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Fusarium head blight of barley : resistance evaluation and identification of resistance mechanisms

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FUSARIUM HEAD BLIGHT OF BARLEY: RESISTANCE EVALUATION AND IDENTIFICATION OF RESISTANCE MECHANISMS

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ABSTRACT

An evaluation of nineteen barley lines using three artificial inoculation methods concluded that spray inoculation was the most reproducible method and provided the greatest discrimination of resistance. Six of the nineteen barley lines were used for proteomic studies to identify defense responses following *F. graminearum* infection. All lines responded by inducing an oxidative burst and pathogenesis-related proteins. Differences in response magnitude and the proteins activated could be attributed to varying levels of FHB resistance amongst the barley lines. RNA microarray profiling and iTRAQ technology were used to study the interaction between two barley lines under five different treatments testing the effect of the fungus, trichothecene, and their interaction. Resistance was differentiated by the early induction of defense-related genes and the activation of the JA and ethylene defense pathways in Chevron, compared to the induction of a less efficient defense pathway in Stander; observed intra- and inter-cultivar differential responses are discussed.

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2-DE	Two-dimensional electrophoresis
A. cepa	*Allium cepa*
A. thaliana	*Arabidopsis thaliana*
AAFC	Agriculture and Agri-Food Canada
ABC	ATP-Binding Cassette
ACN	Acetonitrile
AFLP	Amplified Fragment Length Polymorphism
amu	Atomic Mass Unit
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
C. arietinum	*Cicer arietinum*
CapLC	Capillary Liquid Chromatography
CapTrap	Capillary Trap Cartridge
CDC	Center for Disease Control
cDNA	Complementary Deoxyribonucleic Acid
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo (Spanish)
CMC	Carboxymethylcellulose
CoA	Coenzyme A
cRNA	Complementary Ribonucleic Acid
CTP	Cytodine Triphosphate

d	Day
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
dpi	Days Post Inoculation
DTT	(D,L)-1,4-Dithiothreitol
ECORC	Eastern Cereal and Oilseed Research Center
ELISA	Enzyme-Linked ImmunoSorbent Assay
ESTs	Expressed Sequence Tags
F. culmorum	*Fusarium culmorum*
F. graminearum	*Fusarium graminearum*
FA	Formic acid
FBP	Fructose Bisphosphate Aldolase
FHB	Fusarium Head Blight
FKBP	FK506 Binding Protein
FNR	Ferredoxin-NADP(H) Reductase
GSH	Glutathione Synthetase
GSr1	Glutamine Synthetase isoform 1
GST	Glutathione-S-Transferase
h	Hour
H. annuus	*Helianthus annuus*
H. vulgare	*Hordeum vulgare*
hpi	Hours Post Inoculation
HPLC	High Pressure Liquid Chromatography

ICARDA	International Center for Agricultural Research in the Dry Areas
	International Maize and Wheat Improvement Center (English)
ID	Inside Diameter
iTRAQ	Isobaric Tags for Relative and Absolute Quantitation
JA	Jasmonic Acid
L. perenne	*Lolium perenne*
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LSD	Least Significant Difference
LTP	Lipid Transfer Protein
MAPKs	Mitogen Activated Protein Kinases
MB	Manitoba
min	Minute
M_w	Molecular weight
MS	Mass Spectrometry
NADPH	Nicotinamide Adenine Dinucleotide
NCBI NR	National Center for Biotechnology Information Non-Redundant
NRC	National Research Council
nsLTP	Non-Specific Lipid Transfer Protein
O. sativa	*Oryza sativa*
OD	Outside Diameter
P. oppositifolius	*Pteropogon oppositifolius*
P. sylvestris	*Pinus sylvestris*
PAL	Phenylalanine-Ammonia Lyase

PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PepMap	Peptide Mapping
pH	Power of Hydrogen
pI	Isoelectric Point
PI	Proteinase Inhibitor
PMT	Photomultiplier tube
ppm	Parts Per Million
PR	Pathogenesis-Related
PSII	Photosystem II
QTL	Quantitative Trait Loci
Q-ToF API	Quadruple Acceleration Time-of-Flight Atmospheric Pressure Ionization Source
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RMA	Robust Multi-Array Average
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
rpm	Revolutions Per Minute
RUBISCO	Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase
s.d.	Standard Deviation
SA	Salicylic Acid
SAR	Systemic Acquired Resistance

SAS	Statistical Analysis System
SCX	Strong Cation Exchange
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
SOD	Superoxide Dismutase
spp.	Species
SSR	Simple Sequence Repeat
Sti1	Stress-induced protein 1
T. aestivium	*Triticum aestivium*
TCA	Trichloroacetic Acid
TIGR	The Institute for Genomic Research
TLP	Thaumatin-Like Protein
TOF	Time of Flight
Tri5	Trichothecene 5 gene
U of S	University of Saskatchewan
U.S.A.	United States of America
UDP	Uridine Diphosphate
UTP	Uridine Triphosphate
V. repens	*Veronica repens*
Z. mays	*Zea mays*

CHAPTER 1 LITERATURE REVIEW

1.1 Fusarium head blight

Fusarium head blight (FHB), or scab, is a devastating disease of wheat and barley often grown in humid and semi-humid climates worldwide (Schroeder and Christensen, 1963; Steffenson, 2003). Although many *Fusarium* species can cause FHB, *Fusarium graminearum* Schwabe [teleomorph = *Gibberella zeae* (Schwein) Petch] is the primary pathogen responsible for recent epidemics in the United States, Canada, and China. Disease symptoms associated with *Fusarium* spp. infection develop in the spike tissue and are marked by premature necrosis and brown/grey discoloration. FHB in cereals causes significantly lower grain yield, lower test weight, reduced grain quality, and reduced milling yield (Stack, 1999). Infection with *Fusarium* spp. leads to destruction of the cell walls and starch granules, affecting endosperm storage proteins, resulting in a poor quality product (Snijders, 2004).

1.1.1. Inoculum and Infection

The genus *Fusarium* was named in 1809 by the German mycologist, Link, for a fungus having fusiform spores (Booth, 1971). *F. graminearum* is a saprophyte and facultative parasite. It is capable of colonizing living host tissue such as wheat, corn, barley, soybean, and rice tissues efficiently at specific stages of the host life cycle as well as establishing itself in senescent tissue and crop debris, building up an inoculum in the soil (Xu and Chen, 1993; Miller, 1994; Shaner, 2003). Ascospores, macroconidia, chlamydospores, and hyphal fragments can all serve as inocula (Bai and Shaner, 1994).

Ascospores released from soil surface debris form the primary inoculum responsible for initiating epidemics; *F. graminearum* completes its sexual cycle by the formation of perithecia on exposed spikes (Xu and Chen, 1993; Bai and Shaner, 1994; Shaner, 2003). The ascospores are released in a diurnal pattern, correlated with a rise in relative humidity during early evening hours (Paulitz, 1996). Rainfall is required to trigger the release of ascospores, but may not be needed for their formation or to ensure crop maturity (Paulitz, 1996). The abundance of a primary inoculum and weather conditions, mainly moisture and temperature, during and after anthesis determine the severity of FHB (Hart *et al.,* 1984; Bai and Shaner, 2004).

In wheat and barley, heads are most susceptible and infection levels are highest during anthesis (Sutton, 1982). Figure 1.1.1 shows the anatomy of a barley spike and phenotypic symptoms associated with FHB of barley. Airborne spores released from crop residue are deposited on or inside wheat florets where they germinate and initiate infection. In wheat, the fungus initially does not penetrate directly through the epidermis; rather, hyphae develop on the exterior surfaces of florets and glumes, allowing the fungus to grow toward the stomata and other susceptible sites within the spike, possibly leading to direct penetration of the epidermal cells (Bushnell *et al.,* 2003). Other avenues for direct entry include the stomata and underlying parenchyma, partially or fully exposed anthers, openings between the lemma and palea of the spikelet, and through the base of the wheat glumes where the epidermis and parenchyma are thin-walled (Lewandowski and Bushnell, 2001; Bushnell *et al.,* 2003). In wheat, spread of the fungus among florets is through the vascular bundles in the rachis and rachilla (Ribichich *et al.,* 2000). As the

A) B)

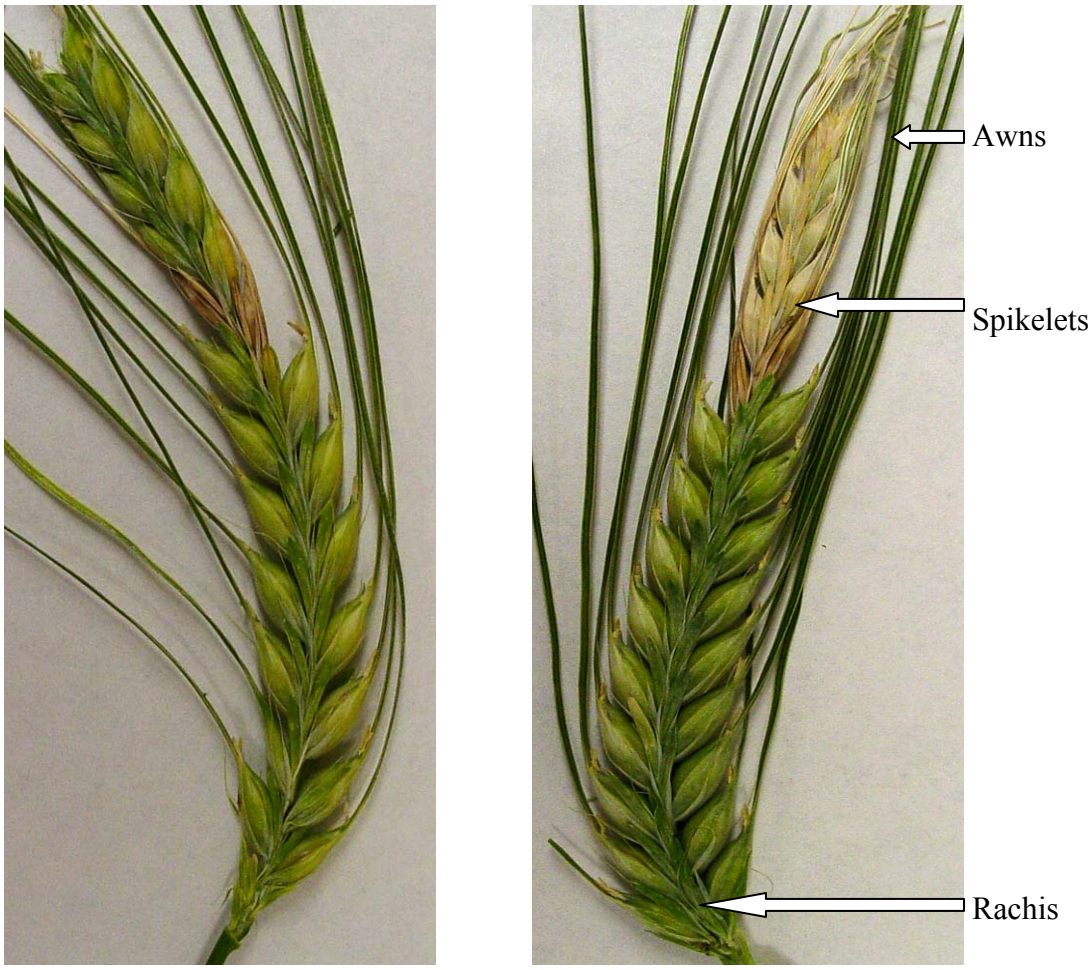

Figure 1.1.1 Anatomy of a barley spike and phenotypic symptoms associated with Fusarium head blight of barley from point inoculation with *Fusarium graminearum* strain N2 macroconidia. A) Moderately resistant two-row variety Morrison; figure shows the brown discoloration and shriveling of point inoculated spikelets. B) Intermediate resistant two-row variety CDC Bold; figure shows the brown discoloration and shriveling of spikelets following point inoculation and the bleaching of upper spikelets and awns due to clogging of vascular tissues.

fungus spreads within a spike, browning of the plant usually extends into the rachis and eventually down into the stem tissues.

In barley, the fungus is known to spread on the exterior of the spike under wet conditions; internal spread through the rachis is more limited (Bushnell *et al.,* 2003). *F. graminearum* infection of barley floral structures likely proceeds through direct penetration of cells via infection-peg-like and coralloid structures, through stomata, and by growing from the abaxial to the adaxial side of floral bracts (Bushnell *et al.,* 2003). Boddu *et al.* (2006) observed a difference in timing of infection between wheat and barley; infection-related structure development and other morphological changes were observed between 12 to 24 h earlier in wheat than in barley. The delayed germination of fungal structures in barley could provide an opportunity for the plant to respond to infection and restrict spread of the disease.

In both cereal crops, minor clogging of the vascular tissues via the rachis can cause the head to ripen prematurely; even grains not directly infected become shriveled due to a shortage of water and nutrients (Schroeder and Christensen, 1963; Bai, 1995). If infection is extensive at a very early stage, then kernels may fail to develop properly, significantly reducing grain yield and quality. The fungus appears to have a brief biotrophic relationship with the host during the initial 48 to 72 h post inoculation (hpi) before switching to the necrotrophic phase at approximately 72 hpi (Kang and Buchenauer, 1999; Bushnell *et al.,* 2003). It has been suggested that the shift from the biotrophic to the necrotrophic stage is mediated in part, by deoxynivalenol (DON) and related phytotoxic trichothecene mycotoxins produced by head blight fungi (Bushnell *et al.,* 2003). The necrotrophic stage is often associated with an increase in fungal

colonization strength and eventually plant death, allowing for further colonization of the host substrate (Goswami and Kistler, 2004).

1.1.2. Economic and Social Impacts

FHB was first described in 1884 in England by W. G. Smith (Arthur, 1891) and within a decade, the disease was found in North America and throughout Europe. Arthur (1891) reported a 75% yield reduction in a wheat field in Indiana following the 1890 epidemic. By 1917, FHB had gained world recognition, being identified in 31 states in the U.S.A., throughout Europe (Atanasoff, 1920), and in Japan (Nisidako, 1959). Outbreaks of FHB continued in North America during the 1920s and 1930s, and in 1940 FHB lead to epidemics in Eastern Canada and Ontario (Sutton, 1982). From 1928 to 1937, large yield losses were reported in many states of the U.S.A. (Dickson, 1942). In China, FHB outbreaks in the Yangtse River Valley have occurred every two to four years during the past three decades with yield losses as high as 40% in severely infected fields (Anonymous, 1989). Severe epidemics have also been reported in Hungary since 1970 (Mesterhazy, 1984) and in other European countries since 1998 (Ellner, 1999). In 1993, scab struck the tri-state area of Minnesota, North Dakota, and South Dakota, and the Canadian prairie province of Manitoba, devastating the wheat and barley industries (McMullen *et al*., 1997). An average direct yield loss of more than 30% of wheat was reported in Minnesota with an economic loss of more than $400 million U.S. (Busch and Wiersma, 1994). In total, economic losses since 1990 have been estimated at $3 billion U.S. for wheat and $0.4 billion U.S. for barley (Windels, 2000). Part of the devastation caused by FHB is a result of its ever-changing impact on the cereal industry; fluctuations

in infection frequency, and the lack of knowledge regarding disease significance (Comeau, 1999).

1.2 Mycotoxins

During the infection process, *F. graminearum* produces mycotoxins, which adversely affect grain quality, create toxic dust, and make the grain unsuitable for human or livestock consumption. Mycotoxins are toxic secondary metabolites produced by moulds during their growth period. Mycotoxins produced by *F. graminearum* belong to the zearalenone and trichothecene families. Trichothecenes cause vomiting, diet refusal, diarrhea, hemorrhage, blood and nerve disorders, edema, weakening of the immune system and death in small animals (Ueno, 1977; Anonymous, 1989). DON remains intact during the malting and brewing processes (Schwarz *et al.,* 1996), and as a consequence, the food and malting industries have set very low tolerance levels for trichothecenes. In 1980, DON first appeared in Canada, originating from *F. graminearum* infections of wheat in Ontario and Quebec (He *et al.,* 1992). The presence of DON in grains created an enormous challenge for cereal breeders, producers, and the food processing industry. The Food and Drug Administration established the following advisory levels for DON in food and feed in 1993: 1 part per million (ppm) for finished grain products used for human consumption, 10 ppm for feed given to cattle over four months old and poultry, 5 ppm for swine and all other animals, and no standard has been set for raw grain going into milling processes (Stack, 1999).

In cereals, *F. graminearum* and *F. culmorum* are the major mycotoxin-producing species (Parry *et al.,* 1995). The quantity of toxic metabolites varies among seedlings

exhibiting similar scab levels and it appears that regulation of DON accumulation depends on complicated interactions among the host and fungal genotypes as well as on ecological and environmental conditions (Mesterhazy *et al.,* 1999). To date, it is not possible to develop forecasting models based on toxin contamination; however, Mesterhazy *et al.* (1999) observed a close association between DON accumulation, FHB symptoms, and relative grain weight.

1.2.1 Trichothecenes

Trichothecenes are tetracyclic sesquiterpenoids; their biosynthesis diverges from general isoprenoid metabolism with the formation of trichodiene via the cyclization of farnesyl pyrophosphate (Desjardins *et al.,* 1993). Trichodiene undergoes a series of oxygenations, cyclizations, isomerizations, and esterifications to yield bioactive trichothecenes such as DON and acetylated DON (Desjardins *et al.,* 1993). Trichothecenes share the same basic chemical structure as a 12, 13-epoxytrichothec-9-ene ring system; DON specifically is 3α, 7α, 15-trihydroxy-12, 13-epoxytrichothec-9-en-8-one (He *et al.* 1992; Bretz *et al.,* 2005). Based on type B trichothecene production there are three chemotypes: the nivalenol chemotype produces nivalenol and acetylated derivatives; the 3-acetyl-deoxynivalenol (3A-DON) chemotype produces DON and 3A-DON; and the 15-acetyl-deoxynivalenol (15A-DON) chemotype produces DON and 15A-DON (Miller *et al.,* 1991). Figure 1.2.1 shows the chemical structure of trichothecene, three chemotypes, and the conversion of farnesyl pyrophosphate to deoxynivalenol.

The trichothecene mode of action involves blocking peptidyl transferase activity at the 60S ribosomal subunit in eukaryotes; thereby interfering with initiation, elongation

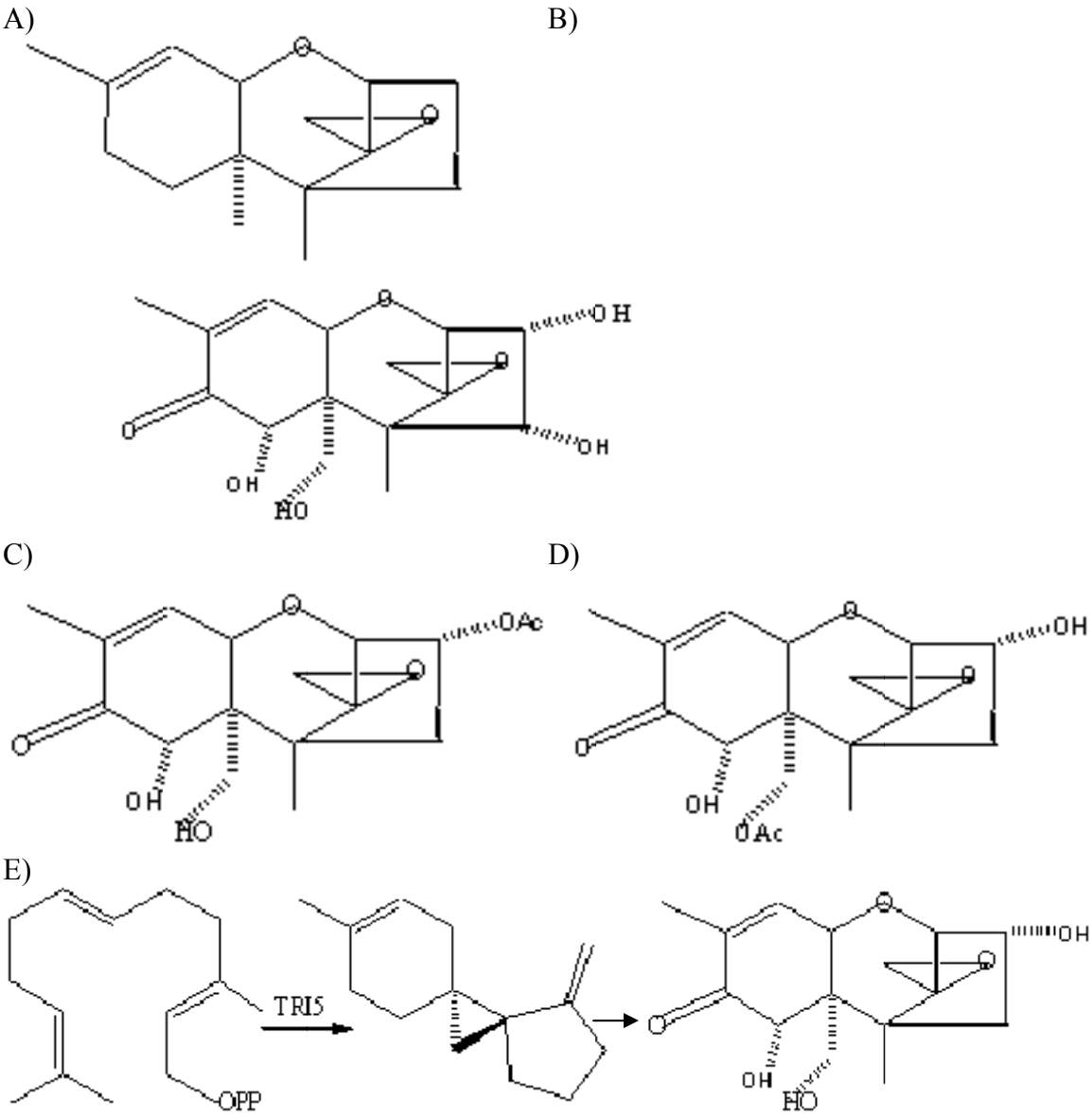

Figure 1.2.1 Chemical structure of mycotoxins. A) Chemical structure of trichothecene. B) Chemical structure of nivalenol chemotype. C) Chemical structure of chemotype 3-acetyldeoxynivalenol (3A-DON). D) Chemical structure of chemotype 15-acetyldeoxynivalenol (15A-DON). E) Conversion of farnesyl pyrophosphate to trichodiene using trichodiene synthase (TRI5) and then the conversion to deoxynivalenol.

or termination of protein synthesis (McLaughlin *et al.,* 1977). Trichothecenes also have multiple effects in plant cells, inhibiting DNA and RNA synthesis and altering membrane properties (Khachatourians, 1990). Blocking of ribosomal activity can cause inhibition of nucleic acid synthesis (Thompson and Wannemacher, 1986; Minervini *et al.,* 2004) and mitochondrial function (Pace *et al.,* 1988; Minervini *et al.,* 2004), as well as having a negative impact on cell division (Rocha *et al.,* 2005) and membrane integrity (Kang and Buchenauer, 1999).

Some researchers have reported a strong association between FHB intensity and DON concentration in infected grain (Hart *et al.,* 1984; Wang and Miller, 1988; Snijders and Krechting, 1992; Wong *et al.,* 1994) while others have failed to detect an association (Snijders and Perkowski, 1990). Savard *et al.* (2000) described the pattern of accumulation and relative concentration of DON in different parts of wheat heads at various times after single point inoculation with *F. graminearum*. Results showed that DON was first detected 4 days post inoculation (dpi) in both the floral parts and the rachis; and DON concentration (average = 298 ppm) was highest in the inoculated spikelets (Savard *et al.,* 2000). The concentration of DON in the rachis increased rapidly, surpassing that found in the floral tissue within 9 days; 19 dpi, DON concentrations of up to 1000 ppm were recorded in the peduncle, and levels in the floral tissue below the inoculation point peaked at 500 ppm of DON (Savard *et al.,* 2000).

1.2.2 Virulence

Diversity in trichothecene production contributes to *Fusarium* spp. virulence and genetic variability. The capacity of the trichothecene to produce different toxin isolates may be the determining factor for pathogenicity (Mesterhazy *et al.,* 1999). Arsenuik *et al.* (1999) showed that an inoculum containing a mixture of *Fusarium* spp. produced a more severe FHB reaction than inocula containing individual species, suggesting that diversity of trichothecenes could also contribute to severity of FHB. Wang and Miller (1988) suggested that correlating cultivar susceptibility with reaction to a single purified trichothecene for all genotypes would be difficult, since studies have shown that several trichothecenes are involved with infection (Lemmens *et al.,* 1997). Reports in barley indicate that within a field, mixtures of *F. graminearum* strains or other *Fusarium* spp. often coexist within the same head and sometimes in the same seed (McCallum *et al.,* 1999; McCallum and Tekauz, 2002).

Trichothecene virulence has been well studied by Proctor *et al.* (1995) and Desjardins *et al.* (1996). *Tri5*, of the trichothecene biosynthesis gene cluster was the first virulence gene of *F. graminearum* to be identified and verified by gene disruption. *Tri5* encodes a trichodiene synthase that catalyses the first committed reaction in the trichothecene biosynthetic pathway. Proctor *et al.* (1995) and Desjardins *et al.* (1996) used molecular genetic techniques to produce mutant fungal strains (GZT40) lacking the ability to synthesize trichothecenes. These mutants were pathogenic, but compared to the parental wild types (GZ3639), produced a reduced incidence and severity of infection, less bleaching of heads, less yield reduction, and less spread from single spikelet inocula (Proctor *et al.,* 1995; Desjardins *et al.,* 1996; Eudes *et al.,* 1997; Mirocha *et al.,* 1997).

The trichothecene-producing strain was more aggressive than the trichothecene non-producing strain in a Fusarium seedling blight when assayed in wheat, oats, and winter rye, and in a head blight assayed in wheat (Proctor *et al.,* 1995; Desjardins *et al.,* 1996; Bai *et al.,* 2001a; Eudes *et al.,* 2003); however, no significant difference was observed between the strains in maize (Proctor *et al.,* 1995; Harris *et al.,* 1999). Further studies comparing the effects of trichodiene synthase-producing strains and trichodiene synthase mutants must be done to better understand the effects of the fungus and the mycotoxins relative to plant defense and FHB resistance.

1.3 Control methods

1.3.1 Culture practices

FHB epidemics occur when inoculum and humidity levels are elevated at anthesis (Parry *et al.,* 1995). Management practices that prevent inoculum build-up, or interfere with dispersion and infection of spikes can greatly reduce the severity of FHB epidemics (Parry *et al.,* 1995). Crop rotation is a simple and effective method for reducing the amount of *Fusarium* inoculum available on wheat residue (Atanasoff, 1920; Dickson, 1929; Jones, 1994; McMullen and Luecke, 1996). An early survey of 182 wheat fields across seven states in the U.S.A. indicated that corn-wheat rotations increased inoculum levels of FHB; whereas wheat-clover rotations decreased inoculum levels (Koehler *et al.,* 1924). This can be attributed most likely to the fact that both wheat and corn are susceptible to the same FHB pathogens and extensive corn residue contributes to the survival of these pathogens and subsequent wheat infection (Parry *et al.,* 1995). An

alternative method for reducing inoculum potential of the fungus is through tillage practices since fungal survival is best on the residue left on or above the soil surface. Soil tillage buries grain residues, thus reducing fungal survival (Stack, 1999). Staggered planting of small grain crops or planting of cultivars differing in days to maturity is also advised, so that a producer's entire crop is not at risk of flowering during a period favorable for FHB (Stack, 1999). At the time of harvest, machinery may be adjusted so that light-weight FHB kernels are removed, however this will not remove *Fusarium* spp.-infected kernels that have managed to develop properly (Stack, 1999).

1.3.2 Chemical controls

A fungicide spray program may help reduce FHB damage, but chemical control of *Fusarium* spp. infection has had limited success (Parry *et al.,* 1995; McMullen *et al.,* 1997). Reductions in FHB severity of 50 to 60% can be achieved when fungicides are applied at the early flowering stage for wheat and durum, and at the early heading stage in barley (Stack, 1999). Unfortunately, results with heading time application of fungicides have been variable; these inconsistencies may occur due to a lack of disease forecasting information (Mauler-Machnik and Zahn, 1994; Milus and Parsons, 1994; Parry *et al.,* 1995). In the mid-1990s, the protectant Mancozeb and systemic Benomyl fungicides were registered for heading time application in wheat and barley (Stack, 1999); since this time other fungicides have become available to producers. The systemic fungicide Tilt (Syngenta Crop Protection Canada) and Folicur (Bayer Crop Science) were used for heading time application in wheat and barley, and the fungicides Tebuconazole and Carbendazim have been shown to substantially reduce both *Fusarium* and mycotoxin

levels in wheat (Stack, 1999; Cromey *et al.,* 2001; Simpson *et al.,* 2001). However, the potential risk of increased DON contamination of grain following treatment with Azoxystrobin to control head blight in susceptible wheat cultivars was reported by Simpson *et al.* (2001). Although fungicide applications for managing FHB have provided some desirable results, the environmental effects and relationship between cost and return per acre are limiting factors (McMullen *et al.,* 1997). In the future, improved application technology and disease forecasting information may allow producers to effectively use fungicides in conjunction with other disease management strategies (Mauler-Machnik and Zahn, 1994).

1.3.3 Genetic Resistance to Disease

Christensen *et al.* (1929) believed that the development of resistant wheat varieties was the only effective method for controlling scab. Repeated scab epidemics and large economic losses have resulted in increased emphasis on development of resistant cultivars. Progress has been made and today, new sources of resistance have been identified (Wang *et al.,* 1982; Bai and Shaner, 1994; Busch, 1995; Lipps and Johnston, 1996). Studies conducted by Mesterhazy (1995) have lead to a better understanding and greater knowledge regarding FHB-resistance, while Bai and Shaner (1994) have developed molecular tools for incorporating resistance into various crops. Stack (1999) reported improved collaboration in the exchange and evaluation of breeding materials and the evolution of a longer-term commitment to funding FHB-resistance research. Breeding and pathology programs are actively screening and developing genetic material with improved resistance to FHB (Stack, 1999). Although there are no reported sources

of immunity, considerable genetic variability for resistance to FHB exists in some cereal species.

In China, FHB resistance research began in the 1950s and continues today (Liu and Wang, 1990). In the 1960s, Japanese scientists initiated ongoing FHB resistance research programs in barley (Takeda and Heta, 1989). FHB is also a major focus of breeding programs in several European countries including Hungary, Poland, Austria, Germany, and the Netherlands (Bai and Shaner, 1994; Gilbert *et al.,* 1997; Miedaner, 1997; Stack *et al.,* 1997), and in North America (Bai and Shaner, 1994; Gilbert *et al.,* 1997; Stack *et al.,* 1997; Rudd *et al.,* 2001). The current strategy employed by many breeding programs is to recombine different types and sources of resistance gradually, using conventional breeding strategies. The best characterized and most widely used source of wheat FHB resistance is Sumai 3 (Rudd *et al.,* 2001). Lines derived from Sumai 3 have stable resistance and more suitable agronomic traits. Unfortunately, high yielding progeny of crosses from Sumai 3 and other resistant wheat cultivars have never reached the resistance levels of the original sources (Chen, 1983). This may account for the limited number of resistant cultivars registered in North America and Europe.

Sources of FHB resistance in barley are more limited than in wheat and the highest level of resistance is modest. Although FHB in barley does not spread from spikelet to spikelet within a spike, barley seems to be susceptible to initial infection (Bai and Shaner, 2004). The non-malting barley cultivar, Chevron, from Switzerland, exhibits the best known source of FHB resistance and lowest DON content of six-rowed genotypes evaluated to date, while CI4196, from China, exhibits the highest level of FHB resistance known to date in a two-rowed variety (Prom *et al.,* 1996; Rudd *et al.,* 2001;

Steffenson, 1998; 2003; McCallum *et al.,* 2004). Unfortunately, these cultivars tend to be undesirable from an agronomic point of view, susceptible to other disease, have unacceptable malting and feed quality, and their resistance to FHB has been shown to break down if infection levels are high enough (Legge *et al.,* 2004). Other sources of FHB resistance in barley are Zhedar #1, Gobernadora, Harbin, Svanhals, Svansota, Shenmai 3, and HDE84194-622-1. Sources of moderate and intermediate FHB resistance are Morrison, Island, CDC Sisler, AC Metcalfe, TR253, TR04281, TR04282, and TR04283. Two sources of susceptibility to FHB are the two-rowed variety CDC Bold and the six-rowed variety Stander (Tucker *et al.,* 2003).

1.3.3.1 Types of Resistance

In 1963, Schroeder and Christensen proposed two types of FHB resistance in wheat: resistance to initial infection (type I) and resistance to spread of infection within a spike (type II). In wheat, type II resistance has been extensively studied and appears to be more stable and less affected by non-genetic factors than type I resistance (Bai and Shaner, 1994). In barley, although type II resistance has been reported (Zhu *et al.,* 1999), type I resistance is more important (Steffenson, 2003) due to the absence of fungal spread within the spike among infected spikelets (Bushnell *et al.,* 2003). In wheat and barley, different artificial inoculation methods are used to distinguish between the two types of resistance.

Type I resistance is challenged by spraying a spore suspension over flowering spikes and counting the diseased spikelets, a technique referred to as spray inoculation. Type I resistance may be tested in the greenhouse or in the field where, corn residue

inoculated with *Fusarium* spp. is dispersed on the ground underneath the flowering crop and diseased spikelets are counted. Type II resistance is assessed in a greenhouse by point inoculation; delivering macroconidia into a floret of a spike by direct injection and counting the blighted spikelets after a period of time. Spray inoculation mimics natural infection while controlling environmental factors and inoculum dosage and requires less time and labor for inoculation and rating. It is possible that point inoculation results in the over powering of the plants' defenses, making it difficult to distinguish symptom severity among the lines tested. Field trial results are greatly impacted by environmental conditions and may vary significantly from year to year. In barley, the proportion of blighted spikelets on an infected spike (disease index) may be a good measurement of type I resistance (Bai and Shaner, 2004).

Four other types of resistance have been described (Ban, 2000). Type III and IV resistance encompass the ability of the host to degrade and tolerate DON, respectively (Miller *et al.,* 1985; 1986; Wang and Miller, 1988). Mesterhazy *et al.* (1999) added type V and type VI resistance as the resistance to kernel infection and tolerance to FHB, respectively. Resistance to kernel infection is measured by threshing infected spikes and observing damage to the kernels (Mesterhazy, 1995). Type VI resistance is assessed by measuring grain yield of naturally or artificially inoculated spikes or plots and comparing the data with spikes or plots that do not show disease symptoms (Mesterhazy, 1989; 1995; Mesterhazy *et al.,* 1999). Challenges associated with evaluating type I resistance include difficulties in quantifying the amount of applied inoculum, varying environmental factors, and confounding effects that type II, III, and IV resistance have on

disease assessment. The confounding effects of type III and IV resistance also make accurate disease assessment for type II resistance very difficult.

Type III and IV resistance could be assessed together by measuring DON concentration at a given level of FHB or *in vitro* using a tissue assay (Wang and Miller, 1988). Regardless of a cultivar's resistance to FHB, infected grain will usually contain DON; however DON content can differ greatly among cultivars (Bai *et al.,* 2001b). Low DON content in an infected kernel could result from three possible causes: a) a low level of DON produced by the fungus, b) degradation of DON by plant metabolites during kernel development, or c) a high level of DON in spike tissue other than kernels, and failure of DON to move into kernels during their development (Bai and Shaner, 2004). Miller *et al.* (1986) reported that disease-resistant cultivars of wheat such as Frontana had lower *F. graminearum* biomass and a lower concentration of DON than susceptible cultivars such as Casavant. Further studies concluded that there was proportionally less DON in Frontana relative to Casavant than biomass measurements indicated, suggesting that some resistant cultivars have factors that prevent the synthesis and/or promote degradation of DON (Miller *et al.,* 1986). Mesterhazy *et al.* (2002) concluded that the level of resistance in a given cultivar is more important in governing DON accumulation than the aggressiveness of a mycotoxin producing isolate. Collectively, these results suggest that accumulation of resistance from numerous sources could eliminate toxin contamination of infected grains.

1.3.4. Phenotypic Traits

Some morphological traits in wheat are reported to be associated with observed FHB incidence (Mesterhazy, 1995). Head anatomy or positioning that contributes to higher humidity around the spikelets is often associated with higher disease levels. Generally, *Fusarium* spp. spreads more rapidly in awned lines with a short peduncle and a compact spike than in awnless lines possessing a long peduncle and lax spike (Mesterhazy, 1995; Miedaner, 1997). In one wheat population infertile lateral florets were associated with FHB resistance and low DON content (Zhu *et al.,* 1999). In addition, short-stature lines with a long grain-filling duration are generally more susceptible than tall genotypes that have rapid grain fill (Mesterhazy, 1995; Miedaner, 1997). Factors interfering with cereal maturation can also be important in head blight epidemiology; those plants that have later heading dates may avoid infection under conditions favorable for fungal invasion (Castonguay and Couture, 1983). Such morphological attributes of the spike and plant have also been associated with FHB resistance in barley (Zhu *et al.,* 1999). It has been reported that two-rowed barley varieties are generally more resistant to FHB than six-rowed varieties (Gocho and Hirai, 1987; Takeda and Heta, 1989). This correlates to mapping studies reporting relatively minor quantitative trait loci (QTL) conferring resistance to FHB, coincidental with a QTL determining head type (Zhu *et al.,* 1999; Mesfin *et al.,* 2003).

1.4 Molecular and Biochemical Mechanisms of Resistance

Resistance in wheat and barley to FHB is a complex, quantitative trait as demonstrated by the work of Zhou *et al.* (2005; 2006). Resistance most likely involves complex, interacting networks of signaling pathways. New technologies may allow for an in-depth

look at this network of signaling pathways and its potential association with FHB resistance. Microarray analysis allows for monitoring of genome-wide gene expression in a single experiment and enables large-scale examination of transcript accumulation as a function of temporal and spatial events (Schena *et al.,* 1995; Kagnoff and Eckmann, 2001). The Barley1 Affymetrix GeneChip probe array representing 22,439 genes was developed by Close *et al.* (2004). Global monitoring of defense-related gene expression and activation of protein pathways can lead to a better understanding of the molecular basis of defense against infection by *F. graminearum*, provide insight into defense-related signal pathways, and facilitate identification of key genes involved in these pathways (Bai and Shaner, 2004). Proteomic and metabolomic studies have also been used to better understand plant defense against various environmental stress, including pathogens.

Plants exhibit a variety of resistance mechanisms when attacked by a pathogen. Common downstream responses include activation of defense-related genes, an oxidative burst, programmed cell death, induction of the phenylpropanoid pathway (resulting in production of metabolites utilized for the biosynthesis of cellular compounds and lignin structures), and walling off of the pathogen (Hammond-Kosack and Jones, 1996; Birch *et al.,* 1999; Kawasaki *et al.,* 1999; Heath, 2000; Shirley, 2001; Apel and Hirt, 2004). In wheat Zhou *et al.* (2005; 2006) reported the significance of proteins found to be more abundant, less abundant, or newly induced following *F. graminearum* inoculation. Metabolic studies in wheat by Hamzehzarghani *et al.* (2005) reported higher levels of phenylalanine-ammonia lyase and cinnamic acid, precursors for salicylic acid, and sugars such as *myo*-inositol in the resistant wheat cultivar Sumai 3 than in the susceptible

cultivar Roblin. These findings suggest that defense-related genes in wheat and barley are activated after fungal infection and defense-related proteins play a role in general defense against *Fusarium* infection, but may not be the only genes responsible for resistance. Several other enzymes such as superoxide dismutase, catalase, ascorbic acid peroxidase, and ascorbic acid oxidase have also been related to FHB resistance in wheat (Zhou *et al*., 2005; 2006).

Studies in *Arabidopsis* show that pure trichothecenes have an elicitor-like activity, including the activation of MAPKs (mitogen activated protein kinases), induction of defense genes, and the accumulation of salicylic acid and reactive oxygen intermediates (Nishiuchi *et al.,* 2006). Genes that may have a role in detoxifying or transporting trichothecenes in barley have also been reported (Boddu *et al.,* 2006). The identification of several UDP-glucosyltransferases indicate that barley may have the potential to alter the structure of the trichothecenes (Poppenberger *et al.,* 2003); identification of ABC transporters and multi-drug and toxic compound exclusion factors indicates that plant cells obtain the ability to reduce concentrations of these compounds in the cytoplasm by transferring trichothecenes into a vacuole or out of a cell (Boddu *et al.,* 2006). The combination of transcriptomic, proteomic, and metabolomic studies make it possible to identify groups of compounds associated with resistance and possibly explain the functions of the identified genes, proteins, and metabolites in wheat and barley plant defense against *F. graminearum*.

