The expression profile of Chi-1, Glu-2, Glu-3 and PR1.2 genes in Scab-resistant and susceptible wheat cultivars during infection by Fusarium graminearum

Hassan Soltanloo*1, Effat Ghadirzade Khorzoghi1, S. Sanaz Ramezanpour1, Mehdi Kalateh Arabi2, Mohammad Hadi Pahlavani1

1Plant breeding and Biotechnology Dept., Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
2Gorgan Agricultural Research Centre, Gorgan, Iran

*Corresponding author: soltanlooh@gau.ac.ir

Abstract

Fusarium head blight (FHB) is one of the most destructive diseases of wheat (Triticum aestivum L.) in the world. Importance of this disease is due to yield reduction and contamination of grains to FHB mycotoxin harvested from infective ears which are dangerous to human and animals. The goal of the present study was evaluation of few famous PR proteins in wheat defense reaction to Fusarium graminearum in ‘Falat’ as a highly susceptible and ‘Sumai3’ as a resistant cultivar. The expression pattern of Chi-1, Glu-2, Glu-3 and PR1.2 genes have been studied during wheat defense reaction to Fusarium graminearum in Falat and Sumai3 as susceptible and resistant wheat cultivars, respectively. Infected spikes are sampled in 1, 3 and 7 days after artificial inoculation and subjected to total RNA extraction and cDNA synthesis. Quantitative Real-time PCR analysis showed Chi-1, Glu-2, Glu-3 genes was significantly down regulated in the susceptible cultivar, whereas their expression were significantly increased in the resistant cultivar. The transcript of PR1.2 gene exhibited a particular pattern and was induced significantly in 7 days after inoculation in the resistance cultivar. Result showed that respected encoded PR proteins might play important roles in defense reaction of wheat to Fusarium graminearum.

Keyword: Chitinase; Fusarium graminearum; glucanase; PR-1.2; QRT-PCR; Triticum aestivum.

Introduction

Fusarium head blight (FHB) is economically one of the most important fungal diseases of wheat throughout the world. It also is an important disease of wheat in different areas of Iran, such as Mazandaran, Gorgan, Gonbad and Moghan regions (Moosawi et al., 2007). In most cases, F. graminearum was considered to be the predominant species responsible for the disease (Parry et al., 1995). Grains infected by Fusarium graminearum often are shriveled, with significantly lower kernel weight and can be easily blown away with the chaff during threshing (Bai and Shaner, 2004). Additional losses come from contamination of grains with mycotoxins produced by F. graminearum (Bernardo et al., 2006), which are hazardous to both humans and animals (Steiner et al., 2008). To control this dangerous disease, several strategies could be employed, such as application of fungicides or biological antagonists and breeding of genetically resistant cultivars. The most efficient strategy to control FHB in wheat is through the development of resistant cultivars. Resistance to FHB exhibits quantitative variation and its inheritance involves many loci on different chromosomes (Steiner et al., 2008). Recently, several studies have shown that infection by F. graminearum induces transcript accumulation of several classes of biotic and abiotic stress-related genes in both partially resistant and susceptible cultivars (Li et al., 2001; Pritsch et al., 2000, 2001; Kruger et al., 2002). The expression of biotic and abiotic stress-related genes may result in reduced FHB severity in wheat, but the relationship and mode of action between FHB resistant cultivar and F. graminearum has not been clearly addressed. Identifying host genes differentially expressed in response to the pathogen may help illustration of the cellular processes activated or repressed during the early phase of host-pathogen interactions that ultimately determine the extent of fungal colonization (Kong et al, 2005). The best studied quantitative trait loci (QTL) which confer FHB resistance are those of chromosomes 3BS (Fhb1 syn. Qfhs.ndsu-3BS) and 5A (Qfhs.ifa-5A) (Anderson et al, 2001; Buerstmayr et al, 2002, 2003). Despite several studies, the molecular events during early stages of the infection process resulting in resistance or susceptibility of wheat are still poorly understood. Clarification of molecular mechanisms involved in defense reaction of FHB-resistant cultivar in response to disease infection could open windows for plant breeders to reconstruct associated physiological traits in susceptible cultivars. Wheat responds to Fusarium infection by inducing various defenses including activation of pathogenesis-related (PR) genes has been reported by several studies (Pritsch et al., 2000; Li et al., 2001; Kruger et al.; 2002; Han et al., 2005; Zhou et al., 2005; Golkari et al, 2007; Bernardo et al, 2006). The induction of PR genes is a general
response to FHB, but it has been observed that few PR proteins are up-regulated, earlier, faster and/or more in resistant genotypes than in susceptible genotypes (Steiner et al., 2008). Between various quantification methods of measuring gene expression, QRT-PCR is the most sensitive and flexible and can be used to compare the levels of mRNAs in different sample populations, characterize patterns of mRNA expression, discriminate between closely related mRNAs and analyze RNA structure. In current study, we have employed QRT-PCR technique for investigation of expression pattern of Chi-1, Glu-2, Glu-3 and PR1.2 genes of wheat in response to FHB. The goal of the present study was evaluation of few famous PR proteins to determining initiation time point of wheat defense response to Fusarium graminearum in resistant and susceptible cultivars.

