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

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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 response to *Fusarium graminearum* in 'Falat' as a highly susceptible and 'Sumai3' as a resistant cultivar. The expression pattern of C*hi-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 educed 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; Buerstmayer *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-resistance 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 10^5 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 after inoculation (dai). The mock inoculation was made by distilled water in both 'Sumai3' and 'Falat' for all time points. Immediately, the sampled spikes were placed on liquid nitrogen and transferred into a -80 ˚C freezer for storage until RNA extraction. The lemma, palea and subtending section of the rachis were pooled and ground into fine powder in liquid nitrogen using mortar and pestle. Total RNA was isolated from the *F. graminearum* conidia suspension-inoculated (tester) and mock inoculated (control) glumes of 'Sumai3' and 'Falat' using RNX-PLUS kit (Cinagen, Iran). Extracted RNA was quantified by spectrophotometer and its quality was verified by 1% agarose gel electrophoresis. RNA was treated with *DNase*I (Fermentase™, Germany) to remove DNA contamination 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 (denature 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 specifity.

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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-3* 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

Gene	Gene description	Accession No.	Sequences	Amplified fragment (bp)
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	*EF592180*	tcaccaccgactacatgacc Acagcaacctccttctcacc	120
Chi-1	basic chitinase	*AB029934*	gggctactgcttcaaggaaga acactaggtctgggttgctca	146
PR-1.2	neutral PR-1	*AJ007349*	cgtcttcatcacctgcaacta caaacataaacacacgcacgta	144
Glu-2	β -(1,3; 1,4)-glucanase	*Z22874*	agcagaactggggactcttct Cacatacgtaccgcatacacg	150
Glu-3	β-(1,4)-glucanase	*AY091512*	ccttgcctctttgtatgctga tcatcttttgtgggttcttgc	146

