uOttawa

Characterizing differentially expressed genes from the *Thinopyrum elongatum* 7EL chromosome region that is responsible for FHB resistance, after introgression in *Triticum aestivum*

Aparna Haldar

Thesis submitted to the Faculty of Graduate and Postdoctoral Studies in partial fulfillment of the requirements for the degree of

Master of Science in Biology

Supervised by:
Dr. Thérèse Ouellet

Department of Biology
Faculty of Science
University of Ottawa

January 2019

ABSTRACT

Triticum aestivum (bread wheat) is an important cereal crop not only in Canada but also worldwide. The pathogen *Fusarium graminearum* is responsible for causing the disease fusarium head blight and generates yield loses and mycotoxin contaminated grains, including in wheat. A strategy used to mitigate this problem is through the production of FHB resistant wheat varieties by crossing strongly resistant germplasms from closely related wheat species. *Thinopyrum elongatum* is a wild wheat grass that carries genetic resistance to FHB on the long arm of its chromosome 7E (7EL). Previous work has developed genetic material by crossing Chinese Spring (CS) ph1b line with a CS-7E(7D) substitution line to facilitate introgression of 7E fragments from *Thinopyrum* into the 7D chromosome of wheat. In the first part of this project a genetic order for previously designed 7EL- and 7D- specific markers was proposed using IWGSC RefSeq v1.0 and was used to characterize the introgressed material from the above cross. Progeny from BC_1F_7 and BC_1F_5 families of different lineages were genotyped and phenotyped to characterize regions of introgression which were estimated to be at least 42 and 22 Mbp respectively. Gene expression analysis was also performed for selected 7EL genes. Results showed that the expression of selected 7EL genes present within the introgressed fragments were highly variable between the three families characterized as well as within families. It was also observed that the 7EL introgressed progeny had variable expression when compared to the addition line CS-7EL. Additionally gene expression analyses were also performed using 7D genes. These results showed that there was variation in 7D gene expression between the 7EL introgressed progeny and the controls CS-Fg and addition line CS-7EL-Fg. Possible explanations regarding the variation in gene expression includes differential methylation patterns, silencing of

genes in the progeny, alteration of repetitive sequences or activation of transposable elements. Further research will be needed to test these hypotheses.

ACKNOWLEDGEMENTS

I would like to thank many people who generously contributed their time and efforts to make this thesis possible. First and foremost I would like to thank my thesis advisor Dr. Thérèse Ouellet for giving me the opportunity to work in her lab and for her continued support and kindness. This project would not have been feasible without her help. I would also like to thank our lab technicians Margaret Balcerzak and Hélène Rocheleau for their advice and invaluable help in troubleshooting my experiments. It was a pleasure to work with everyone in the lab.

I am also grateful to my committee members Dr. Doug Johnson and Dr. Johann Schernthaner for their time and feedback on my research reports. Additionally, I would like to thank the University of Ottawa and Agriculture and Agri-Food Canada for funding and facilitating this project.

I would also like to thank my friends for their support and sense of humour which helped me throughout this process. Lastly, my special and warmest thanks go to my parents, who supported me through this journey and for their encouragement during tough times.

Table of Contents

List of Figures

List of Tables

List of Appendices

List of Abbreviations

Abbreviations	Explanation
7EL	Long arm of chromosome 7E
AAFC	Agriculture and Agri-Food Canada
AFLP	Amplified Fragment Length Polymorphism
$BC_1F_{(1-7)}$	First Back Cross, (First-Seventh) Family
BLAST	Basic Local Alignment Search Tool
CMC	Carboxy-Methyl-Cellulose
Cq	Quantification Cycle
CS	Chinese Spring
CS-7E	CS addition line (full wheat genome & full 7E chromosome)
CS-7E(7D)	CS substitution line (7E chromosome replaced wheat 7D chromosome)
CS-7EL	CS addition line (full wheat genome & long arm of chromosome 7E)
CS-7ES	CS addition line (full wheat genome & short arm of chromosome 7E)
CS-ph1b	CS carrying an inactive ph1 locus
DNA	Deoxyribonucleic Acid
DON	Deoxynivalenol

