Early events involved in barley response to phytopathogenic fungi with different lifestyles

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Abstract

Broad spectrum of different fungal pathogenic lifestyles can infect barley plants, most of which are responsible for significant annual crop losses. Understanding of gene expression that take place at the earliest stages of infection would be a necessary step for describing the initial mechanism between barley and the pathogen interactions. The purpose of the present work was to monitor the expression of some well-identified genes PR1, PR2, PR3, PR5, PAL and SGT1 during interaction of resistant barley plants with three economically important diseases viz, spot blotch (Cochliobolus sativus), scald (Rhynchosporium secalis) and powdery mildew (Blumeria graminis) at very early stages of disease development. Data demonstrated a remarkable contradiction in the gene expression patterns between barley and pathogens interactions 12 and 24 hours post inoculation (hpi), and all of them showed significant differential expressions compared to the control plants. The most significant differences were balanced in SGT1 expression which was 3.86 (C. sativus) and 2.5 (R. secalis and B. graminis) folds higher at 12 hpi as compared with the corresponding control treatments. The results revealed that barley plants activated various resistance mechanisms against the three pathogens 12 hpi and increased dramatically at 24 hpi, and the same defense-related genes expression were changed in adaptation to the each fungus. Overall, this work provides insight into a signaling pathway that accounts for classical gene expression changes at very early times of infection, elicited during barley interaction with fungal pathogens having various lifestyles.

Keywords: Barley, Defense response, Powdery mildew, Quantitative PCR, Scald, Spot blotch.

Introduction

Research into plant defense responses at early infection stages by fungal pathogen is considered very important for pathogenesis studies and plant breeding programs. Recently, with the applying proteomics, genomics and transcriptomics techniques, different biochemical events can occur at very early stages of plant pathogen interactions (Kumar and Kirti, 2015; Zhao et al., 2019). Since plants have innate immune systems that can at early stages distinguish the presence of pathogens and thus activate defense responses (Chowdhury et al., 2017). Therefore, the biochemical mechanisms involved in plant resistance to these fungal pathogens are highly dynamic inducing both direct and indirect defense responses. As a consequent, increasing our understanding of these protective mechanisms is still needed.

It has been reported that plant leaves motivate the defense mechanisms 3 hpi in maize inoculated with Colletotrichum graminicola, 24 hpi in wheat challenged with yellow rust, and 12 hpi in rice (Liu et al., 2016). These examples showed that plants responses against pathogens started rapidly after coming in touch with each other and then various signal pathways to achieve disease resistance were triggered. Therefore, defense mechanisms and signaling pathways remain to be investigated.

Spot blotch caused by the necrotrophic fungus Cochliobolus sativus, scald caused by the hemibiotroph fungus Rhynchosporium secalis, and powdery mildew caused by the biotrophic fungus Blumeria graminis f. sp. hordei, are among the most important fungal pathogens of barley causing economic crop losses globally (Gangwar et al., 2018). Barley plants have developed a complex defense system against these diverse pathogens; however, the molecular events at very early stages of infection are not yet completely understood (Glazebrook, 2005).

Changes in the expression levels of a large number of defense-related genes can be estimated in barley plants during early stages of fungal pathogen infection, which considered a key defense genes (Stephens et al., 2008; Shen et al., 2017). Therefore, it is useful to improve our understanding concerning the changes of these genes in barley challenged by the four fungal pathogens with different lifestyles. Quantitative PCR (qPCR) is reported to be an effective method for measuring the relative expression level of particular genes in plant species infected with different fungal pathogens (Nolan et al., 2006; Derveaux et al., 2010).

Activation defense responses have been considered to be as one of the first reaction levels that usually noticed after disease infection (Kumar et al., 2016). These examples showed that plant leaves motivate the defense mechanisms 3 hpi in maize inoculated with Colletotrichum graminicola, 24 hpi in wheat challenged with yellow rust, and 12 hpi in rice (Liu et al., 2016). These examples showed that plants responses against pathogens started rapidly after coming in touch with each other and then various signal pathways to achieve disease resistance were triggered. Therefore, defense mechanisms and signaling pathways remain to be investigated.
al., 2002). A number of plant defense-related genes including PR genes like PR1, PR2, PR3, PR5, PAL and SGT1 were identified after fungal pathogen infection. These genes having various functions for instance systemic acquired resistance (PR1), beta-1,3-glucanase (PR2), chitinases (PR3), thaumatin like (PR5), secondary phenylpropanoid metabolism (PAL) and R protein accumulation (SGT1). However, since PR1, PR2, PR3 and PR5 genes coding key enzymes in the PAL and due to these functional roles they were chosen in this work.

