ROLE OF *PYRENOPHORA TERES* TOXINS IN NET BLOTCH OF BARLEY

Submitted by

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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July 2007

Declaration

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Abolfazl Sarpeleh

07/07/2007

Abstract

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*. Both forms induce a combination of brown necrotic spots and extensive chlorosis in susceptible barley cultivars. Although a number of low molecular weight compounds (LMWCs) have been previously isolated from *P. teres* culture filtrates, they only induced certain components of symptoms.

Fungal metabolites were extracted from culture filtrates of both forms of the pathogen and separated into low (<3kDa) and high molecular weight compounds (HMWCs, >10 kDa) with each fraction inducing a component of the net blotch symptoms in a barley leaf toxicity assay. Inactivation of both LMWCs (<1kDa) and HMWCs resulted in loss of activity confirming their potential role in symptom development.

Low molecular weight compounds induced chlorosis and water soaking but not the brown necrotic spots or lesions usually seen during the infection of barley by *P. teres*. The high molecular weight compounds (>10 kDa) induced the brown necrotic spots or lesions with no chlorosis evident. Further characterisation of LMWCs showed that they are not host specific while HMWCs exhibited host specificity.

LMWCs were purified and further analysed using high voltage paper electrophoresis, staining and mass spectrometry. Electrophoretic properties and staining of the LMWCs with ninhydrin indicated that both forms of *P. teres* produced similar LMWCs in the conditions grown. Each form produced eight ninhydrin-positive compounds with the same

relative mobilities. Each individual compound was shown to induce chlorosis in excised barley leaves.

All compounds except the one isolated in this study appear to be derivatives of or are the previously described compounds; *N*-(2-amino-2carboxyethyl) aspartic acid (Toxin A), aspergillomarasmine A, anhydroaspergillomarasmine A and aspergillomarasmine 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 HMWCs (>10kDa) were proteinaceous since they were identifiable using Coomassie staining. Additionally, the loss of activity that occurred with incubation at 40, 60, and 80 ^{o}C for 30 and 60 min followed a pattern fairly typical for protein denaturation. Further, treatment with protease decreased their phytotoxicity in proportion to the amount of enzyme used.

Enzyme and heat treatment of proteins extracted from each form showed that proteins of *P. teres* f. *teres* are more resistant to heat and enzyme treatment compared with those of *P. teres* f. *maculata.* This suggests the protein(s) involved in symptom induction by *P. teres* f. *teres* and *P. teres* f. *maculata* are different which contributes to the difference in the symptom expression during the interaction between the pathogens and barley.

Proteinaceous metabolites extracted from *P. teres* f. *teres* and *P. teres* f. *maculata* ranged from 10 to 100 kDa. Fractions purified using gel filtration had biological activity when they contained eight proteins when extracted from *P. teres* f. *maculata* (90, 80, 75, 55, 48, 35, 14 and 12 kDa) and six proteins when extracted from *P. teres* f. *teres* (90, 80, 55, 48, 14 and 12 kDa). 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 12 kDa) and *P. teres* f. *teres* (80, 48 and 14 kDa). Automated MS/MS sequencing of the biologically active proteins showed no resemblance to the sequences or conserved domain information available in public databases and as a consequence, these proteins were considered as novel proteins for *P. teres*. However, exact short matches with fragments resulting from the 80, 48 and 14 kDa proteins, showed considerable homology with ATP-binding cassette (ABC) transporters and their components, cellulases, serine proteinases as well as some hypothetical proteins isolated from different fungal species.

Reaction of six plant species including one susceptible barley cultivar (Sloop) and one resistant line (CI9214) to *P. teres* showed that partially purified proteins induce the symptoms selectively in barley cultivars where the proteinaceous metabolites only induced brown necrotic spot/lesions in barley with a greater response seen on the susceptible cultivar Sloop when compared to the resistant line CI9214. 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.

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 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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Acknowledgements

I am grateful to the Agricultural Research and Education, Plant Protection Research Institute and the Ministry of Science, Research and Technology of Iran for their sponsorship toward a Ph.D degree and my principal supervisor Dr Amanda J. Able for the extra funding that supported this research from a Grains, Research and Development Corporation grant. I would also like to thank her for her guidance, patience, inspiration and valuable discussion throughout the project. I also wish to thank my co-supervisors Dr. Hugh Wallwork and Professor David Catcheside for their advice in this project. I am especially grateful from Professor Max E. Tate, who taught me many skills in chemistry.

I would also like to thank all members of the Able lab for their help and advice in different parts of the project. Thanks to Dr. Alan Little and Annette Boettcher for their help in the molecular biology work. I appreciate Mr. Jelle Lahnstein and Timothy March, for their advice in the protein works. I also would like to thank Dr Christopher Bagley from the Adelaide Proteomics Centre for sequencing and Professor Richard Oliver from Murdoch University for providing access to the database of *Stagonospora nodorum* sequences. I appreciate Mr. Mark Butt, Dr. Ester Cappio and Robert Asenstorfer for their advice and providing some of the materials used in this project.

I would like to thank all my family especially my parents and my parents in-law for their encouragement and support. Finally, I would like to express my great appreciation to my wife Fataneh for her patience, understanding and love.

Abbreviations

Ala	Alanine	µg	Micrograms
Asp	Aspartic acid	µg/mL	Micrograms per millilitre
º C	Degree Centigrade	µL	Micro litre
bp	Base pair	µM	Micro molar
BSA	Bovine serum albumin	ml	Millilitre
cDNA	Complementary DNA	Ml/min	Millilitre per minute
cm	Centimeter	Ms	Mass spectophotometery
cv	Cultivar	MW	Molecular weight
DAP	Diaminopropionic acid	*m/z*	Mass to charge ratio
DNA	Deoxyribonucleic acid	PCR	Polymerase chain reaction
EDTA	Ethylene diamine tetraacetic acid	PDA	Potato dextrose agar
EST	Expressed sequence tag	PFCM	Phosphate buffered FCM
FCM	Fries culture medium	pH	Potential of Hydrogen
g	Gram	PR-Protein	Pathogenesis related-protein
HMWC	High molecular weight compound	QTL	Quantitative trait loci
HST	Host specific toxin	RNA	Ribonucleic acid
HVPE	High voltage paper electrophoresis	R_F	Retention factor
IPTG	Isopropylthiogalactosidase	R_m	Relative mobility
IWF	Intercellular washing fluid	rpm	Revolutions per minute
KV	Kilo Volt	RT-PCR	Reverse transcriptase PCR
l	Litre	SDS	Sodium dodecyl sulphate
LMWC	Low molecular weight compound	SDW	Sterile distilled water
LSD	Least significant difference	TAE buffer	Tris-acetate EDTA buffer
M	Molar	TE buffer	Tris EDTA buffer
mg	Milligram	TLC	Thin layer chromatography

Tris	Hydroxymethyl amino methane	WA	Water agar
UV	Ultra violet	w/v	Weight for volume
V	Voltage	×	Times
v/v	Volume for volume		