1.5 Inheritance

Inheritance of type II resistance in wheat has been extensively studied (Nakagawa, 1955; Bai *et al.,* 1989; Liu and Wang, 1991; Bai *et al*. 2000; Ban, 2001; Buerstmayr *et al.,* 2002). Classic genetic research indicates that a combination of major and minor genes control type II resistance with relatively high heritability (Nakagawa, 1955; Liao and Yu, 1985; Bai *et al.,* 1989; Bai and Shaner, 1994; Van Ginkel *et al.,* 1996); and in most cases additive and non-additive gene effects play an important role in heritability (Bai *et al.,* 1989; 2000; Snijder, 1990a; 1990b). The most important non-additive component appears to be dominance (Bai *et al.,* 1990; Snijder, 1990a); although epistatic effects were also detected in some studies (Bai *et al.,* 2000).

Analysis of chromosome substitutions indicate that resistance genes from different Chinese and Japanese wheat cultivars are distributed over the entire wheat genome, except for chromosome 1A (Lu *et al.,* 2001); however different studies have proposed different numbers of genes for the same resistant cultivars (Lu *et al.,* 2001). Kolb *et al.* (2001) stated that such inconsistencies may be due to polygenic control of FHB resistance in wheat, effect of different genetic backgrounds, different resistance evaluation methods, genotype and environment interactions, heterogeneous sources of a resistant parent, or inoculation techniques used in the different studies. Chen (1983) and Yu (1990) reported on polygenic control of resistance, while more recent reports demonstrated that a few genes with major effects might be involved in resistance to spread of scab in a spike (Bai, 1995; Van Ginkel *et al.,* 1996). Molecular marker technology may be able to provide more precise information on the number and location of QTL for FHB resistance.

1.6 QTL Mapping in Wheat and Barley

A QTL is a region of DNA that is associated with a particular phenotypic trait. QTL mapping is the statistical study of the alleles occurring at a locus and the phenotypes that they produce. It is a powerful tool to dissect quantitative traits into single Mendelian factors that can be handled more efficiently in practical breeding programs. Following the determination of linkage between a QTL and molecular marker, marker-assisted selection can be used to transfer the QTL into different genetic backgrounds. Molecular mapping has been successfully used to elucidate the scab resistance QTL. Restriction length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), and simple sequence repeat (SSR) markers linked to scab resistance QTLs have been reported (Bai *et al.,* 1999; Waldron *et al.,* 1999; Anderson *et al.,* 2001; Buerstmayr *et al.,* 2002; Zhou *et al.,* 2002). SSR markers linked to major scab resistance QTLs provide a new approach for selection of resistant plants from segregating populations and provide fast, affordable, accurate, and efficient methods for tagging QTLs with agronomic importance in breeding programs.

In wheat and barley, genetic resistance to FHB is partial and is quantitatively controlled by many loci. In wheat, several markers have been identified for scab resistance QTLs, using RFLPs and random amplified polymorphic DNAs (RAPDs) (Bai, 1995; Moreno-Sevilla *et al.,* 1997); however, all of these markers only accounted for a small portion of the observed variation. A low level of polymorphism of RAPDs and RFLPs within wheat may be a barrier for the identification of markers that are closely linked to major scab resistance genes (Chao *et al.,* 1989; Bai, 1995). In Sumai 3, a major QTL on chromosome 3BS explained up to 50% of the phenotypic variation and was

found to be primarily associated with type II resistance (Buerstmayr *et al.,* 2003). In contrast, the QTL on chromosome 5A contributed more to type I resistance than to fungal spread (Buerstmayr *et al.,* 2002; 2003). Zhou *et al.* (2002) monitored the performance of the 3BS QTL in different genetic backgrounds, using several flanking SSR markers and reported that the substitution of a pair of susceptible marker alleles with resistance marker alleles at the same locus reduced scab severity two-fold and concluded an additive effect of scab resistance at the 3BS QTL. Studies to date have revealed multiple QTLs that confer partial resistance during mapping of FHB resistance in wheat (Bai *et al.,* 1999; Waldron *et al.,* 1999; Anderson *et al.*, 2001; Buerstmayr *et al.,* 2002; Otto *et al.,* 2002; Zhou *et al.,* 2002).

Only a few studies report molecular mapping of resistance QTLs in barley. QTLs for FHB resistance have been identified on each of the seven barley chromosomes (de la Pena *et al.,* 1999; Zhu *et al.,* 1999; Ma *et al.,* 2000; Dahleen *et al.,* 2003; Mesfin *et al.,* 2003). Bai (1995) found that RAPD markers linked to two QTLs explained 21% of the phenotypic variation. Dahleen *et al*. (2003) and Mesfin *et al*. (2003), using populations derived from two-rowed and six-rowed crosses, reported a QTL for FHB resistance near the *vrs1* locus (controlling row type) on chromosome 2H; however, they could not clarify whether FHB resistance was due to the multiple effects of the *vrs1* locus or to linkage with the *vrs1* locus. Molecular mapping of a population of recombinant inbred lines derived from the cross between the six-rowed barley cultivars Chevron and M69 identified that QTLs 10, 11, and 4 were associated with FHB severity, kernel discoloration score, and low DON content, respectively (de la Pena *et al.,* 1999). These QTLs are distributed over all seven barley chromosomes; three QTLs on 2H and 7H for

low DON are associated with low FHB severity; several QTLs for low kernel discoloration score are mapped near the QTL for low severity or DON content, and two QTLs on 2H and one on 6H might have a large effect on low kernel discoloration and could be used for marker-assisted selection (Nduulu *et al.,* 2002; Canci *et al.,* 2003).

In another study, Chevron did not show any QTLs with major effects on FHB resistance, but markers linked to QTLs on chromosomes 1, 2, and 4 were proposed for marker-assisted selection (Ma *et al.,* 2000). Two QTLs from Gobernadora, on chromosomes 4 and 5, each explain 7% and 10% of phenotypic variation, respectively (Zhu *et al.,* 1999). QTLs for type I resistance have been located on chromosomes 1, 3, and 4; QTLs for low DON content were detected on chromosomes 1, 2, 3, 4, and 5, but only the QTL on chromosome 4 was detected in more than one experiment (Zhu *et al.,* 1999). The discovery that QTLs for low DON content and type II resistance were at the same positions on chromosomes 2 and 4, suggested that the same QTL may control both traits (Zhu *et al.,* 1999). Smith *et al.* (2004) concluded that the Stander allele at a QTL on chromosome 3 resulted in lower DON accumulation under controlled conditions. In the previous studies, most of the FHB resistant QTLs have been mapped to the same locations as those associated with morphological traits such as heading date, plant height, lateral floret size, spike angle, and kernel plumpness; it is unknown if these QTLs are linked or whether the same QTL shows multiple effects.

1.7 Breeding for FHB Resistance

Using genetics to control plant diseases is the most desirable option; however breeding for FHB resistance is particularly difficult as the pathogen is not one unique strain or

species, but rather a collection of species that show a high degree of intra- and inter-species variability and produces a large array of trichothecenes important in pathogenesis. In addition, complex interactions among the fungus, trichothecenes, environments, and genotypes provide challenges for the development of resistant cultivars in wheat and barley since resistance is not a single trait. A simple, efficient, and reliable method for screening lines for such polygenic traits remains to be developed. Current breeding strategies look to recombine type II resistance with type I in wheat and barley, and in addition to select for low DON content in the kernels. There are conflicting reports regarding the ability to select for resistant cultivars. Mesterhazy *et al.* (1999) proposed that breeding should concentrate on visual symptoms, since low kernel infection, yield loss, and low DON contamination are only moderately correlated with the displayed level of resistance; Bai *et al.* (2001b) claimed that selecting resistant cultivars based on visual FHB symptoms of infected spikes and grains should lead to new cultivars with low DON levels.

The challenge for breeders and pathologists is to develop effective screening methods, to cooperatively standardize current methods of evaluation, and to better understand the inheritance behind partial resistance. Significant progress has been made in breeding for resistance to FHB during the past 30 years; breeding commercial wheat cultivars that combine desired agronomic traits and a high level of FHB resistance remains a significant challenge (Bai and Shaner, 2004). Difficulties are most likely associated with the polygenic control of disease resistance, the association of undesirable agronomic traits in available resistant sources, complicated disease evaluation procedures, and the effect of the environment on resistant phenotypes (Bai and Shaner,

2004). Moreover, resistance is not a single trait; it involves resistance to primary infection of the spike, subsequent spread of the pathogen, kernel invasion, and to DON accumulation.

1.8 Future Directions

Significant progress has been made over the past century towards the assessment of disease severity and development of FHB-resistant barley cultivars. One objective of the research reported in this thesis was to compare the efficacy and reproducibility of point inoculation and spray inoculation methods in a mist-irrigated greenhouse, with artificial inoculation in a nursery. The goal of this comparison was to identify consistently resistant barley lines regardless of the inoculation method used based on *F. graminearum* infection levels and recorded DON content. Nineteen barley lines and cultivars representing various sources of resistance and susceptibility to FHB were evaluated. Additionally, a reliable method for screening FHB-resistant and FHB-susceptible barley lines is recommended.

To date, many pathologists and biologists have evaluated resistance in wheat and barley, but the mechanisms associated with fungal virulence and the pathways associated with resistance have not yet been identified. Objectives put forth in chapters three and four of the thesis are based on the hypothesis that fungal invasion of a host will elicit a plant defense response that should discriminate between incompatible interactions observed in FHB-resistant lines and compatible interactions observed in FHB-susceptible lines. In chapter three, the objective was to compare and identify differentially expressed proteins in FHB-resistant and FHB-susceptible barley lines under infected and uninfected

conditions, as well as to describe possible mechanisms of resistance utilized by the plant in response to fungal invasion. In chapter four, data from transcriptomal and proteomic studies are combined to achieve the objectives of identifying and comparing differentially transcribed genes and expressed proteins with respect to induction timing in FHB-resistant and FHB-susceptible barley lines under infected and uninfected conditions. Additionally, various treatments were used to distinguish plant defense responses based on the effects of fungal infection, trichothecene production, and their subsequent interactions. In combination with current knowledge regarding FHB-resistance in barley and the results provided in chapters 2, 3, and 4 from these studies, significant progress towards the understanding of resistance mechanisms and pathways activated during incompatible interactions between *F. graminearum* and barley has been made; the information available to breeders and pathologists could lead to the successful growth and harvest of cereal crops in regions previously devastated by the FHB epidemic.

CHAPTER 2 Evaluation of inoculation methods on *Fusarium graminearum* infection and deoxynivalenol production in barley[*].

2.1 Introduction

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum* Schwabe (teleomorph = *Gibberella zeae* (Schwein) Petch), has resulted in yield and quality losses due to sterility of the florets and the formation of discolored, shriveled kernels containing mycotoxins in small cereal grains in North America, China, and Europe (McMullen *et al.,* 1997; Stack, 1999). *F. graminearum*, a saprophyte and facultative parasite, is able to colonize living barley tissue efficiently at specific stages during its life cycle, thus building up inoculum within the spike (Miller, 1994). The process of natural infection starts with *Gibberella zeae* ascospores being released from the perithecia and landing on floral pieces (Paulitz, 1996). In barley, the kernels show a grey or brown discoloration several days after infection and premature bleaching of the spikelets (Bai, 1995; Stack, 1999; Tekauz *et al.,* 2000). Since 1990, outbreaks of FHB have affected cereal crops in twenty-six states in the U.S.A. and five provinces in Canada; these severe outbreaks of FHB have had a significant economic impact on the cereal industry with losses totaling $3 billion U.S. in wheat and $0.4 billion U.S. in barley (Windels, 2000).

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The incorporation of FHB resistance into new cultivars presents a large challenge because of the polygenic nature of disease resistance, the association of undesirable agronomic traits, the complicated disease evaluation procedures, and the effect of the environment on resistant genotype screening (Galich, 1997; Bai and Shaner, 2004). Current barley breeding strategies look to combine resistance to initial infection and spread, and to select for low deoxynivalenol (DON) content in the kernels (Steffenson, 2003). Fungal secondary metabolites known as mycotoxins (e.g. DON) are produced during the progression of FHB phenotypic symptoms. Mycotoxin contamination greatly reduces grain quality and market value because of detrimental effects on livestock, humans, and fermentation processes (Ueno, 1977; Anonymous, 1989). Studies by Miedaner *et al.* (2003a) have reported a positive correlation between DON concentration and the level of FHB resistance in barley plants. As observed in wheat, prevention of trichothecene accumulation as a result of DON degradation or detoxification may explain DON tolerance in resistant barley lines and may result in an overall increase of FHB resistance (Miller *et al.,* 1985; Mesterhazy *et al.,* 2002).

Type I resistance was defined by Schroeder and Christensen (1963) as resistance to initial infection, and type II resistance was defined as resistance to the spread of infection. In barley, although type II resistance has been reported (Zhu *et al.,* 1999), type I resistance is more important (Steffenson, 2003) due to the absence of fungal spread within the spike among infected spikelets (Bushnell *et al.,* 2003). The genetic diversity of barley displaying resistance to FHB might largely be associated with resistance to initial infection (Prom *et al.,* 1996; Tekauz *et al.,* 2000; Steffenson, 2003; Lewandowski *et al.,* 2006). Type I resistance may be tested in the greenhouse or in the field. In the

greenhouse, type I resistance is challenged by spraying a spore suspension over flowering spikes and counting the diseased spikelets; a technique referred to as spray inoculation. Type I resistance in the field, is assessed by dispersing corn residue inoculated with *Fusarium* spp. on the ground beneath the flowering crop and counting the diseased spikelets. Type II resistance is challenged in a greenhouse by point inoculation; delivering macroconidia into a floret of a spike by direct injection and counting the blighted spikelets after a period of time.

The main objective of this research was to compare the efficacy and reproducibility of three inoculation methods: point inoculation and spray inoculation methods in a mist-irrigated greenhouse and artificial inoculation in a nursery. A second objective of this study was to identify consistently FHB-resistant barley lines regardless of the inoculation method used and record DON levels. Nineteen barley lines and cultivars representing various sources of resistance and susceptibility to FHB were evaluated for levels of resistance to *F. graminearum* and for DON content.

2.2 Materials and Methods

2.2.1 Indoor experimentation

Nineteen barley breeding lines and cultivars representing various levels of resistance and susceptibility to FHB were tested in greenhouses at Agriculture and Agri-Food Canada, Lethbridge, Alberta, in 2004-2005 (Table 2.2.1). Seeds were planted in 15 cm pots and placed in a greenhouse at 21/18°C with a day/night cycle using a 16 h photoperiod until anthesis (McCallum and Tekauz 2002). Plants were treated with Tilt™ (2.5 mL/L

propiconazole, Syngenta Crop Protection Canada, Guelph, ON) during the tillering stage and also with Intercept™ (0.004 g/L of soil, Imidacloprid, Bayer Crop Science Canada, Toronto, ON) once sufficient root development had established to prevent powdery mildew and aphids, respectively.

Table 2.2.1 Barley breeding lines and cultivars displaying varying degrees of resistance and susceptibility to fusarium head blight.

Barley Line	Row Type	Source	IR*
Chevron	6-row	Switzerland	R
Gobernadora	2-row	ICARDA/CIMMYT, Mexico	R
HDE-84194-622-1	2-row	China	R
Harbin	2-row	China	R
Svanhals	2-row	Sweden	R
Svansota	2-row	Sweden	R
Shenmai 3	2-row	China	R
Zhedar #1	2-row	China	R
CI4196	2-row	China	R
Morrison	2-row	AAFC-ECORC	MR
TR04281	2-row	AAFC-Brandon	MR
TR04282	2-row	AAFC-Brandon	MR
TR04283	2-row	AAFC-Brandon	MR
Island	2-row	AAFC-ECORC	MR
TR253	2-row	AAFC-Brandon	I
AC Metcalfe	2-row	AAFC-Brandon	I
CDC Sisler	6-row	U of S	I
CDC Bold	2-row	CDC, U of S	S
Stander	6-row	United States of America	S

* IR= Infection response, where R= resistant, MR= moderately resistant, I= intermediate resistance, and S= susceptibility.

A single isolate of *F. graminearum* strain N2, from an infected wheat head, (J. Gilbert, Winnipeg, MB, Canada) was cultured on potato dextrose agar (PDA) for 5 d at room temperature. A macroconidial suspension of *F. graminearum* was produced by transferring four PDA plugs (1 cm x 1 cm) of the established fungal culture to 500 mL of CMC broth (carboxymethylcellulose 15 g, NH_4NO_3 1 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, yeast 1 g, and H_2O 1 L). The culture was incubated on a rotary shaker (150 rpm) at 22°C for two weeks (McCallum and Tekauz, 2002). A hemocytometer was used to count macroconidia. The *F. graminearum* culture was diluted with water to produce a suspension of 40,000 *F. graminearum* macroconidia per mL. A mock inoculum was prepared by diluting sterile CMC broth to the same extent as the *F. graminearum* culture.

To check the developmental stage of barley spikes, the leaf sheath was carefully pulled back from the spike, without damaging the spike and leaf. The macroconidial suspension of *F. graminearum* was applied either by floret injection or spray application to the entire spike. For floret injection treatments, two florets per head were inoculated. At anthesis, the spikelet was carefully opened by spreading the palea and lemma and injecting 10 µL of the spore suspension inside two central spikelets using a micropipette (Evans *et al.,* 2000; McCallum and Tekauz, 2002). For spray inoculation treatments at anthesis, approximately 504,000 macroconidia/m^2 of a *F. graminearum* suspension was sprayed (125 mL) from 35 cm above the canopy, using a spray cabinet (Model # 822-

1547 Research Instrument MFG. CO. LTD., Guelph, ON). Following both inoculation methods, pots were placed inside a mist-irrigated greenhouse (95% relative humidity) for 72 h at 25/21°C with a 16 h photoperiod and then subsequently returned to the original greenhouse (no mist-irrigation). At eighteen days post-inoculation (dpi), the number of spikelets or kernels with visible symptoms of *F. graminearum* infection was counted to determine FHB phenotypic symptoms. Symptoms included the grey or brown discoloration of the spikelets and kernels, shriveling of the kernels, and showed a mycelial film covering the kernels and rachis. For DON analysis, infected barley heads were cut from the stems and placed inside paper envelopes for storage at -20°C (McCallum and Tekauz, 2002). Days to heading was also recorded on every inoculated plant as an internal control. Point and spray inoculation experiments 1, 2, and 3 were run over two to four months in fall 2004, spring 2005, and summer 2005, respectively. Greenhouse experiments, using either inoculation method, were conducted three times with three replicates. The experimental unit was the plant, each arranged in a randomized block design, and on average three spikes were sampled per plant. Number of infected spikelets was processed by ANOVA and mean differences were tested using the LSD test from SAS (SAS Institute Inc. 1988, Cary, NC).

2.2.2 Nursery experimentation

Plants in the Brandon (MB) nursery from 2000-2005 were inoculated with grain spawn (corn seed infected with three isolates of *F. graminearum*) dispersed on the soil surface in 3-4 weekly applications, beginning before heading of the earliest barley lines in the nursery and irrigated to promote fungal development (Legge *et al.,* 2004). Nursery plots

were 0.9 m rows of barley, tested in a replicated experimental design (n=3). FHB symptoms were rated using a 0-5 scale; 0 represented no symptoms and 5 represented severe symptoms. Ratings were recorded about three and a half weeks after plants were 50% headed (Legge *et al.,* 2004). For five lines, data were collected in adjacent experiments in the nursery where the repeated check AC Metcalfe showed a similar infection level. An ANOVA and LSD mean comparison were completed on the annual FHB means (SAS Institute Inc.).

2.2.3 Deoxynivalenol quantification

Eighteen dpi-infected barley heads from greenhouse experiments and mature barley heads from the Brandon (MB) nursery were collected for DON analysis (McCallum and Tekauz, 2002). The greenhouse and nursery samples were lyophilized for 48 h, hand threshed, and the kernels were separated from the awns and rachis (Savard *et al.,* 2000). Kernels were ground, and a 1 g sub sample was analyzed using Enzyme-Linked ImmunoSorbent Assays (ELISA, Sinha and Savard, 1997). DON concentrations were measured in point inoculation experiments 2 and 3; nursery DON concentrations were measured on composite samples consisting of three replicates. An ANOVA and LSD mean comparison were completed on nursery composite samples and replicated samples from indoor studies (SAS Institute Inc.).

2.3 Results

2.3.1 Point inoculation

Statistical analysis (ANOVA) for the number of infected spikelets following point inoculation was completed for each experiment separately, due to large variability across experiments ($P<0.0001$). Significant differences in FHB phenotypic symptoms were observed among the 19 barley lines in each experiment ($P<0.0001$). All spikelets were inoculated at anthesis, but heading time varied among the lines within the experiments, possibly contributing to the inter-experimental variation observed ($P<0.0001$). Table 2.3.1 shows the average days to heading and the mean rate of infection. Table 2.3.2 shows DON concentrations for each barley line following point inoculation. In point inoculation experiment 1 (F value = 23.0, $P<0.0001$), the following lines were the most resistant and not significantly different: CI4196, Harbin, Island, Shenmai 3, TR04282, TR04281, TR04283, TR253, Morrison, Gobernadora, HDE84194-622-1, AC Metcalfe, and Svansota. The most susceptible lines were CDC Bold, CDC Sisler, Chevron, and Stander. The mean number of spikelets with symptoms varied from 0.2 to 10.0. In experiment 2 (F value = 5.7, $P<0.0001$), the most resistant lines were Harbin, Island, Shenmai 3, Morrison, TR04282, TR04281, AC Metcalfe, Svansota, Chevron, and Svanhals. The most susceptible lines were HDE84194-622-1, CDC Bold, and Stander. The mean number of infected spikelets varied from 0 to 4.8. In experiment 3 (F value = 11.3, $P<0.0001$), the most resistant lines were CI4196, Harbin, Island, TR04283, TR253, Morrison, Gobernadora, TR04282, TR04281, AC Metcalfe, Svansota, CDC Sisler, and Chevron. The most susceptible lines were Shenmai 3, HDE84194-622-1, and Stander. The mean number of infected spikelets varied from 0 to 5.8. The average mean number of infected spikelets across all point inoculation experiments varied from 0.1 to 6.8.

In point inoculation experiment 2, DON concentrations were an average of pooled replicates and statistical analysis could not be conducted. The lowest DON concentrations were observed in TR04283, TR04282, CDC Bold, and AC Metcalfe. The highest DON concentrations were observed in Gobernadora, Shenmai 3, Stander, and Harbin. In experiment 3, statistical analysis on DON concentrations indicated significant differences

Table 2.3.1 Fusarium head blight phenotypic symptoms of 19 barley lines following *Fusarium graminearum* point inoculation.

	Experiment 1		**Experiment 2**		**Experiment 3**	
Line	**Mean days to heading (s.d.)**	**Mean infected spikelets**	**Mean days to heading (s.d.)**	**Mean infected spikelets**	**Mean days to heading (s.d.)**	**Mean infected spikelets**
AC Metcalfe	67 (5.5)	1.7ab	61 (1.6)	0.8abc	48 (0)	1.0ab
CDC Bold	68 (7.0)	3.6bc	54 (1.5)	1.8c	43 (0)	2.0b
CDC Sisler	58 (3.1)	3.9c	54 (1.6)	1.6bc	44 (0)	1.6ab
Chevron	60 (3.8)	5.1c	46 (2.8)	1.0abc	50 (0)	1.0ab
CI4196	94 (0)	0.2a	59 (0)	.	62 (0)	0.5a
Gobernadora	59 (6.2)	0.9ab	44 (2.6)	0.9b	42 (0)	0.0a
Harbin	86 (8.0)	0.5ab	61 (3.7)	0.2ab	55 (1.5)	0.3a
HDE84194-622-1	40 (0)	1.3ab	38 (2.2)	1.9c	35 (0.6)	5.8d
Island	57 (6.4)	0.5a	53 (3.5)	0.3ab	47 (3.8)	1.2ab
Morrison	66 (5.7)	0.7ab	52 (1.8)	0.2ab	46 (2.1)	0.4a
Shenmai 3	56 (6.9)	0.6a	39 (1.4)	0.0a	35 (0)	4.0c
Stander	59 (5.5)	10.0d	49 (2.7)	4.8d	45 (2.0)	4.1c
Svanhals	83 (0)	.	52 (4.6)	0.4ab	48 (0)	2.5bc
Svansota	90 (4.6)	1.8ab	57 (0)	0.1ab	59 (2.2)	1.0ab
TR04281	58 (4.4)	1.1ab	56 (0)	0.7abc	50 (1.7)	1.5ab
TR04282	78 (7.6)	1.0ab	55 (1.5)	0.5abc	44 (0)	1.0ab
TR04283	70 (4.4)	0.6ab	55 (1.4)	1.2bc	44 (4.6)	1.2ab
TR253	71 (6.8)	0.7ab	55 (1.5)	1.3bc	49 (0)	0.0a
Zhedar #1	89 (4.6)	2.1b	57 (0)	1.5bc	56 (0)	.

[a]Values with shared lettering do not significantly differ.

Table 2.3.2 Fusarium head blight deoxynivalenol content of 19 barley lines following *Fusarium graminearum* point inoculation.

Line	Experiment 2 Mean DON[1] (ppm)	Experiment 3 Mean DON (ppm)
AC Metcalfe	1.1	7.5ab
CDC Bold	1	8.7ab
CDC Sisler	3.5	6.9ab
Chevron	3.3	6.9ab
CI4196	.	15.2b
Gobernadora	5.6	4.5a
Harbin	9.6	11.4ab
HDE84194-622-1	2.4	61.5c
Island	1.4	10.7ab
Morrison	.	8.6ab
Shenmai 3	7.5	17.1b
Stander	9.1	12.5ab
Svanhals	2	6.0ab
Svansota	.	9.5ab
TR04281	1.9	6.2a
TR04282	0.8	9.4ab
TR04283	0.4	6.4a
TR253	3.5	9.6ab
Zhedar #1	.	.

[1]Replicate samples were pooled together. Only mean DON values available.

[a]Values with shared lettering do not significantly differ

among barley lines (P<0.0001). The lowest DON concentrations were reported for Harbin, Island, TR04283, TR253, Morrison, Gobernadora, TR04282, TR04281, AC Metcalfe, Svansota, CDC Bold, CDC Sisler, Chevron, Stander, and Svanhals. HDE84194-622-1 had the highest DON concentration. In point inoculation experiment 3, a positive correlation between FHB symptoms and DON content (r=0.719) was observed.

2.3.2 Spray inoculation

Statistical analysis for the number of infected spikelets following spray inoculation was performed on combined data from three spray inoculation experiments; inter-experimental variation was not significant (P=0.9217). Significant differences in the mean number of infected spikelets were observed among the 19 barley lines (F value = 14.8, P<0.0001), and are presented in Table 2.3.3. The following barley lines were the most resistant: Harbin, Chevron, Svanhals, Island, TR04281, TR253, Svansota, Zhedar #1, HDE84194-622-1, and AC Metcalfe. The most susceptible lines were CDC Sisler, Gobernadora, and Stander. The mean number of infected spikelets varied from 0.8 to 7.6.

DON concentrations were recorded for spray inoculation experiments 2 and 3 at 18 dpi. The statistical analysis did not show a significant difference in DON concentration among the barley lines (P=1.00) in intra-experimental comparisons.

HDE84196-622-1, Shenmai 3, CDC Bold, TR04281, Chevron, Harbin, Svanhals, TR04283, TR253, Gobernadora, Zhedar #1, AC Metcalfe, Svansota, CDC Sisler, and Island had the lowest DON concentrations, respectively. Morrison, TR04282, and Stander had the highest DON concentrations at 18 dpi. No correlation was found between

Table 2.3.3 Fusarium head blight phenotypic symptoms and deoxynivalenol content of 19 barley lines following *Fusarium graminearum* spray inoculation recorded for 3 experiments.

Lines	Mean days to Heading (s.d.)	Mean infected spikelets	Mean DON[1] (ppm)
Harbin	75 (17.5)	0.8a	0.6a
Chevron	56 (18.5)	1.2ab	0.3a
Svanhals	71 (17.6)	1.3ab	0.6a
Island	59 (10.9)	1.4ab	2.1a
TR04281	60 (11.1)	1.6ab	0.3a
TR253	58 (10.5)	1.6ab	0.7a
Svansota	68 (13.2)	1.8ab	1.0a
Zhedar #1	83 (12.9)	2.0ab	0.9a
HDE84194-622-1	39 (2.8)	2.0ab	0.2a
AC Metcalfe	63 (11.2)	2.1ab	0.9a
CI4196	75 (12.3)	2.3abc	2.6ab
TR04282	56 (14.3)	2.3b	5.6ab
Shenmai 3	46 (9.4)	2.6b	0.2a
TR04283	59 (11.3)	2.7bc	0.6a
CDC Bold	63 (15.1)	2.7bc	0.2a
Morrison	59 (11.2)	2.9bc	8.1b
CDC Sisler	59 (10.3)	4.0c	1.0a
Gobernadora	50 (6.5)	4.9c	0.8a
Stander	54 (4.3)	7.6d	4.8ab

[1]DON data available for experiments 2 and 3 only.

[a]Values with shared lettering do not significantly differ

the FHB phenotypic symptom levels and DON content in the spray inoculation experiments (r=0.360).

2.3.3 Nursery inoculation

Statistical analysis on the combined data from the *Fusarium* spp.-infected spikelets from the Brandon (MB) nursery (2000-2005) was performed (Table 2.3.4). Significant differences in FHB symptom levels were observed among the barley lines (F value = 17.8; P< 0.0001). The days to heading varied significantly (P< 0.0001) for the nursery experiments; however the statistical analysis was completed only on the annual means and no conclusion regarding the effect of heading time on FHB symptom levels could be determined. The following were the most resistant lines according to statistical analysis: Svansota, CI4196, Chevron, Island, Harbin, Zhedar #1, and TR04283. The most susceptible lines were TR253, Shenmai 3, Morrison, HDE84194-622-1, Stander, and CDC Bold. The FHB rating ranged from 1.5 to 3.9 based on the five-point scale, and disease severity was noted to vary significantly through the years. The reported mean DON concentrations ranged from 8.0 ppm in HDE84194-622-1 to 31.2 ppm in CDC Bold. There was a positive correlation found between the observed FHB phenotypic symptoms and DON concentrations (r=0.678).

Table 2.3.4 Fusarium head blight phenotypic symptoms and deoxynivalenol content of 19 barley lines following *Fusarium* spp.-inoculation in Brandon (MB) nursery from 2000-2005.

Lines	Mean days to Heading (s.d.)	Mean Ratings (0-5)	Mean DON (ppm)
Svansota	57 (4.9)	1.5a	10.4a
CI4196	63 (7.1)	1.6a	10.3a
Chevron	56 (4.7)	1.6a	9.9a
Island	50 (2.3)	1.6a	10.5ab
Harbin	57 (4.6)	1.8ab	10.6a
Zhedar #1	61 (4.8)	1.8ab	10.9a
TR04283*	53 (5.3)	2.1abc	11.1a
Svanhals	60 (5.8)	2.1b	11.2a
TR04282*	56 (3.2)	2.2bc	9.6a
TR04281*	58 (7.4)	2.6bc	9.4a
AC Metcalfe	55 (4.3)	2.7c	15.6ab
CDC Sisler	54 (4.4)	2.8c	11.2a
Gobernadora	51 (5.3)	2.8c	13.2ab
Shenmai 3[1]	44 (0)	2.9bcd	23.6ab
TR253*	59 (5.6)	3.2cd	22.6ab
Morrison	56 (5.8)	3.3d	15.0ab
HDE84194-622-1	46 (4.1)	3.3d	8.0a
Stander	52 (3.3)	3.8d	20.5ab
CDC Bold*	54 (4.4)	3.9d	31.2b

*Data on these entries came from adjacent nursery environments and AC Metcalfe was used as a check reference in all tests.

[1]Only two years of data were available.

[a]Values with shared lettering do not significantly differ

2.4 Discussion

2.4.1 Point inoculation

Of the 19 barley lines tested for phenotypic reaction to *F. graminearum*, 9 of the lines were known sources of resistance to FHB (Table 2.2.1). The results concluded that under point inoculation conditions Harbin and Svansota represented sources of FHB resistance, and TR04281, TR04282, and Island represented moderate sources of resistance. AC Metcalfe and Chevron were shown to provide intermediate resistance while Stander was the most susceptible source. According to previous studies, two-row barley varieties are more resistant to infection by *F. graminearum* than the six-row varieties used in malting (Chelkowski *et al.,* 2000; Bai and Shaner, 2004). From field experiments, the best available source of resistance from a two-row variety was CI4196 and from a six-row variety, was Chevron (Prom *et al.,* 1996; Bai and Shaner, 2004). However, these lines are undesirable from an agronomic point of view as they tend to be susceptible to other diseases and resistance to FHB has been shown to break down if infection levels are high enough (Legge *et al.,* 2004).

Significant experimental variability was observed for FHB phenotypic symptom levels and heading date ($P<0.0001$). As well, in point inoculation experiment 1, Chevron displayed lower than expected levels of resistance to fungal infection, but showed higher resistance in point inoculation experiments 2 and 3. These differences may be attributed

to the delayed heading time observed in this line during experiment 1. In all other point inoculation experiments Chevron supported its recognized resistance to FHB. Despite the absence of statistical analysis for CI4196 in point inoculation experiment 2, this line displayed moderate levels of resistance in all remaining point inoculation experiments, supporting its use as a resistant source to FHB. The average FHB phenotypic symptom levels recorded for point inoculation experiments 1, 2, and 3 ranged from 0.1 to 6.8; indicating that the point inoculation method was appropriate for discriminating FHB resistance and susceptibility.

In point inoculation experiment 3, a positive correlation between the FHB phenotypic symptoms and DON content (r=0.719) was observed. The observed positive correlation was consistent with previous reports in barley (Miedaner *et al.,* 2003b), although no such correlation existed in experiment 2 (r=0.266). Perhaps, quantifying samples individually for DON content would have highlighted differences among the lines and provided a more accurate correlation in experiment 2. The reported DON concentrations quantified from 18 day old *F. graminearum*-infected kernels varied between point inoculation experiments 2 and 3. Statistical analysis of point inoculation experiment 3 reported that no significant differences in DON concentration were observed (P=1.00); however, DON values showed high levels of variation. Such deviation may be attributed to seasonal variation during plant development or the possible instability of resistance in some of the tested lines.

2.4.2 Spray inoculation

Spray inoculation at anthesis was an effective method for evaluating the importance and stability of type I resistance in *F. graminearum*-infected barley lines. Lewandowski *et al.* (2006) reported pathways of fungal entry in barley heads, mainly through the crevices between the overlapping lemma and palea or through the apical floret mouth, which reinforce the importance of resistance to initial infection. Many of the barley lines evaluated for type I resistance displayed levels of resistance consistent with those reported in Table 2.2.1. Harbin, Chevron, Svanhals, Island, TR04281, TR253, Svansota, Zhedar #1, HDE84194-622-1, AC Metcalfe, TR04282, CI4196, and Shenmai 3 all reported high to moderate levels of resistance following infection, whereas Stander consistently reported susceptibility. These results indicated a high level of reliability regarding the spray inoculation method. Chevron and HDE84194-622-1 showed high levels of resistance during spray inoculation experiments versus lower resistance levels in their respective point inoculation experiments. As the point inoculation method by-passes resistance to initial infection, type I resistance may be the major resistance mechanism in these lines. The natural resistance displayed by Chevron supports its use worldwide as a standard for breeding and evaluating FHB resistance

Similar to point inoculation experiments, heading time was used as an internal control to assess differences in plant development among the three experiments. Across the spray inoculation experiments the number of days to plant heading significantly varied ($P< 0.0001$), but this variation in heading time did not appear to be associated with the intra-experimental variability observed in FHB symptom levels; supporting the reliability of the spray inoculation method. The inter-experimental variation was not significant ($P=0.9217$), indicating a high level of reproducibility among the experiments.

The mean infection rates ranged from 0.8-7.6 and provided good discrimination of FHB resistance and susceptibility. The statistical analysis on the mean number of infected spikelets reported for each line was clearly able to differentiate between resistance, moderate resistance, intermediate resistance, and susceptibility, supporting the efficacy of the evaluation method.

No correlation was found between the FHB symptom levels and DON content in the spray inoculation experiments (r=0.360). The lack of correlation may be attributed to genetic differences among the lines. The results supported the need for both DON assessment and FHB assessment of the infected barley lines in the greenhouse for a reliable inference regarding FHB resistance in the tested lines.

2.4.3 Nursery inoculation

The nursery inoculation experiments were conducted post-anthesis, when the barley spike had emerged from the leaf sheath, and were an effective method for evaluating the importance and stability of type I resistance in *Fusarium* spp.-infected barley lines. Many of the barley lines evaluated for type I resistance displayed levels of resistance and susceptibility consistent with those reported in Table 2.2.1. Svansota, CI4196, Chevron, Island, Harbin, Zhedar #1, TR04283, Svanhals, TR04282, and TR04281 all reported high to moderate levels of resistance following infection, whereas Stander and CDC Bold consistently reported susceptibility. Heading time was used as an internal control to assess differences in plant development among the three experiments. Across the nursery inoculation experiments the number of days to plant heading significantly varied ($P < 0.0001$); however the statistical analysis was completed only on the annual means and no

conclusion regarding the effect of heading time on FHB symptom levels could be determined. The FHB phenotypic mean ratings reported from nursery trials ranged only from 1.5-3.9; such a narrow range of separation among the barley lines tested made it difficult to discriminate FHB resistance and susceptibility.

The annual mean DON concentrations varied greatly through the years; making it difficult to correlate differences in observed DON content to overall resistance of the barley lines (P=0.6614). The variation in DON concentration could be correlated to differences among the barley lines tested in their ability to tolerate DON; or possibly as a result of environmental variation throughout the years tested. Variation in levels of precipitation and temperature could greatly increase experimental variation, making it difficult to identify trends among DON concentrations.

2.4.4 Comparison of methodology

The heading time was noted in every experiment to serve as an internal control for experimental reproducibility of the three phenotypic assessment methods. Heading time varied significantly (P<0.0001) for the three experiments. However, such heading time differences have only contributed to significant inter-experimental variation during point inoculation, suggesting that this method of inoculation may be more sensitive to minor fluctuations in plant development. It would be expected that heading time would also contribute to significant differences observed during field experiments because the timing of inoculation is less controlled by the plant development stage; however, results reported inter-experimental variation to be correlated with FHB phenotypic symptom levels and annual variation.

Three phenotypic evaluation methods were compared to measure the efficacy and reproducibility of inoculation methods and DON content in 19 barley lines. The evaluation of type I resistance using spray and nursery inoculation methods combined with the evaluation of type II resistance using point inoculation supported the presence of dual resistance and highlighted the importance of both type I and II resistance towards avoiding and tolerating infection. The following lines showed dual levels of resistance: Svansota, CI4196, Chevron, Island, Harbin, Zhedar #1, and TR04283. Reproducibility and reliability of phenotypic evaluation varied among the methods. Point inoculation showed variability among the experiments that was not observed with the indoor spray inoculation method. The use of a controlled instrument, the spray cabinet, increased the chance of consistent inoculum delivery among experiments.

Differences in phenotypic assessments could be found within the barley genetics. Langevin *et al.* (2004) reported that barley had a much lower susceptibility to fungal spread than durum wheat, common wheat, and triticale, and no significant genotypic differences. Genetic diversity for barley resistance to FHB might largely be associated with resistance to initial infection, challenged by spray and nursery inoculations, which would explain the broader range of visual disease symptoms observed with indoor spray inoculation relative to the point inoculation method. The apparent low range of FHB symptom levels in the nursery was due to a different rating scale from 0 to 5. Such differences in observed resistance could also be correlated to differences in inoculation timing; greenhouse inoculations were conducted at anthesis, whereas nursery inoculations occurred later in the plant development stage, once the spike had emerged from the boot. Shenmai 3, HDE84194-622-1, and CDC Bold showed varying levels of resistance across

the three inoculation experiments; indicating a lack of stable resistance influenced by the method of inoculation. Finally, Stander showed consistent levels of susceptibility to FHB across all experiments, indicating a significant challenge of type I and type II resistance.

The method of spray inoculation provided the most reproducible phenotypic assessment (P=0.9217) of barley lines based on the limited variation among experiments; when comparing FHB symptom levels the spray inoculation method addressed the challenges associated with reliably assessing FHB resistance and susceptibility. Spray inoculation also reported the broadest range of FHB phenotypic symptoms of 0.8 to 7.6; indicating that spray inoculation provided the greatest discrimination of FHB resistance and susceptibility. The results of the three inoculation methods also indicated that phenotypic evaluation of resistance within the intermediate sources varied among each experiment and recognized Harbin, Island, CI4196, Chevron, Zhedar #1, TR04283, and Svansota as being consistently resistant when compared across the three phenotypic evaluations and Stander as being consistently susceptible in all three phenotypic evaluations.