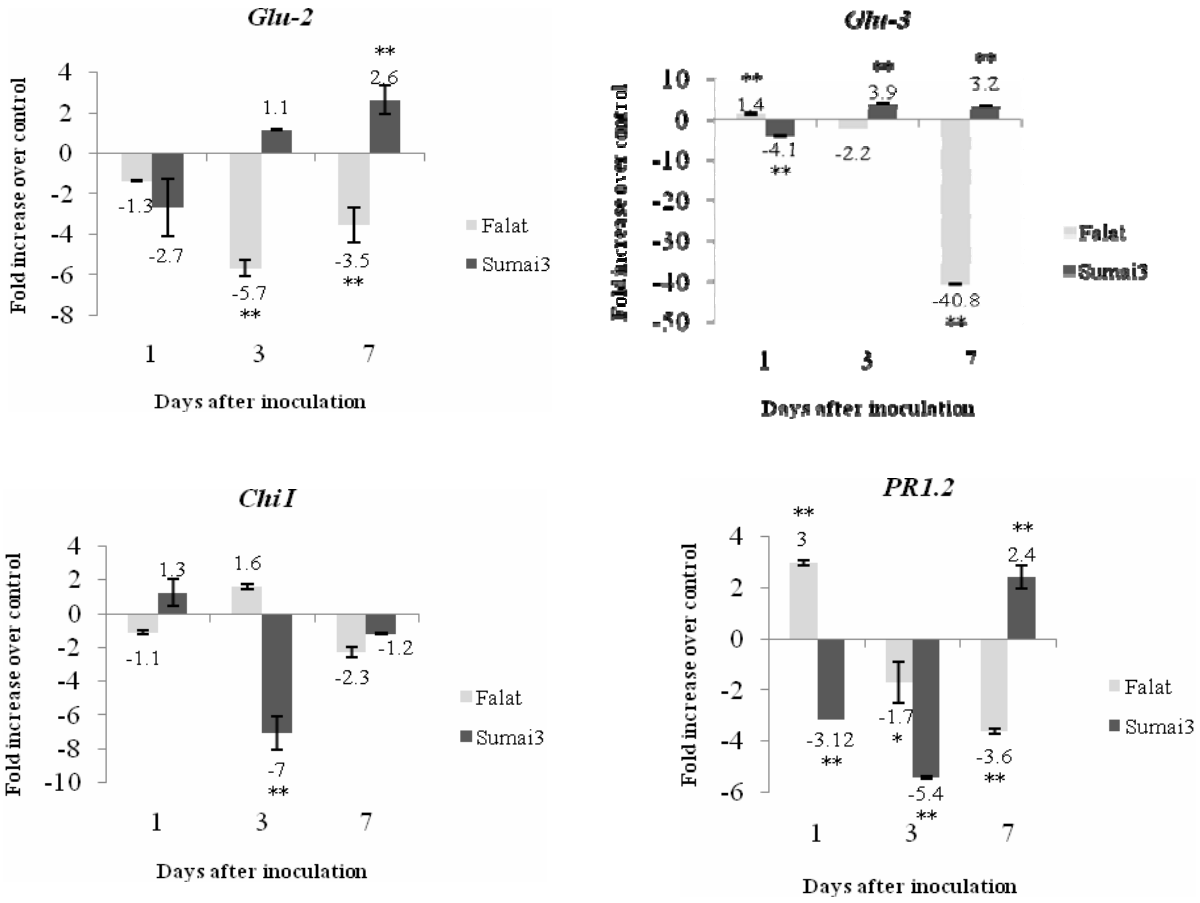

Fig 1. Fold changes in accumulation of *Glu-2, Glu-3, Chi-1 and PR1.2* transcripts in FHB-resistant ('Sumai3') and FHB-susceptible ('Falat') wheat cultivars at different days after inoculation (dai) with *F. graminearum*. The relative fold change of target gene transcripts was calculated using the comparative 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: $^{*}< 0.05$; $^{**}< 0.01$.

inoculation. In Falat cultivar, expression of the *PR1.2* gene significantly increased at 24 hours after inoculation. Induction PR1.2 gene in the susceptible cultivar considerably decreased at 3 days after inoculation while primary depletion of *PR1.2* in the resistant cultivar, 'Sumai3', was followed with significant increase 7 days after inoculation. Primary depletion of *PR1.2* could be occurred due to existence of Fusarium mycotoxin.

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 *F. 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; Shinshi *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 rot (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

In this study, we have evaluated expression patterns of some pathogenesis related proteins of two wheat cultivars including *Glu-2, Glu-3, Chi-1 and PR1.2*, during several time points after infection by *fusarium graminearum*, causal agent of fusarium head blight disease. The artificial inoculation has been conducted under field condition on 'Sumai3', as a well-known FHB-resistance cultivar and 'Falat', as highly FHB-susceptible check. Quantitative real-time PCR analysis showed that studied PR genes are regulated specifically and distinctively in resistance and susceptible cultivars. The expression profile of studied PR genes showed rapid accumulation of related mRNA and sometime higher up-regulation in resistance cultivar in response to *fusarium graminearum* infection. In conclusion, we demonstrated that general expression of *Glu-2, Glu-3, Chi-1 and PR1.2* genes was significantly increased and/or decreased under field condition. However, *PR1.2* gene had different particular pattern and induced significantly in resistant cultivar at 7 days after inoculation. Our results indicated that *Glu-2, Glu-3* and *PR1.2* genes are involved in defense reaction pathways of resistant cultivar during 3 and 7 day of *Fusarium graminearum* attack. The expression of these genes increased significantly in resistance cultivar is an evident of their role in defense reaction to FHB disease. Increment of *PR1.2* transcript 24 hour after inoculation in FHB-susceptible cultivar was unusual remarkable event and need to be addressed in future research.

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References

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno- sevilla B, Mitchell Fetch J, Sony QJ, Cregan PB, Ferohberg RC (2001) DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor Appl Genet 102:1164-1168.

Bai GH, Shaner G (1994) Scab of wheat: prospects for control. Plant Dis 78:760-776.

Bai GH, Shaner G (2004) Management and resistance in wheat and barley to Fusarium head blight. Annu Rev Phytophathol 42:135-161.

Bartnicki-Garcia S (1968). Cell wall chemistry, morphogenesis, and taxonomy of fungi. Annu Rev Microbiol 22:87–108.

Bernando A, Bai G, Guo P, Xiao K, Guenzi AC, Ayobi B (2006) *Fusarium graninearum*-induced changes in gene expression between Fusarium head blight-resistant and susceptible wheat cultivars. Funct Integr Genomics 7:69-77.

Boller T, Gehri A, Mauch F, Vogeli V (1983) Chitinase in leaves: induction by ethylene, purification, properties, and possible function. Planta 157:22- 31.

Broekarert WF, Peumans WJ (1988) Pectic polisacharides elicit chitinase accumulation in tobacco. Physiol Plant 74:740-744.

Buerstmayer H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (2003) Molecular mapping of QTL for Fusarium head blight resistance in spring wheat. II. Resistant to fungal penetration and spread. Theor Appl Genet 107:503-508.

Buerstmayer H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. resistance to fungal spread (Type II resistance). Theor Appl Genet 104:84-91.

Collinge DB, Kragh KM, Mikkelsen JD, Nielsen KK, Rasmussen U, Vad K (1993) Plant chitinases. Plant J 3:31–40.

Danhash N, Wagemakers CAM, Van Kan JAL, de Wit PJGM (1993) Molecular characterization of four chitinase cDNA obtained from *Cladosporium fulrum*- infected tomato. Plant Mol Biol 22:1017-1029.