DPI	Days Post Inoculation
FHB	Fusarium head blight
IDT	Integrated DNA Technologies
IWGSC	International Wheat Genome Sequencing Consortium
LTR	Long terminal repeat
MITEs	Miniature Inverted Transposable Elements
Mbp	Millions of base pair
NRC	National Research Council
PCR	Polymerase Chain Reaction
PFT	Pore forming toxin
Ph1	Pairing homoeologous 1
Ph2	Pairing homoeologous 2
QTL	Quantitative Trait Loci
RefSeq	Reference Sequence
RNA	Ribonucleic Acid
RNA-Seq	Ribonucleic Acid-Sequencing
RT-qPCR	Reverse Transcription-quantitative Polymerase Chain Reaction
SLAF-Seq	Specific Length Amplified Fragment sequencing

SNA	Spezieller-Nährstoffer Agar
TAE	Tris-acetate EDTA

Chapter 1: Introduction

Cereal crops comprising wheat, barley, rice, and maize are the main sources of food in many parts of the world. According to the Food and Agriculture Organization of the United Nations, world cereal utilization for 2017/18 is forecast at 2,641 million tonnes (FAO of the UN, 2018)(http://www.fao.org/worldfoodsituation/csdb/en/, date accessed August 2018). In 2016/17 a total of 752 million metric tonnes of wheat was produced worldwide (USDA, https://apps.fas.usda.gov/PSDOnline/app/index.html#/app/home, date accessed August 2018). Canada is the fifth largest producer of wheat in the world and the second largest in North America behind the United States (USDA, 2018). In 2017, Canada was responsible for producing a total of 30 million metric tonnes of wheat, which is approximately 1.1% of total world production. Figure 1 shows the percent breakdown of winter wheat, spring wheat and durum wheat produced in each of the provinces.

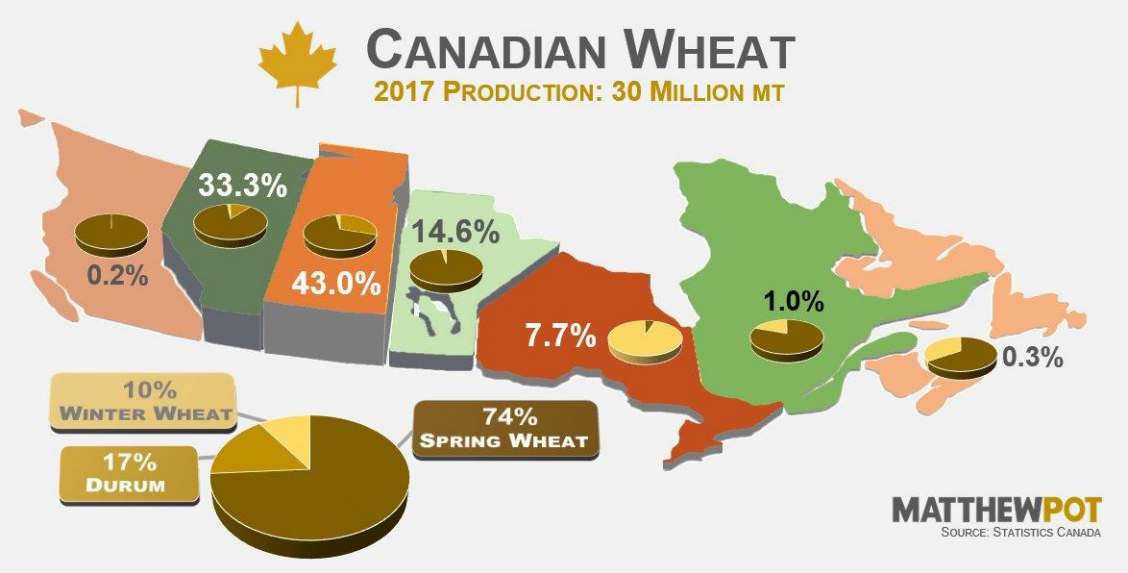


Figure 1: Percentage of wheat produced in 2017 across all provinces in Canada.
Figure courtesy of: (Pot, 2017).

Besides production, wheat is also an important export of Canadian markets. Therefore, maintaining a certain level of crop yields is important for the Canadian economy. Crop yields can be affected by abiotic factors such as droughts or increases in temperatures or by biotic factors such as bacteria, viruses or fungi causing diseases. Fusarium head blight (FHB) is a major fungal disease affecting wheat in Canada. The economic loss resulting from FHB in Alberta was estimated to be around $12 million in 2016 (Komirenko, 2018) (https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex92#grading, date accessed August 2018).Therefore, mitigating losses due to FHB is important not only for wheat production but also for Canada's economy. In this project the addition of a novel source of resistance to wheat was examined, and its effect towards FHB resistance.