There is still a great deal to be learned concerning the defense mechanisms at very early stages of barley infection with different fungal pathogenic lifestyles (Al-daooude et al., 2016; Jawhar et al., 2017a, b). Therefore, we evaluated here the expression changes of some important genes PR1, PR2, PR3, PR5, PAL and SGT1 at very early interaction periods 12 and 24 h after pathogens begin to contact with barley plant leaves surfaces.

Materials and Methods

Experimental design

The barley cv. Banteng from Germany was considered to be highly resistant to all B. graminis and C. sativus isolates available so far under extensive field trials for 15 years under greenhouse and field conditions (Arabi and Jawhar, 2004; 2012), therefore, it was used in the present work. Seeds were grown in plastic pots filled with peat moss, 10 seeds/pot with three replicates of each treatment. They were kept in a growth chamber at temperatures 18–22 °C and a relative humidity of 90%.

Inoculation with C. sativus

The high virulent isolate of C. sativus (pt4) described by Arabi and Jawhar (2004) was used in this study. Culture was grown on Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI, USA) at 20 °C for 10 days in an incubator. Seedlings were uniformly inoculated with conidia mL⁻¹ as reported previously by Arabi and Jawhar (2004).

Inoculation with B. graminis

Barley seedlings were infected with the virulent B. graminis conidiospores isolate (Pt1m) as described previously by Arabi and Jawhar (2012). Plants were placed under growth chamber, while control uninoculated plants were transferred to another growth chamber to keep away from infection with B. graminis.

Inoculation with R. secalis

The most virulent Syrian pathotype R. secalis (Rs46) described by Arabi et al. (2010) was used in this study. Mycelia was grown on Petri dishes containing lima bean agar (LBA), and the fungal suspension used to inoculate barley seedling was adjusted to 0.5 × 10⁶ (Zadoks et al., 1974).

RNA extraction and cDNA synthesis

Third barley leaves were collected at 12 and 24 hpi using liquid nitrogen, and control non-inoculated plants were collected at each time period. mRNA from each sample was isolated using Nucleo Trap mRNA mini kit (Macherey-Nagel, Düren, Germany). cDNA was synthesized according to Quanti Tect Reverse Transcription Kit (Qiagen, Germany).

Quantitative real-time PCR (qPCR)

Expressions of the selected genes PR1, PR2, PR3, PR5, PAL and SGT1 were performed in Step One Plus using SYBR Green Master Mix (Roche, USA). The PCR primer sequences are shown presented in Table 1. The threshold cycle (Ct) was determined according to Livak and Schmittgen (2001). Raw data of fluorescence levels were checked by qPCR dissociation curve analysis using StepOne™ Software v2.3. Tukey's test was used for statistical significance at 0.05 level.

Results and Discussion

In order to investigate the defense mechanisms present in resistant barley plants at very early stages of infection with three fungal pathogens having different lifestyles C. sativus, R. secalis and B. graminis (Table 2), the expression of six genes viz. PR1, PR2, PR3,PR5, PAL and SGT1 was assayed in the resistant barley cv. Banteng. Oligonucleotides designed from NCBI database demonstrated consistent results across replicates and gave differential amplification profiles (Table 1).

Results revealed significant changes in the gene expressions after infection with the three pathogens 12 and 24 hpi in barley resistant plants as compared with mock inoculated controls. However, the expression of the same genes was changed due to each fungus (Fig. 1). The most noticed differences were recorded in SGT1 gene expression, which was highly regulated for all the pathogens which were 3.86 (C. sativus) and 2.5 (R. secalis and B. graminis) folds higher 12 hpi as compared with the respective controls. These results indicate that this gene has different roles in response to various biotic pressures. Similarly, SGT1 was bound to enhance the resistance of Nicotiana benthamiana to the necrotrophic fungus Botrytis cinerea (El Oirdi and Bouarab, 2007), while compromised resistance was observed when barley and H. villosa infected with the biotroph B. graminis fungus (Shen et al., 2003; Xing et al., 2013).

Interestingly data showed that some of the studied genes were related with a multi-gene resistance which removes the current credence that identical responses are implicated in defense mechanisms to different fungal pathogenic lifestyles. For instance, PR1, PR2 and PR3 expressions were higher for both the necrotrophic Cs and hemibiotrophic Rs as compared with biotrophic ones.
B. graminis (Fig. 1), and probably is speedily targeting secondary mycelia growth of C. sativus and R. secalis than B. graminis This variation might be attributed to the fact that biotrophy demands a suitable period to suppress programmed cell death over effector secretion. Spanu and Pansu (2017) reported that the high stress of plant defenses might motivate the alteration from biotrophy to necrotrophy. On opposite, the change to necrotrophy and hemibiotrophic such as C. sativus and R. secalis could also be attributed to the fungal requires for improved nutrient acquirement (Kabbage et al., 2015). It has also reported that biotrophic Uromyces vignae and hemibiotrophic Mycosphaerella graminicola have suppressed the host defenses post fungal pathogen infection through the biotrophic phase (Doehlemann et al., 2008). The PRs functions in plant cell walls have been well documented (Golshani et al., 2015).