Although spray and nursery inoculation methods both assessed type I resistance in barley lines, no correlation between FHB symptoms and DON content was observed following spray inoculations; although their was a positive correlation following the nursery inoculations (r=0.678). These correlation differences could be attributed to differences in inoculation timing, and in DON sample collection timing; point and spray inoculations were collected 18 dpi, whereas nursery inoculations were collected at maturity, which could possibly influence the reliability of rating barley lines based on a single criterion. Further studies would be needed to confirm the absence of correlation

between the FHB symptom levels and DON content following spray inoculation in the greenhouse.

Traditionally, DON concentrations were preferred over FHB phenotypic symptom evaluations in nurseries for decision making, mainly due to the economic impact of mycotoxins (Liu *et al.,* 1997; Arseniuk *et al.,* 1999). Although there is a positive correlation between FHB phenotypic symptoms and DON concentrations, the variation observed in DON content in the field suggested that the more reliable and accurate measurement of disease assessment should be based on visual disease symptoms supported by DON concentrations. The experimental means varied greatly in all three inoculation methods and no trend could be found across all DON data; indicating that when DON quantification is not absolutely necessary it would be more economical and efficient to screen the barley lines based on visual symptoms.

2.5 Conclusion

The evaluation of 19 barley lines combining three artificial inoculation experiments concluded that indoor spray inoculation was the most effective and reproducible method for screening barley lines for FHB phenotypic symptoms based on the limited variation among experiments, the consistent report of resistance levels, and the ability to differentiate infection levels among the lines. Spray inoculation mimics natural infection while controlling environmental factors and inoculum dosage, and requires less time and labor for inoculation and rating. The indoor spray inoculation method, using a mist-irrigated greenhouse allows for the possibility of rapidly screening advanced material, as well as early screening of F_{2-4} generations and double haploid lines. Spray inoculation

also offers the possibility of running phenotypic evaluations throughout the year, which would not be possible in a nursery; and has the ability to exclude competing organisms (e.g., *Bipolaris sorokiniana*) during line evaluations. Occasional occurrence of competing organisms has made it difficult to distinguish symptoms and proper FHB ratings because of competition and thus displacement of *F. graminearum*. However, there is the issue of limited space for barley germplasm experiments in the greenhouse compared to the greater space potential of a field nursery. Spray inoculation experiments in the greenhouse would also allow for pathological studies, due to the sensitivity and reproducibility of this method, thereby allowing the study of many aspects of fungal infection and spread of the disease. Other opportunities benefiting for the reported reliability and reproducibility of spray inoculation techniques include genomic and proteomic studies that would allow for a better understanding of the effect of *Fusarium* spp.-infection on gene expression and possible protein pathways associated with resistance.

CHAPTER 3 Differential expression of proteins in response to the interaction between the pathogen *Fusarium graminearum* and its host, *Hordeum vulgare*[†].

3.1 Introduction

Fusarium head blight (FHB) or scab, caused mainly by *Fusarium graminearum* Schwabe (teleomorph = *Gibberella zeae* (Schwein) Petch), is a severe disease of barley and wheat grown in humid and semi-humid climates (Parry *et al.,* 1995; McMullen *et al.,* 1997). Disease symptoms develop in spike tissue and are marked by premature necrosis and brown/grey discoloration. FHB causes significantly lower grain yield, lower test weight, reduced grain quality, and reduced milling yield (Stack, 1999). The fungus also produces trichothecene mycotoxins such as deoxynivalenol (DON) that are detrimental to both humans and livestock (Bai and Shaner, 2004). These mycotoxins have been implicated in pathogenesis, phytotoxicity, and the induction of apoptosis in eukaryotic cell cultures (Desjardins and Hohn, 1997; Kang and Buchenauer, 1999; Shifrin and Anderson, 1999). Shriveled grains contaminated with mycotoxins are commonly observed in susceptible cultivars infected by *Fusarium* spp. (Bai and Shaner, 1994). Infection results in a significant loss in value for both the producer and the barley milling industry (McMullen *et al.,* 1997).

Partial control of FHB in barley is through a combination of management practices and partially resistant varieties. Introduction of FHB-resistant barley cultivars would contribute to improved food safety and reduce losses suffered by barley growers and milling industries. In barley, resistance to FHB appears to be less variable than in

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wheat due to fewer visual symptoms following infection; however resistance in wheat has been studied more extensively and has lead to the development of highly resistant cultivars such as Sumai 3. As a result, the development of FHB-resistant cultivars is a high-priority breeding objective for many barley breeding programs worldwide. However, breeding for FHB-resistance has proven to be a challenge due to the limited understanding of the biochemical and molecular mechanisms involved in plant resistance against infection and spread of *F. graminearum*.

Plants delay pathogen growth or resist pathogen attack by mobilizing a variety of biochemical and molecular defenses (Bowles, 1990). An incompatible interaction between the host and the pathogen results in the triggering of defense responses through signaling pathways; these include the production of reactive oxygen species (ROS) (e.g. O_2-, H_2O_2, and OH), nitric oxide, salicylic acid (SA), jasmonic acid (JA), and ethylene (Lu *et al.,* 2006). Signaling pathways activate a broad series of defense responses that control or eliminate the pathogen. These responses include hypersensitive response, deposition of cell wall reinforcing materials, and the synthesis of a wide-range of antimicrobial compounds including pathogenesis-related (PR) proteins and phytoalexins (Veronese *et al.,* 2003).

Host response to *F. graminearum* infection has been studied mainly in wheat (Pritsch *et al.,* 2000; 2001; Li *et al.,* 2001). Molecular characterization of cDNA clones and expressed sequence tags (ESTs) from *Fusarium* spp.-infected wheat spikes revealed an increase in transcript levels of many PR-genes (Pritsch *et al.,* 2000; 2001; Li *et al.,* 2001). Different classes of PR-proteins including PR-1, PR-2 (β-1,3-glucanases), PR-3 and PR-4 (chitinases), PR-5 (thaumatin-like protein), and PR-9 (peroxidases) were

induced within 6-12 h following infection (Pritsch *et al.,* 2000; 2001; Li *et al.,* 2001). In monocots, exogenous application of JA to rice has resulted in the accumulation of transcripts for PR-1, -2, -3, -5, and -9 which are associated with hypersensitive cell death (Schweizer *et al.,* 1997). Moreover, SA treatments in monocots have resulted in the up-regulation of PR-2, -3, and -5 (Molina and Garcia-Olmedo, 1993a; Wasternack and Hause, 2002; Lu *et al.,* 2006). These findings suggest the occurrence of cross-talk between the JA and SA pathways during plant response to pathogen invasion. This also suggests that defense-related proteins in monocots are activated after fungal infection and may play a role in the general defense against *Fusarium* spp. infection. Similar to findings in wheat, a recent transcriptomal study on the interaction between barley and *F. graminearum* has reported the induction of transcripts encoding defense-related proteins, oxidative burst-associated enzymes, and phenylpropanoid pathway enzymes (Boddu *et al.,* 2006). A recent study based on metabolome profiling led to the identification of groups of compounds that were able to discriminate resistance, and suggested plausible functions for metabolites in wheat plant defense against *F. graminearum* (Hamzehzarghani *et al.,* 2005).

Proteomic techniques provide tools for studying plant stress responses and possible mechanisms of plant resistance. Using a 2-dimensional electrophoresis (2-DE)-based protein separation method, a global protein expression profile can be generated and compared. One of the major advantages of the 2-DE technique is that differentially expressed proteins can clearly and reproducibly be detected when infected and uninfected plant conditions are compared. Proteins showing differential expression between treatments may have an important role in the response of the plant to stress. Identification

of these differentially expressed proteins by LC-MS/MS technology can provide insight into the molecular mechanisms of resistance and underlying functions of these proteins in determining resistance in barley plants. In this study, a systemic comparison of protein profiles among barley spikelets from six cultivars inoculated with *F. graminearum* or a mock control was made at 1 and 3 days post inoculation (dpi). The objective of this study was to identify differentially expressed proteins in FHB-resistant and FHB-susceptible barley lines under infected and uninfected conditions, as well as describe the possible mechanisms of resistance.

3.2 Materials and Methods

3.2.1 Plant growth

Six barley lines showing a wide range of phenotypic responses to point inoculated FHB (Chapter 2 of this thesis) were used in this study: *i.e.,* three barley lines representing FHB-resistant sources (Harbin, CI4196 and Svansota), two cultivars of intermediate-resistance to FHB (Chevron and CDC Bold), and one very susceptible cultivar (Stander). Seeds were planted in 15 cm pots and placed in a greenhouse at 21/18°C with a 16 h photoperiod until anthesis (McCallum and Tekauz, 2002). Plants were watered daily and treated once with Tilt™ (2.5 mL/L propiconazole, Syngenta Crop Protection Canada, Guelph, ON) during the tillering stage and Intercept™ (0.004 g/L of soil, Imidacloprid, Bayer Crop Science Canada, Toronto, ON) once sufficient root development was established to prevent powdery mildew and aphids, respectively.

3.2.2 Preparation of macroconidia inoculum

A single isolate of *F. graminearum* strain N2, from an infected wheat head, (J. Gilbert, Winnipeg, MB, Canada) was cultured on potato dextrose agar (PDA) for 5 d at room temperature. A *F. graminearum* macroconidial suspension was produced by transferring four PDA plugs (1 cm x 1 cm) of the established fungal culture to 500 mL of CMC broth (carboxymethylcellulose 15 g, NH_4NO_3 1 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, yeast 1 g, and H_2O 1 L). The culture was incubated on a rotary shaker (150 rpm) at 22°C for two weeks (McCallum and Tekauz, 2002). A hemocytometer was used to count macroconidia. The *F. graminearum* culture was diluted with water to produce a suspension of 40,000 *F. graminearum* macroconidia per mL. A mock inoculum was prepared by diluting sterile CMC broth to the same extent as the *F. graminearum* culture.

3.2.3 Barley spike inoculations

To check the developmental stage of the barley plant, the leaf sheath was pulled back from the spike, without damaging the spike and leaf. The macroconidial suspension of *F. graminearum* was applied to the spikelet using point inoculation at the anthesis stage. Spikelets were inoculated by carefully spreading the palea and lemma and injecting 10 µL of 40,000 *F. graminearum* macroconidia/mL suspension inside the spikelets using a micropipette (Evans *et al.,* 2000; McCallum and Tekauz, 2002). Diluted CMC broth was similarly inoculated into spikelets on a separate plant to serve as a control. Every second group of spikelets per head was inoculated in fall 2005. Following inoculation, pots were placed inside a mist-irrigated greenhouse (95% relative humidity) for 72 h at 25/21°C with a 16 h photoperiod. Infected heads were harvested at 1 and 3 dpi. Harvested spikes

were immediately placed in liquid nitrogen and transferred to a -80°C freezer for storage until protein extraction.

3.2.4 Protein extraction and quantification

Protein samples were extracted using the acetone and trichloroacetic acid (TCA) method described by Wang *et al.* (2003) as reported by Zhou *et al.* (2006) with some modifications. Barley spikelets that received either *F. graminearum* or mock treatment were ground in liquid nitrogen in a pre-chilled mortar. Finely ground powder was collected in a 50 mL Falcon tube and weighed. Five mL of 10% (w/v) TCA (T0699, Sigma-Aldrich) and 0.07% (v/v) 2-mercaptoethanol (M-3148, Sigma-Aldrich) was made up in cold (-20°C) acetone and was added to 0.5 g of ground tissue. The samples were incubated for 1 h at -20°C to precipitate proteins and then centrifuged for 20 min at 12,000 rpm. The pellet of precipitated proteins and debris was washed several times with 5 mL cold 90% acetone containing 0.07% (v/v) 2-mercaptoethanol until the pellet was colorless. A 20 min centrifugation at 12,000 rpm was used to pellet the proteins after each wash. Pellets were air dried for 20 min, and the proteins were resuspended in 1 mL of lysis buffer for 20 min. Lysis buffer contained 8 M urea (161-0731, Bio-Rad Laboratories Ltd., Mississauga, ON, Canada), 2% CHAPS (BP571-5, Bio-Rad Laboratories Ltd.), 50 mM dithiothreitol (DTT, 161-0611, Bio-Rad Laboratories Ltd.), and 0.2% Bio-lyte carrier ampholytes pH 3-10 (ZM0021, Invitrogen Canada Inc., Burlington, ON, Canada). After centrifugation at 12,000 rpm for 20 min to remove debris, the supernatant was collected and immediately cleaned using the Bio-Rad ReadyPrep 2-D cleanup kit (163-2130, Bio-Rad Laboratories Ltd.) according to the

manufacturer's instructions. A 5 μL sample was removed for protein assay and the remaining supernatant was stored at -80°C until protein electrophoresis. The Bradford method (1976) was used to quantify protein concentration. Three biological replicates were completed for each line and treatment.

3.2.5 Isoelectric focusing and SDS-PAGE

A solubilized protein sample (50 μg for analytical and preparative gels) was mixed with lysis buffer to a total volume of 300 μL and loaded on a 17 cm pH 4-7 Bio-Rad Ready Gel Strip (163-2008, Bio-Rad Laboratories Ltd.) with the in-gel rehydration method according to the manufacturer's instructions. For the second dimension separation, the strips were equilibrated for 10 min on a rotary shaker (60 rpm) with 2% DTT and 2.5% iodacetamide (163-2109, Bio-Rad Laboratories Ltd.). The strips were positioned on top of a 12.5% polyacrylamide gel in the presence of SDS and sealed with 1% agarose. The gels were run for 30 min at 30 mA followed by 5 h at 60 mA using a Bio-Rad Protean II Cell.

3.2.6 Staining of SDS-PAGE

Protein spots were stained with Sypro Ruby (S-12000, Invitrogen Canada Inc.) and quantified according to the manufacturer's instructions. Images from all Sypro Ruby stained gels were captured using a Typhoon 9400 scanner (GE Healthcare, Baie D'Urfe, QC, Canada) with the following scanning settings: scan resolution: 300 dots/cm; photomultiplier (PMT): 600 V; normal sensitivity; filters: 610 BP30/Green (532 nm).

Protein spots with significantly altered expression following *F. graminearum* infection were manually excised for LC-MS/MS analyses.

3.2.7 Image analysis

Computer software Phoretix 2D Expression (v2005, from Nonlinear Dynamics Durham, NC 27703, USA) was used to analyze images of Sypro Ruby-stained gels. Three images for 1 and 3 dpi with *F. graminearum* or mock inoculum, and for each of the six barley lines were grouped to calculate the average volume of all the individual protein spots. To reduce experimental variation arising during processing of 2-DE, a normalized volume for each individual protein spot was calculated using 100 times the volume of the protein divided by the total volume of all proteins detected on the same image. Warping, matching, and volume comparisons of proteins among the treatments were generated by the software and confirmed manually. Both 1 and 3 dpi samples were compared for each of the six lines separately; at each time point averaged gels of the mock treatment were subtracted from averaged gels of the *F. graminearum* treatment, and gels from 3 dpi were further analyzed. Significantly more abundant or less abundant proteins were defined by a minimum 2-fold change in their average expression volumes over triplicate images in the *F. graminearum* versus mock treatments, at one or more time points. I defined *de novo* proteins as those proteins only present in the *F. graminearum* treatment, although this kind of change theoretically describes a maximal increase in abundance. Proteins showing altered expression were compared among the resistant and susceptible barley lines. Several protein homologs were identified, indicating limited variance of protein positioning across the 2-DE gels.

3.2.8 LC-MS/MS

Excised Sypro Ruby-stained protein spots were stored in 2 mL microcentrifuge tubes and shipped on dry ice to the University of British Columbia Laboratory of Molecular Biophysics (URL: http://www.lmb.ubc.ca/analytical.html) or to the National Research Council of Canada (NRC, Ottawa, ON, Canada) for LC-MS/MS analysis. According to the NRC protocol, the proteins were destained and reduced with DTT, alkylated with acrylamide, and digested with trypsin (Promega, Madison, WI, USA). The resulting peptide solution was analyzed on a Micromass CapLC and Q-ToF API US (Manchester, United Kingdom) LC-MS system. A peptide CapTrap (Michrom Bioresources, Auburn, CA, USA) was used for online desalting, followed by back flushing onto a 0.075 x 100 mm PepMap C_{18} column (LC Packings, Amsterdam, Netherlands). Peptides were eluted from the column with a 30 min linear gradient of 3-45% solvent B (solvent A: 97.9% H_2O, 2% ACN, 0.1% formic acid; solvent B: 97.9% ACN, 2% H_2O, 0.1% formic acid) at a flow rate of ~300 nL/min. The standard micromass nanospray source with blunt-tip 90 μm OD, 20 μm ID fused silica emitter was held at 80°C, capillary voltage +3.4 kV, cone voltage 32V. Data acquisition was performed in data dependent mode, with up to 3 precursors for MS/MS selected from each MS survey scan. The .pkl files generated by Micromass ProteinLynx software were searched against the NCBI NR and the TIGR protein databases using the Mascot MS/MS Ion Search (www.matrixscience.com).

3.3 Results

3.3.1 Protein identification

Approximately 600 protein spots were resolved in the pH 4-7 range on all of the 2-DE gels. Protein profile changes observed were more significant and only reported for 3 dpi. No results were presented from *F. graminearum* versus mock inoculation at 1 dpi. Comparison of 2-DE images indicated there were both qualitative and quantitative differences between protein profiles from *F. graminearum* and mock-inoculated spikelets in barley lines CI4196, Harbin, Svansota, Chevron, CDC Bold, and Stander. These six barley lines were evaluated for resistance to FHB using three inoculation methods (Chapter 2 of this thesis); CI4196, Harbin, and Svansota were consistently resistant across the point, spray, and nursery inoculation experiments, Chevron and CDC Bold were susceptible in at least one evaluation and were categorized as intermediate-resistance sources, and Stander was consistently susceptible across all experiments.

In total, 116 protein spots were further analyzed by LC-MS/MS for protein identification, and were characterized according to their potential functional roles through annotational analyses. These 116 protein spots were hand-selected based on average differences between mock-inoculated samples and *F. graminearum*-inoculated samples, differences between 1 dpi and 3 dpi gels, and the protein spots were present in at least two out of three gel replicates. Seventy-six of the 116 protein spots selected were significantly more or less abundant among the mock-inoculated and *F. graminearum*-inoculated gel replicates. The majority of the non-significantly different protein spots were associated with metabolism and regulation. Appendix 1 shows the 71 different

proteins that were grouped according to an oxidative burst and defense response, pathogenesis-related proteins, and proteins associated with metabolism, regulation, and those of unknown function. The 116 protein spots identified represented approximately 26% of the total protein volume among all six barley lines.

Seventeen proteins were detected only in the *F. graminearum* treatment 3 dpi and categorized as *de novo* in 1 or more cultivars. Forty-six proteins were more abundant while 53 proteins were less abundant after *F. graminearum* treatment 3 dpi. Changes in abundance of defense-related protein spots were more frequent in CDC Bold, Harbin, CI4196, and Stander than in Svansota and Chevron. Figure 3.3.1 shows the total protein expression profile from FHB-resistant barley line, Harbin, following *F. graminearum* or mock point inoculation at 1 and 3 dpi. A total of 43 proteins were identified in Harbin; 9 proteins associated with an oxidative burst and defense response had decreased abundance while 1 protein had increased abundance, and 1 *de novo* PR-protein was identified. Also in Harbin, 24 proteins identified to be associated with metabolism, regulation, or of unknown function had decreased abundance, 3 proteins had increased abundance, and 4 showed *de novo* expression. Figure 3.3.2 shows the total protein expression profile from FHB-intermediate resistance barley line CDC Bold, following *F. graminearum* or mock point inoculation 1 and 3 dpi. A total of 35 proteins were identified in CDC Bold; 2 proteins associated with an oxidative burst and defense response had decreased abundance while 12 proteins had increased abundance, and 1 more abundant PR-protein was identified. Also in CDC Bold, 1 identified protein was associated with metabolism, regulation, or of unknown function had decreased

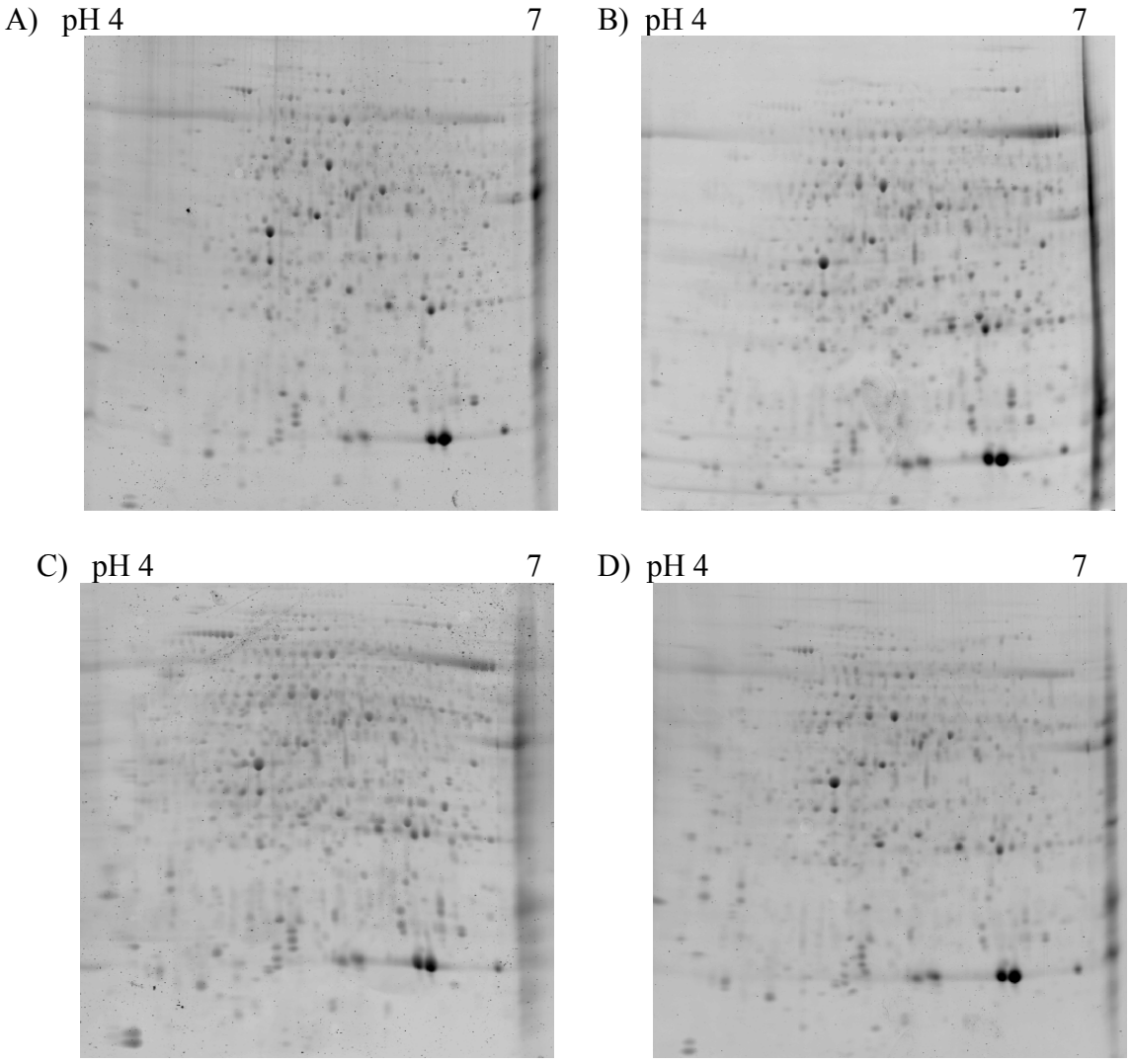

Figure 3.3.1 Protein expression profile of samples extracted from spikelets of fusarium head blight-resistant barley line, Harbin, which were harvested 1 (A, B) and 3 (C, D) dpi. Inoculated samples with *F. graminearum* (B, D) and control (A, C) were compared. This is a representative figure from three biological replicates.

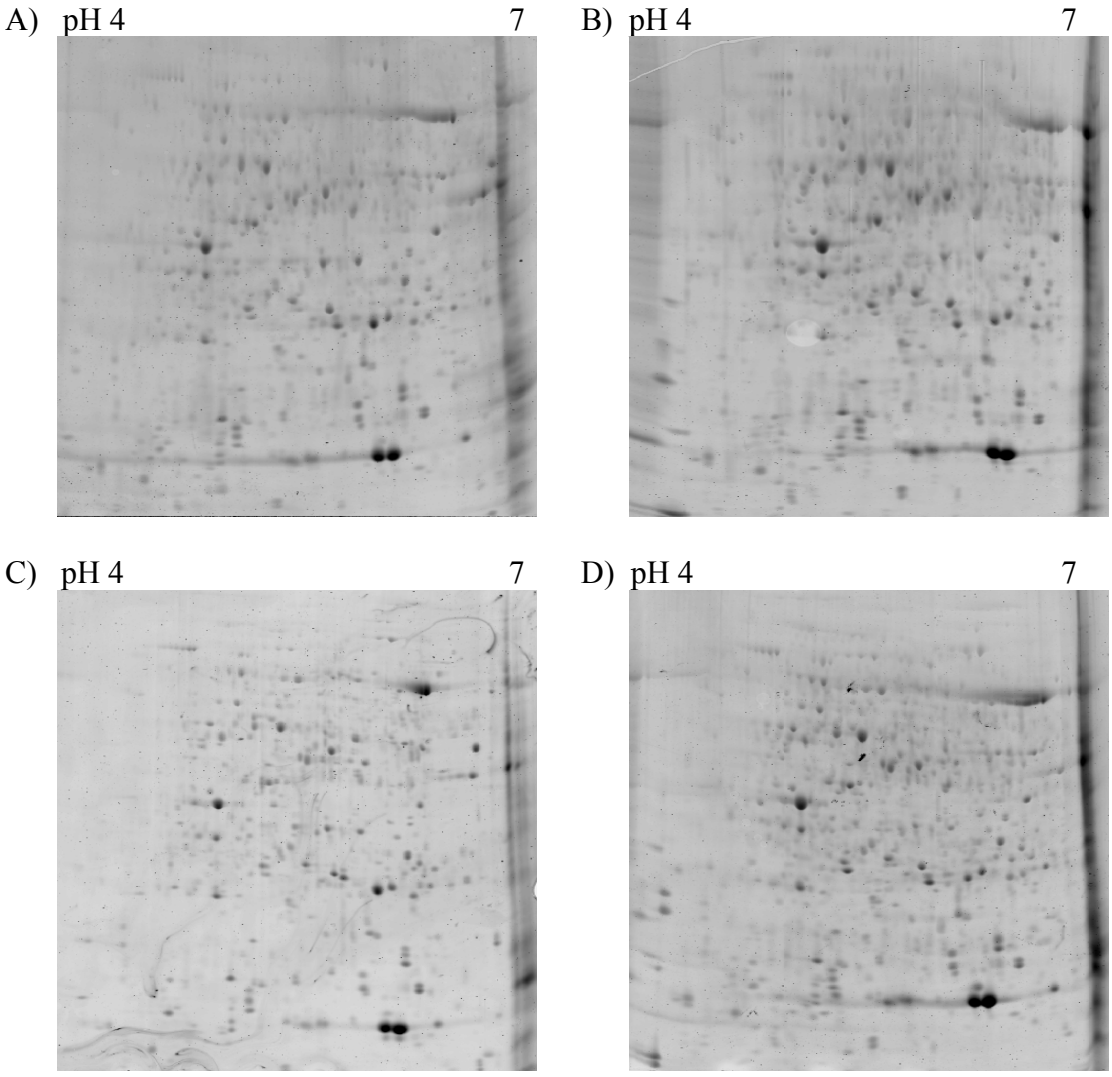

Figure 3.3.2 Protein expression profile of samples extracted from spikelets of fusarium head blight-intermediate resistance barley line, CDC Bold, which were harvested 1 (A, B) and 3 (C, D) dpi. Inoculated samples with *F. graminearum* (B, D) and control (A, C) were compared. This is a representative figure from three biological replicates.

abundance, while 14 proteins had increased abundance, and 5 showed *de novo* expression.

3.3.2 Proteins associated with an oxidative burst and defense response

Among the 76 significantly distinct protein spots analyzed, 19 were associated with an oxidative burst response, leading to the identification of 18 different proteins. Changes in abundance of these proteins were observed in the barley lines, however two dramatically different responses were observed among the six lines. CDC Bold, Stander, and CI4196 presented lots of similarities with a higher abundance of peroxidase precursors, peroxidases, malate dehydrogenases, and NADPH:isoflavone oxidoreductase. On the contrary, protein profiles from Chevron showed little change, while Svansota and Harbin showed reduced abundance of peroxidases and malate dehydrogenases.

Protein profiles at 3 dpi with *F. graminearum* showed altered abundance in the barley lines. NADPH:isoflavone oxidoreductase (gi|17949) showed increased abundance in the resistant line CI4196 (22.8 fold). Ferredoxin-NADP(H) oxidoreductase (gi|20302471) showed a -5.8 fold decrease in abundance in the resistant line Svansota. In the intermediate-resistant line CDC Bold, peroxiredoxin Q (Q5S1S6) was 14.2 fold more abundant. Malate dehydrogenases were identified in all six barley lines. Cytosolic malate dehydrogenase (gi|37928995) showed decreased abundance in the resistant lines Harbin (-2.8 fold) and Svansota (-2.2 fold), and in the intermediate resistant line, CDC Bold (-3.2 fold); in resistant line CI4196, intermediate-resistant line Chevron, and in the susceptible line Stander a 2.3, 2.9, and 14.8 fold increase in abundance, respectively, was observed. For cytoplasmic malate dehydrogenases (gi|18202485 and gi|37535388), the susceptible

line Stander showed -2.5 fold lower abundance, while the intermediate-resistant line CDC Bold showed 19.7 fold higher abundance. The putative malate dehydrogenase (gi|34911788) had lower abundance in the resistant lines Harbin and Svansota (-2.2 fold for each line), lower abundance in the susceptible line Stander (-2.3 fold), and higher abundance in both the intermediate-resistant line CDC Bold (3.7 fold) and in the resistant line CI4196 (13.4 fold). Gamma hydroxybutyrate dehydrogenase-like protein (gi|29368238) showed a -6.5 fold lower abundance in the resistant line Harbin.

CI4196 showed a -8.1 fold lower abundance of glutathione transferase (gi|18479038). For glutathione transferase F5 (gi|23504745) Stander showed a -6.4 fold lower abundance. Cytosolic superoxide dismutase (Cu-Zn) 4 (TC262714) was -3.2 fold less abundant in Harbin. Figure 3.3.3A shows the decrease in abundance of superoxide dismutase (SOD) from resistant line Harbin at 3 dpi with *F. graminearum*. The histogram for SOD expression does not show a significant difference in protein abundance between the mock inoculated sample and the *F. graminearum* inoculated sample. Dehydroascorbate reductases, enzymes associated with peroxidases, were identified in three barley lines; three GSH-dependent dehydroascorbate reductase 1 proteins (gi|6939839) showed -4.9 and -2.0 fold lower abundance in Chevron and Svansota, respectively, and a 4.7 fold higher abundance in CI4196. One dehydroascorbate reductase (gi|28192421) was 18.2 fold more abundant in CI4196.

Four different peroxidases were identified in the barley lines; Chevron and CDC Bold showed a -4.9 fold lower abundance and a 3.1 fold higher abundance, respectively, for peroxidase gi|22587. The ascorbate peroxidase gi|3688398 had lower abundance in

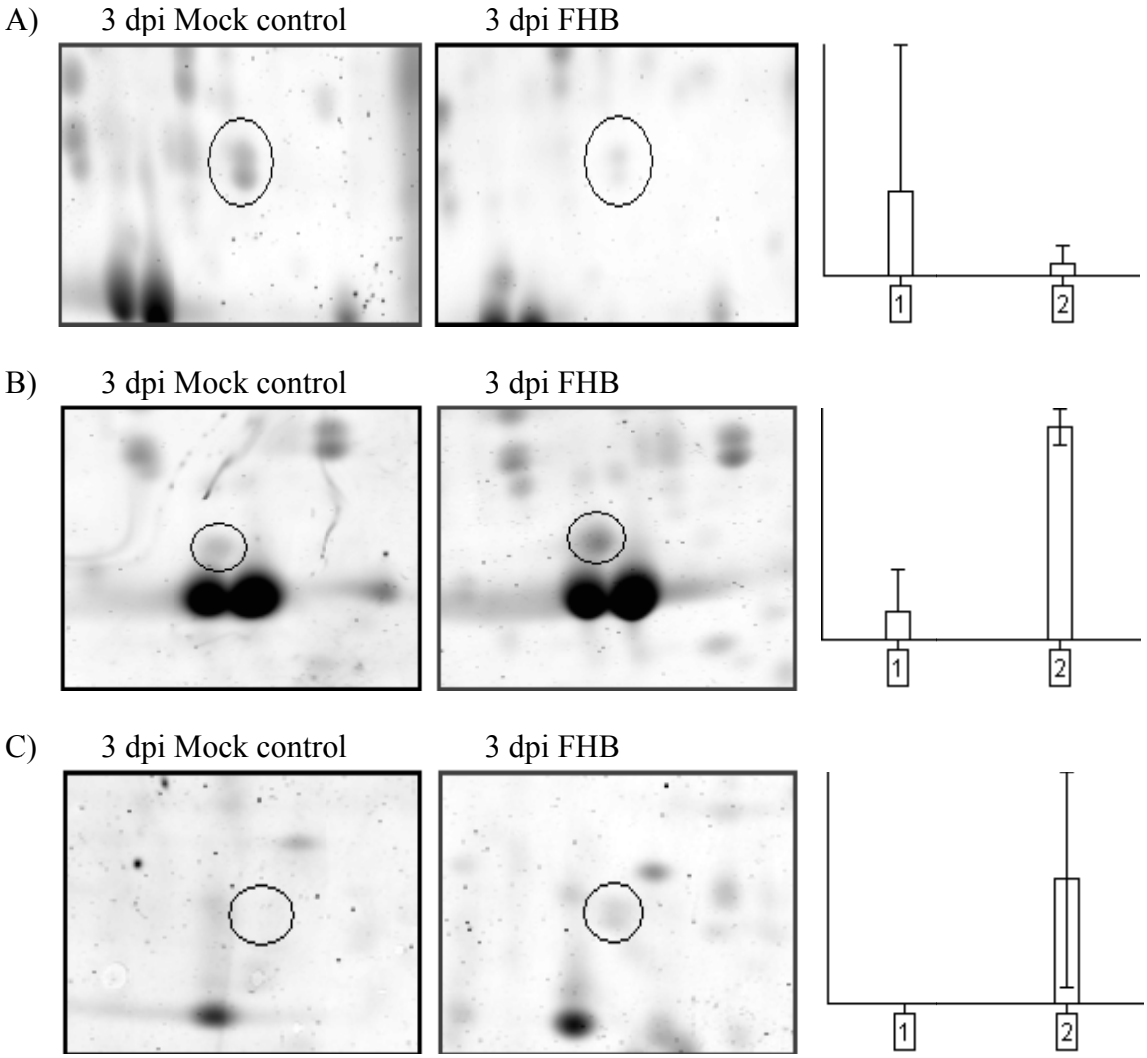

Figure 3.3.3 Enlarged inlets and expression histograms of proteins differentially expressed following *F. graminearum* infection. A) Superoxide dismutase (Cu-Zn) from resistant line Harbin with decreased abundance (expression profile: 1 is mock protein 3 dpi and 2 is FHB protein 3 dpi). B) Thaumatin-like protein 4 from intermediate-resistant line CDC Bold with increased abundance (expression profile: 1 is mock protein 3 dpi and 2 is FHB protein 3 dpi). C) Putative immunophilin/FKBP-type peptidyl-prolyl cis-trans isomerase from susceptible line Stander as *de novo* protein (expression profile: 1 is mock protein 3 dpi and 2 is FHB protein 3 dpi). Y-axis scale is from 0.0 to 0.35 (normalized volumes).

Harbin (-2.9 fold), and higher abundance in CDC Bold (2.9 fold) and CI4196 (6.7 fold). Another ascorbate peroxidase gi|15808779 showed -2.3 and -2.2 fold lower abundance in Harbin and Svansota, respectively; CDC Bold and Stander showed a 2.4 and a 3.0 fold higher abundance, respectively. Harbin showed -3.7 fold lower abundance of a stromal ascorbate peroxidase (gi|32879781), while CDC Bold showed an 8.7 fold higher abundance. A jasmonate-induced protein (gi|400094) showed decreased abundance in Stander and Harbin (-6.4 and -2.3 fold, respectively), and increased abundance in Chevron and CI4196 (2.0 and 2.4 fold, respectively). A universal stress-like protein (gi|53791695) was identified in Harbin with a -2.7 fold lower abundance and in CI4196 and CDC Bold with 2.0 and 2.7 fold higher abundance, respectively.

3.3.3 Pathogenesis-related proteins

Among the 76 significantly distinct protein spots analyzed, 4 were associated with pathogenesis, leading to the identification of 4 different proteins following sampling of tissues 3 dpi with *F. graminearum*. Three different PR-5, thaumatin-like proteins (TLPs), were identified. TLP3 (gi|75103125) was *de novo* expressed in the resistant line Harbin; and TLP4 (gi|5609013) was more abundant in the susceptible line Stander (2.3 fold) and in the intermediate-resistant line CDC Bold (7.3 fold). Figure 3.3.3B shows the increase in abundance of TLP4 from the intermediate-resistant line CDC Bold 3 dpi with *F. graminearum*. TLP7 (Q94649) showed a decrease in abundance in the susceptible line Stander (-16.2 fold) and an increase in abundance in the resistant line Svansota (2.5 fold). One PR-3 protein was identified; chitinase 2b (gi|563489) was 33.3 fold more abundant in the resistant line CI4196.

3.3.4 Proteins associated with plant metabolism, regulation, and unknown functions

Among the 76 significantly distinct protein spots analyzed, 12 were associated with metabolism, leading to the identification of 12 different proteins; 37 were associated with regulation, leading to the identification of 34 different proteins; and 4 were associated with unknown functions, leading to the identification of 3 different proteins. A change in abundance of these protein spots was observed in the barley lines. Putative tyrosine phosphatases were more abundant in CI4196 (6.7 fold) and Chevron (9.1 fold). Bet vI allergen showed higher abundance in Stander (2.0 fold), while Harbin showed lower abundance of two Bet vI allergens (-7.7 and -3.2 fold). S-adenosylmethionine:2-demethylmenaquinone methyltransferase-like protein was 3.1 fold more abundant in CI4196. β-cyanoalanine synthase was less abundant in Stander and Harbin (-2.6 and -2.2 fold, respectively). Nucleoside diphosphate kinase was less abundant in Harbin (-2.5 fold). A Riekse Fe-S protein and its precursor were -10.5 fold less abundant in Harbin and 3.4 fold more abundant in CDC Bold, respectively. An inorganic pyrophosphatase was -2.3 fold less abundant in Harbin; and two chloroplast fructose bisphosphate aldolase (FBP) precursors were -2.6 and -3.3 fold less abundant in Harbin and -2.2 fold less abundant in Stander. Finally, one cytosolic triose phosphate isomerase was less abundant in CDC Bold (-7.4 fold).

At three days post-inoculation, all six barley lines showed changes in abundance with respect to proteins associated with cellular regulation. Several prohibitin proteins were identified; CI4196, Harbin, and Stander were less abundant, while CI4196 and Stander also showed higher abundance. Six photosystem II (PSII) oxygen-evolving

complex proteins were identified; two were *de novo* in CDC Bold and Chevron, and three (one from each line) showed higher abundance in Stander, CI4196, and Chevron. Four proteins associated with ATPase were less abundant in Harbin, Stander, and CI4196, and more abundant in Svansota. Ten proteins were associated with RUBISCO; one phosphoribulokinase was less abundant in Harbin, and Harbin showed lower abundance, while CDC Bold, Chevron, CI4196, Stander, and Svansota showed higher abundance of RUBISCO activases and synthases. A putative immunophilin/FKBP-type peptidyl-prolyl cis-trans isomerase was identified as *de novo* in Stander. Figure 3.3.3C shows the novel expression of a putative immunophilin/FKBP-type peptidyl-prolyl cis-trans isomerase from the susceptible line Stander at 3 dpi with *F. graminearum.*

3.4 Discussion

3.4.1 Protein identification

A total of 18 different acidic defense proteins and precursors of ascorbate peroxidases were identified in the comparative 2-DE gel-analysis of six barley lines. These proteins were most likely the result of plant defense response induction, as they were observed at 3 dpi with *F. graminearum*. I did not observe enzymes involved in the synthesis of precursors of the jasmonic acid (JA), salicylic acid (SA) or phenylalanine-ammonia lyase (PAL) pathways, nor the altered expression of β-1,3-glucanases (PR-2) at either 1 or 3 dpi, suggesting that these pathways were activated within the first 24 h. Limitations of the methodology used could also explain the absence of such observations: e.g., use of a

narrow pH range of 4 to 7. However, the acidic defense proteins identified provide insights into the diverse responses of barley to *F. graminearum* infection.