1.1 Structure of the thesis

This thesis is divided into four chapters. The most relevant literature and recent research progress in the field is described in the first chapter. The second chapter focuses on detailed objectives and methodologies. The third chapter will include experimental results and data analysis. Finally the fourth chapter will focus on a broader discussion of the results, conclusions and directions for future work.

1.2 Wheat – A genetic overview

Wheat, otherwise known as *Triticum*, is part of the grass family *Poaceae*. Of the cultivated wheats, hexaploid common wheat (*Triticum aestivum*, AABBDD genomes, 2n=6x=42) and tetraploid durum wheat (*Triticum turgidum*, AABB genomes, 2n=4x=28) are the two main types. *T. aestivum* is generally used for the production of bread and cakes while *T. turgidum* is used to produce pasta products. The ancestors that comprise the A and B genomes of *T. aestivum* and *T. turgidum* are *Triticum urartu* and *Aegilops speltoides* respectively. A subsequent hybridization

with diploid plant (*Aegilops tauschii*) contributed the D genome which led to the production of the hexaploid *Triticum aestivum* species (IWGSC, 2014). Each of the diploid A, B and D genomes contain seven pairs of homologous chromosomes. Homologous chromosomes are the same size and shape; they also contain the same genes in the same order but may contain different alleles. Aside from homologous chromosomes, the wheat genome also contains homoeologous chromosomes. These chromosomes have the same number but are derived from different ancestors such as 2A, 2B and 2D (Figure 2). Homoeologous chromosomes have similar gene content and order but have different repetitive DNA content.

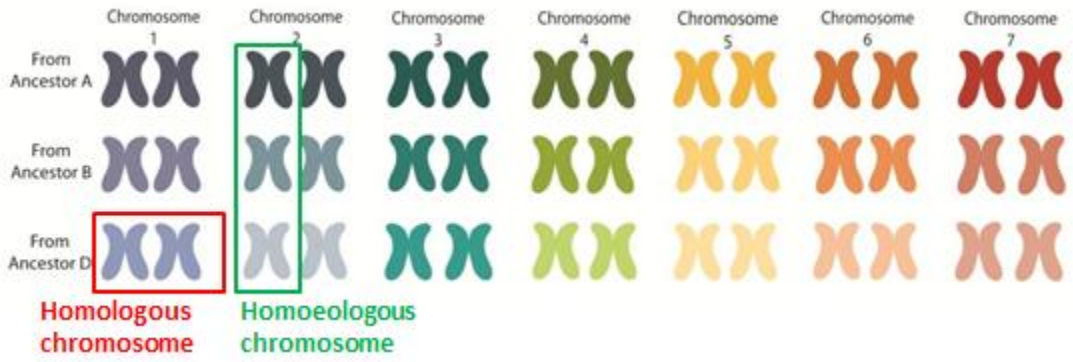


Figure 2: Structure and organization of *Triticum aestivum* chromosomes.
Note chromosomes are not drawn to scale. Modified from (Colorado Wheat, 2018).

The size of the wheat genome has been shown to be 16 giga-base pairs, while containing approximately 107,891 high confidence protein coding loci across the A, B and D subgenomes (IWGSC, 2018). In addition the wheat genome contains many repetitive DNA sequences, totalling around 80% of the genome. These characteristics made sequencing the wheat genome rather challenging. Recently the International Wheat Genome Sequencing Consortium (IWGSC) were able to generate a high quality genome sequence for *T. aestivum* cv. *Chinese Spring* (CS) using next generation sequencing and physical maps integrated with other sequence resources (IWGSC, 2018). In December 2015, a whole genome assembly of CS was produced from

Illumina short sequence reads. Following this, a further refined version of the reference sequence v0.4 was released in June 2016. In January 2017 the IWGSC Reference Sequence (RefSeq) v1.0 was released which contained pseudomolecules for each chromosome in the wheat genome. These pseudomolecules were generated by integrating information from physical maps, BAC sequencing and whole genome profiling tags. This was followed by the official release of RefSeq version 1.0 to the public on August 2018 (IWGSC, 2018). This RefSeq database was particularly helpful in determining positions of DNA markers present throughout the genome.

