A proteomics approach to dissect SnToxA effector mode-of-action in wheat

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GRDC Grains Research & Development Corporation

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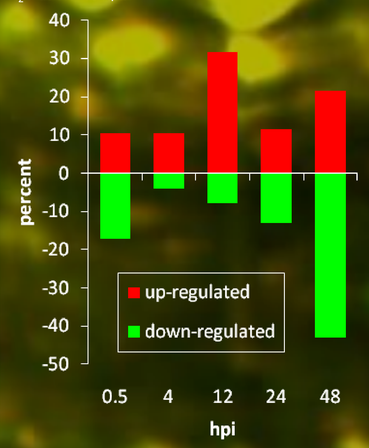
Abstract

Stagonospora nodorum is a necrotrophic fungal pathogen of wheat causing devastating foliar damage resulting in significant yield losses globally. *S. nodorum* operates in inverse gene-for-gene manner through the interaction of a secreted effector and a dominant host susceptibility protein resulting in disease development. The effector protein SnToxA and its corresponding host gene Tsn1 follow such a system. This study aims at deciphering the molecular responses triggered by SnToxA in the susceptible wheat cultivar BG261 over a 48h time course using a gel-based proteomics strategy. Infiltrated leaves were sampled at 0, 0.5, 4, 12, 24, and 48 hours post-infiltration (hpi); soluble proteins were extracted and separated using two-dimensional electrophoresis (2-DE). Acidic proteins were labelled using DIGE CyDyes prior to isoelectrofocusing (IEF) along 4-7 pH range and studied across the whole 0-48h time course. Basic proteins, not labelled, were studied at 24-48 hpi. Significantly differentially-expressed proteins were identified using tandem mass spectrometry. Pathogenesis-related proteins are up-regulated at 48 hpi while antioxidant enzymes participate to host defense reaction at early stages. Many proteins are involved in carbon fixation, most of them localised in the chloroplast, and accumulate at 12 hpi followed by a repression at 48 hpi. SnToxA mode-of-action influences carbon fixation by manipulating C_1, C_2, and C_3 cycles in the host.

Figure 2: Percentage of differentially-expressed proteins (fold change >1.5, converted into percents) in the susceptible wheat cv. BG261 after infiltration with SnToxA and compared to an infiltration with H_2O at 0.5-48 hpi.



Results

SnToxA infiltration causes chlorosis of susceptible wheat leaves **(Fig. 1)**. In ToxA-infiltrated leaves, most of the proteins are up-regulated at 12 hpi and down-regulated at 48 hpi **(Fig. 2)**. Basic proteins respond more to ToxA infiltration **(Fig. 3)** due to the predominance of pathogenesis-related (PR) proteins such as chitinases, thaumatin-like proteins and glucanases. Aside from PR proteins, other proteins that were reported in various fungal pathogen-wheat systems are differentially regulated in our study, suggesting that SnToxA on its own is able to initiate a host reaction akin to disease response. Most of ToxA-responsive proteins (58%) are chloroplastic, mainly involved in photosynthesis via Calvin-Benson C_3 cycle and thylakoid electron transfer **(Fig. 4)**. Linked with chlorosis symptoms, chlorophyll would start to degrade rapidly (after 12 hpi), as attested from proteins physically connected to it or acting in its biosynthesis. Photorespiration, or C_2 cycle, is also affected, involving two peroxisomal enzymes as well as enzymes participating to methyl C_1 cycle. Because enzymes of the antioxidant system, mainly detoxifying hydrogen peroxides and preventing lipid peroxidation, are induced by ToxA treatment, we assume an oxidative burst occurs shortly after infiltration (12-24 hpi).

Materials and Methods

Plant culture (*T. aestivum* cv. BG261) and leaf infiltration with SnToxA in growth chamber

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Post-infiltration leaf sampling over time course:
- 2 treatments (H_2O, ToxA)
- 6 time points (0, ½, 4, 12, 24, and 48h)
- 3 biological replicates (1BL=pool of 10 leaves)

→ 36 samples

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Extraction of soluble proteins (TCA/acetone)

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Protein abundances determined using 2-DE

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	Acidic proteins	Basic proteins
Samples	36 (all)	12 (24/48h)
Protein load	0.1 mg	0.5 mg
pH range	4-7	7-10
Labelling	DiGE minimal kit	none

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Differentially-regulated proteins analysed using statistical analyses (ANOVA, PCA, HCA)

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Identification of significant spots by tandem mass spectrometry (MS/MS)

Figure 1: Toxicity assay over 0-72h post-infiltration (hpi) following wheat leaf infiltration with A/ mock (H_2O, blue) solution (n=5), or B/ a 1.22 µg/mL ToxA (red) solution (n=10). C/ Chlorosed area within the infiltrated region (marked with horizontal black lines) was quantified using Scion Image software and converted into per cents. Significance levels (*** P<0.001) were obtained using independent one-way ANOVAs for each time points.