3.4.2 Proteins associated with an oxidative burst and defense response

The high proportion of identified proteins associated with an oxidative burst and/or oxidative stress response in the six barley lines investigated, indicated that such a plant defense response was prevalent. The plasma membrane of plant cells produces reactive oxygen species, H_2O_2 and O_2-, in response to both biotic and abiotic stimuli that play an important role in plant-pathogen interactions (Foyer and Mullineaux, 1994a; Levine *et al.,* 1994). The identification of dehydroascorbate reductases, peroxidases, NADPH:isoflavone oxidoreductase, ferredoxin-NADP(H) oxidoreductase (FNR), peroxiredoxin, and malate dehydrogenases provided the first direct evidence for differential expression of proteins involved in an oxidative burst following infection with *F. graminearum* in barley spikelets. Pathogen-induced production of oxygen free radicals by the plant has several effects: a) it hinders penetration of the pathogen by stimulating peroxidase activity and by cross-linking cell walls at the site of contact; b) it poses a stress on the pathogen as well as the host cell generating the oxidative burst; and c) it acts as a diffusible signal that leads to systemic acquired resistance (SAR) (Noctor and Foyer, 1998). High intracellular levels of H_2O_2 cause the activation of plant cell death and defense mechanisms during pathogen invasion (Takahashi *et al.,* 1997).

Altered patterns of expression of individual proteins at 3 dpi with *F. graminearum* were observed in the barley lines. CI4196 showed a significant increase in abundance of NADPH:isoflavone oxidoreductase, which generate superoxide radicals needed to induce

an oxidative burst. FNR, known to eliminate toxic radicals produced during oxidative stress (Foyer and Mullineaux, 1994b), showed a decrease in abundance in Svansota, indicating that prolonged exposure of the plant to ROS may be correlated with its resistance against fungal invasion. An increase in abundance of peroxiredoxin Q in CDC Bold may enhance the antioxidant and ROS detoxification capacity of this line. The increased abundance of malate dehydrogenases in Chevron, CI4196, and Stander greatly increased their capacity to reduce O_2- to H_2O_2 and O_2 (Ostergaard *et al.,* 2004). Lower abundance of SOD, as seen in Harbin, can be explained in cells undergoing apoptosis or those using an alternative method for ROS detoxification. Harbin showed an increase in abundance of glutathione transferase, which may be induced in response to oxidative stress, where H_2O_2 acts as a local signal for the hypersensitive death of challenged cells (Tenhaken *et al.,* 1995). Harbin may rely on glutathione transferase as an alternative to SOD for protection from oxidative damage following fungal invasion. Ascorbate peroxidases (PR-9) associated with the JA signaling pathway is part of the main ROS-removing system for cellular protection against oxidative stress (Moller, 2001). A massive increase in abundance of peroxidases in CI4196 and CDC Bold, and a slight increase in abundance in Stander could contribute to the elimination of H_2O_2. On the contrary, a slight decrease in abundance of these same peroxidases and their associated enzymes in Svansota, Harbin, and Chevron could be mechanisms designed to increase the oxidative burst action towards the fungus, or as a strategy to remobilize resources to develop an alternative defense response.

Overall, I could identify two main oxidative response patterns. In Svansota and Harbin, and to a lesser extent in Chevron, I observed a significant decrease in the

abundance of all proteins associated with an oxidative burst and oxidative stress responses; *i.e.,* ascorbate peroxidase and other peroxidases, NADPH:isoflavone oxidoreductase, and malate dehydrogenases. A reduction in abundance of these proteins can be explained by down-regulation of the oxidative stress defense response, which could reduce plant cell death, and favor reallocation of energy for the development of other active mechanisms of defense: e.g., PR-proteins. In contrast, CI4196, CDC Bold, and Stander, showed dramatic increases in abundance (3.0 to 23.0 fold increase of specific proteins) of the oxidative stress defense response proteins: e.g., peroxidase precursors and peroxidases, NADPH:isoflavone oxidoreductase, and malate dehydrogenases. Such a massive oxidative burst and plant defense against free oxygen radicals may have direct oxidative action on *F. graminearum*, but could also lead to a hypersensitive reaction and massive plant cell death. The hypersensitive reaction is a common mechanism used by plants to contain and confine pathogens (Dixon, 2001); however, as a saprophyte, *F. graminearum* infection would progress even in the presence of dead plant tissue. Genetic differences among the 6 barley lines may provide a possible explanation for the observed disparity among FHB-resistance (Takeda, 1992).

3.4.3 Pathogenesis-related proteins

Although no protein precursors of the JA, SA or PAL pathways were identified, a classic model of cereal defense response was reported (Li *et al.,* 1999) and the analysis of acidic PR-proteins observed in this study could provide valuable information regarding the barley response to *F. graminearum* attack. The SA pathway activates selective PR-proteins, and induces H_2O_2 accumulation as a signal for SAR (Tenhaken *et al.,* 1995;

Lamb and Dixon, 1997; Spoel *et al.,* 2003). PR-3 (chitinases) and PR-5 (Thaumatin-like proteins) are induced in cereals following treatment with either JA or SA, and PR-1 and PR-9 are only activated by the JA pathways (Molina and Garcia-Olmedo, 1993a; Schweizer *et al.,* 1997; Wasternack and Hause, 2002; Lu *et al.,* 2006).

Svansota and Harbin showed no change or a decrease in abundance of the acidic PR-1 and PR-9 proteins, while CDC Bold, CI4196, Chevron, and Stander showed increases in abundance for one or both of these PR–proteins, indicating the use of the JA antioxidant signaling pathway in pathogen defense. As acidic PR-1 and PR-9 had lower abundance in two out of three resistant lines it also suggested that activation of the JA pathway was not an absolute requirement for the induction of an incompatible interaction and FHB-resistance. However, I could not eliminate the possibility that a complete analysis of acidic and basic PR-proteins may reveal activation of basic PR-proteins and inhibition of acidic PR-proteins.

Resistant line CI4196 showed a significant increase in abundance of a chitinase 2b protein (gi|563489) (abundance: 0.26% of total protein). The corresponding gene *cht2b* was discovered in the barley cultivar Pallas, inoculated with powdery mildew (Genbank accession # X78672). Chitinases are hydrolytic enzymes that inhibit the growth of many fungi *in vitro* by hydrolyzing the chitin of fungal cell walls. The oligomeric products of digested chitin can also act as signal molecules to stimulate further defense responses (Mauch *et al.,* 1988; Pritsch *et al.,* 2001). An expression profile for ESTs from highly homologous barley chitinase II (*cht2*) indicated high expression in leaf, spike, and stem, moderate expression in the sheath, and no expression in the root seed and flower (http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Hv&CID=173). CI4196

combined induction of a very strong oxidative burst, response to oxidative stress, and chitinase which could explain its high level of stable resistance.

Three different TLPs were reported in four barley lines. A *de novo* produced TLP3 (gi|75103125) was observed in resistant line Harbin, which showed lower abundance for the oxidative burst response. Interestingly, a TLP7 (Q94649) was 2.5 fold more abundant in the resistant line Svansota, which also had a lower abundance for an oxidative burst response; expression of TLP7 was considerably reduced (-16.2 fold) in the susceptible line Stander. An increase in abundance of a TLP4 (gi|56090131) was observed in the intermediate-resistant line CDC Bold and in the susceptible line Stander; while both appeared to exhibit an activated oxidative burst response. Chevron did not show activation of any acidic PR-proteins associated with the SA signaling antioxidant pathway, possibly indicating exclusive use of the JA signaling pathway for defense or the use of an alternative mode of defense against *F. graminearum* invasion. Genetic differences among the 6 barley lines may provide a possible explanation for the observed disparity among FHB-resistance (Takeda, 1992).

3.4.4 Proteins associated with plant metabolism and regulation

Proteins associated with metabolism, regulation, and protein structure also presented altered expression patterns following *F. graminearum* infection. A modification in expression patterns of these proteins was most likely a by-product of invasion while the fungus attempted to acquire resources from the plant, for growth and survival, in a compatible interaction. Rieske Fe-S protein, nucleoside diphosphate kinase, and FBP aldolase identified in CDC Bold, CI4196, and Harbin are involved in basic cellular

metabolism; ß-cyanoalanine synthase, identified in Stander and Harbin, is involved in the synthesis of cysteine, suggesting that the alteration of the amino acid synthesis and nitrogen metabolism was a result of *F. graminearum* infection. A decrease in abundance of RUBISCO, PSII, Mg-chelatase, ATP synthase, and chlorophyll was reported as a result of the reduced photosynthetic potential of the plant following oxidative stress (Palatnik *et al.,* 1997). Finally, prohibitin, a type III membrane protein, putative immunophilin/FKBP-type peptidyl-prolyl cis-trans isomerase, ribosomal protein L18, and profilin 1 which have roles in cell growth, transportation, protein folding and synthesis, and protein structure, respectively, also showed altered expression patterns due to fungal infection and the resulting plant oxidative stress.

3.4.5 Comparison of defense response among barley lines

FHB resistant barley line CI4196 had the strongest increase in abundance of PR-1, PR-3 and PR-9 proteins, and the strongest active oxidative burst and oxidative stress response, among all barley lines studied. This is typical of an incompatibility interaction between a pathogen and the host plant and indicative of activation of strong and diversified defense responses. Early-defense responses and the continued and prolonged production of ROS during an oxidative burst, may have contributed to activation of chitinase 2b in the spikelets. The FHB susceptible line Stander followed the classic model of a compatible interaction, response to oxidative stress and lack of or delays in PR-protein induction, and in this particular case a decrease in abundance of PR-1 and PR-5. TLP is a multi-gene family that has been quite well studied in barley. Reiss and Horstmann (2001) have reported eight isoforms that accumulated in leaves infected with *Drechslera teres*

(associated with net blotch disease). Interestingly, four were acidic (TLP1-TLP3) and four were basic (TLP5-TLP8).

Reported acidic protein profile changes suggested different defense mechanisms in the other 4 barley lines. The most interesting observations were made in Svansota and Harbin, which have reduced abundance of acidic peroxidases, NADPH, SOD, and cytosolic and cytoplasmic malate dehydrogenases. Only acidic PR-5 proteins had increased abundance or were *de novo* expressed. Inhibition of fungal growth by PR-5 has been reported, but varies depending on the TLP isoform and the fungal species. TLP antifungal activity differences among isoforms might depend on the binding capacity to various fungal (1,3)-β-D-glucans *in vivo*, and the interaction with other PR-proteins: e.g., PR-2 and PR-3 (Osmond *et al.,* 2001).

CDC Bold presented lots of similarities with resistant line CI4196, but showed an increase in abundance of TLP4 instead of TLP7 and a decrease in abundance of cytosolic malate dehydrogenase rather than an increase. The findings suggested that TLP7 might be more efficient than TLP4 against *F. graminearum* mycelia. A study of basic proteins or the timing of the defense response would highlight more phenotypic differences associated with resistance between these two lines. Chevron had very few significant changes in defense-related acidic protein abundance; *i.e.,* a slight reduction in the abundance of peroxidase and its precursors, and a slight increase of cytosolic malate dehydrogenase and PR-1. Again, a study of the basic proteins might reveal resistance mechanisms not identified in this study at pH 4-7.

3.5 Conclusion

To the best of my knowledge, this is the first report of the application of proteomic techniques in studying the interaction between a series of barley lines representing various levels of resistance to *F. graminearum* infection. This proteomic investigation of resistant and susceptible genetically-unrelated barley lines to FHB revealed a complex cellular network in the barley cells in response to fungal invasion. The network encompassed an oxidative burst, JA and SA antioxidant signaling pathways, induction of PR-proteins, protein synthesis, photosynthesis, regulation, and other metabolic pathways. The results indicated that the plant defense responses following fungal infection were diverse among resistant and susceptible barley lines. I was able to detect several components of SAR in the susceptible line Stander; such as the production of antioxidant proteins, and a decrease in abundance of PR-proteins. Resistant lines CI4196, Harbin, and Svansota differed in oxidative stress response, but showed a common induction response of acidic PR-3 and PR-5 proteins. An increase in abundance of oxidative responses and cell death in susceptible and intermediate lines, induced by the trichothecene producing fungus, might prepare the terrain for invasion by the saprophytic *F. graminearum*. Transcriptomal analysis and proteomic studies of acidic and basic proteins, in response to the fungus, the trichothecenes, and their interactive effect in FHB pathogenesis would help to complete the picture of resistance put forth by these 2-DE proteomic studies.

CHAPTER 4 Barley defense response to *Fusarium graminearum* and deoxynivalenol stresses.

4.1 Introduction

Fusarium head blight (FHB) or scab, caused mainly by *Fusarium graminearum* Schwabe [teleomorph = *Gibberella zeae* (Schwein) Petch], is a severe disease of barley and wheat grown in humid and semi-humid climates (Parry *et al.,* 1995; McMullen *et al.,* 1997). Disease symptoms develop in the spike tissue and are marked by spikelet discoloration and premature necrosis. *F. graminearum* not only reduces the yield and quality of infected grain, but also produces a number of mycotoxins, including zearalenone and the trichothecene, deoxynivalenol (DON), in infected grain (Cook, 1980; 1981). Trichothecenes have been implicated in pathogenesis, virulence, phytotoxicity, and with mycotoxicosis in humans and animals (Marasas *et al.*, 1984; Sharma and Kim, 1991; Desjardins and Hohn, 1997; Kang and Buchenauer, 1999; Shifrin and Anderson, 1999). Trichodiene synthase, encoded by the gene *Tri5,* catalyzes the first reaction in the trichothecene pathway; disruption of this gene blocks all trichothecene production (Desjardins and Hohn, 1997). Studies have shown that *F. graminearum* without a functional *Tri5* gene does not produce DON in wheat kernels and does not show fungal spread from inoculated spikelet to uninoculated spikelets, confirming that trichothecene toxins are virulence factors for FHB (Desjardins *et al.,* 1996; Eudes *et al.,* 1997; Bai, 2001).

Following pathogen attack, plants delay pathogen growth or resist pathogen spread by mobilizing a variety of biochemical and defense responses (Bowles, 1990). A

recent transcriptomal study on the interaction between barley and *F. graminearum* has reported the induction of transcripts encoding defense-related proteins, oxidative burst-associated enzymes, and phenylpropanoid pathway enzymes from 24 to 96 h (Boddu *et al.,* 2006). In wheat, recent metabolome profiling studies identified groups of compounds that discriminated resistance, and elucidated plausible functions of metabolites in defense against *F. graminearum* (Hamzehzarghani *et al.,* 2005). Studies in wheat and barley have also identified 14 classes of pathogenesis-related (PR)-proteins, PR-1 through PR-14 (van Loon and van Strien, 1999; Li *et al.,* 2001; Pritsch *et al.,* 2000; 2001; Chapter 3 of this thesis). Moreover, results from studies on *Fusarium* infections in wheat and barley have suggested the occurrence of cross-talk between the jasmonic acid and salicylic acid antioxidant signaling pathways and activation of defense-related proteins (Spoel *et al.,* 2003; Lu *et al.,* 2006)

Transcriptomal and proteomic techniques provide powerful tools for studying plant stress responses and uncovering possible mechanisms of plant resistance. Microarray profiling enables the large-scale examination of transcript accumulation as a function of temporal and spatial events. Caldo *et al.* (2004) identified transcripts that accumulated differentially in compatible and incompatible interactions between barley and *Blumeria graminis* (powdery mildew) and demonstrated the power of the Barley1 GeneChip to study barley-pathogen interactions. iTRAQ (Isobaric Tags for Relative and Absolute Quantitation) technology is a stable method for relative protein quantitation using mass spectrometry; a global protein expression profile is generated and compared. iTRAQ technology may provide insight into the molecular mechanisms of resistance and underlying roles of these proteins in FHB-resistance of barley.

In this study, comparisons of RNA transcripts and protein profiles among barley spikelets of resistant and susceptible lines inoculated with trichothecene producing and non-producing *F. graminearum* strains, DON, trichothecene non-producing *F. graminearum* supplemented with DON, or mock inoculum, were made using RNA microarray profiling and iTRAQ technology. The main objective of this study was to describe barley responses to fungal stress, trichothecene stress, and the interaction of the fungus and the trichothecene. The hypothesis was that trichothecene, a virulence factor, would inhibit or delay plant defense responses. A second objective was to identify factors associated with resistance or susceptibility. The hypothesis was that the resistant barley line, Chevron, would be able to activate a defense response earlier than the susceptible line, Stander, and utilize a distinct defense pathway.

4.2 Materials and Methods

4.2.1 Plant development

Two barley cultivars: Chevron, an FHB-resistant source, and Stander, an FHB-susceptible source, were grown in greenhouses at Agriculture and Agri-Food Canada, Lethbridge, Alberta, in 2006. The resistance levels of Chevron and Stander were evaluated following artificial inoculation with *Fusarium* spp. (Chapter 2 of this thesis). Seeds were planted in 15-cm pots and grown in a greenhouse at 21/18ºC with a 16 h photoperiod until anthesis (Evans *et al.,* 2000; McCallum and Tekauz, 2002). Plants were watered daily and treated with Tilt™ (2.5 mL/L propiconazole, Syngenta Crop Protection Canada, Guelph, ON) during the tillering stage and Intercept™ (0.004 g/L of soil,

Imidacloprid, Bayer Crop Science Canada, Toronto, ON) once sufficient root development was established to prevent powdery mildew and aphids, respectively.

4.2.2 Preparation of inoculum

Single isolates of *F. graminearum Tri5-* (trichothecene non-producing, strain GZT40) and *Tri5+* (trichothecene producing, strain GZ3639) were cultured on potato dextrose agar (PDA) for 5 d at room temperature (Desjardins and Hohn, 1997). Individual macroconidial suspensions of each *F. graminearum* strain were produced by transferring four PDA plugs (1 cm x 1 cm) of the established fungal cultures to 500 mL of CMC broth (carboxymethylcellulose 15 g, NH_4NO_3 1 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, yeast 1 g, and H_2O 1 L). The culture was incubated on a rotary shaker (150 rpm) at 22ºC for two weeks (McCallum and Tekauz, 2002). A hemocytometer was used to count macroconidia. The *F. graminearum Tri5-* and *Tri5+* cultures were diluted with water to produce a suspension of 40,000 *F. graminearum* macroconidia per mL. Similarly diluted sterile CMC broth was used as a control or mock inoculum.

Deoxynivalenol (D0156, Sigma-Aldrich, Oakville, ON, Canada) was dissolved in 95% ethanol to a concentration of 1 mg/mL. The mycotoxin was diluted in ddH_2O to working concentrations of 2 parts per million (ppm), 10 ppm, and 25 ppm for inoculation at day 0, 1, and 2, respectively. For the *Tri5-*(DON) treatment, a 10 mL macroconidial suspension of the *F. graminearum* strain GZT40 at 40,000 macroconidia per mL was supplemented with DON at 2 ppm.

4.2.3 Barley spike inoculations

To check the developmental stage of the barley plant, the leaf sheath was pulled back from the spike, without causing damage to the leaf or spike. Five inocula were applied by spikelet injection at the anthesis stage, treatments are referred to as *Tri5*- (strain GZT40), *Tri5*+ (strain GZ3639), DON, *Tri5*-(DON) (GZT40 supplemented with DON), and mock treatment. Every second spikelet per head was inoculated. Spikelets were carefully opened by spreading the palea and lemma and 10 µL of one inoculum was injected into the spikelets (Evans *et al.,* 2000; McCallum and Tekauz, 2002). For iTRAQ experiments, 10 ppm of DON was injected at 24 h, and 25 ppm of DON was injected at 48 h for treatments DON and *Tri5*-(DON). Table 4.2.3 summarizes the five treatments used for inoculation experiments and the effect on the plant being evaluated.

Following inoculations, pots were placed inside a mist-irrigated greenhouse (95% relative humidity) for up to 72 h at 25/21°C with a 16 h photoperiod. Infected heads were harvested at 0, 8, and 24 hpi for RNA experiments and at 0 and 3 dpi for iTRAQ experiments. For RNA experiments, the spikelets were separated from the rachis before freezing and storing; harvested spikes for the iTRAQ experiments were immediately placed in liquid nitrogen and then, transferred to a -80ºC freezer for storage until protein extraction. Two replicates for each line and each treatment were conducted for both the RNA and iTRAQ experiments, with one plant per pot, each arranged in a randomized block, with up to two spike samples collected per plant.

Table 4.2.3 A summary of the five treatments applied by point inoculation to Chevron and Stander and the evaluated effect of each treatment on the plant.

Treatment	*F. graminearum* strain	Effect
Tri5-	GZT40	Fungus
Tri5+	GZ3639	Interaction between the fungus and the mycotoxin
DON	n/a	Mycotoxin
Tri5-(DON)	GZT40 supplemented with DON	Interaction between the fungus and the mycotoxin
Mock	n/a	Control

4.2.4 RNA experimentation

4.2.4.1 RNA extraction and processing, Barley1 GeneChip hybridization, and data acquisition

Spikelets from each treatment, time point, and replication were ground in liquid nitrogen. Total RNA was extracted from approximately 1 g of tissue using the Qiagen RNeasy Mini Protocol for Isolation of Total RNA from Plant Cells and Tissues and Filamentous Fungi (Qiagen Inc., Mississauga, ON, Canada) according to the manufacturer's instructions. Genomic DNA was digested using RNasefree DNase and the RNA was purified on RNeasy columns (Promega Corp., Madison, WI, U.S.). Prior to labeling, total RNA quality was examined on an Agilent 2100 bioanalyzer (Agilent, Palo Alto, CA, U.S.A.). cDNA synthesis was conducted with 15 µg of total RNA and T7-Oligo(dT) primer (Proligo, Boulder, CO, U.S.A.) using the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen Canada Inc., Burlington, ON, Canada). The cDNA was purified with the Affymetrix Gene-Chip sample cleanup module (Affymetrix, Santa Clara, CA, U.S.A.). To produce biotinylated cRNA, the cDNA was transcribed *in vitro* using the Enzo BioArray HighYield RNA transcript labeling kit (Enzo Life Sciences, Farmingdale, NY, U.S.A.) in the presence of biotinylated UTP and CTP. The biotin-labeled cRNA was purified with the Affymetrix Gene-Chip Sample Cleanup Module. Labeled RNA (15 µg) was chemically fragmented using the Affymetrix GeneChip sample cleanup module and used for hybridization. The chip hybridizations, washes, and chip readings were conducted at Genome Quebec Innovation Centre (McGill University, Quebec, Canada) following the standard Affymetrix procedures.

4.2.4.2 Barley1 Affymetrix GeneChip probe array and data analysis

The Affymetrix Barley1 GeneChip probe array consisted of 22,792 probe sets (Close *et al.,* 2004) corresponding to 22,439 barley genes. Exemplar sequences for the GeneChip design were derived from approximately 350,000 barley ESTs. Probe sets consisted of pairs of 11 matched and 11 mismatched 25-mer oligonucleotides designed primarily from the 3′ end of each exemplar sequence (Close *et al.,* 2004). Hybridization of labeled RNA to each probe set was determined and raw numerical values representing the amount of transcript accumulation were obtained at the McGill University and Genome Quebec Innovation Centre.

GeneChip data was obtained from Chevron and Stander spikelets at 0, 8, and 24 hpi with *Tri5*-, *Tri5*+, DON, *Tri5*-(DON), and mock treatments. Data analysis was conducted using Robust Multi-array Average (RMA) analysis (Irizarry *et al.,* 2003) algorithm on the Microarray Platform website (McGill University and Genome Quebec Innovation Center) for the condensation and normalization. The RMA files were then downloaded into Microsoft Excel (Microsoft Canada Co, Mississauga, ON) and the $\log_2$-transformed data with a detection quality of 1, was used to identify a two-fold differential regulation of genes by treatment effect, time effect, and the treatment–time interaction. A detection quality of 1 provided a value for each transcript regardless of the level of the signal. Transcripts showing differential accumulation, mainly due to developmental effect (0 h no treatment vs. 8 and 24 h treatments), were used as controls and were excluded from further analysis. Treatment transcripts were normalized to mock inoculations at their relative time points to eliminate transcripts associated with development or impact of inoculation. Differential regulation patterns were classified

quantitatively depending on transcript abundance differences and clustered based on time-dependent regulation patterns. The differentially transcribed genes were classified into seven functional groups based on the gene target description (identified proteins) of the probe sets at the Affymetrix NetAffex Analysis Center web site (https://www.affymetrix.com/analysis/netaffx/index.affx); oxidative burst activity, phenylpropanoid pathway, jasmonic acid pathway, ethylene defense pathway, PR, deoxynivalenol defense, and general plant defense. Differentially transcribed genes also identified gene targets associated with metabolism, regulation, kinase activity, transportation, and unknown functions.

4.2.5 iTRAQ experimentation

4.2.5.1 Protein sample preparation

Barley spikes inoculated with *F. graminearum* strains GZT40, GZ3639, DON, GZT40 supplemented with DON, and mock inoculum were ground in liquid nitrogen in a pre-chilled mortar. Finely ground powder was collected in a 50 mL Falcon tube and weighed. Two replicates each consisting of four groups of four samples (sample Stander day 0 was an internal control for all intra-replicate groupings) were shipped on dry ice to the Victoria Proteomics Centre (University of Victoria, Vancouver Island Technology Park, Victoria, BC, Canada) for iTRAQ analysis. The proteins were extracted using an acetone precipitation protocol (Applied Biosystems, Foster City, CA, U.S.A.). The samples were then applied to the iTRAQ Reagents Multiplex kit (4352135, Applied Biosystems) according to the manufacturer's instructions. The multiplex kit required the reduction of

proteins and blocking of cysteines, a trypsin digestion, labeling of the protein digests with iTRAQ reagents, and combining of the iTRAQ reagent-labeled digested samples.

4.2.5.2 LC-MS/MS

Prior to performing LC-MS/MS analysis, the sample mixtures were cleaned using cation exchange chromatography with a Vision Workstation (Applied Biosystems) equipped with a Polysulfoethyl A (Poly LC, Colombia, MD) 100 mm X 4.6 mm, 5 μm, 300 A SCX column. The flow rate was set to 0.5 mL/min. Samples were brought up to 2 mL with buffer A (10 mM KH_2PO_4, pH 2.7 and 25% acetonitrile (ACN)) and injected onto the column. The column was allowed to equilibrate for 20 min in buffer A before a 0-35% gradient of buffer B (10 mM KH_2PO_4, 25% ACN, 0.5 M KCl) was applied over 30 min. Fractions were collected every minute after injection. The collected fractions were then reduced in volume in a Speed-Vac (Savant Instruments, Holbrook, NY) and transferred to autosampler vials (LC Packings, Amsterdam, Netherlands).

LC-MS/MS analysis was performed using an integrated Famos autosampler, SwitchosII switching pump, and UltiMate micro pump (LC Packings) system with Hybrid Quadrupole-ToF LC/MS/MS (QStar Pulsar i) equipped with a nano-electrospray ionization source (Proxeon, Odense, Denmark) and fitted with a 10 μm fused-silica emitter tip (New Objective, Woburn, MA, U.S.A.). Chromatographic separation was achieved on a 75 μm x 15 cm C18 PepMap Nano LC column (3 μm, 100 A, LC Packings) and a 300 μm x 5 mm C18 PepMap Nano LC column (5 μm, 100 A, LC Packings) was in place before switching inline with the analytical column and the mass spectrometer. The mobile phase (solvent A) consisted of water:ACN (98:2 (v/v)) with

0.05% formic acid (FA) for sample injection and equilibration on the guard column at a flow rate of 100 µL/min. A linear gradient was created upon switching the trapping column inline by mixing with solvent B which consisted of ACN:water (98:2 (v/v)) with 0.05% FA and the flow rate was reduced to 200 nL/min for high resolution chromatography and introduction into the mass spectrometer.

Samples were brought up to 20 µL with 5% ACN and 3% FA and transferred to autosampler vials (LC Packings). Ten µL of sample was injected in 95% solvent A and allowed to equilibrate on the trapping column for 10 min to wash away any contaminants. The MS was switched inline and a linear gradient from 95% to 40% of solvent A was established for 40 min; the composition of the mobile phase was increased to 95% of solvent A before decreasing to 20% for a 15 min equilibration prior to the next sample injection. MS data was acquired automatically using Analyst QS 1.0 software Service Pack 8 (BI MDS SCIEX, Concord, ON, Canada). An information dependent acquisition method consisting of a 1 s ToF-MS survey scan of mass range 400-1200 atomic mass unit (amu) and two 2.5 s product ion scans of mass range 100-1500 amu. The two most intense peaks over 20 counts, with charge state 2-5 were selected for fragmentation and a 6 amu window was used to prevent the peaks from the same isotopic cluster from being fragmented again. Once an ion was selected for MS/MS fragmentation it was put on an exclude list for 180 s. Curtain gas was set at 23, nitrogen was used as the collision gas and the ionization tip voltage used was 2700 V. Protein identifications were determined by searching an all-species mass spectrometry (MS) database and a *Fusarium* database (Dr. Linda Harris, AAFC-Ottawa, ON, Canada). Data files were processed using the Protein Pilot software version 1.0 (Applied Biosystems). All values were normalized to

the internal control (Stander at 0 dpi); the replicates for each treatment were then averaged and those proteins were used for further analysis. Chevron treatment values were also compared to Chevron at day 0 no treatment.

4.3 Results

4.3.1 RNA experimental results

Microarray profiling identified 22,840 RNA transcripts from Chevron and Stander spikelets at 0, 8, and 24 hpi with treatments *Tri5*-, *Tri5*+, DON, and *Tri5*-(DON), and mock inoculations. Using the Affymetrix Netaffex web site, a total of 368 significantly-altered gene transcripts were associated with a plant defense response at 8 and 24 hpi in at least one treatment; 31 identified gene transcripts were associated with an oxidative burst, 56 were associated with the phenylpropanoid pathway, 24 were associated with the jasmonic acid (JA) antioxidant signaling pathway, 8 were associated with the ethylene defense pathway, 134 were associated with pathogenesis, 24 were associated with DON defense, and 91 were associated with general plant defense response. Refer to Appendix 2 (Chevron results) and Appendix 3 (Stander results) for a complete list of gene transcripts, the probe set IDs associated with each gene transcript, and numerical values for the changes in observed regulation patterns.

4.3.1.1 Gene transcripts associated with an oxidative burst

Among the 31 identified gene transcripts associated with an oxidative burst, only those transcripts coding for oxalate oxidases have been reported. Four transcripts coding for

germin proteins were identified; Chevron and Stander showed down-regulation in the DON treatment, and up-regulation in Chevron treatment *Tri5*-(DON) and in Stander *Tri5*-. One oxalate oxidase 2 precursor was up-regulated in Chevron treatments *Tri5*-, DON, and *Tri5*-(DON) and in Stander treatments *Tri5*- and *Tri5*-(DON). Eight transcripts coding for oxalate oxidase were also identified; Chevron *Tri5*+ and DON treatments and Stander *Tri5*-, *Tri5*+, and DON treatments showed down-regulation of 3 genes; all treatments in Chevron, and Stander *Tri5*-, DON, and *Tri5*-(DON) treatments showed up-regulation of 6 genes.

4.3.1.2 Gene transcripts associated with the phenylpropanoid pathway

Fifty-six identified gene transcripts were associated with the phenylpropanoid pathway. Eight gene transcripts coding for phenylalanine ammonia-lyase (PAL) were identified; Chevron *Tri5*+ and Stander DON treatments showed down-regulation of 5 genes, while Chevron *Tri5*- and Stander *Tri5*- and *Tri5*-(DON) showed up-regulation of all 8 genes. In the Chevron and Stander DON treatments, a caffeic acid O-methyltransferase was down-regulated, compared to its up-regulation in Chevron *Tri5*-. Chevron showed down-regulation of a chalcone synthase in *Tri5*+ and up-regulation in *Tri5*-(DON), while Stander showed up-regulation in the *Tri5*-, DON, and *Tri5*-(DON) treatments. Two coumarate-CoA ligases were down-regulated in Stander DON and up-regulated in *Tri5*-; and three cinnamoyl-CoA reductases were down-regulated in all Chevron treatments and in Stander *Tri5*+, DON, and *Tri5*-(DON) treatments, whereas 2 genes were up-regulated in Stander *Tri5*+ and DON treatments. Three cinnamoyl alcohol dehydrogenases were

identified; Chevron *Tri5*+ and Stander DON showed down-regulation, while Chevron *Tri5*-(DON) and Stander *Tri5*- and *Tri5*-(DON) showed up-regulation.

4.3.1.3 Gene transcripts associated with the jasmonic acid antioxidant signaling pathway

Twenty-four identified gene transcripts were associated with the JA signaling pathway. One 12-oxophytodienoate reductase was up-regulated in Stander *Tri5*-; and two 12-oxophytodienoic acid reductases were down-regulated in Chevron *Tri5*+, and up-regulated in Chevron DON and in Stander *Tri5*- and *Tri5*-(DON). Two allene oxide synthases were down-regulated in Chevron *Tri5*- and *Tri5*-(DON) and in Stander DON, whereas Stander *Tri5*- showed up-regulation. Four gene transcripts coding for lipoxygenases were down-regulated in Chevron *Tri5*- and *Tri5*-(DON), and in Stander DON, whereas they were up-regulated in Stander *Tri5*-. Two gene transcripts coding for methyljasmonate-inducible lipoxygenase 2 were down-regulated in Chevron *Tri5*-(DON) and in Stander *Tri5*+ and DON, compared to their up-regulation in Chevron *Tri5*+ and in Stander *Tri5*-. Four gene transcripts coding for jasmonate-induced proteins were down-regulated in all Chevron treatments and in the Stander DON treatment; whereas in Chevron DON and *Tri5*-(DON) and in Stander *Tri5*- and *Tri5*-(DON) treatments three gene transcripts coding for jasmonate-induced proteins were up-regulated.

4.3.1.4 Gene transcripts associated with the ethylene defense pathway

Among the 8 identified gene transcripts associated with the ethylene defense pathway only 3 have been reported. One gene transcript coding for a 1-aminocyclopropane-1-

carboxylate oxidase was up-regulated in Chevron DON and in Stander *Tri5*+ and *Tri5*-(DON). Gene transcripts coding for an ethylene-responsive transcriptional co-activator and an ethylene-responsive protein were up-regulated in Chevron DON, and down-regulated in Stander DON, respectively.

4.3.1.5 Pathogenesis-related gene transcripts and PR-precursors

One hundred and thirty-four identified gene transcripts were associated with pathogenesis. Gene transcripts coding for PR-1 protein precursors were down-regulated in Chevron *Tri5*-, DON, and *Tri5*-(DON) and in Stander *Tri5*- and *Tri5*-(DON), and up-regulated in Stander *Tri5*+. Five gene transcripts coding for PR-1 proteins were down-regulated in Stander *Tri5*+ and up-regulated in Chevron and Stander *Tri5*-, DON, and *Tri5*-(DON) treatments. Stander *Tri5*+ and DON showed down-regulation of a gene transcript coding for a PR-2 precursor, whereas Chevron *Tri5*- and Stander *Tri5*- and *Tri5*-(DON) showed up-regulation. Chevron *Tri5*-(DON) and Stander *Tri5*+ and DON treatments showed down-regulation of a gene transcript coding for a β-glucanase, while Stander *Tri5*-(DON) showed up-regulation.

A gene transcript coding for a chitinase was down-regulated in Chevron *Tri5*+ and in Stander DON, whereas all other Chevron treatments and Stander *Tri5*-(DON) showed up-regulation. Gene transcripts coding for chitinase 2a and 2b precursors were down-regulated in Stander *Tri5*+, and up-regulated in Chevron DON and *Tri5*-(DON) and in Stander *Tri5*- and *Tri5*-(DON). Six gene transcripts coding for PR-5 proteins were identified; TLP4 (thaumatin-like protein) and TLP8 were up-regulated in Chevron *Tri5*-, DON, and *Tri5*-(DON); and TLP4, TLP7, and TLP8 were up-regulated in Stander *Tri5*-

and *Tri5*-(DON). A gene transcript coding for a proteinase inhibitor was down-regulated in Chevron *Tri5*+ and in Stander *Tri5*-, DON, and *Tri5*-(DON), whereas it was up-regulated in Chevron *Tri5*- and *Tri5*-(DON).

Gene transcripts coding for PR-9 proteins and their precursors were altered in various treatments. A gene transcript coding for a peroxidase precursor was down-regulated in Chevron *Tri5*+ and DON, and in Stander *Tri5*+, DON, and *Tri5*-(DON) treatments; and up-regulated in Chevron *Tri5*- and *Tri5*-(DON). Fifteen gene transcripts coding for peroxidases were identified; Chevron *Tri5*+ and DON and all Stander treatments showed down-regulation of 13 genes, whereas Chevron *Tri5*-, DON, and *Tri5*-(DON) and Stander *Tri5*- and *Tri5*-(DON) showed up-regulation of 7 genes. Three gene transcripts coding for PR-10a proteins were identified: Chevron *Tri5*+ and Stander *Tri5*+ and DON showed down-regulation, whereas Chevron and Stander *Tri5*-, DON, and *Tri5*-(DON) treatments showed up-regulation.

Gene transcripts coding for a Defensin J1-2 precursor protein were down-regulated in Chevron *Tri5*-, *Tri5*+, and DON, whereas Stander *Tri5*- showed up-regulation. Gene transcripts coding for a PR-13, thionin protein, were down-regulated in Stander *Tri5*+, DON, and *Tri5*-(DON), and up-regulated in Chevron DON. A total of eight gene transcripts coding for nonspecific lipid transfer protein (nsLTP) precursors were identified; two genes were down-regulated in Stander *Tri5*+ and DON, and two genes were up-regulated in Chevron *Tri5*- and DON and in all Stander treatments. One gene transcript coding for an nsLTP was down-regulated in Stander *Tri5*- and *Tri5*-(DON), and up-regulated in Chevron *Tri5*+; and one gene transcript coding for an LTP

was down-regulated in Chevron *Tri5*+ and DON, compared to up-regulation in Stander *Tri5*+.

4.3.1.6 Gene transcripts associated with DON defense

Among the 24 identified gene transcripts associated with DON defense, only those previously shown to be involved in DON detoxification were reported. Interestingly, very few genes showed altered patterns of regulation in the resistant line Chevron, while three gene transcripts coding for sucrose-UDP glucosyltransferases were down-regulated in the Stander DON treatment.

4.3.1.7 Gene transcripts associated with plant defense response

Among the 91 identified gene transcripts associated with plant defense response, only regulation trends were reported. Gene transcripts coding for pectin-associated proteins were down-regulated in all Chevron treatments except *Tri5*-(DON), compared to up- and down-regulation in all Stander treatments. Gene transcripts coding for cell-wall associated proteins; *i.e.,* glycine-rich, proline-rich, hydroxyproline-rich, extension-like, xyloglucan endo-1,4-β-D glucanases, and xylanase inhibitors showed altered patterns of regulation in both lines and under all treatments. A total of 21 gene transcripts coding for glutathione transferases were identified and showed altered regulation patterns in both lines and under all treatments. Several gene transcripts coding for pathogen-induced proteins were down-regulated in Stander *Tri5*+ and DON, and up-regulated in Chevron DON and *Tri5*-(DON) and in Stander *Tri5*- and *Tri5*-(DON). No significant differences

in gene transcripts coding for alcohol dehydrogenases were observed in Chevron, whereas Stander showed altered regulation patterns under all treatments.

4.3.2 iTRAQ experimental results

On average, 700 protein spots were identified per treatment for each line using iTRAQ technology. Comparison of LC-MS/MS data concluded that both qualitative and quantitative differences were identified between protein profiles from *F. graminearum* treatments *Tri5*-, *Tri5*+, DON, *Tri5*-(DON), and mock-inoculated spikelets in the resistant line Chevron and in the susceptible line Stander. A total of 47 proteins identified were associated with defense pathways, pathogenesis, and defense response at 3 dpi; 11 proteins showed significantly higher abundance and 16 proteins showed significantly lower abundance across various treatments. Seventeen identified proteins were associated with an oxidative burst, and the SA and JA defense pathways, 18 proteins were associated with pathogenesis, and 12 were associated with DON defense and general plant defense response. Refer to Appendix 4 (Chevron results) and Appendix 5 (Stander results) for a complete list of proteins identified, along with their designated accession numbers and magnitude of expression changes.

4.3.2.1 Proteins associated with an oxidative burst, and the SA and JA antioxidant signaling pathways

Among the 4 identified proteins associated with an oxidative burst only a coproporphyrinogen oxidase protein had lower abundance in Chevron *Tri5*+. Among the 7 identified proteins associated with the phenylpropanoid pathway; two PAL proteins

showed lower abundance in all Chevron treatments, one caffeic acid O-methyltransferase showed lower abundance in Stander *Tri5*-, and *Tri5*-(DON) treatments, and one chalcone synthase protein showed lower abundance in Chevron *Tri5*+. Among the 6 identified proteins associated with the JA antioxidant signaling pathway; only a lipoxygenase protein in Stander *Tri5*+ treatment was significantly less abundant.