Materials and methods

Two wheat (Triticum aestivum L.) genotypes with contrasting levels of resistance and susceptibility to Fusarium head blight (FHB) were used. An Iranian spring wheat cultivar, Falat, as a highly susceptible to FHB along with a Chinese originated FHB resistance cultivar, Sumai3 which known for Type I and II FHB resistance (Bai and Shaner, 1994) have been employed. To prepare inoculums, fungal isolate was collected from 2008 field trap nursery and cultured on potato dextrose agar medium. About 5 gr straw powder were added to 125 ml of distilled water into 250 ml flask. Mixtures were autoclaved at 120 °C and 1 atmosphere for 30 minutes two times during 48 hours. Then, each flask was inoculated with an agar plug from a clean F. graminearum isolate in a laminar flow hood. The flasks were swirled gently at 120 rpm at 25 °C for 96 hours. The number of conidiospores per ml was determined by counting spores using a hemacytometer and adjusted to the desired spore concentration of 105 conidia spores/ml with distilled water. Plants were grown in field at Gorgan Agricultural Research Station in 2009 and inoculation was conducted in 6 to 7 weeks after germination at anthesis according to Zadoks stages 65-69 (Zadoks et al., 1974). Either 10 μl of F. graminearum suspension or distilled water was injected between the palea and lemma of 10 central spikelets per each spike. The infected glumes were collected for RNA isolation at 1, 3 and 7 days before cDNA synthesis according to manufactures instructions. The first strand of cDNA was synthesized from 2 μg total RNA as the template using M-MuLV Reverse Transcriptase (Fermentase, Germany) and oligo(dT) 18 primer. The forward and reverse primers for QRT-PCR were designed by Primer3 online software (Rozen and Skaletsky, 2000). Table 1 shows properties and sequences of primers for the Glu-2, Glu-3, Chi-1 and PR1.2 genes and also for the reference gene, GAPDH. The quantity of mRNA correspond to each gene was measured by SYBR Green method using SYBRBIOPARS™ Kit (Gorgan University, Iran). After an initial activation step of the DNA polymerase at 95°C for 10 min, samples were subjected to 40 cycles of amplification (denaturation at 95 °C for 10 sec, annealing at 60 °C for 10 sec and extension at 72 °C for 10 sec) and a terminal extension step at 72 °C for 5 min. Each sample was evaluated in 3 technical and two biological replications. Relative gene expression was calculated by Pfaffl formula (Pfaffl, 2001). The ratio between the target gene and housekeeping genes was analyzed by the REST software (Pfaffl et al., 2002). Melting curve was used to check primer specificity.

Results

Wheat genotypes Sumai3 and Falat, respectively as FHB resistant and susceptible, were investigated to determine the correlation between infection in spike tissues and timing of transcript accumulation of four defense response genes including Glu-2, Glu-3, Chi-1 and PR1.2 which encode β- (1,3; 1,4)-glucanase, β-(1,4)-glucanase, basic chitinase and neutral PR-1 protein, respectively. Figure 1 shows fold increase of Glu-2, Glu-3, Chi-1 and PR1.2 transcripts in FHB-resistant, ‘Sumai3’ and FHB-susceptible cultivar, ‘Falat’, at different time points after inoculation with F. graminearum. The y-axis values indicate the relative expression of differentially expressed genes in ‘Sumai3’ and ‘Falat’ inoculated with F. graminearum compared to control (mock inoculated) at each time point after inoculation (x axis). As shown in Figure 1, the expression of Glu-2 gene showed significant reduction 24 hours after inoculation in resistant cultivar ‘sumai3’. But three days after inoculation, in spite of primary depletion there was significant increase in gene expression and was continued for 7 days after inoculation. But the highest level of gene expression was observed 3 days after inoculation. This is consistent to the results of Pritsch et al., (2000). According to their results the maximum expression of defense genes in 36 to 48 hours after inoculation was synchronized with production of many branched hypha. There was significant depletion in expression of Glu-2 gene at 3 and 7 days after inoculation in ‘Falat’. Regardless of increasing, there was no significant difference for expression of this gene in resistant cultivar ‘sumai3’ at 3 days after inoculation but 7 days after inoculation expression of the gene was significantly upregulated. No significant differences were observed in gene expression of Chi-1 in FHB-susceptible cultivar ‘Falat’ all times after inoculation. Although in Sumai3 cultivar, there were no increase in gene expression 24 hours after inoculation but 3 days after inoculation expression of the gene was significantly decreased and this reduction continued until 7 days after inoculation. Regardless of Chi-1 transcript in FHB-resistance cultivar transcription of ‘Falat’ was upregulated 24 hours after inoculation but it wasn’t significant (Figure 1). In this case, Kong et al., (2005) obtained the same results for susceptible cultivar. The PR1.2 gene had different particular pattern and induced significantly in ‘sumai3’ at 7 days after
Table 1. Properties and Nucleotide sequences of primers used in QRT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene description</th>
<th>Accession No.</th>
<th>Sequences</th>
<th>Amplified fragment (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>glyceraldehyde-3-phosphate dehydrogenase</td>
<td>EF592180</td>
<td>tcaccaacgactacatgacc</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>agaaactgatctactagacaa</td>
<td></td>
</tr>
<tr>
<td>Chi-1</td>
<td>basic chitinase</td>
<td>AB029934</td>
<td>gggaactgctgaagagaga</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>aacttagctctactggctctg</td>
<td></td>
</tr>
<tr>
<td>PR-1.2</td>
<td>neutral PR-1</td>
<td>AJ007349</td>
<td>egcttctacacctcaacta</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>caacataacacgcaacta</td>
<td></td>
</tr>
<tr>
<td>Glu-2</td>
<td>β-(1,3; 1,4)-glucanase</td>
<td>Z22874</td>
<td>acagaactggaacactctcct</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cacatactgcaactcagacg</td>
<td></td>
</tr>
<tr>
<td>Glu-3</td>
<td>β-(1,4)-glucanase</td>
<td>AY091512</td>
<td>etctgctgttgatgctga</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tcacatgtggtgctggtg</td>
<td></td>
</tr>
</tbody>
</table>

