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Research article

Are ineffective defence reactions potential target for induced resistance during the compatible wheat-powdery mildew interaction?

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ABSTRACT

Powdery mildew caused by *Blumeria graminis* f.sp. *tritici*, an obligate aerial biotrophic fungus, would be one of the most damaging wheat (*Triticum aestivum*) diseases without the extensive use of conventional fungicides. In our study, the expression levels of some basal defence-related genes were investigated during a compatible interaction in order to evaluate wheat reactions to infection, along with the different stages of the infectious process in planta. As fungal conidia initiated their germination and developed appressorial germ tube (AGT), early defence reactions involved the expression of a lipoxygenase (LOX)- and an oxalate oxidase (OXO)-encoding genes, followed by activations of corresponding LOX (EC 1.13.11.12) and OXO (EC 1.2.3.4) activities, respectively. When penetration of AGT took place, up-regulation of chitinases (CHI) and PR1-encoding genes expression occurred along with an increase of CHI (EC 3.2.1.14) activity. Meanwhile, expression of a phenylalanine ammonia-lyase-encoding gene also took place. Up-regulation of a phospholipase C- and lipid transfer proteins-encoding genes expression occurred during the latest stages of infection. Neither the phi glutathione S-transferase (GST)-encoding gene expression nor the GST (EC 2.5.1.13) activity was modified upon wheat infection by powdery mildew. Whether these defence reactions during such a compatible interaction are markers of immunity or susceptibility, and whether they have the ability to contribute to protection upon modulation of their timing and their intensity by resistance inducers are discussed.

1. Introduction

Powdery mildew caused by *Blumeria graminis* f.sp. *tritici* (DC.) E.O. Speer (*Bgt*), an obligate biotrophic ascomycete fungus that invades wheat (*Triticum aestivum*) aerial parts, is an important disease in wheat-growing areas worldwide. Corresponding yield losses ranged from 3 to more than 30% over the last 15 years (Briceño-Felix et al., 2008). So far, the use of host plant resistance has been the most cost-effective and environmentally safe method for the control of wheat powdery mildew (Hsam et al., 2002). However, the durability of wheat resistance conferred by some powdery mildew (*Pm*) major resistance genes is impaired since new virulence genes may arise within the pathogen population, resulting in the overcome of cultivar resistance (Jones, 2001). The consistency and the severity of the damage caused by *Bgt* therefore render necessary a systematic and extensive use of conventional fungicides. Again, powdery mildew populations can develop resistance, leading to the loss of efficacy of many systemic fungicides, such as strobilurins and sterol demethylation inhibitors (DMIs) (Hollomon and Wheeler, 2002). New strategies are currently being considered, that match the growing concern about the consequences of the use of fungicides on both health and environment. Among them, emerging alternative control strategies are based on the activation of the plant defence responses by the application of resistance inducers (Tayeh et al., 2014a). A given elicitor may trigger specific signal transduction pathways within the plant that could affect particular steps of the infectious process leading to a substantial level of resistance. Therefore, the knowledge of plant general defence responses, occurring in a susceptible

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wheat genotype but unable to confer total resistance on its own against powdery mildew, became of a crucial importance. Understanding corresponding mechanisms should lead to improved protection of plants from this agronomically important disease.

However, all plants, whether they are resistant or susceptible, respond to a pathogen attack by the induction of a coordinated defence strategy. The innate ability of plants to detect pathogens is essential for their immunity (Altenbach and Robatzek, 2007). This is made possible by the recognition of nonself molecular structures termed pathogen/microbe-associated molecular patterns (PAMPs/MAMPs) through pattern recognition receptors (PRR) (Nürnberger et al., 2004). As a result of PAMPs/MAMPs recognition, defence reactions are activated in the plant and the pathogen has to overcome the PAMP-triggered immunity (PTI) in order to establish a compatible interaction and successfully colonize the plant (Chisholm et al., 2006). The inactivation of defence mechanisms *via* the release of effectors that can suppress host immune responses is widely described as effector-triggered susceptibiliy (ETS) (Dry et al., 2010). However, it was not reported yet for *Bgt*. The time-course and the extent of the plant response to intracellular signaling resulting from infection determine the outcome of the interaction. The successful colonization of wheat by *Bgt* in susceptible genotypes is likely to be caused either by weak and/or delayed plant defence expression as suggested by Dixon et al. (1994). Furthermore, little attention has been paid so far to the plant genes expressed during the early phase of infection.

