres, the causal agent of net blotch of barley (Hordeum vulgare L.), exists in two forms; P. teres f. teres and P. teres f. maculata. P. teres f. teres (the net form) produces brown necrotic streaks, which form a net-like pattern, whereas P. teres f. maculata (the spot form) produces circular or elliptical lesions (1). Chlorosis is also observed during both net and spot type of net blotch. Although a number of low molecular weight compounds (LMWCs) have been previously isolated from P. teres culture filtrates, they only induced certain components of the symptoms. In an attempt to fully understand this disease, we have isolated and characterised both LMWCs and proteinaceous metabolites to establish their role in chlorosis and necrosis development respectively.

MATERIALS AND METHODS

Toxin isolation Isolates of Pyrenophora teres f. teres (Ptt) and P. teres f. maculata (Ptm) were grown in Fries culture medium (FCM) and phosphate buffer FCM (PFCM) over 40 days at 24°C. Proteins and LMWCs were extracted from cell free filtrates every four days by passing through Whatman No.1 filter paper using a Buchner funnel followed by a 0.45 μm Millipore filter. Extracts were then concentrated using Amicon centrifplus filter devices (YM-10) such that retentates and filtrates were used for protein and LMWCs isolation respectively. Freeze-dried filtrates (containing LMWCs) were desalted using a strong acid ion exchange in hydrogen form and a strong basic ion exchange column in acetate form eluted respectively with ammonia (2 N) and a gradient of ammonium acetate (0.1 to 1 M). Ninhydrin positive LMWCs were visualised by high voltage paper electrophoresis (HVPE) in acetic-formic acid buffer (pH=1.75) and characterised further using thin layer chromatography (TLC) and mass spectrometry (MS). The retentates (>10 kDa) were shown to be proteinaceous since they were identifiable using Coomassie staining. Proteins with biological activity were purified using gel filtration and then characterised using automated MS/MS sequencing.

Bioassay Proteinaceous metabolites and LMWCs were either injected into barley leaves (cv. Sloop) using a Høgborg device at the three leaf growth stage or leaves were excised and allowed to take up the toxins by placing the cut end of leaves in the solution. The Høgborg device was also used to inject toxins into leaves from rye, faba bean, wheat and the resistant barley line C19214. The expression of symptoms was monitored every 24 hours.

RESULTS AND DISCUSSION

Electrophoretic properties and staining of the LMWCs with ninhydrin indicated that both forms of P. teres produced the same eight ninhydrin-positive LMWCs. Each individual compound was shown to induce chlorosis in excised barley leaves and to also be non-host-specific. All LMWCs except one isolated in this study appear to be derivatives of or are the previously described compounds; N-(2-amino-2-carboxyethyl) aspartic acid (Toxin A), aspergillogramarasmine A, anhydroaspergillogramarasmine A and aspergillogramarasmine B. The exception is a bioactive UV absorbing LMWC which appears to be a reductive conjugation of the α-keto acid of phenylalanine with Toxin A.

The partially purified proteinaceous metabolites induced brown necrotic lesions selectively with a greater response seen on the susceptible barley cultivar Sloop when compared to the resistant line CI 9214. No symptoms were seen on other plant species employed in this study suggesting that the proteinaceous metabolites isolated in this study are host specific phytotoxins. Eight proteins from P. teres f. maculata (90, 80, 75, 55, 48, 35, 14 and 13 kDa) and five proteins from P. teres f. teres (90, 80, 55, 48, and 14 kDa) were identified as toxic agents. Additionally, intercellular washing fluids (IWF) extracted from barley plants inoculated with both forms of P. teres, contained proteins of the same size as those in the biologically active fractions extracted from culture filtrates of P. teres f. maculata (80, 14 and 13 kDa) and P. teres f. teres (80, 48 and 14 kDa). No resemblance to sequences or conserved domain information available in public databases was identified and as a consequence, these proteins are considered to be novel.

CONCLUSIONS

This research has allowed the first isolation of proteinaceous host-specific toxins from P. teres as well as the identification of a UV-sensitive LMWC phytotoxin not previously described. Proteinaceous toxins induced brown necrotic spots/lesions specific to the susceptible host while the LMWCs induced chlorosis in a number of different plant species. This contributes significantly to the body of knowledge defining how symptoms are caused during the pathogenicity process in the interaction between P. teres and barley.

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REFERENCES