Fig 1. Fold changes in accumulation of Glu-2, Glu-3, Chi-1 and PR1.2 transcripts in FHB-resistant ('Sumai3') and FHB-susceptible ('Falat') wheat cultivars at different days after inoculation (dai) with F. graminearum. The relative fold change of target gene transcripts was calculated using the comparative cycle threshold method. The infected samples were quantified relative to the controls (mock inoculated) at the same time points. GAPDH was used as an endogenous control to normalize the data for input RNA difference between the various samples. Mean values and standard deviation (S.D.) for four independent assays are shown. Asterisks indicate significant difference between control and infected samples: *< 0.05; **< 0.01.

Discussion

In this study we tried to analyze the role of some defense genes in response to Fusarium inoculation by applying the QRT-PCR method. The results showed rapid accumulation of Chi-1, Glu-2 and Glu-3 in the resistant cultivar comparing to the susceptible cultivar due to their role in host defense to F. graminearum.
This is in agreement with a previous study by Pritsch et al., (2000) that showed infection of wheat spikes with *Fusarium graminearum* induced a large set of biotic stress-related genes, including PR-1, PR-2, PR-3, PR-4, PR-5, and peroxidase in both type II resistant cv. Sumai3 (resistant to fungal spread in the spike) and the susceptible cv. Wheaton (Pritsch et al., 2000). Another study by Li et al., (2001) showed that expression of biotic stress-related genes encoding PR-2 and PR-3 was earlier and greater in ‘Sumai3’ than a susceptible ‘Sumai3’ mutant.

Further studies showed a systemic induction of biotic stress-related gene encoding PR-1, PR-3, PR-5, and peroxidase (Li et al., 2001). Chitinase is induced by various factors including fungal (Danhash et al., 1993; Van Kan et al., 1992), bacterial (Broekaert and Peumans, 1998), viral infection (Margis-Pinhero et al., 1993; Vogeli-Long et al., 1988), fungal elicitors (Hedrick et al., 1988; Mauch et al., 1988), treatment with plant hormones (Boller et al., 1983; Shimshi et al., 1987), abiotic factors (Roby et al., 1986; Yeh et al., 2000). Induction of chitinase is often coordinated with the induction of specific β-1,3-glucanase and PR proteins (Collinge et al., 1993). Previous study has indicated that chitinases and β-1,3-glucanases inhibit fungal growth by degrading chitin and (1,3)-β-glucan, both major structural cell-wall polysaccharides in growing hyphae (Bartnicki-Garcia, 1968). In this study, induction of chitinase, β-1,4-glucanase in high levels showed defense reaction of resistant cultivar to Fusarium infection. Antifungal properties of chitinase were also demonstrated by in vitro inhibition of purified chitinase in arrested growth of *Trichoderma reesei* and *Fusarium Sporotrichioides*, a barley seed root (Leah et al., 1991). Additionally, the role of chitinase can be synergistically enhanced by β-1,3-glucanases (Mauch et al., 1988). Therefore, the degradation of the fungal cell walls by the host chitinase may be, in part, an active defense mechanism of disease resistance in wheat.

Conclusions


Pfaffl MW, Horgan GW, Dempfle L (2002) Relative expression software tool (REST) for group-wide comparison and statistical analysis of relative expression results in real-time PCR. Nucl Acid Res 30: e36