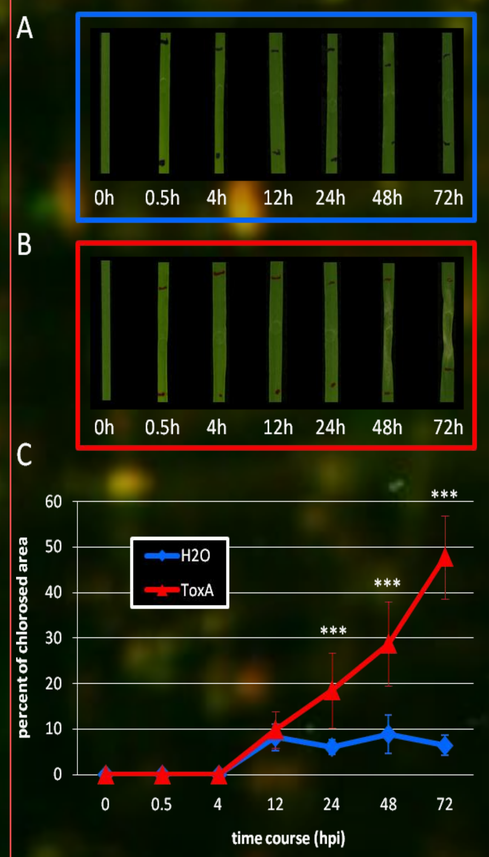


Figure 3: 2-D gels along A/ acidic range (4-7) following DIGE labelling and B/ basic range (7-10). Spots excised and MS-analysed are indicated by an arrow when present or an open circle when missing.

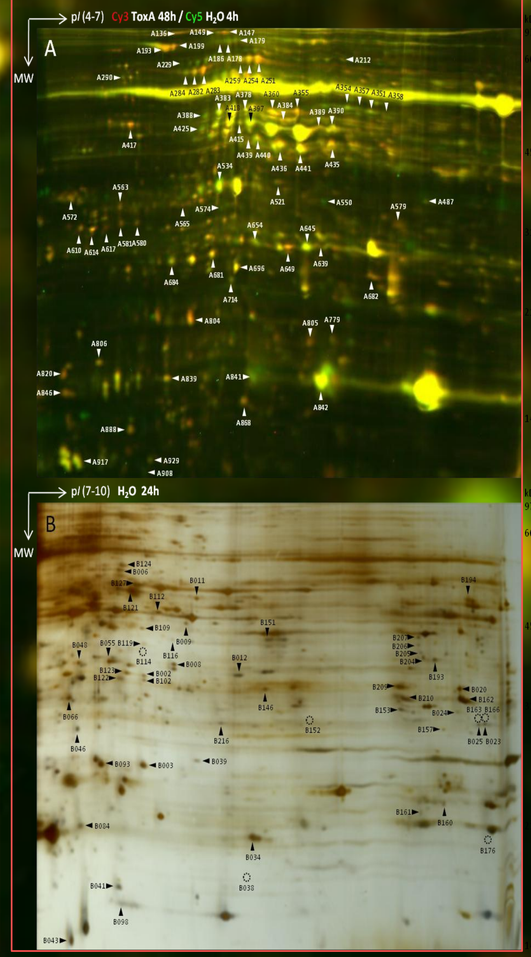
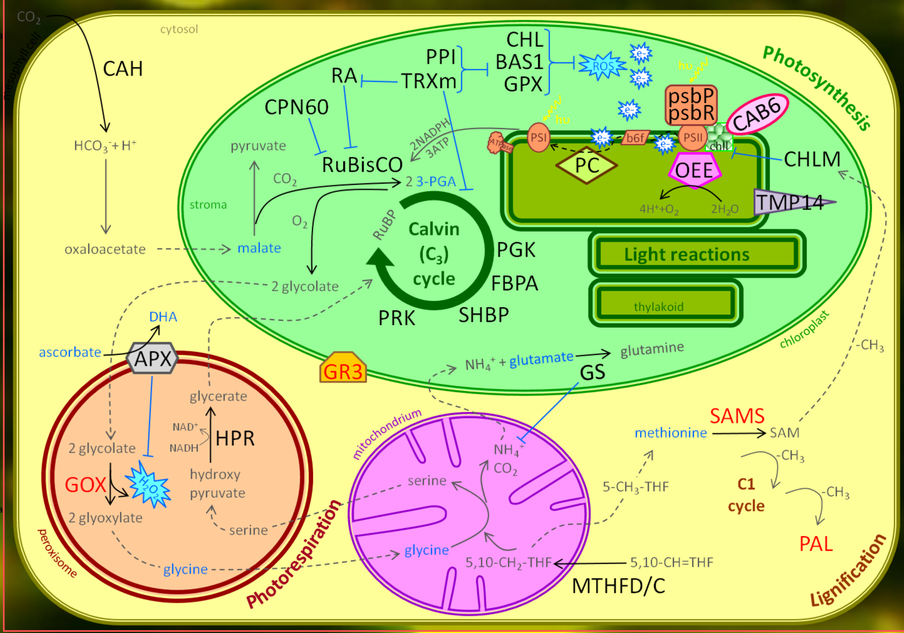


Figure 4: Proteins responsive to SnToxA infiltration in wheat mesophyll cells and involved in photosynthesis, photorespiration, and lignification. Proteins in red are up-regulated by ToxA treatment. Blue lines are potential feed-backs, based on the literature.



Conclusions

This work demonstrates that the effector SnToxA alone, independent of the pathogen, is able to trigger all the molecular events leading to cell death in the susceptible wheat host , which satisfies *S. nodorum* necrotrophic lifestyle. As observed previously (Manning *et al.* 2007), ToxA targets the chloroplasts where it seems to initiate photosynthesis collapsing and the oxidative burst preceding the host hypersensitive response, while reinforcing cell walls through enhanced lignification.