4.3.2.2 Pathogenesis-related proteins and PR-precursors

Eighteen identified proteins were associated with pathogenesis. One PR-1a protein showed higher abundance in Chevron *Tri5*+ and in all Stander treatments. Chitinase precursor proteins showed higher abundance in Stander *Tri5*- and *Tri5*-(DON) and in Chevron *Tri5*+. Chevron showed higher abundance of a PR-4 protein in *Tri5*-, *Tri5*+, and *Tri5*-(DON). Chevron also showed higher abundance of TLP3 and TLP8 under the DON treatment. One dehydroascorbate reductase was less abundant in Chevron *Tri5*-, and in Stander *Tri5*-, *Tri5*+, and *Tri5*-(DON); and Chevron showed lower abundance for an ascorbate peroxidase in *Tri5*+. One LTP was identified in Stander, showing increased abundance in the *Tri5*- and *Tri5*+ treatments.

4.3.2.3 Proteins associated with DON defense and general plant defense response

Four identified proteins were associated with DON defense. One UDP-glucose dehydrogenase showed lower abundance in all Chevron treatments. Eight identified proteins were associated with general plant defense response; only expression trends were reported. Several 14-3-3 proteins were identified and showed higher abundance in Stander *Tri5*+ and in Chevron *Tri5*- and *Tri5*+.

4.4 Discussion

4.4.1 Gene transcripts and proteins associated with an oxidative burst, the SA and JA antioxidant signaling pathways, and the ethylene defense pathway

The plant defense responses observed in both barley lines using RNA microarray profiling and iTRAQ experiments identified differential regulation patterns and differential expression patterns of 35 gene transcripts and proteins, respectively, which were associated with an oxidative burst. Since 1993, germin proteins have been associated with an oxidative burst response and have been known to display oxalate oxidase activity, an activity which degrades oxalic acid into H_2O_2 and CO_2 (Dumas *et al.,* 1993; Lane *et al.,* 1993); H_2O_2 is required for peroxidase mediated cross-linking reactions of the cell wall, a potent defense mechanism (Dumas *et al.,* 1995; Zhang *et al.,* 1995; Hurkman and Tanaka, 1996).

Chevron and Stander showed both an up- or down-regulation of gene transcripts coding for oxidases in all treatments, and the magnitude of responses was comparable. Microarray and iTRAQ results suggested that oxalate oxidases and other oxidases were associated with general plant defense and an oxidative burst occurred following fungal or mycotoxin inoculations. The effect of the fungus alone (*Tri5*- treatment) induced an oxidative burst as early as 8 hpi in Chevron, but not until 24 hpi in Stander; the rapid oxidative burst response observed in Chevron may support its resistance towards FHB. The DON treatment induced an oxidative burst as early as 8 hpi in both Chevron and Stander, but did not show continued induction at 24 hpi in Stander; indicating the ability of the mycotoxin alone to initiate an oxidative burst in the susceptible line, but not the

ability to provide continuous protection to the plant; it could also be the result of protein synthesis inhibition in the susceptible line, sending a negative feed-back to DNA transcription. The interaction between the fungus and the mycotoxin of the *Tri5+* treatment showed an oxidative burst only occurring in Chevron at 8 hpi, which suggested that the interaction between the fungus and DON interfered with the induction of an oxidative burst in Stander and did not provide continual protection of the plant in either line. In contrast, the *Tri5-*(DON) treatment showed the induction of an oxidative burst at 8 hpi and a continued response at 24 hpi in both lines, suggesting that the trichothecene non-producing inoculum supplemented with DON was not equivalent to the wild strain. Synthesis of trichothecenes, and their diversity, produced by the wild strain might be critical for virulence; reducing the plants ability to adequately and efficiently respond to the fungal attack.

The RNA microarray profiling and iTRAQ experiments identified a total of 63 differentially regulated gene transcripts and differentially expressed proteins associated with the phenylpropanoid (or salicylic acid) defense pathway. The phenylpropanoid pathway uses phenylalanine ammonia lyase (PAL), cinnamic acid, and various flavonoid-related compounds for the biosynthesis of anthocyanins, lignins, phytoalexins, salicylic acid, and other stress-induced phenylpropanoids. Figure 4.4.1 outlines the compounds and processes associated with the phenylpropanoid defense pathway. PAL, a well-characterized enzyme, is responsible for converting phenylalanine to cinnamic acid in the first step of the pathway. The up-regulation of PAL in Chevron and Stander in the *Tri5-* and *Tri5-*(DON) treatments indicated that the fungus alone and the interaction between the fungus and the trichothecene, respectively, was sufficient to induce the first step of

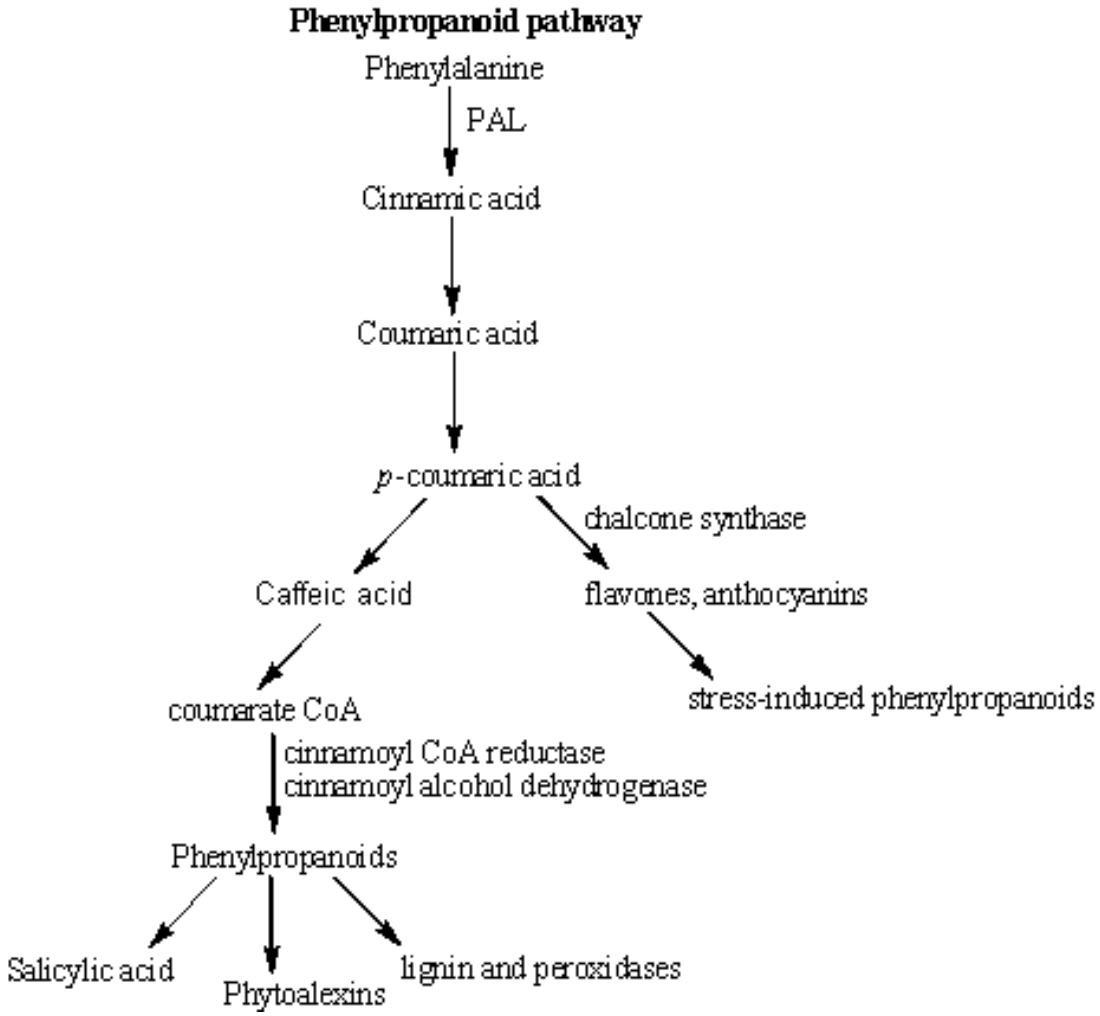

Figure 4.4.1 The phenylpropanoid or salicylic acid antioxidant signaling defense pathway. The phenylpropanoid pathway utilizes phenylalanine-ammonia lyase, cinnamic acid, and various flavonoid-related compounds for the biosynthesis of anthocyanins, lignins, phytoalexins, salicylic acid, and other stress-induced phenylpropanoids.

the SA pathway in both barley lines. Chevron also showed up-regulation of a caffeic acid O-methyltransferase mRNA at 8 hpi in the *Tri5*- treatment, another enzyme involved in the pathway; however, there was no further induction of the phenylpropanoid pathway in Chevron. The lack of further pathway induction indicated that this mode of defense was not essential for FHB-resistance in Chevron.

In contrast, Stander showed up-regulation of gene transcripts coding for several enzymes and metabolites associated with the phenylpropanoid pathway; indicating the possible activation of SA in response to pathogen attack. All treatments in Stander were capable of inducing the SA pathway; indicating that such a response was central during a compatible interaction. Previous studies in monocots have stated that SA is not the first line of defense and is often not the most efficient method of defense response, especially against saprophytic fungi, but may provide basal defense responses in place of an alternative (Molina and Garcia-Olmedo, 1993a; Wasternack and Hause, 2002; Lu *et al.,* 2006). The activation of the SA pathway in Stander may not be the most efficient method of protection and may support the observed susceptibility of Stander to FHB. In Chapter 3, I used 2-DE-based proteomics to show an increase in abundance of proteins associated with SA and its subsequent defense response in Stander, while Chevron appeared to rely on an alternative method. These results were consistent with those observed from the RNA microarray profiling experiments.

An alternative defense response may utilize proteins induced by JA and the JA antioxidant signaling pathway. The RNA microarray profiling and iTRAQ experiments identified 30 differentially regulated gene transcripts and differentially expressed proteins, respectively, to be associated with the JA pathway (also referred to as the

octadecanoid pathway). The JA pathway utilizes lipoxygenase to convert linolenic acid to 13-hydroperoxide, which utilizes cycloxygenase, allene oxide synthase or cyclase for the conversion to 12-oxophytodienoic acid, which then utilizes 12-oxophytodienoic acid reductase to form JA. Figure 4.4.2 outlines the compounds and processes associated with the JA defense pathway. Chevron showed up-regulation of a gene transcript coding for 12-oxophytodienoic acid reductase in the DON treatment at 8 hpi, the up-regulation of a gene transcript coding for a methyljasmonate-inducible lipoxygenase 2 protein at 8 hpi in the *Tri5*+ treatment, and up-regulation of four gene transcripts coding for jasmonate-induced proteins in the DON and *Tri5*-(DON) treatments; indicating that the fungus alone did not activate the JA pathway, and that most likely, it was the presence of DON which was responsible for a JA-associated defense response. Stander showed up-regulation of gene transcripts associated with the JA defense pathway at 24 hpi; indicating that the response observed in Stander was different than that observed in Chevron, and was delayed. In Stander, the fungus alone was responsible for the induction of JA-associated defense, and in both barley lines, the interaction observed from the *Tri5*-(DON) treatment was sufficient to induce a JA-associated defense response.

The RNA microarray profiling and iTRAQ experiments identified 8 differentially regulated gene transcripts and differentially expressed proteins, respectively, to be associated with the ethylene defense pathway. Figure 4.4.3 outlines the compounds and processes associated with the ethylene defense pathway. One significant difference observed between the barley lines was the up-regulation of a gene transcript coding for a 1-aminocyclopropane-1-carboxylate oxidase, a precursor of the ethylene defense

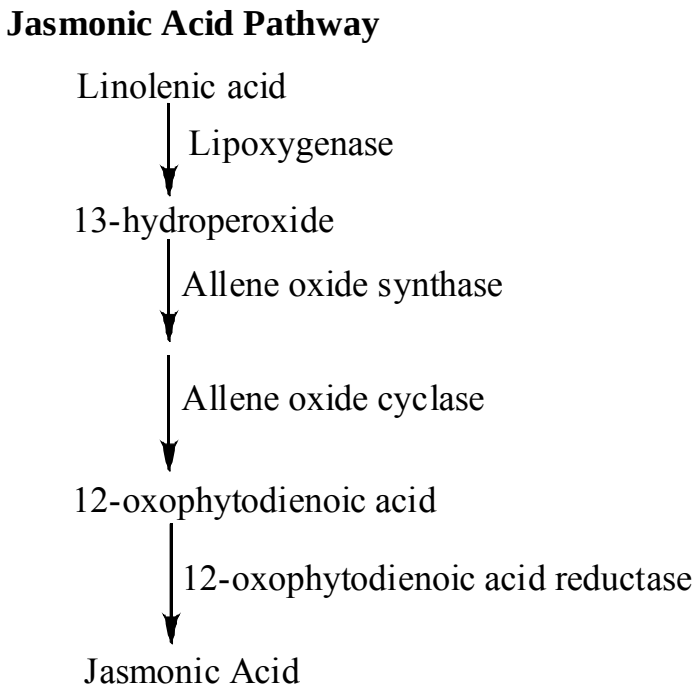

Figure 4.4.2 The jasmonic acid antioxidant signaling defense pathway. The pathway converts linolenic acid to jasmonic acid using lipoxygenase, allene oxide synthase and cyclase, and 12-oxophytodienoic acid reductase.

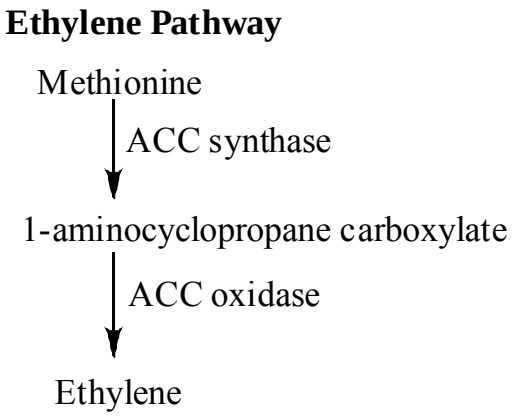

Figure 4.4.3 The ethylene defense pathway. The pathway converts methionine to ethylene using 1-aminocyclopropane-1-carboxylate (ACC) synthase and oxidase.

pathway, and the up-regulation of a gene transcript coding for an ethylene-responsive transcriptional co-activator protein in Chevron under the DON treatment at 8 hpi. These results showed that the ethylene defense pathway was only induced in Chevron following treatment with pure DON; indicating that the presence of neither the fungus nor the interaction between the fungus and the mycotoxin elicited such a response. Ethylene is a volatile messenger in plant defense, it is an endogenous plant hormone that influences many aspects of plant growth and development, such as germination, senescence, and it also participates in a variety of biotic and abiotic stresses (Abeles *et al.,* 1992). Control of these processes by ethylene involves regulation of its biosynthesis and transcription factors, which regulate ethylene-responsive genes; and exogenous applications of ethylene have been shown to induce transcription of various PR-genes (Ohme-takagi and Shinshi, 1990; Eyal *et al.,* 1993; Hart *et al.,* 1993). The early timing of ethylene defense pathway activation was important for the overall plant defense in Chevron and may be associated with its observed resistance.

4.4.2 Gene transcripts and proteins associated with pathogenesis

The RNA microarray profiling and iTRAQ experiments identified 152 differentially regulated gene transcripts and differentially expressed proteins, respectively, associated with pathogenesis. Response to infection varied between the resistant line Chevron and the susceptible line Stander, across all treatments, and at the different time points. RNA microarray profiling experiments were conducted at 8 and 24 hpi and identified gene transcripts associated with early plant defense responses following infection. iTRAQ experiments were conducted at 0 and 3 dpi and identified proteins that had been altered

following infection and following the subsequent induction of their respective RNA transcripts.

The first class of PR-proteins (PR-1) are induced by pathogens, JA, SA, or ethylene and are commonly used as a marker for systemic acquired resistance (SAR); however their functions are not well known (Schweizer *et al.,* 1997). The up-regulation of gene transcripts coding for PR-1 proteins and their precursors was observed in Chevron as early as 8 hpi, whereas the response in Stander was not identified until 24 hpi under the *Tri5*+ treatment; these results were consistent with studies of PR-1a-transformed tobacco plants, which have demonstrated significantly reduced disease development upon exposure to pathogens (Alexander *et al.,* 1993). The results also indicated that the trichothecene non-producing fungus and DON showed up-regulation of gene transcripts coding for PR-1 proteins and their precursors in Chevron; however in Stander the opposite plant response was observed between *Tri5*+ and *Tri5*-(DON), suggesting that the trichothecene non-producing fungus supplemented with DON, according to my method, was not equivalent to the trichothecene-producing fungal wild strain. The same differential response between these two treatments was observed in other reported transcripts in Stander and Chevron. Protein profile expression analysis at 3 dpi showed significantly higher abundance of a PR-1a in the Chevron *Tri5*+ treatment, and in all Stander treatments; indicating that up-regulation of gene transcripts coding for several PR-1a proteins under particular treatments may not be detectable in the first 24 hpi.

There were few differences in regulation of gene transcripts coding for PR-2 proteins and their precursors in either barley line. In Chevron and Stander, *Tri5*- showed

slight up-regulation of a precursor, and Stander *Tri5*-(DON) showed up-regulation of a gene transcript coding for a PR-2 protein. β-1,3-glucanases have been associated with degradation of fungal and bacterial cell walls. Previous studies using two-dimensional gel electrophoresis in barley to study the effect of *F. graminearum* infection at 3 dpi did not report significant differences in the abundance of PR-2 proteins, suggesting that these proteins were not used as the primary mode of defense (Chapter 3 of this thesis).

Chitinases are described in four classes of PR-proteins: PR-3, PR-4, PR-8, and PR-11. Chitinases are hydrolytic enzymes that inhibit the growth of many fungi *in vitro* by hydrolyzing the chitin of fungal cell walls. The oligomeric products of digested chitin can also act as signal molecules to stimulate further defense responses (Mauch *et al.,* 1988; Pritsch *et al.,* 2001). Chevron and Stander reported the up-regulation of gene transcripts coding for chitinases and their precursors. These results indicated that under all treatments Chevron utilized chitinase as a form of plant protection; however under the *Tri5*+ and DON treatments in Stander, chitinases were not elicited and may have resulted in less plant protection against invading pathogens. An mRNA precursor of chitinase 2b was up-regulated in Chevron under the DON treatment and in both lines under the *Tri5*-(DON) treatment. The *cht2b* gene was discovered in the barley cultivar Pallas, inoculated with powdery mildew (Genbank accession # X78672) and an expression profile of ESTs of highly homologous barley chitinase II (*cht2*) genes indicated high expression in leaf, spike, and stem, moderate expression in the sheath, and no expression in the root seed and flower (http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Hv&CID=173). Protein profile expression analysis at 3 dpi showed higher abundance of a chitinase 2b protein in Stander *Tri5*- and *Tri5*-(DON). The 2-DE studies described in Chapter 3

showed induction of the chitinase 2b protein in the barley line CI4196 at 3 dpi with a trichothecene producing *F. graminearum* strain. The up-regulation of gene transcripts coding for chitinases in both Chevron and Stander relative to induction timing implied that plant resistance from this PR-gene was not differentiated prior to 24 hpi; moreover, as Stander did not up-regulate chitinases under the *Tri5+* and DON treatments it is likely that a more complex interaction is responsible for the observed defense responses.

In monocots, transcripts of TLPs have accumulated following exogenous applications with JA and SA (Molina and Garcia-Olmedo, 1993a; Schweizer *et al.,* 1997; Wasternack and Hause, 2002; Lu *et al.,* 2006). A possible mode of defense associated with TLPs included their direct inhibitory activity against the hyphae and/or germinating spores of the pathogen; they have been shown to accumulate in response to fungal attack (Uknes *et al.,* 1992). Gene transcripts coding for TLPs were up-regulated in Chevron at 8 hpi and in Stander at 24 hpi; an increase in abundance of one TLP was also observed in the proteome of Chevron following treatment with DON, whereas Stander showed no changes. The transcriptomal results were consistent with the accumulation of TLPs following pathogen attack; however, no up-regulation of gene transcripts coding for TLP was observed in either line following *Tri5+* treatment, indicating that *Tri5*-(DON) and *Tri5+* did not elicit equivalent responses and showed differences between the interactions observed following both treatments. It is also possible that the timing of induction of specific TLPs and the period of increased abundance, as observed in Chevron at 3 dpi may have influenced the plant defense responses.

Gene transcripts coding for pathogenesis-related proteins 6 and 7, proteinase inhibitors and proteinases, respectively, showed up- and down-regulation in both barley

lines during the various treatments. Proteinase inhibitors (PIs) comprise one of the most abundant classes of proteins in plants; their functions include anti-metabolic activity and they are responsible for controlling proteolysis within the cells (Garcia-Olmedo *et al.,* 1987). Gene transcripts coding for PIs were not induced in either line following treatment with *Tri5*+ or *Tri5*-(DON); indicating that such interactions between the fungus and the mycotoxin interfered with the induction of PR-6 genes. The PIs may be responsible for limiting plant resources available to the fungus following infection; the up-regulation of gene transcripts coding for various proteinases from microarray and iTRAQ data demonstrated the ability of the plant to maintain cellular productivity despite the presence of pathogen attack.

The up-regulation of gene transcripts coding for PR-9 proteins and their precursors in both Chevron and Stander following all treatments was indicative of an oxidative burst, a well known plant defense response. The plasma membrane of plant cells produces reactive oxygen species, H_2O_2 and O_2-, in response to both biotic and abiotic stimuli that play an important role in plant-pathogen interactions (Foyer and Mullineaux, 1994a; Levine *et al.,* 1994). Pathogen-induced production of oxygen free radicals by the plant has several effects: a) it hinders penetration of the pathogen by stimulating peroxidase activity and by cross-linking cell walls at the site of contact; b) it creates a stress on the pathogen as well as the host cell generating the oxidative burst; and c) it acts as a diffusible signal that leads to SAR (Noctor and Foyer, 1998). High intracellular levels of H_2O_2 cause the activation of plant cell death and defense mechanisms during pathogen invasion (Takahashi *et al.,* 1997). Chevron showed up-regulation of several gene transcripts coding for peroxidases at 8 hpi, while Stander

showed their up-regulation only at 24 hpi. It is conceivable that the difference in transcript induction timing was responsible for differentiating the levels of resistance between the two barley lines. RNA microarray profiling suggested that Chevron relied on an oxidative burst to defend against the invading pathogen earlier than Stander, possibly resulting in slower progress of the fungus and subsequently, less damage caused to the plant. H_2O_2 has been associated with degradation of DON (Miller *et al.,* 1983); and an early oxidative stress response could lead to a reduction in trichothecene abundance and a possible reduction in fungal virulence. It appears that in both Chevron and Stander, gene transcripts coding for peroxidases were only down-regulated following the *Tri5+* treatments; perhaps the interaction between the fungus and the trichothecene was responsible for inducing a less efficient response by the plant to protect itself from fungal invasion, or the observed decrease in regulation could be due to an increase in the release of reactive oxygen species and the subsequent reduction in plant defense to oxidative stress.

PR-10 proteins are classified as ribonuclease-like; they have been characterized to be small acidic intracellular proteins resistant to proteases (Awade *et al.,* 1991; Warner *et al.,* 1994). Studies in various plants have indicated that PR-10 proteins could play important roles in both plant defense and normal development, although their precise biological functions are not yet known (Crowell *et al.,* 1992; Barratt and Clark, 1993; Sikorski *et al.,* 1999). Chevron and Stander both showed up-regulation of gene transcripts coding for PR-10 proteins, suggesting they play a role in general plant defense, but they may not be responsible for differentiating resistance.

Defensins (PR-12 proteins) are low-molecular weight proteins occurring in seeds, stems, roots, and leaves in a number of plant species that are toxic to bacteria, fungi, and yeast *in vitro*, and they have also been shown to cause permeabilization of fungal membranes, leading to the inhibition of fungal growth (Commue *et al.,* 1992; Florack and Stiekema, 1994; Broekaert *et al.,* 1995; Thevissen *et al.,* 1999). Only Stander *Tri5*- showed up-regulation of a gene transcript coding for a defensin precursor at 8 hpi, but no change in regulation of gene transcripts coding for the defensin peptides; indicating that the fungus alone was sufficient to induce such a response and although induction of the precursor was rapid there was no further defense provided to the plant. In Chevron, a gene transcripts coding for a plant defensin protein was up-regulated in both *Tri5*- and DON at 8 hpi; implying that again the fungus alone was sufficient to induce such a response and that the rapid induction of a PR-12 protein contributed to efficient plant defense. It is interesting that a gene transcript coding for a defensin protein was up-regulated following DON treatment when no fungus was present; perhaps the presence of the pure trichothecene is capable of overwhelming or deceiving the plant into triggering an inappropriate defense response.

PR-13 proteins, thionins and their precursors, are known to have antifungal activity and have been studied extensively in barley. Thionin transcripts have been shown to accumulate with the onset of acquired resistance, and with SA or JA treatments (Wasternack *et al.,* 1994; Kogel *et al.,* 1995). Thionins were induced under all treatments supporting their antifungal roles and suggesting that the differences in resistance between the barley lines may again be correlated with the timing of transcript induction. Additionally, Chevron induced gene transcripts associated with thionin activity in a DON

treated sample when no fungus was present, suggesting that perhaps generic acquired resistance, even without the presence of fungus, was capable of providing sufficient plant defense responses.

Lipid transfer proteins (LTPs) make up class 14 of the pathogenesis-related proteins. PR-14 proteins are known to exhibit antifungal and antibacterial activity, exerting their effect at the level of the plasma membrane of the target microorganism (Garcia-Olmedo *et al.,* 1995). Studies have suggested that nonspecific lipid transfer proteins (nsLTPs) might complement thionins and other defense proteins to form a general barrier against pathogens; they may also combine with thionins for an additive effect (Molina and Garcia-Olmedo, 1993b; Garcia-Olmedo *et al.,* 1995). In Chevron treatments *Tri5*- and DON, gene transcripts coding for nsLTP precursors and an LTP protein were up-regulated at 8 hpi, indicating a highly efficient activation and utilization of plant defense responses; however, the interaction between the fungus and the trichothecene under *Tri5*+ and *Tri5*-(DON) treatments showed a possible inhibition in the ability to utilize PR-14 proteins for defense response. Stander showed the up-regulation of only two gene transcripts coding for PR-14 precursors at 24 hpi; indicating that the inability to fully utilize the defense mechanisms available and the delayed transcript induction may have contributed to its susceptibility.

4.4.3 Plant defense response gene transcripts and proteins

The RNA Microarray profiling and iTRAQ experiments identified 28 differentially regulated gene transcripts and differentially expressed proteins, respectively, to be associated with DON defense. Recently, an UDP-glucosyltransferase was identified in

Arabidopsis thaliana; the enzyme catalyzes the transfer of glucose from UDP-glucose to the 3'OH group of DON, thus inactivating the toxin (Poppenberger *et al.,* 2003). The up-regulation of seven gene transcripts coding for glucosyltransferases observed in Stander suggested that this barley line may allocate a greater quantity of resources for DON detoxification; however the majority of the gene transcripts associated with detoxification were not up-regulated until 24 hpi. The difference in FHB symptoms between Chevron and Stander, and the up-regulation of gene transcripts associated with DON detoxification in Stander could represent a negative correlation between FHB phenotypic symptoms and DON content; such findings have been reported in wheat (Snijders and Perkowski, 1990).

The RNA Microarray profiling and iTRAQ experiments identified 99 differentially regulated gene transcripts and differentially expressed proteins, respectively, to be associated with general plant defense responses. Twenty-seven of these gene transcripts and proteins were associated with cell wall structure and were possibly linked to the strengthening of the cell walls in response to pathogen attack. Chevron and Stander showed up-regulation of gene transcripts coding for pectin-associated proteins; pectin is a major component of the primary cell walls of monocots and dicots and during pathogenesis cell walls act as the first line of defense against pathogens attempting to colonize the plant tissue and obtain nutritional requirements (Gomathi and Gnanamanickam, 2004). Other gene transcripts associated with cell-wall strengthening and plant protection were up-regulated in both barley lines and coded for glycine-rich, proline-rich, and hydroxyproline-rich proteins, as well as extension-like proteins. These results indicated that Chevron and Stander relied on the activation of gene

transcripts associated with cell-wall strengthening for protection against pathogen attack and for lignin biosynthesis; however Chevron showed a uniform response regardless of the treatment, and induction was often at 8 hpi as opposed to 24 hpi in Stander. Other gene transcripts coding for enzymes identified and associated with plant cell walls and defense included β-1,4-glucanases and xylanase inhibitors. Gene transcripts coding for β-1,4-glucanase inhibitors were up-regulated in Stander, most likely in response to fungal invasion; and the up-regulation of gene transcripts coding for xylanase inhibitors indicated the protection of the plant cell wall from degradation enzymes produced by the fungus.

There were a total of 21 up-regulated gene transcripts coding for glutathione transferases following the inoculation treatments. Chevron showed up-regulation of gene transcripts coding for glutathione-S-transferases (GST) in all treatments except under *Tri5+* inoculations; the response in Stander was much broader and occurred under all treatments. Basic research has shown that overall; glutathione appears to play a key role in the protection against oxidative damage arising from a number of stresses, including exposure to a pathogen (Chai and Doke, 1987). The results indicated that gene transcripts coding for GSTs were used in general plant defense, but their regulation did not necessarily differentiate between barley lines and among the treatments.

Other general plant defenses included the up-regulation of gene transcripts coding for pathogen-induced WIR1 proteins, Bowman-birk inhibitor proteins, and various alcohol dehydrogenases. Both lines showed similar regulation of the gene transcripts coding for WIR1 and Bowman-birk proteins, but Chevron did not show any changes in regulation of gene transcripts coding for alcohol dehydrogenases. Protein profile

expression analysis identified the up-regulation of 14-3-3 proteins in both Chevron and Stander; these proteins have been studied in wheat and it was suggested that in response to *F. graminearum* infection the 14-3-3 proteins may be involved in defense response and they may be related to initial infection and mycotoxin accumulation in wheat kernels (Zhou *et al.,* 2005). It is likely that these proteins were altered in response to pathogen attack, but that they did not contribute directly to the observed oxidative burst from the plant and did not account for the observed differences in resistance between the lines. Table 4.4.1 shows a summary of the induction timing of defense-related gene transcripts (8 and 24 hpi) and proteins (3 dpi) following point inoculation with the *Tri5*-, *Tri5*+, DON, and *Tri5*-(DON) treatments in Chevron (red) and in Stander (black). Figure 4.4.4 shows a Venn diagram summarizing the up-regulation of gene transcripts in Chevron and Stander following point inoculation with the *Tri5*- treatment. Figure 4.4.5 shows a Venn diagram summarizing the up-regulation of gene transcripts in Chevron and Stander following point inoculation with the *Tri5*+ treatment. Figure 4.4.6 shows a Venn diagram summarizing the up-regulation of gene transcripts in Chevron and Stander following point inoculation with the DON treatment. Figure 4.4.7 shows a Venn diagram summarizing the up-regulation of gene transcripts in Chevron and Stander following point inoculation with the *Tri5*-(DON) treatment.

Table 4.4.1 Summary of the induction timing of defense-related gene transcripts (8 and 24 hpi) and proteins (3 dpi) following point inoculation with the *Tri5*-, *Tri5*+, DON, and *Tri5*-(DON) treatments in Chevron (red) and in Stander (black).

Protein Identification	8 hpi	24 hpi	72 hpi
PAL			
Chalcone synthase			
12-oxophytodienoic acid reductase			
Methyljasmonate-inducible			
Jasmonic acid inducbile			
1-aminocyclopropane oxidase			
PR-2 precursor			
Chitinase			
Chitinase 2b			
TLP8			
Peroxidase precursor			
Peroxidase			
nsLTP precursor			
Pectin-associated			
Proline-rich			
Xylanase inhibitor			

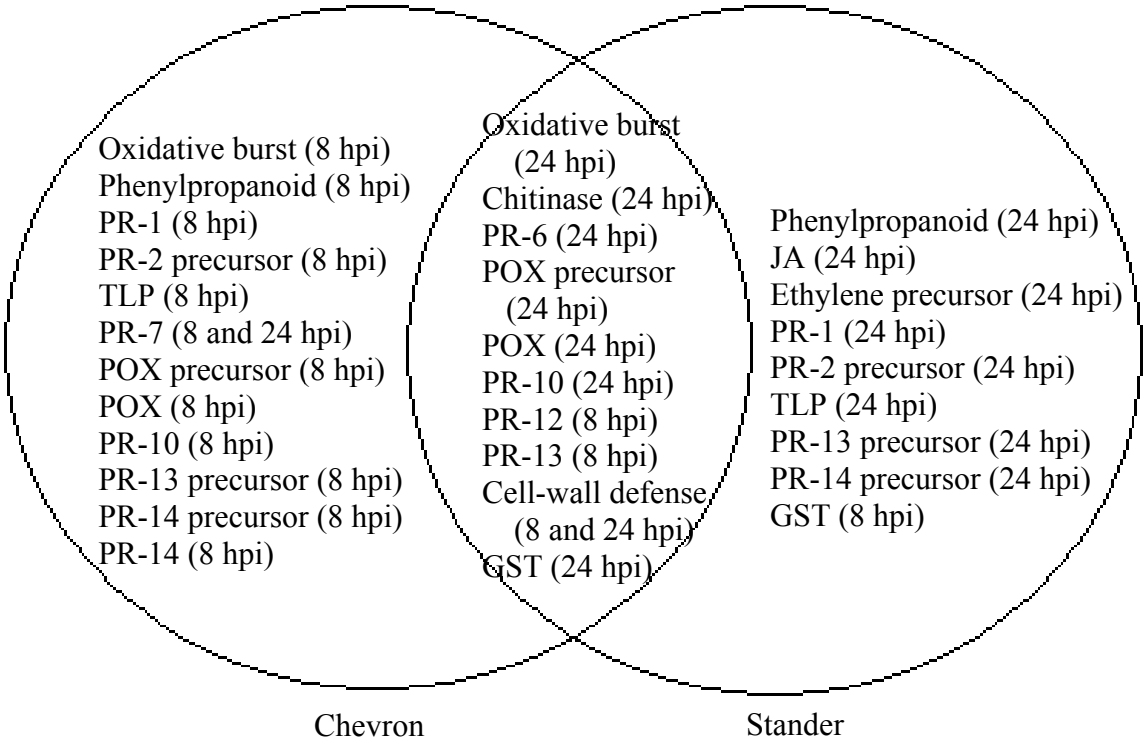

Figure 4.4.4 Venn diagram summarizing the up-regulation of gene transcripts in Chevron and Stander following point inoculation with the *Tri5*- treatment. Evaluating the effect of the fungus on the plant; all gene transcripts in Chevron were induced at 8 hpi, whereas the majority of gene transcripts in Stander were not induced until 24 hpi.

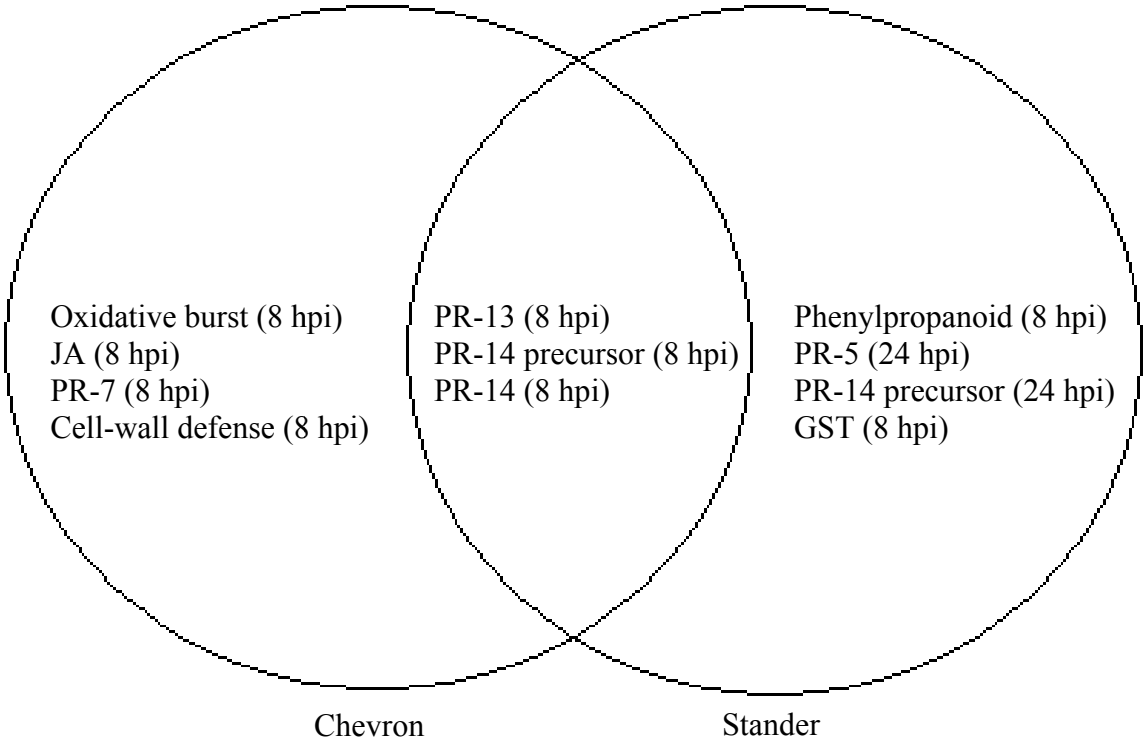

Figure 4.4.5 Venn diagram summarizing the up-regulation of gene transcripts in Chevron and Stander following point inoculation with the *Tri5+* treatment. Evaluating the interaction between the fungus and the mycotoxin on the plant; there were few changes in transcript accumulation following this treatment, indicating that the interaction does not elicit a defense response from the plant within the first 24 hpi.

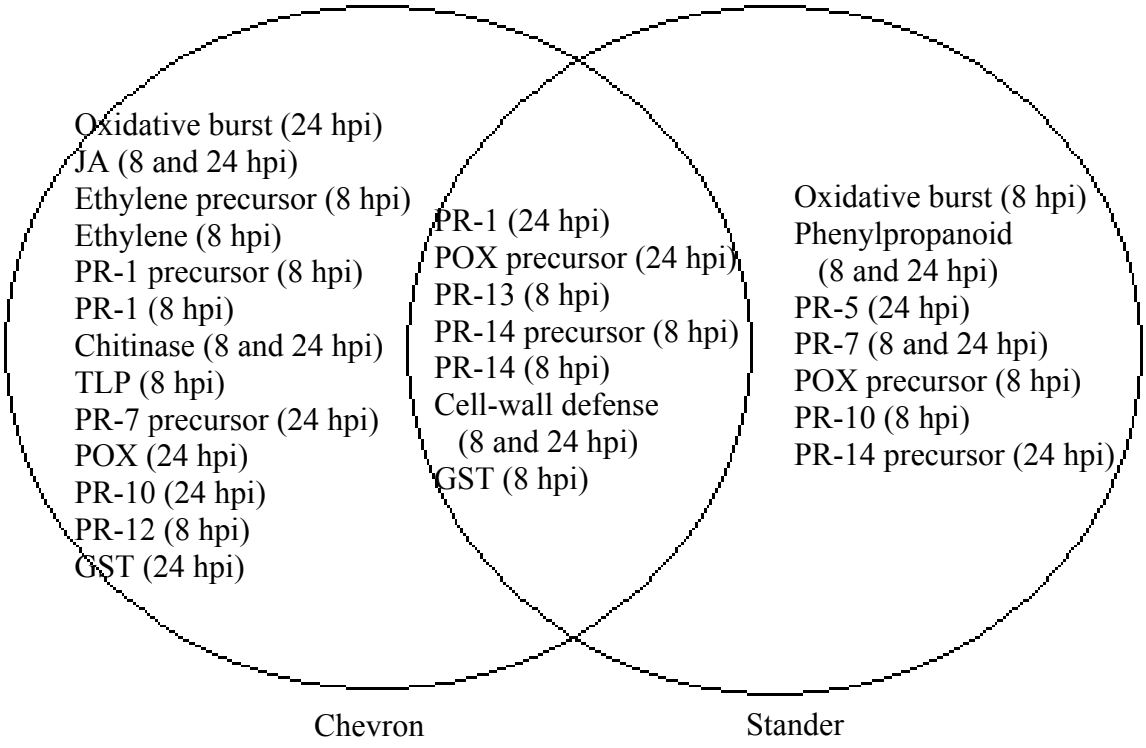

Figure 4.4.6 Venn diagram summarizing the up-regulation of gene transcripts in Chevron and Stander following point inoculation with the DON treatment. Evaluating the effect of the mycotoxin on the plant; the mycotoxin elicits a delayed defense response in Chevron and Stander possibly by deceiving the plant into inducing a less efficient mode of defense.

