Death by toxin net blotch disease of barley




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Barley net blotch is a destructive disease which results in loss of yield (as high as 40%) and quality in barley grain, the world's fourth largest crop. In Australia, the economic losses caused by net blotch disease have been predicted to be greater than $62 million per year[1].

The Ascomycete *Pyrenophora teres* is responsible for net blotch disease of barley (*Hordeum vulgare* L.) and exists in two forms: *P. teres* f. *teres* (*Ptt*) and *P. teres* f. *maculata* (*Ptm*) categorised by the different symptoms they cause (also known as the net and spot forms respectively). The net form is more common and symptoms appear as a blotch, which is irregular in shape, dark brown and consists of a network of narrow lines usually surrounded by a yellow chlorotic area. In contrast, the spot form causes symptoms appearing as small, dark brown spots sometimes surrounded by a chlorotic halo[2]. Although the differences are partially attributable to differences in the life cycle of the two forms of *P. teres*, other factors such as toxin production may also play a role[3,4].

In cell culture, *Pyrenophera teres* produces two types of toxins: low molecular weight compounds (LMWCs) derived from aspergillomarasamine and proteinaceous toxins[2]. The LMWCs cause chlorosis in plant tissue regardless of species[5], whereas the protein mixture causes necrosis on some cultivars of barley and not others, suggesting it contains host-specific toxins[6]. In addition, the profile of proteinaceous toxins differs between the two forms of *P. teres*[7] and between isolates with different aggressiveness (Ismail *et al.*, in preparation).

Research in progress at the University of Adelaide

Our research aims to identify and characterise the proteins that differ between the spot and net form of *P. teres* and between isolates with different aggressiveness (or ability to cause disease). We also aim to identify which individual proteins act as effectors and establish how those proteins contribute to symptoms in susceptible plants.

Protein was extracted from cell culture filtrates of *P. teres* and separated using 2D gel electrophoresis. The protein profiles of a *Ptm* and *Ptt* isolate were compared as were the profiles of a highly aggressive *Ptt* isolate and a low aggressive isolate. Where proteins were present on one gel and not another (for example, Figure 1), the protein spot was extracted from the gel, digested with trypsin and the peptide fragments analysed using tandem mass spectroscopy. Where sequence coverage was greater than 10%, primers designed to the matching sequences in the BLAST database (http://www.ncbi.nlm.nih.gov/) were used to amplify the full length cDNA and gDNA. Reverse-transcriptase (RT)-PCR is being used to confirm gene expression in culture and during the interaction between barley and *P. teres*.

One protein unique to the spot form (SF1) and one unique to the net form (NF1) were identified as conserved hypothetical proteins (Figure 1). SF1 appears to encode for a germin-like protein (or oxalate oxidase). *SF1* was only expressed in *Ptm* cultures and to a greater extent during the interaction of *Ptm* with barley. NF1 appears to be a mutatarose. Interestingly, NF1 also has a RGD motif which has been shown to be present in an effector (host-specific toxin) from *Pyrenophora tritici-repentis*[8] and is thought to reduce plant defence responses by perturbing adhesions between the cell wall and plasma membrane[9]. *NF1* was expressed more in *Ptt* cultures and to a greater extent during the interaction of *Ptt* with barley especially during the early stages.

Two proteins unique to the more aggressive isolate have also been identified as conserved hypothetical proteins. One of the proteins appears to be an isochorismate hydrolase while the other has no known function. Isochorismate hydrolases from *Verticillium dahliae* are only found in aggressive isolates and have been shown to suppress defence responses by inhibiting the formation of salicylic acid[10], a well-known defence response[11]. However, we have observed differences in growth habit (hyphal length and time to germination) for isolates with different aggressiveness, suggesting that a number of factors and not only toxins may contribute to aggressiveness of *P. teres* on susceptible cultivars.

Conclusions and future research

Proteomics has proven to be a useful tool to identify proteins that differ between isolates with varying aggressiveness and between the two forms of *P. teres*. We are now characterising all proteins further by using heterologous expression systems to produce pure protein, which can then be used in bioassays to determine contribution to symptoms and used in biochemical assays to confirm their annotated activity. Which individual proteins contribute to the host-specificity observed for the proteins from culture filtrates [6] is also a focus of our studies.

Based on our knowledge of the wheat-*Stagonospora nodorum* and wheat-*P. tritici-repentis* systems (reviewed by 12), effectors probably target a host protein in susceptible barley plants conferring sensitivity. In resistant plants, the target protein may be absent or differ in a way that prevents alteration by the effector. Alternatively, the alteration of the target by effectors may trigger a defence response. An understanding of these targets within barley will be useful to breed for resistance to *P. teres* and remove the sentence of death by toxin.