1.3 *Fusarium graminearum* causing Fusarium Head Blight (FHB)

Fusarium head blight (FHB) is a fungal disease of cereal crops affecting wheat, barley, oats, rye and many other forage grasses. There are at least 17 *Fusarium* species associated with this disease, but among them *Fusarium graminearum* Schwabe (*Hyocreales: Nectriaceae*) (*F. graminearum*) is the most devastating pathogen for wheat and barley, especially in North America (Wegulo, 2012). Infection by this pathogen not only causes significant yield losses but also a reduction in grain quality due to contamination of the crop with mycotoxins. The most abundant mycotoxin produced by FHB is deoxynivalenol (DON) – a member of the tricothecene family – also referred to as vomitoxin. Therefore, Health Canada has issued recommended advisory levels for DON which is 2.0ppm in uncleaned soft wheat for use in non-staple foods and 1.0ppm in uncleaned soft wheat for use in baby foods (Health Canada, 2016) (http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/contaminants-guidelines-directives-eng.php, date accessed July 2018). Over consumption of infected wheat products in humans leads to symptoms such as vomiting, nausea, abdominal pain, diarrhoea and headaches (Desjardins, 2006). Since wheat is also used in animal feed, contamination with mycotoxins such as DON also leads to adverse effects in animals. In animals, these symptoms manifest as feed refusal and

weight loss. The monetary loss caused by FHB has led to renewed efforts to search for better strategies to reduce this problem.

The *F. graminearum* infection process starts with the deposition of fungal spores on or inside spikelet tissues. Hyphae then develop on the exterior surface of florets and glumes allowing for the fungus to grow towards the stomata and other susceptible sites within the inflorescence (Goswami and Kistler, 2004). Hyphae can form lobed structures between the cuticle and the epidermal cell wall on the surface of inoculated glumes. This subcuticular growth is thought to serve as a mechanism of fungal spread and could lead to direct penetration of epidermal cells. Once inside the floret, the anthers and stigma are easily colonised. In wheat, the principle mode of fungal spread is through the vascular bundles in the rachis and rachilla (Goswami and Kistler, 2004). Several changes in the vascular bundles can cause the xylem and phloem tissues in the infected rachis to become dysfunctional leading to premature death of the spikelet (Brown et al., 2010). The spreading of the fungus is associated with the spreading of DON, a virulence factor responsible for tissue necrosis. This fungus also appears to have a brief biotrophic relationship with its host before switching to a necrotrophic phase. In the necrotrophic phase an increase in colonization by the fungus is seen, that eventually leads to cell death.

1.4 FHB resistance and Quantitative Trait Loci (QTLs)

One of the strategies used to mitigate losses from FHB is the application of fungicides. However, the application cost, pollution to the environment and low level of protection causes farmers to be dissatisfied with fungicides. Therefore, production of FHB resistant wheat cultivars is a good solution towards reducing FHB and minimizing mycotoxin contamination. To produce FHB resistant cultivars, the type of FHB resistance needs to be examined. There are two major types of FHB resistance, type I and type II (Yu et al., 2008). Type I resistance focuses on resistance to

initial penetration by the pathogen while type II resistance focuses on resistance to spread of FHB symptoms within an infected spikelet (Yu et al., 2008). Additionally, three other minor types of FHB resistance exist. Type III resistance deals with accumulation of DON, type IV is associated with kernel infection and type V is related to yield reduction (He et al., 2016). Field spraying and scoring disease incidence in early stages (around 15 days post inoculation) is associated with type I resistance. Type II resistance is measured through point inoculation, where the inoculum is directly injected into the spikelets. Finally, type III and type IV resistance are related to post-harvest traits where DON content and *Fusarium* damaged kernels are taken into account (He et al., 2016).