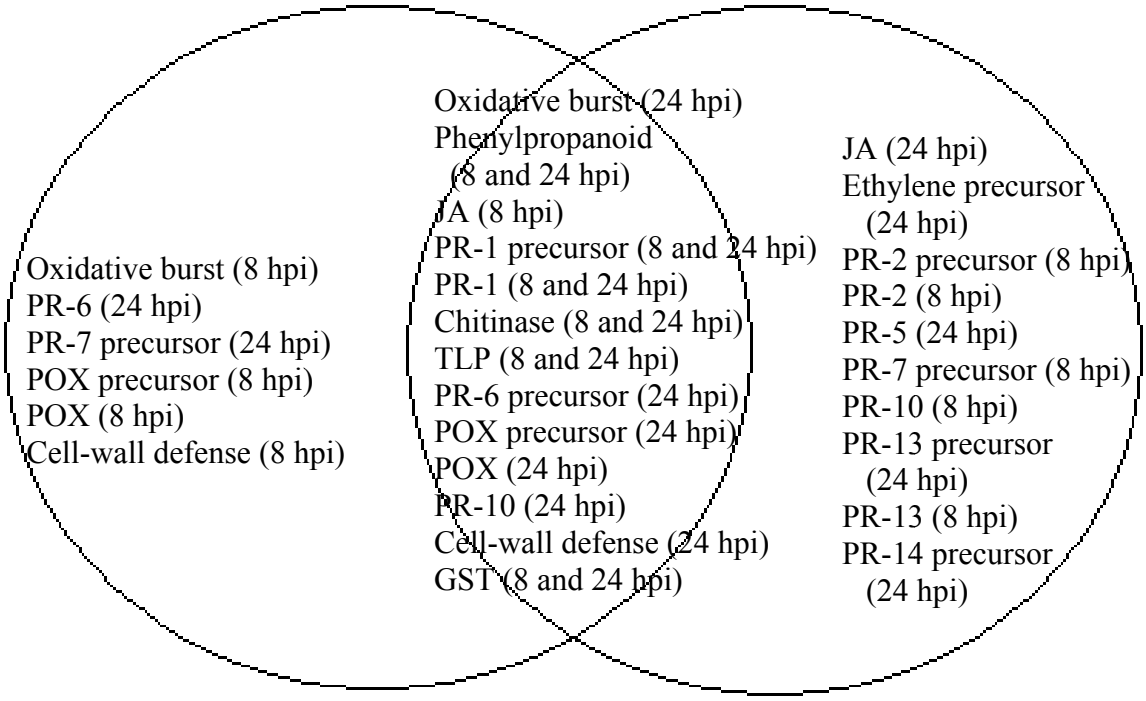

Figure 4.4.7 Venn diagram summarizing the up-regulation of gene transcripts in Chevron and Stander following point inoculation with the *Tri5*-(DON) treatment. Evaluating the interaction between the fungus and the mycotoxin on the plant; peroxidases and an oxidative burst were induced in Chevron at 8 hpi and continued to 24 hpi, whereas Stander showed induction only at 24 hpi, indicating a rapid and continuous defense response in the resistant barley line Chevron.

4.5 Conclusion

To the best of my knowledge, this is the first transcriptomal and proteomic study applied to trichothecene producing and non-producing strains, and DON inoculated barley lines. The RNA microarray and proteomic investigation of FHB resistance in Chevron and Stander, genetically unrelated barley lines, revealed a complex cellular network in the barley cells in response to the fungus, the mycotoxin, and the subsequent interaction between them. Many gene transcripts and proteins identified were associated with an oxidative burst and defense signaling pathways, were pathogenesis-related, and were associated with general defense response. Significant differences observed between the lines included the absence of activation of the phenylpropanoid pathway in Chevron, whereas the susceptible line Stander showed up-regulation of gene transcripts in this pathway; and the apparent up-regulation of the ethylene defense pathway in Chevron which was down-regulated in Stander. Both Chevron and Stander appeared to up-regulate gene transcripts associated with the jasmonic acid pathway; and showed up-regulation of many gene transcripts coding for PR-proteins, but differed in their responses to specific treatments and their induction timing. Specifically, the PR-1 mRNA was more abundant and induced earlier in Chevron than in Stander; gene transcripts coding for chitinases were reported to be more abundant under DON treatments in Chevron and down-regulated in Stander under the interaction of the fungus and the mycotoxin. TLP mRNA was only altered in Stander and did not appear to be induced following DON treatment in either line; mRNA of peroxidases and their precursors, PR-10, PR-13, and LTPs were activated earlier in Chevron than in Stander, supporting the significance of induction timing relative to resistance. Differences in FHB resistance can most likely be attributed

to the presence of an alternative, more efficient mode of defense response in Chevron, determining its resistance to FHB. It could also be attributed to the activation of a less efficient pathway in Stander; which was capable of draining and dispersing energy to various metabolic responses and delaying the induction of the JA/ethylene defense pathways which were observed in Chevron as early as 8 hpi. Further RNA microarray profiling of more time points would assist in completing the picture of pathway induction following infection; and broadening the scope of this experiment to include barley lines with intermediate levels of resistance, or near isogenic lines which may answer questions regarding the significance of gene transcripts and proteins identified in both Chevron and Stander.

CONCLUSION

In chapter 2, nineteen barley lines with varying levels of FHB-resistance were evaluated using three artificial inoculation methods; indoor point inoculation, indoor spray inoculation, and nursery inoculation. From the results of this study, I concluded that indoor spray inoculation was the most reproducible inoculation method and provided the greatest discrimination of resistance based on FHB phenotypic symptoms. Additionally, seven barley lines reported consistent levels of resistance across all three methods of evaluation: Harbin, Island, CI4196, Chevron, Zhedar #1, TR04283, and Svansota; and one barley line, Stander, reported consistent levels of susceptibility. The results also indicated that a reproducible and reliable measurement of disease assessment should be based on FHB phenotypic symptoms supported by measured DON concentrations.

In chapter 3, six barley lines were selected to generate 2-DE protein expression profiles, based on their observed resistance in Chapter 2. The results indicated that the six barley lines relied on an oxidative burst and the induction of oxidative stress as a defense response, but differences in response magnitude and the identification of altered proteins were evident. Resistant lines displayed responses typical of an incompatibility interaction between a pathogen and the host plant, whereas the susceptible line demonstrated a compatible interaction by inducing a smaller oxidative burst and delaying PR-protein induction. The findings also suggested that TLP7 may be more efficient than TLP4 against *F. graminearum* mycelia, a *cht2b* protein may be central to FHB-resistance in CI4196, and that Chevron may rely exclusively on the JA pathway or an alternative mode

for defense against *F. graminearum* invasion; signifying that plant defense responses following fungal infection are diverse among resistant and susceptible barley lines.

In chapter 4, RNA microarray profiling and iTRAQ technology were used to distinguish plant defense responses between two barley lines and to account for a variation in pathway induction as a result of pathogen attack using five treatments. The results showed that Chevron relied on the early induction of gene transcripts coding for PR- and defense-related proteins and the activation of the JA and ethylene pathways for defense, whereas Stander showed delayed induction of gene transcripts coding for PR- and defense-related proteins and activated the phenylpropanoid and JA pathways for defense. The results also showed gene transcript and protein differences among the treatments; gene transcripts coding for chitinases were reported to be up-regulated under DON treatments in Chevron, and gene transcripts coding for TLPs were differentially regulated in all Stander treatments except DON. The differences in FHB resistance between these lines may be due to a more efficient defense response in Chevron compared to the defense response observed in Stander.

The research presented in this thesis examined FHB resistance in barley. A reproducible, efficient, and effective method of screening for FHB-resistance was recommended, and resistance among the 19 barley lines was differentiated. The induction of an oxidative burst response and oxidative stress was shown to occur in barley lines following fungal invasion, and differences in the identification of differentially expressed proteins discriminated resistance among the lines. Finally, the effects on barley lines of the fungus, the mycotoxin, and their subsequent interactions were examined using RNA microarray profiling and iTRAQ technology. These studies identified pathways and

mechanisms associated with resistance in Chevron which were not observed, or showed a delayed response in Stander; further discriminating resistance between these barley lines. The results make it clear that molecular and biochemical differences exist among barley lines tested for resistance to FHB. In the future, continued investigations using RNA microarray profiling and quantitative real-time PCR will enhance the overall significance of the results reported in this thesis and greatly contribute to the understanding of mechanisms associated with FHB-resistance in barley.

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APPENDIX 1

Identification of differentially expressed proteins showing a minimum two-fold change in abundance in response to *Fusarium graminearum* infection in six barley lines associated with oxidative burst and defense response, pathogenesis-related proteins, and proteins associated with metabolism, regulation and unknown functions.

Accession #	Barley Cultivar	Fold Change[1]	Protein Identification	Species	Pred./Exp. M_w (kDa)/pI*
Oxidative burst and Defense response					
gi|18479038	CI4196	-8.1	Glutathione transferase	*H. vulgare*	24.9/5.8 39.0/6.4
gi|29368238	Harbin	-6.5	Gamma hydroxybutyrate dehydrogenase-like	*O. sativa*	48.4/9.8 42.1/5.9
gi|23504745	Stander	-6.4	Glutathione transferase F5	*T. aestivum*	23.4/5.8 28.0/6.0
gi|400094	Stander	-6.4	Jasmonate-induced protein 1	*H. vulgare*	22.9/5.9 32.0/6.7
gi|20302471	Svansota	-5.8	Ferredoxin-NADP(H) oxidoreductase	*T. aestivum*	39.2/8.3 47.2/5.9
gi|22587	Chevron	-4.9	Peroxidase	*H. vulgare*	33.4/6.1 42.0/6.1
gi|6939839	Chevron	-4.9	GSH-dependent dehydroascorbate reductase 1	*O. sativa*	23.7/5.7 34.5/5.7
gi|32879781	Harbin	-3.7	Stromal ascorbate peroxidase	*O. sativa*	38.5/8.8 37.5/8.0
gi|37928995	CDC Bold	-3.2	Cytosolic malate dehydrogenase	*T. aestivum*	24.6/6.6 42.0/5.3
TC262714	Harbin	-3.2	Cytosolic superoxide dismutase (Cu-Zn) 4	*Z. mays*	37.4/7.2 23.0/6.5
gi|3688398	Harbin	-2.9	Ascorbate peroxidase	*H. vulgare*	27.4/5.6 39.0/6.0
gi|37928995	Harbin	-2.8	Cytosolic malate dehydrogenase	*T. aestivum*	24.6/6.6 42.0/5.3
gi|53791695	Harbin	-2.7	Universal stress protein-like	*O. sativa*	49.0/7.5 51.0/5.0

Accession #	Barley Cultivar	Fold Change[1]	Protein Identification	Species	Pred./Exp. M_w (kDa)/pI
Oxidative burst and Defense response					
gi|18202485	Stander	-2.5	Cytoplasmic malate dehydrogenase	*Z. mays*	35.9/5.8 52.0/5.9
gi|15808779	Harbin	-2.3	Ascorbate peroxidase	*H. vulgare*	28.0/5.1 38.0/6.0
gi|34911788	Stander	-2.3	Putative malate dehydrogenase	*O. sativa*	35.4/8.7 48.0/5.8
gi|400094	Harbin	-2.3	Jasmonate-induced protein 1	*H. vulgare*	22.9/5.9 32.0/6.7
gi|15808779	Svansota	-2.2	Ascorbate peroxidase	*H. vulgare*	28.0/5.1 38.0/6.0
gi|37928995	Svansota	-2.2	Cytosolic malate dehydrogenase	*T. aestivum*	24.6/6.6 42.0/5.3
gi|34911788	Harbin	-2.2	Putative malate dehydrogenase	*O. sativa*	35.4/8.7 48.0/5.8
gi|34911788	Svansota	-2.2	Putative malate dehydrogenase	*O. sativa*	35.4/8.7 48.0/5.8
gi|6939839	Svansota	-2.0	GSH-dependent dehydroascorbate reductase 1	*O. sativa*	23.7/5.7 34.5/5.7
gi|400094	Chevron	2.0	Jasmonate-induced protein 1	*H. vulgare*	22.9/5.9 32.0/6.7
gi|53791695	CI4196	2.0	Universal stress protein-like	*O. sativa*	49.0/7.5 51.0/5.0
gi|37928995	CI4196	2.3	Cytosolic malate dehydrogenase	*T. aestivum*	24.6/6.6 42.0/5.3
gi|15808779	CDC Bold	2.4	Ascorbate peroxidase	*H. vulgare*	28.0/5.1 38.0/6.0
gi|400094	CI4196	2.4	Jasmonate-induced protein 1	*H. vulgare*	22.9/5.9 32.0/6.7
gi|53791695	CDC Bold	2.7	Universal stress protein-like	*O. sativa*	49.0/7.5 51.0/5.0
gi|3688398	CDC Bold	2.9	Ascorbate peroxidase	*H. vulgare*	27.4/5.9 37.0/4.7
gi|37928995	Chevron	2.9	Cytosolic malate dehydrogenase	*T. aestivum*	24.6/6.6 42.0/5.3
gi|15808779	Stander	3.0	Ascorbate peroxidase	*H. vulgare*	28.0/5.1 38.0/6.0
gi|22587	CDC Bold	3.1	Peroxidase	*H. vulgare*	33.4/6.1 42.0/6.1

Accession #	Barley Cultivar	Fold Change[1]	Protein Identification	Species	Pred./Exp. M_w (kDa)/pI
Oxidative burst and Defense response					
gi|34911788	CDC Bold	3.7	Putative malate dehydrogenase	*O. sativa*	35.4/8.7 48.0/5.8
gi|6939839	CI4196	4.7	GSH-dependent dehydroascorbate reductase 1	*O. sativa*	23.7/5.7 34.5/5.7
gi|3688398	CI4196	6.7	Ascorbate peroxidase	*H. vulgare*	27.4/5.9 37.0/4.7
gi|32879781	CDC Bold	8.7	Stromal ascorbate peroxidase	*O. sativa*	38.5/8.8 37.5/8.0
gi|34911788	CI4196	13.4	Putative malate dehydrogenase	*O. sativa*	35.4/8.7 48.0/5.8
Q5S1S6	CDC Bold	14.2	Peroxiredoxin Q	*T. aestivum*	23.7/9.3 23.0/7.0
gi|37928995	Stander	14.8	Cytosolic malate dehydrogenase	*T. aestivum*	24.6/6.6 42.0/5.3
gi|28192421	CI4196	18.2	Dehydroascorbate reductase	*T. aestivum*	23.5/5.9 35.1/5.7
gi|18202485	CDC Bold	19.7	Cytoplasmic malate dehydrogenase	*Z. mays*	35.9/5.8 52.0/5.9
gi|17949	CI4196	22.8	NADPH: isoflavone oxidoreductase	*C. arietinum*	35.6/5.9 44.0/5.2
Pathogenesis-Related					
Q94649	Stander	-16.2	Thaumatin-like protein TLP7	*H. vulgare*	24.5/7.4 34.0/7.0
gi|75103125	Harbin	0	Thaumatin-like protein TLP3	*H. vulgare*	18.6/5.0 16.0/4.8
gi|56090131	Stander	2.3	Thaumatin-like protein TLP4	*H. vulgare*	18.4/5.7 19.0/6.0
Q94649	Svansota	2.5	Thaumatin-like protein TLP7	*H. vulgare*	24.5/7.4 34.0/7.0
gi|56090131	CDC Bold	7.3	Thaumatin-like protein TLP4	*H. vulgare*	18.4/5.7 19.0/6.0
gi|563489	CI4196	33.3	Chitinase	*H. vulgare*	26.6/6.1 34.0/5.0
Metabolism, Regulation, and Unknown					
gi|32394644	Harbin	-10.5	Rieske Fe-S protein	*T. aestivum*	42.4/9.4 28.0/6.4
gi|7716458	CI4196	-8.1	Prohibitin	*Z. mays*	45.2/7.2 44.0/6.1

Accession #	Barley Cultivar	Fold Change[1]	Protein Identification	Species	Pred./Exp. M_w (kDa)/pI
Metabolism, Regulation, and Unknown					
gi|21741225	Harbin	-7.7	Bet v I allergen	*O. sativa*	32.9/6.8 31.0/4.4
gi|2507469	CDC Bold	-7.4	Triose phosphate isomerase, cytosolic	*H. vulgare*	26.9/5.4 29.0/5.4
gi|21844	Harbin	-6.4	Oxygen-evolving enhancer protein 1, chloroplast precursor	*T. aestivum*	47.8/6.1 45.0/5.0
gi|1229138	Harbin	-4.8	Low temperature-responsive RNA-binding	*H. vulgare*	15.9/5.5 17.0/5.3
gi|38174807	Harbin	-4.3	Putative immunophilin	*H. vulgare*	16.2/5.4 23.0/5.4
gi|28416583	Harbin	-3.9	Ubiquitin-like protein	*A. thaliana*	33.6/9.5 16.2/5.2
gi|62732953	Harbin	-3.3	Chloroplast fructose-bisphosphate aldolase precursor	*O. sativa*	58.0/9.3 54.0/5.7
gi|21741225	Harbin	-3.2	Bet v I allergen	*O. sativa*	32.9/6.8 31.0/4.4
TC262728	Harbin	-3.2	ATP synthase delta chain, mitochondrial precursor	*O. sativa*	36.1/9.9 35.0/5.7
gi|7716458	Harbin	-3.1	Prohibitin	*Z. mays*	45.2/7.2 44.0/6.1
gi|20790	Chevron	-3.0	Type 1 chlorophyll a/b-binding protein	*P. sylvestris*	22.9/5.9 25.0/5.0
gi|52076093	Harbin	-3.0	Emb|CAB80929.1	*O. sativa*	19.3/10.3 48.0/4.7
gi|31433363	Harbin	-2.8	Cellulose	*O. sativa*	41.6/9.0 45.0/6.3
gi|7716458	Harbin	-2.8	Prohibitin	*Z. mays*	45.2/7.2 44.0/6.1
gi|100796	Harbin	-2.7	Phosphoribulokinase	*T. aestivum*	45.5/5.7 52.0/4.9
gi|7716458	Stander	-2.7	Prohibitin	*Z. mays*	45.2/7.2 44.0/6.1
gi|47607439	Stander	-2.6	Mitochondrial ATP synthase	*T. aestivum*	44.7/8.8 40.0/5.5
gi|51091339	Harbin	-2.6	Chloroplast chaperonin 21	*O. sativa*	36.7/8.8 36.0/4.8

Accession #	Barley Cultivar	Fold Change[1]	Protein Identification	Species	Pred./Exp. M_w (kDa)/pI
Metabolism, Regulation, and Unknown					
gi|55233175	Stander	-2.6	ß-cyanoalanine synthase	*O. sativa*	49.5/9.3 53.0/6.1
gi|62732953	Harbin	-2.6	Chloroplast fructose-bisphosphate aldolase precursor	*O. sativa*	58.0/9.3 54.0/6.1
gi|9652119	Harbin	-2.5	Nucleoside diphosphate kinase	*L. perenne*	16.5/6.3 18.1/6.5
gi|57222134	Harbin	-2.4	Atranbp1b protein (Ran-binding protein 1 homolog b)	*A. thaliana*	45.6/5.4 44.0/4.3
gi|7716458	Harbin	-2.4	Prohibitin	*Z. mays*	45.2/7.2 44.0/6.1
gi|46805452	Harbin	-2.3	Chloroplast inorganic pyrophosphatase	*O. sativa*	42.4/6.6 42.0/4.7
gi|55233175	Harbin	-2.2	ß-cyanoalanine synthase	*O. sativa*	49.5/9.3 53.0/6.1
gi|62732953	Stander	-2.2	Chloroplast fructose-bisphosphate aldolase precursor	*O. sativa*	58.0/9.3 54.0/6.1
gi|28950670	Harbin	-2.1	Legumin-like protein	*Z. mays*	52.7/7.2 52.0/5.9
gi|32352148	CI4196	-2.0	Putative mitochondrial ATP synthase (hypothetical)	*O. sativa*	37.6/9.0 33.1/6.5
gi|4038691	Harbin	-2.0	RUBISCO small subunit	*H. vulgare*	18.6/8.8 18.0/4.7
TC246751	Harbin	-2.0	Profilin-1	*H. vulgare*	44.4/9.8 15.0/4.7
gi|11360993	Chevron	0.0	Glycine dehydrogenase (decarboxylating)	*H. vulgare*	112.0/6.3 110.0/6.3
gi|131344	CDC Bold	0.0	Photosystem II oxygen-evolving complex protein 2 precursor	*T. aestivum*	27.4/8.8 28.0/5.8
gi|131344	CDC Bold	0.0	Photosystem II oxygen-evolving complex protein 2 precursor	*T. aestivum*	27.4/8.8 28.0/5.8

Accession #	Barley Cultivar	Fold Change[1]	Protein Identification	Species	Pred./Exp. M_w (kDa)/pI
Metabolism, Regulation, and Unknown					
gi|131344	Chevron	0.0	Photosystem II oxygen-evolving complex protein 2 precursor	*T. aestivum*	27.4/8.8 17.0/4.2
gi|3121849	Harbin	0.0	Calmodulin	*H. annuus*	16.8/4.1 22.0/4.1
gi|7488889	CDC Bold	0.0	Type III a membrane protein	*V. repens*	40.1/6.2 5.0/5.4
gi|75105223	Harbin	0.0	Ribosomal protein L18	*T. aestivum*	21.4/11.5 25.0/4.9
gi|75133690	CDC Bold	0.0	Putative reversibly glycosylated polypeptide	*O. sativa*	41.7/6.0 45.0/6.2
gi|75136098	Stander	0.0	Immunophilin/FKBP-type peptidyl-prolyl cis-trans isomerase	*O. sativa*	27.6/8.6 26.0/5.3
gi|7528175	Harbin	0.0	Histone H4 Fragment	*A. cepa*	6.6/11.5 6.0/7.0
gi|75308167	CDC Bold	0.0	Putative DNA-binding protein	*O. sativa*	18.7/5.8 23.0/6.4
gi|21741225	Stander	2.0	Bet v I allergen	*O. sativa*	32.9/6.8 31.0/4.4
gi|482311	Stander	2.0	Photosystem II oxygen-evolving complex 1	*O. sativa*	26.6/5.1 40.0/5.1
gi|167096	CDC Bold	2.2	RUBISCO activase isoform 1	*H. vulgare*	47.3/8.6 31.0/5.2
gi|167097	Chevron	2.4	RUBISCO activase A	*H. vulgare*	66.7/9.3 60.0/5.2
gi|167096	CI4196	2.7	RUBISCO activase isoform 1	*H. vulgare*	47.3/8.6 50.0/5.1
gi|28416583	CDC Bold	2.8	Ubiquitin-like protein	*A. thaliana*	33.6/9.5 15.2/5.2
gi|29124123	CI4196	2.9	Actin-depolymerizing factor 3	*O. sativa*	41.6/9.3 23.0/5.8
gi|7716458	CI4196	2.9	Prohibitin	*Z. mays*	45.2/7.2 44.0/6.1
gi|167096	CI4196	3.1	RUBISCO activase isoform 1	*H. vulgare*	47.3/8.6 50.0/5.1

Accession #	Barley Cultivar	Fold Change[1]	Protein Identification	Species	Pred./Exp. M_w (kDa)/pI
Metabolism, Regulation, and Unknown					
gi|46805895	CI4196	3.1	S-adenosylmethionine :2-demethylmenaquinone methyltransferase-like	*O. sativa*	30.5/5.5 25.0/5.4
gi|6683813	Svansota	3.1	UMP/CMP kinase b	*O. sativa*	23.3/5.4 34.0/5.3
gi|847873	CDC Bold	3.3	Mg-chelatase subunit	*H. vulgare*	36.5/4.9 48.0/4.8
gi|32394644	CDC Bold	3.4	Putative Rieske Fe-S precursor protein	*T. aestivum*	24.1/8.5 20.2/6.5
gi|18988	CDC Bold	3.6	Unnamed protein product	*H. vulgare*	47.4/5.1 49.0/5.0
gi|21844	CI4196	4.2	Oxygen-evolving enhancer protein 1, chloroplast precursor	*T. aestivum*	47.8/6.1 45.0/5.0
gi|482311	CI4196	4.7	Photosystem II oxygen-evolving complex 1	*O. sativa*	26.6/5.1 40.0/5.1
gi|11991	Svansota	5.0	ATPase, β-subunit	*H. vulgare*	54.0/5.5 54.0/5.0
gi|7716458	Stander	5.0	Prohibitin	*Z. mays*	45.2/7.2 44.0/6.1
gi|167096	Svansota	5.1	RUBISCO activase isoform 1	*H. vulgare*	51.4/8.0 54.0/5.2
gi|167097	CI4196	5.8	RUBISCO activase A	*H. vulgare*	66.7/9.3 56.0/4.9
gi|29124123	Harbin	6.2	Actin-depolymerizing factor 3	*O. sativa*	41.6/9.3 23.0/5.8
gi|38174807	CI4196	6.6	Putative immunophilin	*H. vulgare*	16.2/5.4 23.0/5.4
gi|20790	CDC Bold	6.7	Type 1 chlorophyll a/b-binding protein	*P. sylvestris*	22.9/5.9 25.0/5.0
gi|8926334	CI4196	6.7	Putative tyrosine phosphatase	*O. sativa*	27.4/6.7 41.0/5.3
gi|4099148	Svansota	7.0	YLP	*H. vulgare*	26.4/6.6 38.0/6.4
gi|167096	CI4196	7.6	RUBISCO activase isoform 1	*H. vulgare*	47.1/8.6 50.0/5.1
gi|1167948	Stander	8.7	RUBISCO activase isoform 1	*H. vulgare*	19.7/9.0 19.0/5.5

Accession #	Barley Cultivar	Fold Change[1]	Protein Identification	Species	Pred./Exp. M_w (kDa)/pI
Metabolism, Regulation, and Unknown					
gi|74049040	CI4196	9.1	Eukaryotic translation initiation factor 5A3	*T. aestivum*	17.6/5.8 28.0/5.8
gi|8926334	Chevron	9.1	Putative tyrosine phosphatase	*O. sativa*	27.4/6.7 41.0/5.3
gi|20790	CDC Bold	11.8	Type 1 chlorophyll a/b-binding protein	*P. sylvestris*	22.9/5.9 25.0/5.0
gi|15232963	CDC Bold	12.8	Unknown protein	*A. thaliana*	18.1/5.7 20.0/5.8
gi|482311	Chevron	14.7	Photosystem II oxygen-evolving complex 1	*O. sativa*	26.6/5.1 40.0/5.1

*Note: Pred. refers to the predicted (theoretical) molecular weights and isoelectric points of the proteins; Exp. refers to the experimental molecular weights and isoelectric points of the proteins.

[1]Fold change represents the change in abundance for proteins identified 3 dpi with *F. graminearum.* A minimum two-fold change was expressed by those proteins identified with LC-MS/MS. The values reported represent the fold changes of proteins following analysis with the Phoretix 2D Expression program.

APPENDIX 2

Identification of differentially transcribed genes associated with an oxidative burst, the phenylpropanoid and jasmonic acid antioxidant signaling pathways, the ethylene defense pathway, pathogenesis, deoxynivalenol defense, and general plant defense-related responses in Chevron following inoculation with *Tri5*- (strain GZT40), *Tri5*+ (strain GZ3639), DON, GZT40 supplemented with DON (*Tri5*-(DON)), and mock inoculum. The differential regulation values presented are log_2-transformed.

		Mock		***Tri5-***		***Tri5+***		**DON**		***Tri5-* (DON)**	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Oxidative burst											
Germin E	AF250937_s_at	-	-	-	-	-	-	-	-	-	-
Germin F	Contig1528_s_at	-	-	-	-	-	-	-	-	-	-
Germin-like 1	Contig2768_s_at	-1.3	-3.0	-	-	-	-	-1.2	-	-	-
Germin 4	Contig9172_at	-	-	-	-	-	-	-	-	-	-
Germin protein 4	Contig9172_s_at	-	-	-	-	-	-	-	-	-	1.6
Oxalate oxidase 2 precursor	Contig3018_at	-	-	-	1.3	-	-	-	1.6	-	2.3
Oxalate oxidase	Contig10847_at	-	-	-	-	-	-	-	-	-	-
Oxalate oxidase	Contig1518_at	-	-	-	-	-	-	-	-	1.0	-
Oxalate oxidase	Contig3017_at	1.5	-	-	3.5	-1.3	-	-	3.2	1.2	4.9
Oxalate oxidase	HVSMEi0004N08r2_at	-	4.2	2.1	-	1.4	-	-	-1.5	-	-
Oxalate oxidase	Contig17901_at	-	2.4	-	-	-	-	-	-	-	-
Oxalate oxidase 7	Contig10860_at	-	-	-	-	-	-	-	-	-	-
Oxalate oxidase-like	Contig3155_s_at	-	-	-	-	-	-	-	-	-	-
Oxalate oxidase-like	Contig3157_at	-	-	-	-	-	-	-	-	-	1.1

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Oxidative burst											
Aldehyde oxidase	Contig4920_s_at	-	-1.4	-	-	-	-	-	-	-	-
Alternative oxidase AOX3 precursor	Contig5887_at	-	-	-	-	-	-	2.3	-	1.3	-
Alternative oxidase	Contig15936_at	-	-	-	-	-	-	1.9	-	-	-
Alternative oxidase	Contig5888_at	-	-	-	-	-	-	1.6	-	-	-
Ascorbic acid oxidase	Contig14651_at	-	-	-	-	-1.0	-	-	-	-	-
L-ascorbate oxidase	Contig16758_at	-	-	-	-	-	-	-	-	-	-1.3
C-4 sterol methyl oxidase	Contig6208_at	-	-	-	-	-	-	-	-1.3	-1.2	-
Cytokinin oxidase	HV_CEa0008F11r2_at	-	-1.5	-	-	-	-	-	-	-	-
Cytokinin oxidase	Contig24300_at	1.1	-	-	-	-	-	-	-	-	-
Fatty acid alpha-oxidase	Contig15882_s_at	-	-	-	-	-	-	2.0	-	1.1	-
Flavin containing polyamine oxidase	Contig19426_at	-	-	-	-	-	-	-	-1.4	-	-1.6
Flavin containing polyamine oxidase	Contig3212_s_at	-	-2.1	-	-	-	-	-	-	-	-
NADPH oxidase	Contig8301_at	-	-1.0	-	-	-	-	-	-	-	-
Oxidase	Contig9320_at	-	-	-	-	-	-	-	-	-	-
Polyphenol oxidase	Contig17722_at	-	-	-	-	1.0	-	1.1	-	-	-
Respiratory burst oxidase	HVSMEn0015O15f_s_at	-	-1.5	-	-	-	-	-	-	-	-
Sarcosine oxidase	Contig17178_at	-	-	-	-	-	-	-	-	-	-
Phenylpropanoid pathway											
Phenylalanine-ammonia lyase	Contig1795_at	1.5	-	-	-	-1.8	-	-	-	-	-
Phenylalanine-ammonia lyase	Contig1799_s_at	-	-	-	-	-	-	-	-	-	-
Phenylalanine-ammonia lyase	Contig1800_at	-	-	-	-	-	-	-	-	-	-
Phenylalanine-ammonia lyase	Contig1800_s_at	-	-	-	-	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Phenylpropanoid pathway											
Phenylalanine-ammonia lyase	Contig1800_x_at	-	-	-	-	-	-	-	-	-	-
Phenylalanine-ammonia lyase	Contig1802_at	2.2	-	-	-	-1.7	-	-	-	-	-
Phenylalanine-ammonia lyase	Contig1803_at	-	-	-	-	-	-	-	-	-	-
Phenylalanine-ammonia lyase	Contig18558_at	-	-	-	-	-	-	-	-	-	-
Phenylalanine-ammonia lyase	Contig1805_s_at	-	-	1.1	-	-	-	-	-	-	-
Caffeic acid O-methyltransferase	Contig2532_at	-	-1.7	-	-	-	-	-1.5	-	-	-
Caffeic acid O-methyltransferase	Contig5311_at	-	-1.0	-	-	-	-	-	-	-	-
Caffeic acid O-methyltransferase	Contig6251_at	-1.2	1.4	2.1	-	-	-	-	-	-	-
Chalcone synthase	HV02B02u_at	-	-	-	-	-	-	-	-	1.1	-
Chalcone synthase 2	Contig11343_at	-	-1.2	-	-	-1.4	-	-	-	-	-
Chalcone synthase 2	Y09233_at	-	-	-	-	-	-	-	-	-	-
Naringenin-chalcone synthase	Contig7356_at	-	-1.3	-	-	-	-	-1.2	-	-	-
Naringenin-chalcone synthase	U43494_at	1.9	-	-	-	-1.6	-	-	-	-	-
Chalcone isomerase	Contig9047_at	-	-	-	-	-	-	-	-	-	-
Chalcone isomerase	Contig9048_s_at	-	-1.1	-	-	-	-	-	-	-	-
Flavonol glucosyltransferase	Contig11602_at	-	-	-	-	-	-	-	-	-	-
Flavonol glucosyltransferase	Contig1826_s_at	-	-	-	-	-	-	-	-	-	-
Flavonol glucosyltransferase	Contig1829_at	1.0	1.4	-	-	-	-	-	-	-	-
Flavonol glucosyltransferase	Contig25368_at	-	-	-	-	-	-	-	-	-	-
Flavonol glucosyltransferase	HY07L14u_at	-	-	-	-	-	-	-	-	1.1	-
Flavonol 3-sulfotransferase	Contig12075_at	-	-	-	-	-	-	2.4	-	2.2	-
Flavonol 4'-sulfotransferase	Contig12910_at	-	-	-	-	-	-	-	-1.3	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Phenylpropanoid pathway											
Flavonol 4'-sulfotransferase	Contig12910_at	-	-	-	-	-	-	-	-1.3	-	-
Flavonol 4'-sulfotransferase	Contig18035_at	-	-	-	-	-	-	-	-	-	-
Flavonone 3-hydroxylase	Contig12724_at	-	-	-	-	-	-	-	-	-	-
Flavonone 3-hydroxylase	Contig11212_at			-	-	-	-	-	-	1.1	-
Flavonoid 3',5'-hydroxylase	HVSMEn0008D16r2_at	-	-1.8	-	-	-	-	-1.1	-	-	-
2'-hydroxyisoflavone reductase	Contig14292_at	-	-	-	-	-	-	-	-	-	-
Dihydroflavonol 4-reductase	Contig8212_at	-	-	-	-	-	-	-	-	-	-
Dihydroflavonol-4-reductase	S69616_s_at	-	-	-	1.6	-	-	-	-	-	-
Anthocyanin 5-O-glucosyltransferase	Contig10670_at	1.1	-	-	-	-	-	-1.1	-	-	-
Anthocyanin 5-aromatic acyltransferase	Contig14295_at	-	-	-	-	1.1	-	-	-	-	-
4-coumarate--CoA ligase	Contig15844_at	-	-	-	-	-	-	-	-	-	-
4-coumarate--CoA ligase	Contig4676_at	-	-	-	-	-	-	-	-	-	-
4-coumarate--CoA ligase 4CL1	Contig4677_at	-	-	-	-	-	-	-	-	-	-
Hydroxymethylglutaryl-CoA lyase	Contig7417_at	-	-	-	-	-	-	-	-	-	-
Cinnamoyl-CoA reductase	Contig11163_at	-	-	-	-	-	-	-	-	-	-
Cinnamoyl-CoA reductase	Contig11163_s_at	-	-	-	-	-	-	-	-	-	-
Cinnamoyl-CoA reductase	Contig2384_at	-	-	-	-	-	-	-	-	-	-
Cinnamoyl-CoA reductase	Contig24449_at	-	-	-	-	-	-	-	-	-	-
Cinnamoyl-CoA reductase	HVSMEn0025O19r2_at	-	-1.5	-1.1	-	-1.1	-	-1.4	-	-2.1	-
Fatty acyl coA reductase	Contig10274_at	-	-	-	-	-1.3	-	-	-	-	-
Cinnamoyl alcohol dehydrogenase	Contig19854_s_at	-	-	-	-	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Phenylpropanoid pathway											
Cinnamoyl -alcohol dehydrogenase	Contig20411_at	-	-	-	-	-	-	-	-	-	-
Cinnamoyl alcohol dehydrogenase	Contig13997_at	-	-	-	-	-	-	-	-	-	-
Cinnamoyl alcohol dehydrogenase	Contig4260_at	1.3	-	-	-	-	-	-	-	-	-
Cinnamoyl alcohol dehydrogenase	HM05N11r_at	1.2	-	-	-	-	-	-	-	-	-
Cinnamoyl alcohol dehydrogenase	HVSMEh0081I20r2_s_at	1.6	-	-	-	-1.1	-	-	-	-	1.1
Cinnamoyl alcohol dehydrogenase 1a	Contig4346_at	-	-	-	-	-	-	-	-	-	-
N-hydroxycinnamoyl /benzoyl transferase	Contig6770_at	-	-1.1	-	-	-	-	-	-	-	-
N-hydroxycinnamoyl /benzoyltransferase-like	Contig19815_at	-1.1	-	-	-	-	-	-	-	-	-
Benzothiadiazole-induced	Contig538_at	-	1.0	-	-	-	-	-	-	-	-
Jasmonic acid pathway											
12-oxophytodienoate reductase	Contig5146_at	-	-	-	-	-	-	-	-	-	-
12-oxophytodienoic acid reductase	Contig2330_x_at	-	-	-	-	-	-	-	-	-	-
12-oxophytodienoic acid reductase	Contig6194_s_at	2.1	-	-	-	-1.3	-	1.4	-	-	-
Allene oxide synthase	Contig12918_at	-	-	-	-	-	-	-	-	-	-
Allene oxide synthase	Contig3096_s_at	-	1.1	-	-1.7	-	-	-	-	-	-1.3
Allene oxide cyclase	Contig4986_at	-	-	-	-	-	-	-	-	-	-
Lipoxygenase	Contig12574_at	-	-	-	-1.0	-	-	-	-	-	-1.3
Lipoxygenase	Contig13288_at	-	-	-	-	-	-	-	-	-	-
Lipoxygenase	Contig1735_s_at	-	-	-	-	-	-	-	-	-	-
Lipoxygenase	HVSMEf0009L21r2_s_at	-	-	-	-1.1	-	-	-	-	-	-1.1
Methyljasmonate-inducible lipoxygenase 2	HVSMEg0005M23r2_at	-	-	-	-	1.3	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Jasmonic acid pathway											
Methyljasmonate-inducible lipoxygenase 2	Contig2306_s_at	-	-	-	-	-	-	-	-	-	-1.3
Jasmonate induced	Contig2899_at	-	-1.1	-	-	-	-	-	-	-	-
Jasmonate induced	Contig2899_s_at	-	-1.6	-	-	-1.1	-	-	-	-	-
Jasmonate induced	Contig2900_at	-	-	-	-1.4	-1.7	-	-	-	-1.4	-1.1
Jasmonate induced	Contig6155_at	-	-	-	-	-	-	1.2	-1.6	-	-
Jasmonate-induced 1	Contig1681_x_at	-	-	-	-	-	-	-	1.1	2.2	-
23 KDa jasmonate-induced 1	Contig1678_s_at	-	1.3	-	-1.3	-	-	1.3	-1.4	-	-
23 KDa jasmonate-induced 1	Contig1679_s_at	-	-	-	-	-1.5	-	-	-	-	-
23 KDa jasmonate-induced 1	Contig1684_x_at	-	-	-	-	-	-	-	1.6	2.2	-
23 KDa jasmonate-induced 1	rbags15p13_s_at	-	-	-	-	-1.3	-	-	-	-	-
32.6 kDa jasmonate-induced	Contig7886_at	-	-	-	-	-1.6	-	-	-	-	-
32.7 kDa jasmonate-induced	Contig7887_at	1.2	-1.7	-	-	-1.8	-	-	-	-	-
Jacalin-like	Contig3504_at	-	-	-	-	-	-	-	-	-	-
Ethylene Defense Pathway											
1-aminocyclopropane-1-carboxylate oxidase	HVSMEm0001O15r2_s_at	-	-	-	-	-	-	-	-	-	-
1-aminocyclopropane-1-carboxylate oxidase	rbasd24g02_s_at	-	-1.1	-	-	-	-	-1.1	-	-	-
1-aminocyclopropane-1-carboxylate oxidase	EBro03_SQ004_E10_at	-	-1.8	-	-	-	-	-	-	-	-
1-aminocyclopropane-1-carboxylate oxidase	Contig19300_at	-	-	-	-	-	-	-	-	-	-
1-aminocyclopropane-1-carboxylate oxidase	Contig2639_at	-	-	-	-	-1.2	-	1.4	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Ethylene Defense Pathway											
1-aminocyclopropane -1-carboxylate oxidase- like	Contig13312_at	-	-	-	-	-	-	-	-	-	-
Ethylene-responsive transcriptional coactivator	Contig18796_at	-	-	-	-	-	-	2.1	-	-	-
Ethylene-responsive	Contig7507_at	-	-	-	-	-	-	-	-	-	-
Pathogenesis-related proteins											
PR	Contig1783_at	-	-	-	-	-	-	-	-	-	-
PR	Contig1783_x_at	-	-	-	-	-	-	-	-	-	-
PR	Contig26466_at	-	-1.4	-	-	-	-	-	-	-1.2	-
PR	Contig5368_at	1.5	-	-	-	-	-	-	-	-	1.2
PR	Contig5369_at	1.2	1.1	-	-	-	-	-	-	-	-
PR	Contig5607_s_at	-	1.3	-	-	-	-	-	-	-	-
Pathogenesis-related 1											
PR-1 precursor	Contig2210_at	-	-	-	-	-	-	-	-	-	-
PRB1-2 precursor	Contig2211_at	-	-	-	-	-	-	-	-	-	1.2
PRB1-3 precursor	Contig2212_s_at	-1.3	-	1.3	-	-	-	1.4	-	1.2	1.5
Acidic PR-1a precursor	Contig24993_at	-	-	-	-	-	-	-	-	-	-
PR-1C precursor	Contig4054_s_at	1.6	2.1	1.0	-	-	-	2.0	-	2.0	1.1
PR-1A/1B precursor	Contig4056_s_at	1.9	2.2	-	-	-	-	1.8	-	1.6	-
PR-1	Contig12046_at	1.3	3.0	1.8	-	-	-	1.8	-	-	-
PR-1	Contig2213_s_at	-	-	-	-	-	-	1.4	2.6	-	3.5
PR-1.2	Contig2208_at	-	-	-	-	-	-	-	1.7	-	2.7
PR-1a	Contig2209_at	-	2.2	2.6	-	-	-	2.2	-	1.7	1.4