Golkari S, Gilbert J, Prashar S, Procunier JD (2007) Microarray analysis of *Fusarium graminearum*-infected wheat genes: identification of organ-specific and differentially expressed genes. Plant Biotechnol J 5:38-49.

Han FP, Fedak G, Ouellet T, Dan H, Somers DJ (2005) Mapping of genes expressed in *Fusarium graminearum*-infected heads of wheat cultivar 'Frontana'. Genome 48:88-96.

Hedrick SA, Bell JN, Boller T, Lamb CJ (1988) Chitinase cDNA cloning and mRNA induction by fungal elicitor, wounding and infection. Plant Physiol 87:325-333.

Kong L, Anderson JA, Ohm HW (2005) Induction of wheat defense and stress-related genes in response to *Fusarium graminearum*. Genome 48:29-40.

Kruger WM, Pritsch C, Chao S, Muehlbauer GJ (2002) Functional and comparative bioinformatic analysis of expressed genes from wheat spikes infected wheat *Fusarium graminearum*. Mol Plant- microbe Interact 5:445-455.

Leah R, Tommerup H, Svendsen I, Mundy J (1991) Biochemical and molecular characterization of three barley seed proteins with antifungal properties. J Biol Chem 266:1564– 1573.

Li WL, Faris JD, Muthukrishnan S, Liu DJ, Chen PD, Gill BS (2001) Isolation and characterization of novel cDNA clones of acidic chitinases and *β*-1, 3-glucanases from wheat spikes infected by *Fusarium graminearum*. Theor Appl Genet 102: 353–362.

Margis-Pinhero M, Martin C, Didierjean L, Burkard G (1993) Differential expression of bean chitinase gene by virus infection, chemical treatment and uv irradiation. Plant Mol Biol 22:659-668.

Mauch F, Hadwiger LA, Boller T (1988) Antifungal hydrolases in pea tissue. Plant Physiol 87:325-333.

Moosawi-Jorf SA, Farokhi-Nejad S, Afarin S (2007) Study of Fusarium head blight of wheat in Khuzestan province in Iran and reporting of *Fusarium Xylaroides* as a new causal agent for disease. J Agron 6:212-215.

Parry DW, Jenkinson P, Mcleod L (1995) Fusarium ear blight (scab) in small grain cereals- a review. Plant pathol 44:207-238.

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

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucl Acid Res 29:2002-2007.

Pritsch C, Muehlbauer GJ, Bushnell WR, Somers DA, Vance CP (2000) Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. Mol Plant Microbe Interact 13:159–169.

Pritsch C, Vance CP, Bushnell WR, Somers DA, Hohn TM, Muehlbauer GJ (2001) Systemic expression of defense response genes in wheat spikes as a response to *Fusarium graminearum* infection. Physiol Mol Plant Pathol 58:1-12.

Roby KC, Toppan A, Esquerre-Tugaye M (1986) Cell surfaces in plant- microorganism interactions. Plant Physiol 81:228-231.

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ, pp 365-386.

Shinshi H, Mohnen D, Meins FJr (1987) Regulation of a plant phatogenesis- related enzyme. Inhibition of chitinase and chitinase mRNA accumulation in cultured tobacco tissues by auxin and cytokinin . Proc Natl Acad Sci 84:89- 93.

Steiner B, Kurz H, Lemmens M, Buerstmayr H (2008) Differential gene expression of related wheat lines with contrasting levels of head blight resistance after *Fusarium graminearum* inoculation. Theor Appl Genet 118:753-764.

Van kan JAL, Joosten MHAJ, Wagemarkers CAM (1992) Differential accumulation of mRNAs encoding extracellular and intracellular PR proteins in tomato induced by virulent and avirulent races of *Cladosporium fulrum*. Plant Mol Biol 20:513-527.

Vogeli-lange R, Hansen-Gehri A, Boller T, Meins FJr (1988) Induction of the defense related glucan hydrolases β- 1,3-glucanase and chitinase, by tobacco mosaic virus infection of tobacco leaves. Plant Sci 54:171-176.

Yeh S, Moffatt BA, Griffith M, Xiong F, Yang DSC, Wiseman SB, Sarhan F, Danyluk J, Xue YQ, Hew CL, Doherty-Kirby A, Lajoie G (2000) Chitinase genes responsive to cold encode antifreeze proteins in winter cereals. Plant Physiol 124:1251–1263.

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stage of cereals. Weed Res 14:415-421.

Zhou WC, Kolb FL, Riechers DE (2005) Identification of protein induced or upregulated by Fusarium head blight infection in the spikes of hexaploid wheat (*Triticum aestivum*). Genome 48:770-780.