Resistance to FHB in wheat is quantitatively inherited and the overall resistance of a given variety arises from the combined effect of several resistance genes. Therefore, various wheat populations have been analysed for the presence of quantitative trait loci (QTL). A QTL is typically related to genes that control a desired phenotype. So far, hundreds of QTL's associated with FHB resistance have been identified in wheat (Buerstmayr et al., 2009). *Fhb1* derived from the Chinese cultivar Sumai 3 has been shown to provide moderately high level of genetic resistance against FHB (Rawat et al., 2016). The *Fhb1* locus is located on chromosome 3BS of wheat and contains a few genes thought to provide broad spectrum resistance against various isolates and species of *Fusarium*. Recent research has identified a potential gene present in this QTL (Rawat et al., 2016). Through mutation analysis, gene silencing and transgenic overexpression experiments it was shown that a pore-forming toxin-like (*PFT*) gene at *Fhb1* confers FHB resistance (Rawat et al., 2016). The predicted PFT protein is a chimeric lectin with two agglutinin domains and a toxin domain. Plant lectins are a heterogeneous group of proteins

that reversibly bind to carbohydrates and play a role in plant defense. However, further studies are required to understand the mechanism of PFT action.

1.5 FHB resistant materials – *Thinopyrum elongatum* introduction

The creation of FHB resistant wheat cultivars is used by breeders not only to prevent FHB and mycotoxin contamination but also used to characterize resistance mechanisms and propose novel improvement strategies. In recent years resistant cultivars *Praag8*, *Sumai 3* and *Sumai 3*-derived lines, *Frontana*, *Wangshuibai*, *Nyu Bai* and *Wuhan 1* were identified as good sources of resistance against FHB (Buerstmayr et al., 2009). There are a limited number of QTLs that are effectively used in breeding programs to improve FHB resistance, thus addition of novel resistant sources can help breeders decrease wheat vulnerability to FHB.

Recently researchers have found that *Thinopyrum elongatum* (*Th. elongatum*) (2n=14; E genome) is strongly resistant to FHB (Shen and Ohm, 2006). *Thinopyrum* is part of the large grass family Poaceae, containing monocotyledon flowering plants including cereals. Figure 3 shows the relationship between *Thinopyrum* and wheat as well as other members in the grass family. Based on the phylogenetic relationship, it is observed that *Thinopyrum* species have a closer evolutionary relationship to *Triticum aestivum* than to *Hordeum vulgare* (barley), *Brachypodium distachyon* or *Oryza sativa* (Rice).