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
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Pathogenesis-related 1											
PR-1a	Contig2214_s_at	-	-	1.5	-	-	-	1.6	-	1.1	1.4
Pathogenesis-related 2											
β-1,3-glucanase precursor	Contig13350_at	-	1.8	1.2	-	-	-	-	-	-	-
β-1,3-glucanase precursor	Contig8262_at	-	1.4	-	-	-	-	-	-	-	-
(1->3,1->4)-β-glucanase isoenzyme II	Contig2834_at	-	-4.6	-3.3	1.4	-1.4	-	-2.2	-	-	-
β-glucanase	Contig1639_at	1.4	-	-	-	-	-	-	-	-1.4	-
Endo-1,3-β-glucanase	Contig11289_at	-	-	-	-	-	-	-	-	-	-
β-1,3-glucanase	Contig5219_s_at	-	-	-	-	-	-	-	-	-	-
β-1,3-glucanase C terminal fragment	Contig17372_at	-	1.2	-	-	-	-	-	-	-	-
Pathogenesis-related 3, -4, -8, -11											
PR-4	Contig15099_s_at	4.6	1.4	-	-	-2.5	-	-	1.9	1.3	2.8
PR-4	Contig2550_x_at	-	-	-	-	-	-	1.2	-	1.1	1.5
PR-4	Contig639_at	-	2.1	1.2	-	-	-	1.1	-	-	-
PR-4	Contig6576_s_at	-	-	-	-	-	-	1.4	-	-	-
Barwin homolog *win2* precursor	Contig2546_at	-	-	-	-1.0	-	-	-	-	-	-
Chitinase	Contig16814_at	-	2.3	-	-	-	-	-	-	-	-
Chitinase	Contig25195_at	-	-	-	-	-1.2	-	-	-	-	-
Chitinase	Contig4173_at	-	-	-	-	-	-	-	-	-	-
Chitinase	Contig9060_at	3.3	-	-	1.2	-2.0	-	-	1.0	-	2.0
Chitinase I	Contig14498_at	1.6	-	-	-	-	-	1.4	-	1.2	1.3
Chitinase II precursor	Contig4324_at	2.4	2.0	-	-	-	-	-	-	-	-
Chitinase II precursor	Contig4324_s_at	2.2	1.8	-	-	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
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Pathogenesis-related 3, -4, -8, -11											
Chitinase cht2a precursor	Contig2990_at	-	-1.7	-	-	-	-	-	-	-	1.3
Chitinase cht2b precursor	Contig2992_s_at	2.3	1.9	-	-	-	-	1.3	-	-	1.8
Chitinase III	Contig23540_at	1.5	-	-	-	-	-	-	-	-	1.4
Chitinase III	Contig5023_at	-	-	-	-	-	-	1.0	-	-	-
Chitinase III	Contig5995_at	-	-	-	-	-	-	-	-	-	2.2
Chitinase III	Contig7001_at	-	-	-	-	-	-	-	-	-	-
Chitinase IV precursor	Contig4326_at	1.4	1.0	-	-	-	-	-	-	-	1.0
Chitinase IV precursor	Contig4326_s_at	2.9	1.8	-	-	-1.1	-	-	-	-	1.3
Pathogenesis-related 5											
PR-5	Contig10686_at	-	1.6	-	-	-	-	-	-	-	-
CsAtPR-5	Contig16303_at	1.0	-	-	-	-	-	-	-	-	-
CsAtPR-5	rbah33e14_at	-	1.3	-	-	-	-	-	-	-	-
CsAtPR-5	rbah33e14_s_at	-	2.2	-	-	-	-	-	-	-	-
TLP	Contig10004_at	-	-	-	-	-	-	-	-	-	-
TLP	Contig2792_s_at	-	-	-	-	-	-	-	-	-	-
TLP4	Contig3947_s_at	1.6	2.0	1.4	-	-	-	2.4	-	1.6	1.7
TLP7	Contig2789_at	-	-	-	-	-	-	-	-	-	-
TLP7	Contig2790_s_at	-	-	-	-	-	-	-	-	-	-
TLP7	rbaak13h13_s_at	-	-	-	-	-	-	-	-	-	-
TLP8	EBem10_SQ002_I10_s_at	-	-1.7	-	-	-	-	-	-	-	1.6
Pathogenesis-related 6											
Proteinase inhibitor-related bsi1 precursor	HD07M22r_s_at	1.1	1.1	-	-	-	-	-	-	-	1.2

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
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Pathogenesis-related 6											
Proteinase inhibitor	Contig34_s_at	1.3	-	-	-	-1.9	-	-	-	-	-
Proteinase inhibitor	Contig50_x_at	-	-	-	-	-1.0	-	-	-	-	-
Proteinase inhibitor	Contig507_x_at	-	-	-	2.1	-	-	-	-	-	1.5
Proteinase inhibitor	Contig5903_s_at	-	-	-	-	-	-	-	-	-	-
Proteinase inhibitor	Contig88_x_at	-	-	-	-	-	-	-	-	-	-
Proteinase inhibitor	HVSMEh0099O01f_s_at	-	1.0	-	-	-	-	-	-	-	-
Cysteine proteinase inhibitor	HT08H03u_s_at	-	-	-	-	-	-	-	-	-	-
Pathogenesis-related 7											
Aspartic proteinase	Contig4566_at	-	2.8	1.7	-	-	-	-	-	-	-
Cysteine proteinase precursor	Contig17638_at	-	-5.9	-2.0	2.3	-	-	-1.4	3.1	-	2.2
Cysteine proteinase	Contig2988_s_at	-	-	-	-	-	-	-	-	-	-
Cysteine proteinase	Contig8896_s_at	-	3.9	-	-1.7	1.6	-	-	-2.0	-	-2.6
Subtilisin-like proteinase	Contig13847_s_at	-1.2	-	-	-	-	-	-	-	-	-
Subtilisin-like proteinase	Contig9015_at	-	3.4	1.9	-	-	-	-	-	-	-
Pathogenesis-related 9											
Peroxidase precursor	Contig1852_at	-	-1.5	-	-	-1.4	-	-1.1	-	-	1.4
Peroxidase precursor	Contig1859_at	-	-	1.9	1.9	-	-	-	1.9	1.1	2.8
Peroxidase precursor	HVSMEf0002E07r2_at	-	-4.0	-	-	-1.2	-	-1.4	1.6	-	-
Peroxidase precursor, pathogen-induced	Contig2118_at	-	-	1.0	-	-	-	-	-	-	-
Peroxidase BP-2A precursor	Contig1854_at	-	3.6	1.5	-	-	-	-	-	-	-
Peroxidase BP-2A precursor	Contig1876_s_at	-	3.0	1.1	-	-	-	-	-	-	-
Peroxidase C2 precursor-like protein	Contig15862_s_at	-	-	-	-	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
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Pathogenesis-related 9											
Peroxidase 2 precursor	Contig2112_at	1.7	-	1.3	-	-	-	-	-	-	1.9
Peroxidase	Contig11361_at	-	-	-	-	-	-	-	-	-	-
Peroxidase	Contig11375_at	-	-1.1	-	-	-	-	-	-	-	-
Peroxidase	Contig12191_at	-	-1.1	-	-	-	-	-	-	-	-
Peroxidase	Contig12191_s_at	-	-	-	-	-	-	-1.0	-	-	-
Peroxidase	Contig1862_at	-	-1.7	-	-	-	-	-	-	-	-
Peroxidase	Contig1864_at	-	-1.6	-	-	-	-	-	-	-	-
Peroxidase	Contig1865_at	-	-	-	-	-1.1	-	-	-	-	-
Peroxidase	Contig1867_at	-	-	-	-	-1.0	-	-	-	-	-
Peroxidase	Contig1871_at	-	-	-	-	-	-	-	-	-	-
Peroxidase	Contig1874_at	-	-	-	-	-1.2	-	-	-	-	-
Peroxidase	Contig21617_at	-	-	-	-	-	-	-	-	-	-
Peroxidase	Contig4337_at	1.9	1.2	-	-	-	-	-	1.1	-	1.9
Peroxidase	Contig6515_at	-	-2.4	-1.0	-	-1.4	-	-	1.1	-	-
Peroxidase	Contig6516_at	-	-	-	-	-1.4	-	-	-	-	-
Peroxidase	Contig7080_at	3.6	-	-	1.4	-2.0	-	-	1.9	1.0	2.8
Peroxidase	HVSMEb0011O12r2_at	-	1.2	-	-	-	-	-	-	-	-
Peroxidase	HVSMEm0005P05r2_at	2.0	-	2.0	-	-	-	-1.3	1.0	1.0	2.5
Peroxidase	rbah13p07_s_at	2.8	-	1.2	-	-1.3	-	-1.0	1.3	-	2.5
Glutathione peroxidase-like	Contig2453_at	-	1.0	-	-	-	-	-	-	-	-
Glutathione peroxidase-like	Contig2454_at	-	-	-	-	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Pathogenesis-related 10											
PR-10a	Contig4402_s_at	1.3	-	1.2	-	-	-	-	-	-	2.8
PR-10a	Contig4405_x_at	1.3	-	-	1.3	-1.3	-	-	1.1	-	3.4
PR-10a	Contig4406_x_at	-	-	1.2	1.9	-	-	-	1.4	-	3.8
Ribonuclease	Contig10672_at	-	-	-	-	-	-	-	-	-	-
Ribonuclease	HD13B05r_s_at	-	-	-	-	-	-	-	-	-	-
Aleurone ribonuclease	Contig3691_at	-	-	-	-	-	-	-	-	-	-
Pathogenesis-related 12											
Defensin J1-2 precursor	Contig24683_at	-1.6	-4.9	-1.5	-	-1.5	-	-1.6	-	-	-
Defensin	Contig3215_s_at	-	-	-	-	-	-	-	-	-	-
Defensin	Contig3216_at	-	-	-	-	-	-	-	-	-	-
Plant defensin	Contig19785_at	-	2.2	1.8	-	-	-	1.2	-	-	-
Pathogenesis-related 13											
Thionin precursor, leaf	Contig1567_x_at	-	-	-	-	-	-	-	-	-	-
Thionin precursor, leaf	Contig1580_x_at	-	-	1.3	-1.7	-	-	-	-	-	-
Thionin precursor, leaf	Contig1582_x_at	-	-2.0	-	-	-	-	-	-	-1.1	-
Thionin precursor, leaf	Contig2653_s_at	-	-	-	-	-	-	-	-	-	-
Thionin	Contig1570_s_at	-	-	-	-	-	-	1.2	-	-	-
Thionin	Contig1579_s_at	-	-	-	-	-	-	-	-	-	-
Thionin	HVSMEb0010B05r2_x_at	-	-	-	-	-	-	-	-	-	-
Thionin-like	Contig25050_at	-1.2	-3.4	-	-	-1.7	-	-1.2	-	-	-
Leaf thionin Asthi3	Contig4731_at	-	4.9	3.0	-	1.1	-	1.5	-	-	-
Gamma-thionin	EBed02_SQ002_A05_at	-	1.7	-	-	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Pathogenesis-related 13											
Gamma-thionin	EBes01_SQ004_M23_at	-	2.0	-	-	-	-	-	-	-	-
Pathogenesis-related 14											
nsLTP precursor	Contig12237_at	-	-	1.1	-	-	-	2.3	-	-	-
nsLTP precursor	Contig2044_at	-	-	-	-	-	-	-	-	-	-
nsLTP precursor	Contig845_s_at	-	2.0	-	-	-	-	-	-	-	-
nsLTP precursor 4	Contig2041_at	-	-	-	-	-	-	-	-	-	-
nsLTP precursor 4	Contig2041_x_at	-	-	-	-	-	-	-	-	-	-
nsLTP precursor 4	Contig2046_at	-	-	-	-	-	-	-	-	-	-
nsLTP precursor 4	HVSMEn0020F17r2_x_at	-	-	-	-	-	-	-	-	-	-
nsLTP precursor 4	Contig3482_s_at	-	4.4	2.8	-	1.6	-	1.7	-	-	-
nsLTP	Contig19992_at	-	-	-	-	1.3	-	-	-	-	-
nsLTP	Contig9857_at	-	2.6	-	-	-	-	-	-	-	-
nsLTP	rbasd16a13_s_at	-	1.1	-	-	-	-	-	-	-	-
Phospho-LTP	AF039024_at	-	-	-	-	-	-	-	-	-	-
Phospho-LTP	Contig150_at	-	4.8	3.0	-	1.5	-	1.3	-	-	-
Phospho-LTP	HA28L22r_s_at	-	4.4	3.0	-	1.4	-	1.5	-	-	-
LTP	Contig3776_s_at	2.0	-	-	-	-1.2	-	-2.1	-	-	-
LTP homolog	Contig3259_at	-	3.1	1.4	-	-	-	1.0	-	-	-
Deoxynivalenol Defense											
Sucrose synthase 1	Contig689_s_at	-	-	-	-	-	-	-	-	-	-
Sucrose synthase 2	Contig23306_at	-	-	-	-	-	-	-	-	-	-
Sucrose-phosphate synthase	Contig19734_at	-1.0	-3.6	-1.4	-	-1.6	-	-1.6	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Deoxynivalenol Defense											
Glucosyltransferase	Contig14830_at	-	-	-	-	-	-	-	-	-	-
Glucosyltransferase	Contig19112_at	-	-	-	-	-	-	-	-	-	-
Glucosyltransferase	Contig19246_at	-	-	-	-	-	-	-	-	-	-
Glucosyltransferase	Contig19290_at	-	-	-	-	-	-	-	-	-	-
Glucosyltransferase	Contig5876_at	1.1	-	-	-	-	-	1.8	-	1.1	-
Glucosyltransferase	Contig9397_at	1.2	-	-	-	-	-	-	-	-	-
Glucosyltransferase NTGT2	HR01O04u_at	-	-	-	-	-	-	-	-	-	-
Glucosyltransferase-13	HVSMEn0025G16r2_at	-	-1.3	-	-	-	-	-	-	-	-
Salicylate-induced glucosyltransferase	Contig9824_at	-	-1.1	-	-	-	-	-	-	-	-
Sucrose-UDP glucosyltransferase	Contig602_at	-	-1.0	-	-	-	-	-	-	-	-
Sucrose-UDP glucosyltransferase 1	Contig361_s_at	-	1.0	-	-	-	-	-	-	-	-
Sucrose-UDP glucosyltransferase 2	Contig481_at	-	-	-	-	-	-	-	-	-	-
Sucrose-UDP glucosyltransferase 2	Contig481_s_at	-	-1.2	-	-	-	-	-	-	-	-
Sucrose-UDP glucosyltransferase 2	Contig823_at	-	-2.7	-	-	-	-	-	-	-	-
Limonoid UDP glucosyltransferase	Contig17260_at	-	-1.1	-	-	-	-	-	-	-	-
Cis-zeatin O-glucosyltransferase	HVSMEm0012J18r2_at	-	-	-	-	-	-	1.3	-	-	-
Betanidin 6-O-glucosyltransferase	Contig9823_at	-	-	-	-	-	-	-	-	-	-
UDP-glucose dehydrogenase	HW06A08u_s_at	-1.0	-	-	-	-	-	-	-	-	-
UDP-Glucose-6-dehydrogenase	rbags10b05_at	-	-	-	-	-	-	-	-	-	-
UDP-glucose 4-epimerase	Contig9721_at	-	1.9	-	-	-	-	-	-	-	-
UDP-glucose 4-epimerase	HVSMEm0008B04r2_s_at	-	1.4	-	-	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Defense Response											
Pectin acetylesterase	Contig9967_at	-	-	-	-	-1.0	-	-	-	-	-
Pectin methylesterase	Contig13335_at	-	-1.2	-	-	-	-	-	-	-	-
Pectin methylesterase-like	Contig15812_at	-	3.7	2.8	-	1.1	-	1.1	-	-	-
Pectin glucuronyl-transferase	Contig5920_s_at	-	-1.1	-	-	-	-	-	-	-	-
Pectate lyase 2	Contig11004_at	-	-1.3	-	-	-	-	-	-	-	-
Pectate lyase homolog	HVSMEg0010G22r2_at	-1.2	-3.4	-1.3	-	-2.1	-	-1.3	-	-	-
Glycine-rich cell wall precursor	Contig10022_at	-	-	-	-	-	-	-	-	-	-
Glycine-rich cell wall structural precursor	Contig1025_s_at	-	1.9	1.4	-	-	-	1.1	-	-	-
Glycine-rich cell wall structural precursor	Contig1039_at	-	1.8	1.4	-	-	-	-	-	-	-
Glycine rich	Contig1071_s_at	2.9	4.6	1.5	-	-	-	-	-	-	-
Glycine rich	Contig9925_at	1.3	1.1	2.1	1.6	-	-	-	-	1.6	2.1
Glycine/proline-rich	HT08F04u_s_at	-	-1.3	-	-	-	-	-	-	-	-
Proline-rich	Contig152_at	-	-	-	-	-	-	-	-	-	-1.3
Proline-rich	Contig17838_at	-	-1.3	-	-	-	-	-	-	-	-
Proline-rich	Contig3774_s_at	-	4.9	1.3	-3.3	-	-1.3	1.4	-2.7	-	-3.2
Proline-rich	Contig3777_at	-	-	-	-	-	-	-	-	-	-
Proline-rich	Contig3782_x_at	-	4.2	1.2	-3.1	-	-1.2	1.3	-2.6	1.1	-3.3
Proline-rich	Contig444_at	-1.2	-5.2	-	1.1	-1.7	-	-2.4	-	-1.4	-
Proline-rich	Contig4725_s_at	-	-	-	-	-1.3	-	-	-	-	-
Proline-rich	Contig603_at	-	-1.4	-	-	-	-	-	-	-	-
Proline-rich	Contig704_at	-	-2.2	-	-	-	-	-	-	-	-
Proline-rich	Contig704_x_at	-	-2.0	-	-	-	-	-	-	-	-
Proline-rich	Contig970_at	-2.0	-3.0	-	-	-	-	-	-	-	-
Proline rich homolog WCOR518	Contig4621_at	-	-	-	-	-1.5	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Defense Response											
Proline rich homolog WCOR518	Contig4622_s_at	-	-1.4	-	1.1	-	-	-	-	-	-
Hydroxyproline-rich glycoprotein	Contig553_s_at	-	-2.2	-	-	-1.4	-	-	-	-	-
Extensin-like	Contig14167_at	-	-	-	-	-	-	-	-	-	-
Extensin-like	Contig15167_at	-	3.2	1.9	-	-	-	1.1	-	-	-
Extensin-like	Contig4320_at	-1.6	-1.6	-	-	-	-	-	-	-	-
Extensin-like	Contig4321_at	-1.1	-1.1	-	-	-	-	-	-	-	-
Extensin-like	Contig9427_at	1.0	-	-	-	-	-	-	-	-	-
Extensin-like	Contig9531_at	-	-	-	-	-1.3	-	-	-	-	-
Endo-1,4-β-glucanase precursor	Contig18702_at	-	-2.2	-1.5	-	-	-	-	-	-	-
Endo-1,4-β-glucanase Cel1	Contig4147_at	-	-1.0	-	-	-	-	-	-	-	-
Xyloglucan endo-1,4-β-D-glucanase	Contig2669_at	-	-	-	-	-	-	-	-	-	-1.4
Xyloglucan endo-1,4-β-D-glucanase	Contig2670_s_at	-	-	-	-	-	-	-	-	-	-
Xyloglucan endo-1,4-β-D-glucanase	Contig2672_at	-	-	-	-	-	-	-	-	-	-
Xyloglucan endo-1,4-β-D-glucanase	Contig2673_at	-	-1.1	-	-	-	-	-	-	-	-
Xyloglucan endo-1,4-β-D-glucanase	HE01I24u_s_at	-	-	-	-	-	-	-	-	-	-
Xyloglucan endo-1,4-β-D-glucanase	HVSMEb0004L16r2_at	-	-	-	-	-	-	-	-	-	-
1,4-β-xylanase	Contig16010_at	-	-1.1	-	-	-	-	-	-	-	-
Xylanase inhibitor	Contig14679_at	2.6	-	1.1	-	-1.3	-	-1.0	1.2	-	1.9
Xylanase inhibitor	Contig5996_s_at	2.8	1.7	-	-	-1.5	-	-1.2	1.6	-	2.6
Xylanase inhibitor	Contig8006_at	-	-	-	-	-	-	-	-	-	-
Xylanase inhibitor I	Contig8905_at	2.5	-	-	1.8	-1.4	-	-	2.5	-	3.3
Glutathione transferase	Contig12776_at	1.6	-	-	-	-1.1	-	-	-	-	1.3

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Defense Response											
Glutathione transferase	Contig12776_s_at	1.6	-	-	-	-1.0	-	-	-	-	1.3
Glutathione transferase	Contig1597_s_at	-	1.1	-	-	-	-	-	-	-	-
Glutathione S-transferase	Contig14304_at	-	-	-	-	-	-	1.3	-	-	-
Glutathione S-transferase	Contig14387_at	-	-	-	-	-	-	-	-	-	-
Glutathione S-transferase	Contig16074_at	-	-	-	-	-	-	-	-	-	-
Glutathione S-transferase	Contig18367_at	-	-	-	-	-	-	-	-	-	-
Glutathione S-transferase	Contig2489_at	1.2	1.3	-	-	-	-	-	-	-	-
Glutathione S-transferase	Contig4044_at	-	-	-	-	-	-	-	-	-	-
Glutathione S-transferase	HO11K23S_s_at	1.2	1.1	-	-	-	-	1.1	-	-	-
Glutathione S-transferase	HV_CEb0004O15r2_s_at	-	-	-	-	-	-	-	-	1.3	-
Glutathione S-transferase	rbah38o04_s_at	1.0	1.0	-	-	-	-	-	-	-	-
Glutathione S-transferase 1	Contig2975_s_at	1.0	-1.2	-	1.1	-1.1	-	-	1.0	-	1.9
Glutathione-S-transferase 2	Contig21640_at	-	-	-	-	-	-	-	-	-	-
Glutathione-S-transferase 2	Contig5838_at	-	-	-	-	-	-	1.1	-	-	-
Glutathione-S-transferase 2	Contig7448_s_at	-	-	-	-	-	-	-	-	-	-
Glutathione S-transferase 24	Contig15264_at	1.6	-	-	-	-	-	2.4	-	1.6	1.2
Glutathione S-transferase 34	Contig6008_s_at	1.2	-	-	-	-	-	-	-	-	1.2
Glutathione-S-transferase Cla47	Contig7171_s_at	-	-	-	-	-	-	1.1	-	-	-
Glutathione-S-transferase Cla47	Contig9764_at	1.4	-	-	-	-	-	1.1	-	-	-
Glutathione transferase F4	Contig6238_s_at	1.0	-	-	-	-	-	-	-	-	1.4
Glutathione S-transferase OsGSTu2	Contig2488_s_at	1.2	-	-	-	-	-	-	-	-	-
Thioredoxin	Contig4022_at	-	-	-	-	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Defense Response											
Thioredoxin	rbaal31m19_s_at	-	1.0	-	-	-	-	-	-	-	-
Thioredoxin-like	Contig4685_at	-	-1.1	-	-	-	-	-	-	-	-
Hypersensitivity-related	Contig17006_at	1.4	-	-	-	-	-	-	-	-	-
Hypersensitivity-related	Contig19684_at	-	-	-	-	-	-	-	-	-	-
Hypersensitive-induced reaction 2	Contig6972_at	-	1.3	-	-	-	-	-	-	-	-
Pathogen-induced WIR1	Contig9917_at	-	1.8	1.2	-	-	-	-	-	-	-
Pathogen-induced WIR1A	Contig2163_at	-	1.7	-	-	-	-	-	-	-	-
Pathogen-induced WIR1A	Contig5974_s_at	-	1.8	1.6	-	-	-	-	-	-	-
Pathogen-induced WIR1A	Contig6519_at	-	-	-	-	-	-	-	-	-	-
Pathogen-induced WIR1A	Contig939_s_at	-	-	-	-	-	-	-	-	-	-
Bowman-birk type trypsin inhibitor	Contig17082_at	-	-	-	1.1	-	-	-	1.5	1.0	3.3
Bowman-birk type trypsin inhibitor	Contig18032_at	-	-1.0	-	1.4	-	-	-	1.6	-	-
Bowman-birk type trypsin inhibitor	Contig2087_s_at	-	-	-	-	-	-	-	-	-	-
Alcohol dehydrogenase	Contig278_at	-	-	-	-	-	-	-	-	-	-
Alcohol dehydrogenase	Contig393_at	-	-	-	-	-	-	-	-	-	-
Alcohol dehydrogenase	Contig431_at	-	-	-	-	-	-	-	-	-	-
Allyl alcohol dehydrogenase	Contig19680_at	-	-1.1	-	-	-	-	-	-	-	-
Allyl alcohol dehydrogenase	Contig5939_at	1.4	-	-	-	-	-	-	-	-	-
Allyl alcohol dehydrogenase	HI04E24r_s_at	-	-	-	-	-	-	-	-	-	-
Allyl alcohol dehydrogenase	HVSMEg0006O20r2_at	-	-	-	-	-	-	-	-	-	-
Short chain alcohol dehydrogenase	Contig20405_at	-	-	-	-	-	-	-	-	-	-
Short chain alcohol dehydrogenase	Contig6362_s_at	-	-	-	-	-	-	-	-	-	-

		Mock		***Tri5-***		***Tri5+***		**DON**		***Tri5-* (DON)**	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Defense Response											
Short-chain type alcohol dehydrogenase	Contig20683_at	-	-	-	-	-	-	-	-	-	-

Note: Pink represents up-regulated transcripts; blue represents down-regulated transcripts.

*PR=pathogenesis-related, TLP=thaumatin-like protein, nsLTP=nonspecific lipid transfer protein, LTP=lipid transfer protein.

APPENDIX 3

Identification of differentially transcribed genes associated with an oxidative burst, the phenylpropanoid and jasmonic acid antioxidant signaling pathways, the ethylene defense pathway, pathogenesis, deoxynivalenol defense, and general plant defense-related responses in Stander following inoculation with *Tri5*- (strain GZT40), *Tri5*+ (strain GZ3639), DON, GZT40 supplemented with DON (*Tri5*-(DON)), and mock inoculum. The differential regulation values presented are log_2-transformed.

		Mock		***Tri5-***		***Tri5+***		**DON**		***Tri5-* (DON)**	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Oxidative burst											
Germin E	AF250937_s_at	-	-	-	1.3	-	-	-	-	-	-
Germin F	Contig1528_s_at	-	-	-	-	-	-	-	-1.0	-	-
Germin-like 1	Contig2768_s_at	-	-1.7	-	-	-	-	-	-	-	-
Germin protein 4	Contig9172_at	-	-	-	1.1	-	-	-	-	-	-
Germin protein 4	Contig9172_s_at	-	-	-	2.3	-	-	-	-	-	1.0
Oxalate oxidase 2 precursor	Contig3018_at	-	1.2	-	2.6	-	-	-	-	-	1.4
Oxalate oxidase	Contig10847_at	-	-1.0	-	-	-	-	-	-1.2	-	-
Oxalate oxidase	Contig1518_at	-	-	-	1.6	-	-	-	-	-	-
Oxalate oxidase	Contig3017_at	1.2	2.5	-	3.4	-	-1.6	-1.3	-1.4	-	2.3
Oxalate oxidase	HVSMEi0004N08r2_at	-	1.6	-	-	-	-	1.3	-	-	-
Oxalate oxidase	Contig17901_at	-	1.8	-	-1.5	-	-	-	-	-	-
Oxalate oxidase 7	Contig10860_at	-	-	-	1.7	-	-	-	-	-	-
Oxalate oxidase-like	Contig3155_s_at	-	-	-	2.7	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Oxidative burst											
Oxalate oxidase-like	Contig3157_at	-	-	-	1.7	-	-	-	-	-	1.2
Aldehyde oxidase	Contig4920_s_at	-	-	-	-	-	-	-	-	-	-
Alternative oxidase AOX3 precursor	Contig5887_at	-	-	-	2.9	-	-	-	-	1.2	1.5
Alternative oxidase	Contig15936_at	-	-	-	2.5	-	-	-	-	1.2	1.2
Alternative oxidase	Contig5888_at	-	-	-	2.2	-	-	-	-	1.3	-
Ascorbic acid oxidase	Contig14651_at	-	-1.7	-	2.0	-	-	-	-	-	-
L-ascorbate oxidase	Contig16758_at	-	-2.0	-	1.2	-	-	-2.0	-	-	-
C-4 sterol methyl oxidase	Contig6208_at	-	-	-	-	-	-	-	-	2.5	-
Cytokinin oxidase	HV_CEa0008F11r2_at	-	-2.0	-	2.2	-	-	-1.1	-	-	1.3
Cytokinin oxidase	Contig24300_at	-	-	-	-	-	-	-	-	-	-
Fatty acid alpha-oxidase	Contig15882_s_at	-	-1.8	-	3.0	-	-	-	-	-	1.4
Flavin containing polyamine oxidase	Contig19426_at	-	-	-	-	-	-	-	-	-	-
Flavin containing polyamine oxidase	Contig3212_s_at	-	-2.1	-	-	-	-	-	-1.0	-	-
NADPH oxidase	Contig8301_at	-	-	-	-	-	-	-	-	-	-
Oxidase	Contig9320_at	-	-1.5	-	-	-	-	-	-	-	-
Polyphenol oxidase	Contig17722_at	-	-	-	-	-	-	-	-	-	-
Respiratory burst oxidase	HVSMEn0015O15f_s_at	-	-1.1	-	-	-	-	-	-	-	-
Sarcosine oxidase	Contig17178_at	-	-	-	-	-	-1.2	-	-2.5	-1.4	-
Phenylpropanoid pathway											
Phenylalanine ammonia-lyase	Contig1795_at	-	1.4	-	2.6	-	-	-	-1.6	1.0	-
Phenylalanine ammonia-lyase	Contig1799_s_at	-	-1.4	-	2.0	-	-	-	-	-	-
Phenylalanine ammonia-lyase	Contig1800_at	-	-2.3	-	2.1	-	-	-1.1	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Phenylpropanoid pathway											
Phenylalanine ammonia-lyase	Contig1800_s_at	-	-	-	1.7	-	-	-	-	-	-
Phenylalanine ammonia-lyase	Contig1800_x_at	-1.0	-1.9	-	1.7	-	-	-	-	-	-
Phenylalanine ammonia-lyase	Contig1802_at	1.2	-	-	3.7	-	-	-	-	-	1.2
Phenylalanine ammonia-lyase	Contig1803_at	-	-	-	-	-	-	-	-1.3	-	-
Phenylalanine ammonia-lyase	Contig18558_at	-	-	-	1.1	-	-	-	-	-	-
Phenylalanine ammonia-lyase	Contig1805_s_at	-	-1.6	-	2.3	-	-	-1.1	-	-	-
Caffeic acid O-methyltransferase	Contig2532_at	-1.5	-1.7	-	-	-	-	-1.0	-1.3	-	-
Caffeic acid O-methyltransferase	Contig5311_at	-	-	-	-	-	-	-	-	-	-
Caffeic acid O-methyltransferase	Contig6251_at	-	-	-	-	-	-	-	-	-	-
Chalcone synthase	HV02B02u_at	-	-	-	2.4	-	-	-	-	-	1.2
Chalcone synthase 2	Contig11343_at	-1.9	-1.6	-	-	-	-	-	-	-	-
Chalcone synthase 2	Y09233_at	-	-	-	1.3	-	-	-	1.4	-	1.5
Naringenin-chalcone synthase	Contig7356_at	-1.1	-1.3	-	-	-	-	-1.1	-1.5	-	-
Naringenin-chalcone synthase	U43494_at	-	-	-	3.3	-	-	-	-	-	1.1
Chalcone isomerase	Contig9047_at	-2.0	-2.0	-	-	-	-	-	-	-	-
Chalcone isomerase	Contig9048_s_at	-1.4	-1.3	-	-	-	-	-	-	-	-
Flavonol glucosyltransferase	Contig11602_at	-1.4	-	-	-	-	-	-	-	-	-
Flavonol glucosyltransferase	Contig1826_s_at	-1.0	-	-	-	-	-	-	-	-	-
Flavonol glucosyltransferase	Contig1829_at	-1.5	-	-	-	-	-	-	-	-	-
Flavonol glucosyltransferase	Contig25368_at	-	-	-	1.2	-	-	-	-	-	-
Flavonol glucosyltransferase	HY07L14u_at	-	-	-	-	-	-	-	-	-	-
Flavonol 3-sulfotransferase	Contig12075_at	-	-	-	-	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Phenylpropanoid pathway											
Flavonol 4'-sulfotransferase	Contig12910_at	-	-	-	-	-	-	-	-	-	-
Flavonol 4'-sulfotransferase	Contig12910_at	-	-	-	-	-	-	-	-	-	-
Flavonol 4'-sulfotransferase	Contig18035_at	-	-1.1	-	-	-	-	-	-	-	-
Flavonone 3-hydroxylase	Contig12724_at	-	-	-	1.1	-	-	-	-	-	-
Flavonone 3-hydroxylase	Contig11212_at			-	-	-	-	-	-	-	-
Flavonoid 3',5'-hydroxylase	HVSMEn0008D16r2_at	-1.1	-1.5	-	-	-	-	-	-	-	-
2'-hydroxyisoflavone reductase	Contig14292_at	-	-1.1	-	-	-	-	-	-1.1	-	-
Dihydroflavonol 4-reductase, putative	Contig8212_at	-	-	-	-	-	-	-	-1.4	-	-
Dihydroflavonol-4-reductase	S69616_s_at	-	-	-	-	-	-	-	-	-	-
Anthocyanin 5-O-glucosyltransferase	Contig10670_at	-	-	-	1.6	-	-	-	-	-	-
Anthocyanin 5-aromatic acyltransferase	Contig14295_at	1.9	-	-	1.0	-1.1	-	-	-	-	-
4-coumarate--CoA ligase	Contig15844_at	-	-1.4	-	1.1	-	-	-	-	-	-
4-coumarate--CoA ligase	Contig4676_at	-	-	-	-	-	-	-	-1.0	-	-
4-coumarate--CoA ligase 4CL1	Contig4677_at	-1.3	-1.9	-	-	-	-	-	-	-	-
Hydroxymethylglutaryl-CoA lyase	Contig7417_at	-1.2	-	-	-	-	-	-	-	-	-
Cinnamoyl-CoA reductase	Contig11163_at	-	-1.2	-	-	-	-	-	-	-	-
Cinnamoyl-CoA reductase	Contig11163_s_at	-	-1.3	-	-	-	-	-	-	-	-
Cinnamoyl-CoA reductase	Contig2384_at	-	1.4	-	-	1.1	-	1.1	-1.2	-	-
Cinnamoyl-CoA reductase	Contig24449_at	-	-1.4	-	-	-	-1.1	-	1.0	-	-1.1
Cinnamoyl-CoA reductase	HVSMEn0025O19r2_at	-	-1.1	-	-	-	-	-	-1.1	-	-
Fatty acyl coA reductase	Contig10274_at	-	-1.1	-	-	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Phenylpropanoid pathway											
Cinnamoyl alcohol dehydrogenase	Contig19854_s_at	-	-1.1	-	-	-	-	-	-	-	-
Cinnamoyl -alcohol dehydrogenase	Contig20411_at	-	-2.0	-	-	-	-	-	-	-	-
Cinnamoyl alcohol dehydrogenase	Contig13997_at	1.2	-	-	-	-	-	-	-	-	-
Cinnamoyl alcohol dehydrogenase	Contig4260_at	-	-	-	1.2	-	-	-	-	-	-
Cinnamoyl alcohol dehydrogenase	HM05N11r_at	-	-1.1	-	-	-	-	-	-	-	-
Cinnamoyl alcohol dehydrogenase	HVSMEh0081I20r2_s_at	-	-	-	1.9	-	-	-	-	-	1.0
Cinnamoyl alcohol dehydrogenase 1a	Contig4346_at	-	-	-	-	-	-	-	-1.0	-	-
N-hydroxycinnamoyl/ benzoyl transferase	Contig6770_at	-	2.2	-	-	1.3	-	1.4	-2.3	2.6	-
N-hydroxycinnamoyl/ benzoyltransferase-like	Contig19815_at	-	-1.1	-	-	-	-	-	-	-	-
Benzothiadiazole-induced	Contig538_at	-	-	-	-	-	-1.5	-	-	-	-
Jasmonic acid pathway											
12-oxophytodienoate reductase	Contig5146_at	-	-	-	1.4	-	-	-	-	-	-
12-oxophytodienoic acid reductase	Contig2330_x_at	-	-	-	2.1	-	-	-	-	-	-
12-oxophytodienoic acid reductase	Contig6194_s_at	-1.0	-1.5	-	2.1	-	-	-	-	1.3	-
Allene oxide synthase	Contig12918_at	-1.1	-1.7	-	-	-	-	-	-	-	-
Allene oxide synthase	Contig3096_s_at	-	-2.5	-	1.8	-	-	-1.1	-1.1	-	-
Allene oxide cyclase	Contig4986_at	-	-1.0	-	-	-	-	-	-	-	-
Lipoxygenase	Contig12574_at	-1.1	-1.0	-	-	-	-	-	-1.7	-	-
Lipoxygenase	Contig13288_at	-	-1.0	-	1.6	-	-	-	-1.0	-	-
Lipoxygenase	Contig1735_s_at	-	-1.7	-	1.2	-	-	-1.4	-2.5	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
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Jasmonic acid pathway											
Lipoxygenase	HVSMEf0009L21r2_s_at	-	-1.9	-	1.5	-	-	-1.5	-3.2	-	-
Methyljasmonate-inducible lipoxygenase 2	HVSMEg0005M23r2_at	-	-2.5	-	-	-1.2	-	-	-	-	-
Methyljasmonate-inducible lipoxygenase 2	Contig2306_s_at	-	-1.5	-	1.4	-	-	-	-1.4	-	-
Jasmonate induced	Contig2899_at	-1.7	-1.8	-	-	-	-	-	-	-	-
Jasmonate induced	Contig2899_s_at	-1.3	-2.3	-	-	-	-	-	-	-	-
Jasmonate induced	Contig2900_at	-1.0	-3.6	-	2.3	-	-	-1.3	-	-	1.1
Jasmonate induced	Contig6155_at	-	-	-	-	-	-	-	-	-	-
Jasmonate-induced 1	Contig1681_x_at	-	-	-	1.0	-	-	-	-	-	-
23 KDa jasmonate-induced 1	Contig1678_s_at	-	-2.0	-	-	-	-	-	-1.6	-	-
23 KDa jasmonate-induced 1	Contig1679_s_at	-	-	-	-	-	-	-1.8	-	-1.0	-
23 KDa jasmonate-induced 1	Contig1684_x_at	-	-	-	1.4	-	-	-	-	-	-
23 KDa jasmonate-induced 1	rbags15p13_s_at	-	-2.2	-	-	-	-	-1.9	-	-1.2	-
32.6 kDa jasmonate-induced	Contig7886_at	-	-3.1	-	4.0	-	-	-3.5	-1.8	-	1.9
32.7 kDa jasmonate-induced	Contig7887_at	1.4	-1.9	-	4.0	-	-	-	-	-	1.9
Jacalin-like	Contig3504_at	-	-1.6	-	-	-	-	-	-	-	-
Ethylene defense pathway											
1-aminocyclopropane-1-carboxylate oxidase	HVSMEm0001O15r2_s_at	-1.5	-1.4	-	-	-	-	-	-1.0	-	1.4
1-aminocyclopropane-1-carboxylate oxidase	rbasd24g02_s_at	-	-	-	-	-	-	-	-	-	-
1-aminocyclopropane-1-carboxylate oxidase	EBro03_SQ004_E10_at	-	-	-	-	-	-	-	-	-1.2	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
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Ethylene defense pathway											
1-aminocyclopropane-1-carboxylate oxidase	Contig19300_at	-	-1.1	-	-	-	-	-	-	-	-
1-aminocyclopropane-1-carboxylate oxidase	Contig2639_at	-1.4	-1.7	-	2.2	-	-	-	-	-	2.3
1-aminocyclopropane-1-carboxylate oxidase-like	Contig13312_at	1.2	-	-	-	-	-	-	-	-	-
Ethylene-responsive transcriptional coactivator	Contig18796_at	-	1.1	-	-	-	-	-	-	-	-
Ethylene-responsive	Contig7507_at	-	-	-	-	-	-	-	-1.1	-	-
Pathogenesis-related proteins											
PR	Contig1783_at	-1.1	-1.1	-	-	-	-	-	-	-	-
PR	Contig1783_x_at	-1.0	-1.2	-	-	-	-	-	-	-	-
PR	Contig26466_at	-	-	-	-	-	-	-	-	-	-
PR	Contig5368_at	1.3	-	1.0	1.4	-	-	-	-	-	-
PR	Contig5369_at	-	-	-	1.7	-	-	-	-	-	-
PR	Contig5607_s_at	-	-	-	-	-	-1.1	-	-	1.1	-
Pathogenesis-related 1											
PR-1 precursor	Contig2210_at	-	-	-	-	-1.4	-1.3	-	-	-	-
PRB1-2 precursor	Contig2211_at	-	-	-	1.5	-1.6	-1.5	-	-	-	1.4
PRB1-3 precursor	Contig2212_s_at	-	-	-	1.7	-1.6	-1.9	-	-	-	1.5
Acidic PR-1a precursor	Contig24993_at	-	-	-	2.7	-	-	-	-	-	-
PR-1C precursor	Contig4054_s_at	-	-	-	1.5	-	-1.4	-	-	1.5	1.2
PR-1A/1B precursor	Contig4056_s_at	-	-	-	1.4	-	-1.2	-	-	1.4	1.1