On the other hand, PAL expression started also 12 hpi and slightly increased after 24 hpi (Fig. 1). However, Huang et al. (2010) reported that domination of phenylalanine to transcinnamate is a crucial regulation point between primary and secondary metabolism. This fact might be the reason of barley cell wall leakage during infection by pathogen. Similarly, Kervinen et al. (1998) found an early increase in PAL expression in response of barley to fungal pathogens and elicitor treatments.

Our results can be supported by the recent works using the development of proteomics, genomics and transcriptomics methods that proved noticeable changes in wheat gene expressions at very early stages of Fusarium graminearum infection (Goswami et al., 2006), and comparative transcriptomics analysis rice and Magnaporthe oryzae (Li et al., 2015).

**Conclusion**

Collectively, this work suggested that in barley resistant plants various defense mechanisms can be activated to strengthen its necrotrophic C. sativus, hemibiotrophs R. secalis and biotrophic B. graminis resistance at very early stages of infections 12 and 24 hpi, and that the same defense-related genes expression were changed in adaptation to the each pathogen. The most observed variations were detected in SGT1 expression which was higher at 12 hpi as compared with controls. The data could be in line with the well-accepted notion that defense strategies are very intense in barley resistant plants.

### Table 1: Properties and nucleotide sequences of primers used in this study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Sequence</th>
<th>Amplified fragment (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF1a</td>
<td>AT1G07920</td>
<td>TGGATTTGAGGGTGACAACA</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCGTTCCAATACCAACCATC</td>
<td></td>
</tr>
<tr>
<td>PR1</td>
<td>AY005474</td>
<td>ACTACCTTTTACCCCAACACGC</td>
<td>182</td>
</tr>
<tr>
<td>PR2</td>
<td>AT3G57260</td>
<td>TTTCTGTCAAACATTCGCC</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCATCCCTGAACCCCTTCCTTG</td>
<td></td>
</tr>
<tr>
<td>PR3</td>
<td>AT3G12500</td>
<td>GGGGCTACTGTTTCAAGCA</td>
<td>187</td>
</tr>
<tr>
<td>PR5</td>
<td>AT1G75040</td>
<td>GGAAGCTGTGGCGGTCTAAG</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GCGTGGAGTCAGAGCACAGCA</td>
<td></td>
</tr>
<tr>
<td>PAL</td>
<td>AT2G14610</td>
<td>CCATTGATGAAGCCAAAGCAAG</td>
<td>123</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATGAGTTGGTTATCGTTGACGG</td>
<td></td>
</tr>
<tr>
<td>SGT1</td>
<td>AF439974</td>
<td>GGCTGTGTCTCTGCTACATCTTC</td>
<td>161</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CGAGGCTGGAATGTTATGTTCTC</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Early interaction between barley and three fungal pathogens used in the study.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>12 hours after inoculation</th>
<th>24 hours after inoculation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. sativus</td>
<td>Germination spores with only a small percentage forming hyphal appressoria.</td>
<td>Appressoria contact with anticlinal epidermal cell walls and stomata.</td>
<td>Rodríguez-Decuadro et al. (2014)</td>
</tr>
<tr>
<td>R. secalis</td>
<td>Germination spores and produce germ tubes, at from which appressoria form in response to stimuli from the leaf surface.</td>
<td>From the appressoria, fungi directly penetrates the cuticle above epidermal cells by means of penetration pegs rather than entering the leaf through stomata.</td>
<td>Jones and Ayres (1974)</td>
</tr>
<tr>
<td>B. graminis</td>
<td>Grows on the epidemis and infecting cells by appressoria.</td>
<td>Haustoria aformation onwards within host cells, enabling the fungus to feed.</td>
<td>Zhang et al. (2005)</td>
</tr>
</tbody>
</table>
Fig. 1: Relative expression profiles of six genes; *PR1* (A), *PR2* (B), *PR3* (C), *PR5* (D), *PAL* (E) and *SGT1* (F) in the resistant barley cv. Banteng during 12 and 24 h following infections with three pathogens (*C. sativus*, *R. secalis* and *B. graminis*). Error bars are representative of the standard error (Mean ± SD, n = 3). Data are normalized to Elongation factor 1α (*EF1α*) gene expression level (to the calibrator, Control 0 h, taken as 0). Significance at *P* < 0.05, **P** < 0.01 and ***P*** < 0.001 within each genotype during different periods comparing with the control.
References


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