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Research article

Metabolomics deciphers the host resistance mechanisms in wheat cultivar Sumai-3, against trichothecene producing and non-producing isolates of *Fusarium graminearum*

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ABSTRACT

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, reduces grain yield and contaminates grains with trichothecene mycotoxins. Host resistance to FHB is quantitatively inherited and more than 100 QTLs have been mapped, but the host resistance mechanisms are poorly understood. Non-targeted metabolic profiling was applied to elucidate the host resistance mechanisms to FHB spread through rachis of wheat cultivar Sumai-3 against both trichothecene producing and non-producing isolates of *Fusarium graminearum*. The accumulation of deoxynivalenol (DON) in Sumai-3 was low, however the resistance to spread was not due to its detoxification into DON-3-*O*-glucoside (D3G), as the proportion of total DON converted to D3G in the resistant was not significantly different from that in the susceptible cultivar Roblin. Instead, the resistance was considered to be due to the accumulation of resistance related (RR) metabolites belonging to the phenylpropanoid pathway that reduced pathogen advancement through increased host cell wall thickening and also reduced pathogen growth due to antifungal and/or antioxidant properties which, in turn, reduced subsequent trichothecene biosynthesis. The RR phenylpropanoids accumulated in Sumai-3 were mainly the preformed syringyl rich monolignols and their glucosides, which are precursors of lignin biosynthesis, as well as antimicrobial flavonoids. The resistant cultivar Sumai-3 inoculated with trichothecene producing *F. graminearum* not only accumulated less RR metabolites but also the abundance of many RR metabolites was lesser than in the trichothecene non-producing *F. graminearum*. This implies repression of host resistance mechanisms by trichothecenes/DON, which is a protein biosynthesis inhibitor. Enhancement of resistance in wheat against FHB can be exploited through stacking of candidate phenylpropanoid pathway genes.

1. Introduction

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch) is one of the most destructive global diseases of wheat (*Triticum aestivum*L.). FHB causes significant reductions in grain yield and deteriorates grain quality by contaminating them with trichothecene mycotoxins. Deoxynivalenol (DON), a trichothecene, is acutely toxic to eukaryotes at very low concentrations (<1 ppm) which inhibit protein synthesis by arresting the host peptidyltransferase, thus posing serious health risks to animals and human beings (Pestka, 2007). Breeding for FHB resistant cultivars is considered a viable and environmentally safe approach to managing FHB. Host resistance to FHB is quantitatively inherited with more than 100 QTLs identified including a few with large effects: *Fhb1*, *Fhb2* and *Fhb5* which are stably inherited (Buerstmayr et al., 2009). However,

Abbreviations: AUDPC, Area Under the Disease Progress Curve; C4H, Cinnamate 4-hydroxylase; CAD, Cinnamoyl alcohol dehydrogenase; COMT, Caffeic acid – *O* - methyl transferase; CS, Chalcone synthase; D3G, DON 3-*O*-glucoside; DON, Deoxynivalenol; dpi, days post inoculation; FC, Fold change; FHB, Fusarium head blight; H_2O_2, Hydrogen peroxide; HCAA, Hydroxycinnamic acid amides; HCT, Hydroxycinnamoyl transferase; LC-HRMS, Liquid chromatography high resolution mass spectrometry; NIL, Near Isogenic line; PAL, Phenylalanine ammonia lyase; PDC, Proportion of DON converted to D3G; PSD, Proportion of spikelets diseased; qPCR, Quantitative polymerase chain reaction; QTL, Quantitative trait loci; ROS, Reactive oxygen species; RR, Resistance related; RRC, Resistance related constitutive; RRI, Resistance related induced; TDP, Total DON produced; UGT, UDP-glycosyltransferase.

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pyramiding of these failed to significantly enhance FHB resistance (McCartney et al., 2007) possibly due to linkage drag. Understanding the host genetic, molecular and biochemical mechanisms against *F. graminearum* would facilitate precise breeding.

Alongside, DON is not only a phytotoxic compound but also a pathogen aggressiveness factor which facilitates the spread of the pathogen from the inoculated spikelet to the adjacent spikelets through rachis (Bai et al., 2002; Jansen et al., 2005). DON was found in cytoplasm, chloroplasts, plasma lemma, cell wall, vacuoles, and endoplasmic reticulum, moving ahead of the intruding fungal hyphae (Kang and Buchenauer, 1999). DON kills the host cells by disrupting the cell membrane, thus causing cellular electrolyte leakage and an increase in cytoplasmic Ca^{2+} ions leading to imbalance in cellular homeostasis (Bushnell et al., 2010; Cossette and Miller, 1995). Damaged host cells systematically transduce defense signals in the host to produce reactive oxygen species (ROS), such as H_2O_2, which trigger cell death (Desmond et al., 2008). Unlike in biotrophs, the ROS induced cell death, instead of preventing the pathogen spread, which further facilitates the necrotrophic phase of *F. graminearum* colonization (Govrin and Levine, 2000), causing bleaching symptoms. Hence, the aggressiveness of pathogen and disease spread depends on its DON producing capacity, which in turn can alter the host defense mechanisms (Mesterházy, 2002).

Following DON inoculation, transcripts related to DON detoxification, host cell death and antioxidant enzymes were up-regulated in barley (Gardiner et al., 2010) and wheat (Foroud et al., 2012; Walter et al., 2008). Contrarily, many host defense-related transcripts were repressed by the eukaryotic protein translation inhibitory action of DON (Foroud et al., 2012; Gardiner et al., 2010). However, determining the appropriate concentrations of DON required, to elucidate the specific host resistance mechanisms is very challenging.