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
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Pathogenesis-related 1											
PR-1	Contig12046_at	1.6	1.3	-	1.4	-1.1	-1.4	-	1.3	1.4	1.1
PR-1	Contig2213_s_at	-1.6	-	-	2.4	-	-1.6	-	1.1	3.1	2.8
PR-1.2	Contig2208_at	-	-	-	2.0	-	-	-	-	1.4	2.2
PR-1a	Contig2209_at	-	1.1	-	1.2	-2.5	-1.8	-	1.3	-	1.5
PR-1a	Contig2214_s_at	-	-	-	1.3	-1.9	-1.7	-	1.1	-	1.4
Pathogenesis-related 2											
β-1,3-glucanase precursor	Contig13350_at	-	-	-	2.4	-	-1.1	-1.0	-	1.2	-
β-1,3-glucanase precursor	Contig8262_at	-	-	-	-	-	-	-	-	-	-
(1->3,1->4)-β-glucanase isoenzyme II	Contig2834_at	1.3	-	-	-	-	-	-	-	-	-
β-glucanase	Contig1639_at	-	-1.6	-	-	-1.2	-	-1.3	-	-	-
Endo-1,3-β-glucanase	Contig11289_at	-	-	-	-	-	-	-	-	1.7	-
β-1,3-glucanase	Contig5219_s_at	-	-1.2	-	-	-	-	-	-	-	-
β-1,3-glucanase C terminal fragment	Contig17372_at	-	1.0	-	-	-	-	-	-	-	-
Pathogenesis-related 3, -4, -8, -11											
PR-4	Contig15099_s_at	3.2	2.2	-	2.1	-	-1.1	-2.0	-1.6	-	-
PR-4	Contig2550_x_at	-	1.5	-	-	-	-1.5	-	-	-	-
PR-4	Contig639_at	-	1.5	-	-	-	-1.5	-	-	1.1	-
PR-4	Contig6576_s_at	-	-	-	-	-	-	-	-	-	-
Barwin homolog win2 precursor	Contig2546_at	-	-	-	-	-	-	-	-	-	-
Chitinase	Contig16814_at	-	1.5	-	-	-	-	-	-	-	-
Chitinase	Contig25195_at	-	-	-	-	-	-	-	-	-	-
Chitinase	Contig4173_at	-	-	-	-	-		-	-	2.0	1.6

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
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Pathogenesis-related 3, -4, -8, -11											
Chitinase	Contig9060_at	1.8	-	-	-	-	-	-1.7	-	-	1.3
Chitinase I	Contig14498_at	-	-	-	1.1	-	-	-	-	-	-
Chitinase II precursor	Contig4324_at	1.4	1.3	-	-	-	-1.2	-1.5	-	-	-
Chitinase II precursor	Contig4324_s_at	1.2	-	-	-	-	-	-1.4	-	-	-
Chitinase cht2a precursor	Contig2990_at	-	-	-	-	-1.2	-1.5	-	-	-	2.0
Chitinase cht2b precursor	Contig2992_s_at	1.2	1.3	-	1.2	-	-1.2	-	-	1.5	1.5
Chitinase III	Contig23540_at	1.2	1.5	-	-	-	-	-1.4	-	-	-
Chitinase III	Contig5023_at	-	-	-	-	-	-1.0	-	-	-	-
Chitinase III	Contig5995_at	-	-	-	1.6	-	-	-	-1.0	-	-
Chitinase III	Contig7001_at	-	-	-	-	-	-1.3	-	-	-	-
Chitinase IV precursor	Contig4326_at	1.4	1.1	-	2.3	-	-1.2	-1.1	-	1.2	-
Chitinase IV precursor	Contig4326_s_at	2.1	1.4	-	2.4	-	-	-	-	-	1.2
Pathogenesis-related 5											
PR-5	Contig10686_at	-	-	-	-	-	-	-	-	-	-
CsAtPR-5	Contig16303_at	-	-	-	1.0	-	1.3	-	1.2	-	1.1
CsAtPR-5	rbah33e14_at	-	-	-	-	-	-	-	-	-	-
CsAtPR-5	rbah33e14_s_at	-	-	-	-	-	-	-	-	-	-
TLP	Contig10004_at	1.2	-	-	-	-	-	-	-	-	-
TLP	Contig2792_s_at	-	-	-	1.1	-	-	-	-	-	-
TLP4	Contig3947_s_at	1.4	1.5	-	2.3	-	-1.2	-	-	2.0	1.6
TLP7	Contig2789_at	-	-1.4	-	1.2	-	-	-	-	1.4	1.0
TLP7	Contig2790_s_at	-	-	-	-	-	-1.3	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Pathogenesis-related 5											
TLP7	rbaak13h13_s_at	-	-	-	-	-	-1.6	-	-	-	-
TLP8	EBem10_SQ002_I10_s_at	-	-	-	1.5	-	-1.4	-	-	-	1.4
Pathogenesis-related 6											
Proteinase inhibitor-related bsi1 precursor	HD07M22r_s_at	-	-	-	-	-	-	-	-	-	1.0
Proteinase inhibitor	Contig34_s_at	-	-1.1	-	2.7	-	-	-2.1	-	-1.6	-
Proteinase inhibitor	Contig50_x_at	-	-	-	2.1	-	-	-1.7	-1.1	-	-
Proteinase inhibitor	Contig507_x_at	-	1.2	-	-1.3	-	-	-	-	-	-
Proteinase inhibitor	Contig5903_s_at	-	-	-	-1.0	-	-	-	-	-	-
Proteinase inhibitor	Contig88_x_at	-	-	-	1.2	-	-	-	-	-	-
Proteinase inhibitor	HVSMEh0099O01f_s_at	-	-	-	-1.1	-	-	-	-	-	-
Cysteine proteinase inhibitor	HT08H03u_s_at	-	-1.2	-	-	-	-	-	-	-	-
Pathogenesis-related 7											
Aspartic proteinase	Contig4566_at	-	1.6	-	-	-	-	-	-	-	-
Cysteine proteinase precursor	Contig17638_at	-1.0	-3.5	-1.7	-	-	-	-1.9	-	2.1	-
Cysteine proteinase	Contig2988_s_at	-	-2.0	-	-	-	-	-	-	-	-
Cysteine proteinase	Contig8896_s_at	1.2	-	-1.4	-	-1.5	-	-	1.2	-2.0	-
Subtilisin-like proteinase	Contig13847_s_at	-	-	-	-1.1	-	-	-	-	-	-
Subtilisin-like proteinase	Contig9015_at	-	2.2	-	-1.2	-	-	1.2	-	-1.0	-
Pathogenesis-related 9											
Peroxidase precursor	Contig1852_at	-	-1.4	-	1.8	-	-	-	-	-	-
Peroxidase precursor	Contig1859_at	2.0	1.3	-	3.5	-	-1.1	-1.8		-1.2	-
Peroxidase precursor	HVSMEf0002E07r2_at	-1.3	-3.0	-	-	-	-	-	-1.2	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Pathogenesis-related 9											
Peroxidase precursor, pathogen-induced	Contig2118_at	-	-	-	2.4	-	-	-	-	-	-
Peroxidase BP-2A precursor	Contig1854_at	-	2.0	-	-2.3	-	-	1.6	1.5	-	-1.3
Peroxidase BP-2A precursor	Contig1876_s_at	-	-	-	-1.0	-	-	-	-	-	-1.0
Peroxidase C2 precursor-like protein	Contig15862_s_at	-	-	-	-	-	-	-	-	-1.1	-
Peroxidase 2 precursor	Contig2112_at	1.1	-	-	2.0	-	-	-1.1	-	-	1.3
Peroxidase	Contig11361_at	-	-	-	2.1	-	-	-	-	-	-
Peroxidase	Contig11375_at	-	-	-	-	-	-	-	-	-	-
Peroxidase	Contig12191_at	-	-1.7	-	-	-	-	-	-	-	-
Peroxidase	Contig12191_s_at	-	-2.2	-	-	-	-	-1.0	-	-	-
Peroxidase	Contig1862_at	-	-1.8	-	-	-	-	-	-	-	-
Peroxidase	Contig1864_at	-	-1.8	-	-	-	-1.0	-	-1.1	-	1.0
Peroxidase	Contig1865_at	-1.1	-	-	-	-	-	-	-	-	-
Peroxidase	Contig1867_at	-	-	-	-	-	-	-	-	-	-
Peroxidase	Contig1871_at	-	-	-	-	-	-	-	-1.4	-	-
Peroxidase	Contig1874_at	-	-	-	-	-	-	-	-	-	-
Peroxidase	Contig21617_at	-	-	-	-1.3	-	-	-	-	-1.0	-
Peroxidase	Contig4337_at	-	-	-	1.4	-	-	-1.1	-	-	-
Peroxidase	Contig6515_at	-	-1.0	-	-	-	-	-	-	-	-
Peroxidase	Contig6516_at	-	-	-	1.8	-	-	-	-	-	-
Peroxidase	Contig7080_at	2.7	1.2	-	2.6	-	-	-1.4	-	-	1.0
Peroxidase	HVSMEb0011O12r2_at	-	-	-	-	-	-	-	-	-	-
Peroxidase	HVSMEm0005P05r2_at	2.4	-	-	3.9	-	-	-2.8	-	-	1.7

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Pathogenesis-related 9											
Peroxidase	rbah13p07_s_at	1.7	1.0	-	2.4	-	-	-1.4	-1.2	-	1.3
Glutathione peroxidase-like	Contig2453_at	-	-	-	-	-	-	-	-	-	-
Glutathione peroxidase-like	Contig2454_at	-	-	-	-	-	-	-	-1.1	-	-
Pathogenesis-related 10											
PR-10a	Contig4402_s_at	1.3	1.5	-	2.4	-	-	-	-	-	1.6
PR-10a	Contig4405_x_at	1.1	1.3	-	2.4	-	-	-	-	-	1.8
PR-10a	Contig4406_x_at	-	1.1	-	2.4	-	-1.3	-1.2	-	1.2	1.4
Ribonuclease	Contig10672_at	-	-1.8	-	-	-	-	-	-	-	-
Ribonuclease	HD13B05r_s_at	-	-1.5	-	-	-	-	-	-	-	-
Aleurone ribonuclease	Contig3691_at	-	-1.3	-	1.3	-	-	1.5	-	-	-
Pathogenesis-related 12											
Defensin J1-2 precursor	Contig24683_at	-1.1	-1.7	1.5	-	-	-	-	-	-	-
Defensin	Contig3215_s_at	-	1.0	-	-	-	-	-	-	-	-
Defensin	Contig3216_at	-	1.1	-	-	-	-	-	-	-	-
Plant defensin	Contig19785_at	1.1	-	-	-	-	-	-	-	-	-
Pathogenesis-related 13											
Thionin precursor, leaf	Contig1567_x_at	-	-2.4	-	-	-	-	-1.5	-	-1.6	-
Thionin precursor, leaf	Contig1580_x_at	-	-4.2	-	1.9	-	-	-1.3	-	-1.4	1.5
Thionin precursor, leaf	Contig1582_x_at	-	-5.0	-	-	-	-	-3.0	-	-3.3	-
Thionin precursor, leaf	Contig2653_s_at	-	-	-	-	-	-	-	-	-1.1	-
Thionin	Contig1570_s_at	-	-5.7	-	-	-2.5	-	-3.3	-	-4.9	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Pathogenesis-related 13											
Thionin	Contig1579_s_at	-	-	-	-	1.8	-	-	-1.1	1.2	-
Thionin	HVSMEb0010B05r2_x_at	-	-1.3	-	-	-	-	-	-	-	-
Thionin-like	Contig25050_at	-	-	1.3	-	-	-	-	-	-	-
Leaf thionin Asthi3	Contig4731_at	-	2.0	-	-1.2	-	-	1.6	-	-	-
Gamma-thionin	EBed02_SQ002_A05_at	-	-	-	-	-	-	-	-	-	-
Gamma-thionin	EBes01_SQ004_M23_at	-	1.8	-	-1.7	-	-1.1	-	-	-	-1.2
Pathogenesis-related 14											
nsLTP precursor	Contig12237_at	1.0	-	-	1.2	-1.5	-	-	-	-	-
nsLTP precursor	Contig2044_at	-	1.7	-	1.2	1.5	1.5	1.5	-1.3	-	1.4
nsLTP precursor	Contig845_s_at	-	1.4	-	-	-	-	-	-	-	-
nsLTP precursor 4	Contig2041_at	-	1.6	-	-	-	1.1	1.5	-1.8	-	-
nsLTP precursor 4	Contig2041_x_at	-	1.7	-	-	1.2	1.1	1.7	-1.7	-	
nsLTP precursor 4	Contig2046_at	-	-2.1	-	-1.8	-2.0	-1.7	-2.0	2.3	-	-1.9
nsLTP precursor 4	HVSMEn0020F17r2_x_at	-	1.2	-	-	-	1.1	-	-	-	-
nsLTP precursor 4	Contig3482_s_at	-	2.9	-	-1.9	-	-	-	-	-	-1.4
nsLTP	Contig19992_at	-	-	-	-	-	-	-	-	-	-
nsLTP	Contig9857_at	-	1.9	-	-1.8	-	-	-	-	-	-1.3
nsLTP	rbasd16a13_s_at	-	-	-	-	-	-	-	-	-	-
Phospho-LTP	AF039024_at	-	3.2	-	-2.1	-	-	1.5	-	-1.4	-1.3
Phospho-LTP	Contig150_at	-	-	-	-	-	-	-	-	-	-
Phospho-LTP	HA28L22r_s_at	-	-	-	-	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Pathogenesis-related 14											
LTP	Contig3776_s_at	-	-	-	-	1.4	-	-	-	-	-
LTP homolog	Contig3259_at	-	1.1	-	-	-	-	-	-1.3	-	-
Deoxynivalenol Defense											
Sucrose synthase 1	Contig689_s_at	-	-	-	-	-	-	-	-1.9	-	-
Sucrose synthase 2	Contig23306_at	-	-1.0	-	-	-	-	-	-	-	-
Sucrose-phosphate synthase	Contig19734_at	-	-	1.2	-	-	-	-	-1.4	-	-
Glucosyltransferase	Contig14830_at	-	-	-	1.1	-	-	-	-	-	-
Glucosyltransferase	Contig19112_at	-	-1.3	-	-	-	-	-	-1.0	-	-
Glucosyltransferase	Contig19246_at	-1.1	-	-	-	-	-	-	-	-	-
Glucosyltransferase	Contig19290_at	-	-	-	1.6	-	-	-	-	-	-
Glucosyltransferase	Contig5876_at	-	-	-	2.8	-	-	1.1	-	1.6	1.1
Glucosyltransferase	Contig9397_at	-	-	-	1.8	-	-	-	-	-	1.3
Glucosyltransferase NTGT2	HR01O04u_at	-	-	-	1.8	-	-	-	-	-	-
Glucosyltransferase-13	HVSMEn0025G16r2_at	-	-	-	-	-	-	-	-	-	-
Salicylate-induced glucosyltransferase	Contig9824_at	-	-	-	-	-	-	-	-	-	-
Sucrose-UDP glucosyltransferase	Contig602_at	-1.7	-2.4	-	-	-	-	-	-1.3	-	-
Sucrose-UDP glucosyltransferase 1	Contig361_s_at	-	-	-	-	-	-	-	-1.3	-	-
Sucrose-UDP glucosyltransferase 2	Contig481_at	-1.2	-1.5	-	-	-	-	-	-	-	-
Sucrose-UDP glucosyltransferase 2	Contig481_s_at	-1.1	-1.8	-	-	-	-	-	-	-	-
Sucrose-UDP glucosyltransferase 2	Contig823_at	-	-1.6	-	-	-	-	-1.0	-1.3	-	-
Limonoid UDP glucosyltransferase	Contig17260_at	-	-	-	1.4	-	-	-	-	-	-
Cis-zeatin O-glucosyltransferase	HVSMEm0012J18r2_at	-	-	-	1.1	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Deoxynivalenol Defense											
Betanidin 6-O-glucosyltransferase	Contig9823_at	-	-	-	-	-	-	-	-	-	-
UDP-glucose dehydrogenase	HW06A08u_s_at	-1.1	-1.0	-	-	-	-	-	-	-	-
UDP-Glucose-6-dehydrogenase	rbags10b05_at	-1.3	-	-	-	-	-	-	-	-	-
UDP-glucose 4-epimerase	Contig9721_at	-	-	-	-	-	-	-	-	-	-
UDP-glucose 4-epimerase	HVSMEm0008B04r2_s_at	-	-	-	-	-	-	-	-	-	-
Defense Response											
Pectin acetylesterase	Contig9967_at	-	-	-	-2.5	-1.3	-2.0	-	1.5	-	-2.4
Pectin methylesterase	Contig13335_at	-1.0	-1.2	-	-	-	-	-	-	-	-
Pectin methylesterase-like	Contig15812_at	-	3.0	-	-1.8	-	-	1.6	-	-1.5	-1.2
Pectin-glucuronyltransferase	Contig5920_s_at	-1.3	-1.5	-	-	-	-	-	-	-	-
Pectate lyase 2	Contig11004_at	-	-	-	-	-	-	-	-	-	-
Pectate lyase homolog	HVSMEg0010G22r2_at	-	-	1.1	-	-	-1.0	-	-1.3	-	-1.0
Glycine-rich cell wall precursor	Contig10022_at	-	-1.4	-	-	-	-	-	-	-	-
Glycine-rich cell wall structural precursor	Contig1025_s_at	-	1.5	-	-	-	-	-	-	-	-
Glycine-rich cell wall structural precursor	Contig1039_at	-	1.4	-	-1.1	-	-	1.1	-	-	-
Glycine rich	Contig1071_s_at	2.2	2.6	-	-	-	-	-	-	-	-
Glycine rich	Contig9925_at	1.1	1.8	-	1.9	-	-	-1.5	-1.1	-	-
Glycine/proline-rich	HT08F04u_s_at	-	-	-	-	-	-	-	-	-	-
Proline-rich	Contig152_at	-1.7	-	1.0	-	-	-1.1	-	-2.5	-	-2.1
Proline-rich	Contig17838_at	-	-	-	-	-	-	-	-	-	-
Proline-rich	Contig3774_s_at	-	-	-	-1.7	-	-	-	-	-	-
Proline-rich	Contig3777_at	-	2.9	-	-	-	-1.4	-	-	-	-2.7

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Defense Response											
Proline-rich	Contig3782_x_at	-	-	-	-	-	-	-	-	-	-
Proline-rich	Contig444_at	-1.4	-3.1	-	-	-	-	-	-	-	-
Proline-rich	Contig4725_s_at	-	-	-	-	-	-1.1	-1.0	-	-1.5	-1.4
Proline-rich	Contig603_at	-	-1.1	-	-	-	-	-	-	-	-
Proline-rich	Contig704_at	-	-1.2	-	-	-	-	-	-	-	-
Proline-rich	Contig704_x_at	-	-1.3	-	-	-	-	-1.2	-	-	-
Proline-rich	Contig970_at	-	-	-	-	-	-	-	-	-	-
Proline rich homolog WCOR518	Contig4621_at	-	-1.5	-	1.1	-	-	-	-	-2.1	-
Proline rich homolog WCOR518	Contig4622_s_at	-	-1.6	-	1.3	-	-	-	-	-1.1	1.4
Hydroxyproline-rich glycoprotein	Contig553_s_at	-	-2.3	-	-	-	-	-1.4	-1.2	-	-
Extensin-like	Contig14167_at	-	-	-	-	-	-	-	-	-1.1	-
Extensin-like	Contig15167_at	-	1.8	-	-1.2	-	-	-	-	-	-
Extensin-like	Contig4320_at	-	-	-	-	-	-	-	-	-	-
Extensin-like	Contig4321_at	-	-	-	-	-	-	-	-	-	-
Extensin-like	Contig9427_at	-	-	-	-	-	-	-	-	-	-
Extensin-like	Contig9531_at	-	-	-	-	-	-	-	-	-	-
Endo-1,4-β-glucanase precursor	Contig18702_at	-	-1.6	-1.9	-	-	-	-1.6	-	-	-
Endo-1,4-β-glucanase Cel1	Contig4147_at	-	-	-	-	-	-	-	-	-	-
Xyloglucan endo-1,4-β-D-glucanase	Contig2669_at	-	-1.6	1.1	-	-	-	-	-1.0	-	1.3
Xyloglucan endo-1,4-β-D-glucanase	Contig2670_s_at	-	-1.1	-	-	-	-	-	-	-	-
Xyloglucan endo-1,4-β-D-glucanase	Contig2672_at	-	-1.8	-	-	-	-	-	-	-	1.3
Xyloglucan endo-1,4-β-D-glucanase	Contig2673_at	-	-	-	-	-	-	-	-	-	-
Xyloglucan endo-1,4-β-D-glucanase	HE01I24u_s_at	-	-1.0	-	-	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Defense Response											
Xyloglucan endo-1,4-β-D-glucanase	HVSMEb0004L16r2_at	-	-2.0	-	-	-	-	-1.1	-	-	-
1,4-β-xylanase	Contig16010_at	-	-	-	-	-	-	-	-	-	-
Xylanase inhibitor	Contig14679_at	1.6	-	-	2.3	-	-	-2.0	-1.0	-	1.3
Xylanase inhibitor	Contig5996_s_at	-	1.1	-	2.1	-	-1.1	-1.2	-1.7	-1.3	1.3
Xylanase inhibitor	Contig8006_at	-	-	-1.1	-1.9	-	-	-	-	-	-1.2
Xylanase inhibitor I	Contig8905_at	1.8	1.5	-	2.6	-	-1.2	-1.8	-1.4	-	1.0
Glutathione transferase	Contig12776_at	-	-	-	2.8	-	-	-	-	1.3	1.4
Glutathione transferase	Contig12776_s_at	-	-	-	2.4	-	-	-	-	-	1.6
Glutathione transferase	Contig1597_s_at	-	-1.1	-	-	-	-	-	-	-	-
Glutathione S-transferase	Contig14304_at	-	-	-	1.6	-	-	-	-	1.1	-
Glutathione S-transferase	Contig14387_at	-	-	-	1.1	-	-	-	-	-	-
Glutathione S-transferase	Contig16074_at	-	-	-	-	-	-	-	-1.1	-	-
Glutathione S-transferase	Contig18367_at	-	-	-	1.0	-	-	-	-	-	-
Glutathione S-transferase	Contig2489_at	-	-	1.0	1.3	1.1	-	1.3	-	-	1.6
Glutathione S-transferase	Contig4044_at	-	-	-	1.1	-	-	-	-	-	-
Glutathione S-transferase	HO11K23S_s_at	-	-	-	1.0	-	-	-	-	-	-
Glutathione S-transferase	HV_CEb0004O15r2_s_at	-	-	-	1.3	-	-	-	-	-	-
Glutathione S-transferase	rbah38o04_s_at	-	-	-	-	-	-	-	-	-	-
Glutathione S-transferase 1	Contig2975_s_at	-	-1.7	1.2	3.2	-	-	-	-	1.2	1.6
Glutathione-S-transferase 2	Contig21640_at	-	1.3	-	-	-	-	1.1	-1.2	-	-
Glutathione-S-transferase 2	Contig5838_at	-	-	-	1.8	-	-	-	-	-	1.0
Glutathione-S-transferase 2	Contig7448_s_at	-	-	-	1.1	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Defense Response											
Glutathione S-transferase 24	Contig15264_at	1.2	-	-	2.1	-	-	-	-	1.1	1.0
Glutathione S-transferase 34	Contig6008_s_at	-	-	-	1.8	-	-	-	-	-	1.1
Glutathione-S-transferase Cla47	Contig7171_s_at	-	-	-	1.2	-	-	-	-	-	-
Glutathione-S-transferase Cla47	Contig9764_at	-	-1.1	-	3.3	-	-	-	-	1.9	1.6
Glutathione transferase F4	Contig6238_s_at	-	-	-	-	-	-	-	-	-	1.3
Glutathione S-transferase OsGSTU2	Contig2488_s_at	-	-	-	-	-	-	-	-	-	1.1
Thioredoxin	Contig4022_at	-1.3	-	-	-	-	-	-	-	-	-
Thioredoxin	rbaal31m19_s_at	-1.7	-	-	-	-	-	-	-	-	-
Thioredoxin-like	Contig4685_at	-	-	-	-	-	-	-	-	-	-
Hypersensitivity-related	Contig17006_at	-	-	-	-	-	-	-	-	-	-
Hypersensitivity-related	Contig19684_at	-	-1.3	-	-	-	-	-	-	-	-
Hypersensitive-induced reaction 2	Contig6972_at	-	-	-	-	-	-	-	-	-	-
Pathogen-induced WIR1	Contig9917_at	-	-	-	-	-2.0	-2.1	-1.4	-	-	-
Pathogen-induced WIR1A	Contig2163_at	-	-	-	-	-2.0	-1.7	-1.4	-	-	-
Pathogen-induced WIR1A	Contig5974_s_at	-	-	-	2.2	-1.1	-1.4	-	1.1	-	-
Pathogen-induced WIR1A	Contig6519_at	-	1.5	-	-	-	-	-	-1.3	-	1.5
Pathogen-induced WIR1A	Contig939_s_at	1.5	-	-	-	-1.2	-1.1	-	-	-	-
Bowman-birk type trypsin inhibitor	Contig17082_at	1.1	1.7	-	1.2	-	-	-1.4	-	-	-
Bowman-birk type trypsin inhibitor	Contig18032_at	-	-	-	2.8	-	-	-1.1	-	-	-
Bowman-birk type trypsin inhibitor	Contig2087_s_at	-	-	-	-	-	-	-	-	1.0	-
Alcohol dehydrogenase	Contig278_at	-	-1.3	-	1.1	-	1.3	-	1.9	-1.6	-
Alcohol dehydrogenase	Contig393_at	-	-	-	1.0	-	-	-	-	-	-

		Mock		*Tri5-*		*Tri5+*		DON		*Tri5-* (DON)	
Gene Target Description*	**Probe Set ID**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**	**8 hpi**	**24 hpi**
Defense Response											
Alcohol dehydrogenase	Contig431_at	-	1.6	-	-1.5	-	-1.4	-	-1.1	2.3	-1.4
Allyl alcohol dehydrogenase	Contig19680_at	-1.1	-1.5	-	-	-	-	-	-	-	-
Allyl alcohol dehydrogenase	Contig5939_at	-	-1.9	-	-	-1.1	-1.1	-1.6	1.8	-	-1.3
Allyl alcohol dehydrogenase	HI04E24r_s_at	-1.1	-1.0	-	-	-	-	-	-	-	-
Allyl alcohol dehydrogenase	HVSMEg0006O20r2_at	-	-1.1	-	-	-	-	-	-	-	-
Short chain alcohol dehydrogenase	Contig20405_at	-	-	-	1.5	-	-	-	-	-	-
Short chain alcohol dehydrogenase	Contig6362_s_at	-	-	-	1.1	-	-	-	-1.5	-	-
Short-chain type alcohol dehydrogenase	Contig20683_at	-	-1.2	-	1.8	-	-	-	-	-	-

Note: Pink represents up-regulated transcripts; blue represents down-regulated transcripts.

*PR=pathogenesis-related, TLP=thaumatin-like protein, nsLTP=nonspecific lipid transfer protein, LTP=lipid transfer protein.

APPENDIX 4

Identification of differentially expressed proteins associated with an oxidative burst, the phenylpropanoid and jasmonic acid antioxidant signaling pathways, pathogenesis, deoxynivalenol defense, and general plant defense responses in Chevron following inoculation with *Tri5*- (strain GZT40), *Tri5*+ (strain GZ3639), DON, GZT40 supplemented with DON (*Tri5*-(DON)), and mock inoculum. Values log_{10}-transformed.

			Mock	***Tri5-***	***Tri5+***	**DON**	***Tri5-* (DON)**
Protein Identification*	**Accession #**	**0 dpi**	**3 dpi**	**3 dpi**	**3 dpi**	**3 dpi**	**3 dpi**
Oxidative burst							
Glycolate oxidase	Q6YT73_ORYSA	-	1.46	-	-	1.69	-
L-ascorbate oxidase homolog	Q70JQ4_WHEAT	-	-	-	-	-	0.68
(S)-2-hydroxy-acid oxidase	T02150	-	-	1.73	1.94	-	1.94
Coproporphyrinogen oxidase	T04486	-	-	0.60	0.48	-	-
Phenylpropanoid pathway							
Phenylalanine ammonia-lyase	Q52QV1_9POAL	-	0.47	-	0.65	0.45	0.46
Phenylalanine ammonia-lyase	T05968	-	0.50	0.49	0.37	0.47	0.46
Caffeic acid O-methyltransferase	Q84N28_WHEAT	-	-	-	-	0.74	-
Chalcone isomerase	Q8S3X0_HORVD	-	0.67	-	0.47	0.62	-
Flavonoid O-methyltransferase	Q38J50_WHEAT	-	0.26	0.31	0.33	0.38	-
Isoflavone reductase	Q9FTN5_ORYSA	-	-	0.60	0.65	-	0.56
2'-hydroxyisoflavone reductase	T02304	-	-	-	-	-	0.74

Protein Identification*	Accession #	0 dpi	Mock 3 dpi	*Tri5-* 3 dpi	*Tri5+* 3 dpi	DON 3 dpi	*Tri5-* (DON) 3 dpi
Jasmonic acid pathway							
Lipoxygenase	T05941	-	1.58	-	1.96	1.48	1.42
Lipoxygenase	T05943	-	-	-	-	-	-
Lipoxygenase 2	T05945	-	1.33	1.39	1.73	1.41	1.49
Lipoxygenase 2	T06190	0.80	0.95	-	-	-	-
Jacalin-like LEM2	Q8L8I5_HORVU	0.16	0.15	0.20	0.12	0.08	0.07
Jasmonate-induced 1	S22514	-	-	-	-	0.92	-
PR-1							
PR-1a	S37166	-	-	-	3.04	-	-
PR-3, -4, -8,-11							
Chitinase cht2b precursor	S48848	-	-	-	-	-	-
Chitinase precursor	T04403	-	-	-	2.15	-	-
PR-4	T06169	-	-	2.43	3.55	-	2.93
PR-5							
Permatin homolog PR5	T05973	-	1.38	-	2.70	1.88	2.34
TLP3	Q5MBN3_HORVU	-	15.80	-	-	8.13	-
TLP8	Q946Y8_HORVU	-	1.48	-	-	2.11	-
PR-7							
Aspartic proteinase	S19697	-	-	-	-	-	-
Aspartic proteinase	T06213	-	3.90	-	-	3.61	-
PR-9							
Dehydroascorbate reductase	Q84UH6_WHEAT	-	-	0.47	0.55	-	0.70
Ascorbate peroxidase	O23983_HORVU	-	0.70	-	-	-	-
Thioredoxin peroxidase	O81480_SECCE	-	-	-	-	-	-
Peroxidase precursor	Q5GMP4_WHEAT	-	-	-	-	-	-

			Mock	*Tri5-*	*Tri5+*	DON	*Tri5-* (DON)
Protein Identification*	**Accession #**	**0 dpi**	**3 dpi**	**3 dpi**	**3 dpi**	**3 dpi**	**3 dpi**
PR-9							
Thioredoxin peroxidase 1	Q5Q0X6_SACOF	-	0.64	-	-	-	-
Ascorbate peroxidase	Q945R5_HORVU	-	-	-	0.42	-	-
PR-13							
Thionin precursor, leaf	S22515	0.43	-	-	-	-	-
PR-14							
LTP	Q9SES6_HORVU	-	1.29	-	1.74	1.04	-
LTP 7a2b	T05950	1.25	-	-	1.37	-	-
Deoxynivalenol Defense							
Sucrose synthase	Q8W1W4_BAMOL	-	-	0.48	0.55	-	0.38
UDP-glucose dehydrogenase	Q9AUV6_ORYSA	-	-	0.34	0.30	0.46	0.43
UDP-glucose dehydrogenase	Q9LIA8_ARATH	-	-	-	0.46	-	0.48
UDP-glucose 6-dehydrogenase	T08818	-	-	-	-	-	-
Defense Response							
Catalase	Q5ZEQ9_SECCE	-	0.72	-	-	-	-
Catalase isoenzyme 1	S62696	-	-	-	1.18	-	-
Superoxide dismutase (Cu-Zn) precursor	T03685	-	-	-	-	-	-
Glutathione transferase F5	Q8GTB8_WHEAT	-	-	-	-	-	-
Thioredoxin H	Q8GVD3_WHEAT	-	-	-	-	-	-
14-3-3b	T04406	-	-	-	-	-	-
14-3-3	T06203	-	-	2.16	2.61	-	1.76
14-3-3 homolog	S18911	-	0.62	0.43	0.45	-	0.48

Note: The differential expression values presented are $\log_{10}$-transformed.

Pink represents proteins with higher abundance; blue represents proteins with lower abundance.

*PR=pathogenesis-related, TLP=thaumatin-like protein, LTP=lipid transfer protein.

APPENDIX 5

Identification of differentially expressed proteins associated with an oxidative burst, the phenylpropanoid and jasmonic acid antioxidant signaling pathways, pathogenesis, deoxynivalenol defense, and general plant defense responses in Stander following inoculation with *Tri5*- (strain GZT40), *Tri5*+ (strain GZ3639), DON, GZT40 supplemented with DON (*Tri5*-(DON)), and mock inoculum. Values log_{10}-transformed.

		Mock	***Tri5-***	***Tri5+***	**DON**	***Tri5-* (DON)**
Protein Identification*	**Accession #**	**3 dpi**	**3 dpi**	**3 dpi**	**3 dpi**	**3 dpi**
Oxidative burst						
Glycolate oxidase	Q6YT73_ORYSA	-	-	-	-	-
L-ascorbate oxidase homolog	Q70JQ4_WHEAT	-	-	-	-	-
(S)-2-hydroxy-acid oxidase	T02150	-	-	-	-	-
Coproporphyrinogen oxidase	T04486	0.75	-	-	0.77	-
Phenylpropanoid pathway						
Phenylalanine ammonia-lyase	Q52QV1_9POAL	0.53	-	-	0.58	-
Phenylalanine ammonia-lyase	T05968	0.56	-	-	0.62	-
Caffeic acid O-methyltransferase	Q84N28_WHEAT	0.49	0.42	-	0.60	0.46
Chalcone isomerase	Q8S3X0_HORVD	-	-	-	-	-
Flavonoid O-methyltransferase	Q38J50_WHEAT	-	0.16	0.19	-	0.17
Isoflavone reductase	Q9FTN5_ORYSA	0.74	-	-	0.57	-
2'-hydroxyisoflavone reductase	T02304	-	-	-	-	-

		Mock	*Tri5-*	*Tri5+*	DON	*Tri5-* (DON)
Protein Identification*	**Accession #**	**3 dpi**	**3 dpi**	**3 dpi**	**3 dpi**	**3 dpi**
Jasmonic acid pathway						
Lipoxygenase	T05941	-	-	-	-	-
Lipoxygenase	T05943	-	-	0.44	-	-
Lipoxygenase 2	T05945	1.42	1.30	-	1.46	1.44
Lipoxygenase 2	T06190	0.94	-	-	1.03	-
Jacalin-like LEM2	Q8L8I5_HORVU	0.62	-	-	0.78	-
Jasmonate-induced 1	S22514	1.73	1.62	-	1.68	1.88
PR-1						
PR-1a	S37166	-	5.36	6.19	3.50	5.15
PR-3, -4, -8, -11						
Chitinase cht2b precursor	S48848	-	3.04	-	-	3.16
Chitinase precursor	T04403	-	-	-	-	-
PR-4	T06169	-	-	-	-	-
PR-5						
Permatin homolog PR5	T05973	2.02	-	-	3.19	-
TLP3	Q5MBN3_HORVU	-	-	-	-	-
TLP8	Q946Y8_HORVU	-	-	-	-	-
PR-7						
Aspartic proteinase	S19697	1.41	-	-	1.30	-
Aspartic proteinase	T06213	-	-	-	-	-
PR-9						
Dehydroascorbate reductase	Q84UH6_WHEAT	-	0.38	0.32	-	0.48
Ascorbate peroxidase	O23983_HORVU	-	-	-	-	-
Thioredoxin peroxidase	O81480_SECCE	-	-	-	1.17	-
Peroxidase precursor	Q5GMP4_WHEAT	-	-	-	-	0.85
Thioredoxin peroxidase 1	Q5Q0X6_SACOF	-	-	-	-	-

		Mock	***Tri5-***	***Tri5+***	**DON**	***Tri5-*** **(DON)**
Protein Identification*	**Accession #**	**3 dpi**	**3 dpi**	**3 dpi**	**3 dpi**	**3 dpi**
PR-9						
Ascorbate peroxidase	Q945R5_HORVU	0.90	-	-	-	-
PR-13						
Thionin precursor, leaf	S22515	1.60	-	-	1.70	-
PR-14						
LTP	Q9SES6_HORVU	1.66	2.05	2.28	1.41	1.87
LTP 7a2b	T05950	-	-	1.48	1.14	-
Deoxynivalenol Defense						
Sucrose synthase	Q8W1W4_BAMOL	0.39	-	-	0.44	-
UDP-glucose dehydrogenase	Q9AUV6_ORYSA	0.51	-	-	0.52	-
UDP-glucose dehydrogenase	Q9LIA8_ARATH	-	-	-	-	-
UDP-glucose 6-dehydrogenase	T08818	0.47	-	-	-	-
Defense Response						
Catalase	Q5ZEQ9_SECCE	1.09	-	1.90	-	-
Catalase isoenzyme 1	S62696	-	-	-	-	-
Superoxide dismutase (Cu-Zn) precursor	T03685	-	-	1.31	-	-
Glutathione transferase F5	Q8GTB8_WHEAT	-	-	0.97	-	-
Thioredoxin H	Q8GVD3_WHEAT	-	-	-	0.79	-
14-3-3b	T04406	-	-	2.97	-	-
14-3-3	T06203	-	-	-	-	-
14-3-3 homolog	S18911	0.66	-	-	-	-

Note: The differential expression values presented are $\log_{10}$-transformed.

Pink represents proteins with higher abundance; blue represents proteins with lower abundance.

*PR=pathogenesis-related, TLP=thaumatin-like protein, LTP=lipid transfer protein.