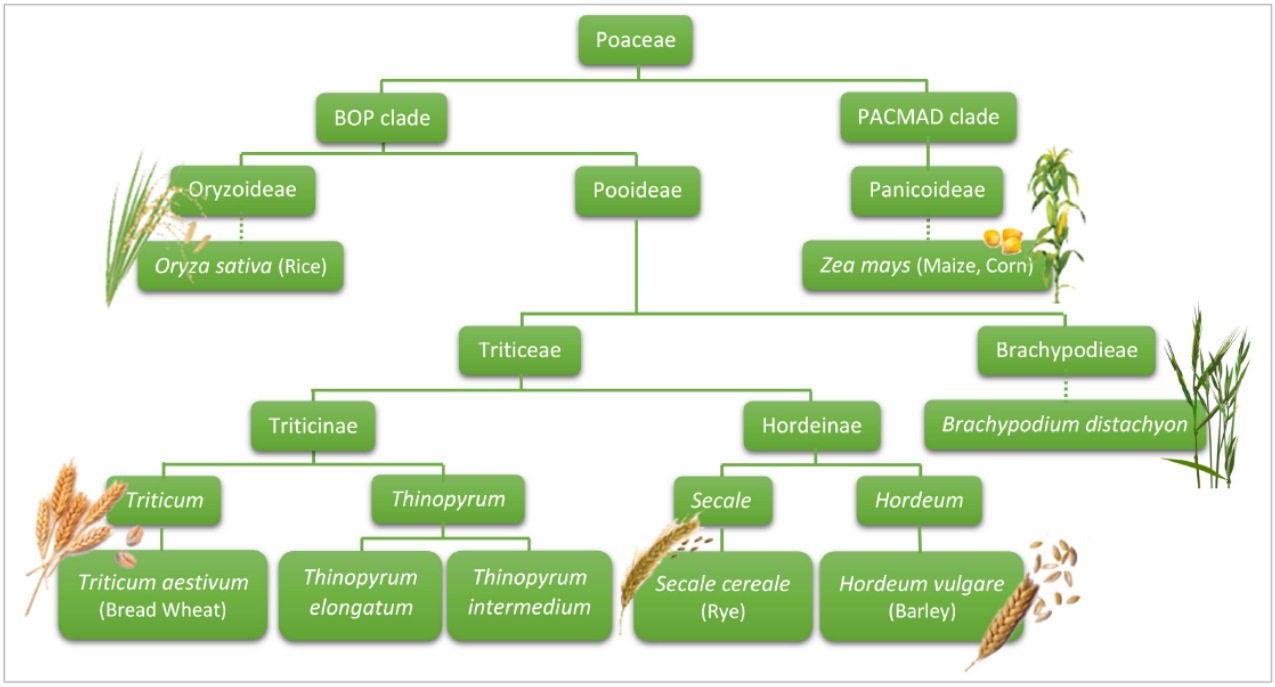


Figure 3: Phylogenetic relationship between *Thinopyrum* and other members of the grass family. Modified from F. Tekieh thesis (Tekieh, 2016).

Th. elongatum is a wild grass commonly referred to as tall wheatgrass, from the tribe *Triticeae* and carries genetic resistance to FHB on the long arm of chromosome 7E (7EL) (Figure 4) (Shen et al., 2004).

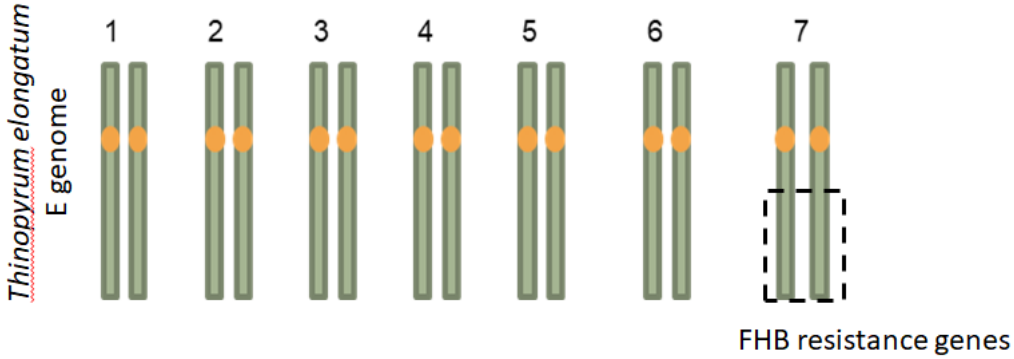


Figure 4: Position of FHB resistance gene(s) on the *Th. elongatum* chromosomes. Note figures not drawn to scale.

Th. elongatum also has good agronomical characteristics such as tolerance to drought and salinity present on chromosome 1 (Garg et al., 2009) as well cold resistance (Roundy, 1985;

Dvorák et al., 1988). It also has high grain protein content and is resistant to leaf and stripe rust (Luo et al., 2009). Secondly, chromosome 4 present in *Thinopyrum* species allows wheat to become resistant to necrotrophic eyespot and root pathogens by increasing the plant's regrowth ability after senescence (Okubara and Jones, 2011). Since *Thinopyrum* is a wild wheatgrass it has lower grain yield when compared to high yield bread wheat varieties. However, these desirable traits from *Th. elongatum* provide a source of potential new genes that can be used by breeders to improve wheat.

1.6 Pairing homoeologous genes and wheat breeding

Because of its attractive disease resistance characteristics, *Th. elongatum* was crossed with wheat in an attempt to produce disease resistant cultivars. Addition of the homoeologous chromosomes from different germplasms can be relatively stable in wheat as additional chromosomes; however introgression and chromosome pairing rarely happened between wheat and alien chromosomes. The pairing homoeologous1 (*Ph1*) locus present on the long arm of chromosome 5B acts as the major regulator for chromosome pairing and recombination between wheat and alien chromosomes (Greer et al., 2012). During meiosis, *Ph1* ensures that recombination occurs only between pairs of homologous chromosomes and prevents recombination between homoeologous chromosomes (Greer et al., 2012). Deletion of the *Ph1* locus in wheat triggers two phenotypic effects. Firstly, chromosome pairing is disrupted which leads to synapsis of homoeologous chromosomes and secondly homoeologous recombination is induced. Therefore, deletion of the *Ph1*locus allows for hybridization between homoeologous chromosomes of wheat and *Th. elongatum*, leading to introgression of *Th. elongatum* DNA into wheat chromosomes (Figure 5).