Alternatively, inoculations with trichothecene producing (*FgTri5*$^+$) and trichothecene non-producing *F. graminearum* (*FgTri5*$^-$) isolates with loss of function of *Tri5* gene were used to study the host response to trichothecenes. The *Tri5* gene encodes trichodiene synthase, the first committed enzyme in the trichothecene biosynthetic pathway. Similar to DON, the inoculation of barley spikelets with *FgTri5*$^+$ led to the up-regulation of transcripts related to DON detoxification, DON sequestration and cell death but not by the *FgTri5*$^-$ mutant strain, signifying host responses specific to trichothecene. However, *FgTri5*$^+$ suppressed the expression of 18 host defense related transcripts in contrast to just one transcript by the *FgTri5*$^-$ mutant. More DON resistance related transcripts were up regulated in FHB resistant wheat near isogenic lines (NILs) than in the susceptible cultivar following *FgTri5*$^+$ inoculation, but not with *FgTri5*$^-$ inoculated samples. In contrast, a higher number of transcripts of ribosomal proteins were down regulated in the susceptible cultivar compared to the resistant NILs (Foroud et al., 2012). However, trichothecenes suppress host defense mechanisms by inhibiting protein translation and a study on the influence of trichothecenes at protein and/or downstream metabolite level could give more insight into the host defense mechanisms.

Analyzing the metabolome, penultimate to the phenome, would reveal more precise host biochemical responses to environmental cues including biotic stress (Kushalappa and Gunnaiah, 2013). Multiple techniques have been employed to detect, identify and quantify the diverse groups of metabolites induced by several diseases and pests. Nuclear Magnetic Resonance (NMR) spectrometry is a rapid and robust method that can detect diverse groups of both primary and secondary metabolites (Kim et al., 2010). NMR spectroscopy has been applied to decipher host biochemical mechanisms against pests (Leiss et al., 2009) and diseases (Figueiredo et al., 2008). Although low sensitivity of NMR has been bettered through improved hardware, its utility in detecting secondary metabolites that are induced against pests and diseases is limited. Non-targeted metabolomics based on chromatography based techniques such as GC–MS and LC–HRMS have revealed quite comprehensive host resistance mechanisms that belong to the phenylpropanoid, flavonoid, fatty acids, terpenoid and alkaloid pathways in barley (Bollina et al., 2010, 2011; Kumaraswamy et al., 2011b) and wheat (Gunnaiah et al., 2012) against *F. graminearum*. In addition, a few metabolites such as phenylalanine, jasmonic acid, and *p*-coumaric acid that were consistently identified as resistance related (RR) metabolites in several FHB resistance cultivars were put forward as potential biomarkers for FHB resistance (Bollina and Kushalappa, 2011; Kumaraswamy et al., 2011a).

Wheat cultivar Sumai-3 and others derived from it are potential sources of FHB resistance in wheat breeding worldwide (Bai and Shaner, 2004). Sumai-3 has been consistently used as a donor parent for the major QTLs *Fhb1* and *Fhb2* and a minor QTL, 3BSc for type II resistance (Buerstmayr et al., 2009). Contrarily, Sumai-3 also contributed negative alleles for FHB resistance at the QTL regions on the chromosomes 2AL and 4B (Anderson et al., 2001; Waldron et al., 1999) and at the QTL 2D (Handa et al., 2008). Elucidating the biochemical host defense mechanisms in Sumai-3 could aid in selecting suitable genes involved in host defense when Sumai-3 is used as a resistance source in breeding. Furthermore, the host deploys distinctive resistance mechanisms in different organs of wheat against *F. graminearum* (Golkari et al., 2007; Gunnaiah et al., 2012); resistance to spread was reported to localize at the rachis (Kang et al., 2008).

Consequently, the present study was designed to elucidate the host biochemical resistance to FHB spread at the metabolite level in the rachis of Sumai-3, in response to trichothecene producing and non-producing isolates of *F. graminearum* using LC–HRMS. The study revealed substantial association of FHB resistance with the phenylpropanoid pathway, especially monomers of syringyl lignin, that were accumulated at higher abundances following inoculation with the trichothecene negative isolates.

2. Material and methods

2.1. Plant material

Seeds of wheat cultivars, Sumai-3 and Roblin, were obtained from Agriculture and Agri-Food Canada, Winnipeg, Canada (Dr. S. Fox). Sumai-3 is a Chinese cultivar, widely used as a breeding source of FHB resistance to spread. Roblin is a high-protein, marquis type quality, early-maturing cultivar for the eastern prairies of Canada, which is highly susceptible to FHB. Plants were grown in the greenhouse at 25 ± 2 °C with 70 ± 10% relative humidity and alternating 16 h of light and 8 h of darkness. Seeds were sown in 6 inches diameter pots filled with Growing Mix-PV20 (Fafard, QC, Canada) and three plants were retained for each pot after germination. Plants were watered as necessary and fortnightly fertilized with 200 mL of 0.3% NPK and 0.035% micronutrients.

2.2. Pathogen, spore production and inoculation

F. graminearum Schwabe, trichothecene producing isolate, GZ3639 (*FgTri5*$^+$) and trichothecene non-producing isolate, GZT40 (*FgTri5*$^-$) (obtained from Dr. Proctor, USDA, USA) (Proctor et al., 1995) were maintained at −80 °C and sub-cultured once on potato dextrose agar. For spore production, the cultures were grown on rye B agar with half the concentration of sucrose, under 16 h of light and 8 h of dark, for three days at 23 ± 2 °C. Later, the culture plates were shifted to UV light chamber and grown under 16 h of UV light and 8 h of dark, for the next four days, at 23 ± 2 °C. From the seven-day-old cultures, mycelium was scraped and suspended