Acknowledgements

This project has been partially supported by the Grains Research and Development Corporation (GRDC). We thank Hugh Wallwork (South Australian Research and Development Institute, SARDI) for provision of isolates and Kelvin Khoo for technical advice. Ismail Ismail is supported by an Iraqi Government Scholarship. We are also evaluating the toxins as a screening tool for net blotch disease resistance in GRDC project UA00125 in collaboration with Hugh Wallwork (SARDI) and Celeste Linde (Australian National University).

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Biographies

Mr Darren Wong is a PhD student at the University of Adelaide. His current research interests include regulation of organic acid metabolism and gene co-expression networks in plants. He performed some of the research presented here while an Honours student.

Mr Ismail Ismail is a PhD student at the University of Adelaide. He was a lecturer in Department of Plant Protection, College of Agriculture, University of Baghdad. His research now focuses on characterisation of proteinaceous toxins in net blotch disease of barley.

Dr Dale Godfrey is a postdoctoral scientist at the University of Adelaide. Her research interests are in molecular plant–fungal interactions, grapevine pathology and organic viticulture.

Assoc Prof Amanda Able is an Associate Professor in Plant Science at the University of Adelaide's Waite Campus. Her research interests are in plant disease resistance, post-harvest pathology, antioxidants in food and post-harvest physiology.

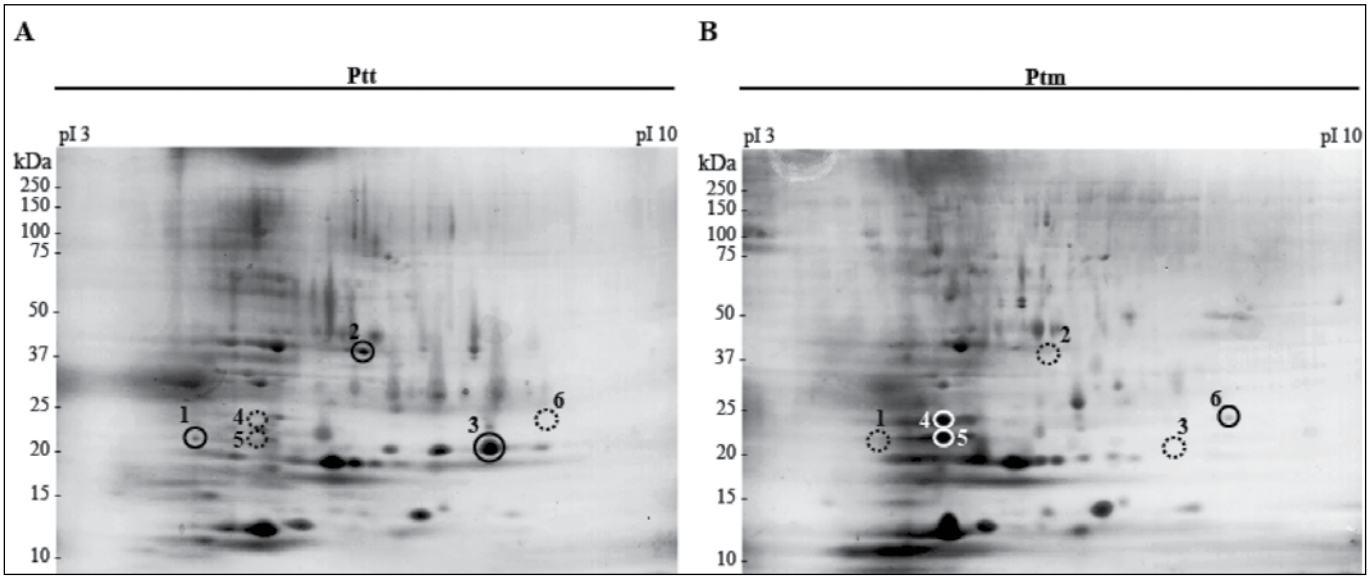

Figure 1. Identification of proteins unique to *Pyrenophora teres* f. *teres (Ptt)* (A) or *P. teres* f. *maculata (Ptm)* (B) using 2D gel electrophoresis. Proteins (100 µg) were separated on the basis of isoelectric potential (pI 3 to pI 10) before separating by molecular weight. Unique protein spots (where complete line is present and dotted line is absent) were identified using tandem mass-spectrometry. Spot 1 and 3 (present in *Ptt* only) were characterised as the same cellulose binding protein. Spot 6 (present in *Ptm* only) was a pectin esterase. Spots 4 and 5 were the same unidentified but conserved hypothetical protein (SF1) while Spot 2 (NF1) was another unique conserved hypothetical proteins. SF1 and NF1 were chosen for further studies. Gels are representative of three independent biological replicates.