Thereby, our work focused on the early phase of a compatible interaction between wheat and powdery mildew. The aims of our study are (a) to describe the wheat response to *Bgt* and (b) to find out markers of defence that could be involved in partial resistance to powdery mildew by examinating a set of defence-related genes. We studied by quantitative real-time PCR (RT-qPCR) the expression of 10 genes over a time-course experiment (including hours after inoculation) to identify defence signal transduction pathways and further activities targeting the pathogen. PR-proteins which accumulate under pathogen attack and inhibit pathogen growth were therefore investigated, such as chitinases (EC 3.2.1.14) and PR1-protein. The *chi* gene (AY437443) expression was shown to be strongly induced in a wheat cultivar susceptible to *Septoria tritici* (*Zymoseptoria tritici*) (Shetty et al., 2009) and the expressions of *chi1* and *chi4 precursor* genes (AB029934 and AF112966) were up-regulated in response to methyl-jasmonate treatment and potentiated upon *Tilletia laevis* infection (Lu et al., 2006). The *PR1* gene (HQ848391) was isolated by Chen et al. (2011) from wheat leaves infected by *Puccinia triticina*. Moreover, reactive oxygen species (ROS) metabolism was studied here at two levels: ROS-generating and -eliminating enzymes, encoded respectively by an oxalate oxidase (OXO, EC 1.2.3.4) and glutathione S-transferase (GST, EC 2.5.1.13) encoding genes. The *gstF* gene (AF387085) was chosen regarding the phi-GST it encodes (cdd 48602); the latter being involved in cellular detoxification of products of oxidative stress as well as in transport of flavonoid pigments. Transcripts of oxalate oxidase (M21962), a ROS-generating enzyme, accumulated in wheat resistant to Hessian fly attack 24 and 48 h after infection (Liu et al., 2010). Lipid metabolism was investigated at 3 levels: lipoxygenases (LOX, EC 1.13.11.12) involved in the synthesis of oxylipins such as jasmonic acid (JA) (Howe and Schilmiller, 2002), phospholipases C (PLC, EC 3.1.4.11) that allow a transient accumulation of phosphatidic acid (PA) (Testerink and Munnik, 2005) and Lipid Transfer Proteins (LTPs) leading to intracellular trafficking of phospholipids and may present antifungal activity (Kirubakaran et al., 2008). A lipoxygenase-encoding gene (U32428) was chosen regarding its early and high responsiveness to several elicitors in wheat tissues, and more specifically its involvement in defence reactions against *Bgt* (Görlach et al., 1996). The wheat lipid transfer protein-encoding gene *Ltp 3F1* (EF432573) encodes an LTP protein that exhibited a broad-spectrum antifungal activity *in vitro*. Moreover, transgenic tobacco expressing *Ltp 3F1* gene showed fungal resistance to *Bipolaris oryzae*, *Cylindrocladium scoparium* and *Alternaria* sp. (Kirubakaran et al., 2008). The *PI-PLC2* gene (HM754653) identified by Khalil et al. (2011) in wheat encodes a phosphoinositide-specific phospholipase C (PI-PLC). The activity and localization of PI-PLCs enzymes have also been shown to regulate membrane trafficking (Thole and Nielsen, 2008) and to play a role in signaling involved in disease resistance in tomato (Vossen et al., 2010). The phenylpropanoid pathway was also examined through the phenylalanine ammonia-lyase (PAL, EC 4.3.1.24) (Vlot et al., 2009). The *pal* gene tested in here was shown to play a key role in generating a successful response in wheat against Diuraphis noxia, the Russian wheat aphid (Van Eck et al., 2010). The expression of the corresponding genes and some enzymatic activities at different stages of early phase of pathogenesis is analyzed and discussed. Although these defence reactions turned to be of a little effectiveness during such a compatible interaction, the modulation of the amplitude and chronology of the examined markers may be conceivable in order to meet the features of an induced resistance.

2. Materials and methods

2.1. Biological materials

Wheat (*T. aestivum* L.) cv. Orvantis provided by Benoits C.C. (Orgerus) was used throughout the experiments. The susceptibility level of Orvantis, a highly productive winter wheat cultivar, to powdery mildew was estimated at 5 on a scale ranging from 1 (most susceptible) to 9 (most resistant) (Arvalis-Institut du Végétal and CTPS, personal communication). The cultivar is susceptible to the MPEBgt1 powdery mildew isolate of *Bgt* used in previous studies (Renard-Merlier et al., 2007). The fungus was inoculated and maintained on Orvantis plants as described by Randoux et al. (2006).

For our experiments, wheat caryopses were soaked overnight in water and then grown on compost in a growth chamber (18 °C day temperature, 12 °C night temperature, and 70% relative humidity) for a 12-h photoperiod. Ten-day-old wheat plantlets were sprayed with *B. graminis* f. sp. *tritici* conidia suspended in Fluorinert FC 43 (heptacosafluorotributhylamine) provided by 3 M. The inoculum concentration was adjusted to 5×10^5 spores mL^{-1} and conidia were sprayed on leaves with a Preval Sprayer (Chicago Aerosol, Illinois, USA).

2.2. RNA extraction and quantification of gene expression by real-time PCR

Non-inoculated (ni) and inoculated (i) wheat leaves were sampled at 0, 3, 6, 9, 12, 15, 18, 21, 24, 48, 72 and 96 h post-inoculation (hpi) and stored at −80 °C until use. Total RNA was extracted from 100 mg of plant tissue using RNeasy Plant Mini Kit (Qiagen, The Netherlands). Genomic DNA contaminating the samples was removed by treatment with DNase using RNase-Free DNase Set (Qiagen, The Netherlands). Reverse transcription of total RNA was carried out using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) according to the manufacturer's protocol. Real-Time qPCR was performed using ABI Prism 7300 detection system (Applied Biosystems, USA). The sequences of primers used are shown in Table 1. The primers pairs were designed using the Primer Express® program (Applied Biosystems, USA) and were tested for secondary structure using NetPrimer® program (Premier Biosoft). The *tub* (U76895) and *act*