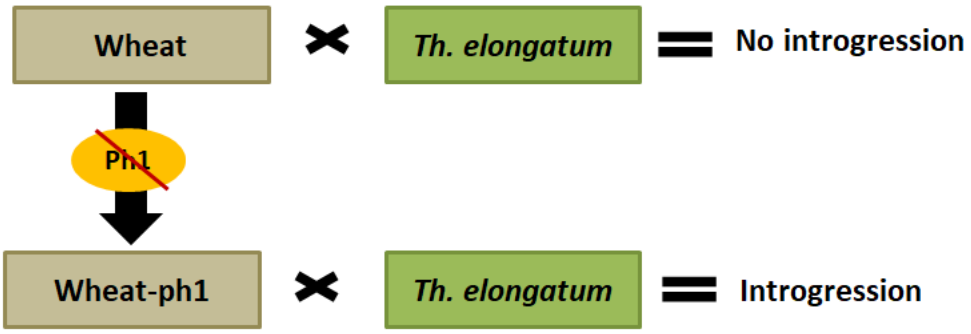


Figure 5: Role of *Ph1* locus in homoeologous chromosome pairing between wheat and *Th. elongatum*.

In addition to the *Ph1* locus, a second suppressor of homoeologous chromosome pairing was identified. This includes the *Ph2* locus which is located on the short arm of chromosome 3D (Sutton et al., 2003). Deletion of the *Ph2* locus induces an intermediate level of homoeologous chromosome pairing in wheat hybrids with alien species but does not affect chromosome pairing in wheat itself (Sutton et al., 2003). Studies have also shown that the *Ph2* locus affects the progression of synapsis, in a similar way to other diploid species (Martinez et al., 2001). The effect on homoeologous pairing in hybrids lacking the *Ph2* locus would be an indirect effect of the action of the *Ph1* locus (Martinez et al., 2001). Therefore, the *Ph2* locus has a secondary role compared to the *Ph1* locus in homoeologous chromosome pairing.

1.7 CS-7E: addition and substitution lines

Chinese Spring (CS) is a moderately FHB susceptible wheat cultivar. CS has been crossed with *Th. elongatum* to develop many addition and substitution lines. Addition lines contain the full complement of wheat chromosomes and one chromosome or parts of a chromosome from *Th. elongatum*. Since FHB resistance is found on chromosome 7E of *Th. elongatum*, the addition lines of interest are CS-7E, CS-7EL (containing only long arm of chromosome 7E) and CS-7ES (containing only the short arm of chromosome 7E) (Dvorak and Knott, 1974; Dvořák, 1979)

(Figure 6). Substitution lines were also created where chromosome 7E replaced one of the wheat chromosomes: CS-7E(7A), CS-7E(7B) and CS-7E(7D) (Dvořák, 1980) (Figure 6).

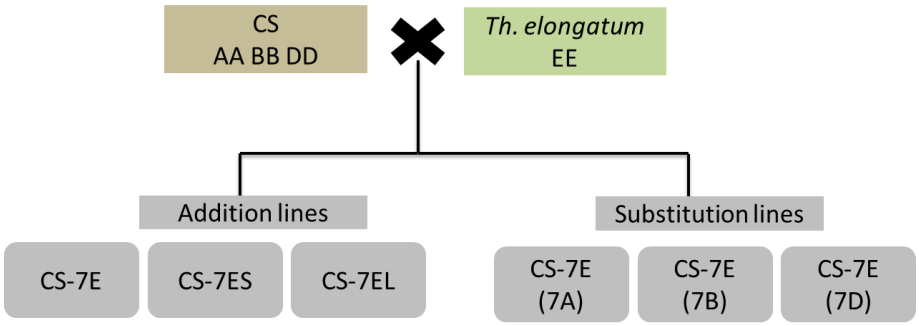


Figure 6: 7E-carrying addition and substitution lines obtained by crossing CS and *Th. elongatum*.

Inoculation experiments have looked at FHB resistance in the addition lines CS-7E, CS-7ES and CS-7EL. Results demonstrated that CS-7E and CS-7EL lines had higher level of resistance to FHB when compared to the CS and CS-7ES lines (Wang et al., 2010). This suggested that there were genetic elements present on the long arm of chromosome 7E of *Th. elongatum* that were responsible for FHB resistance. *F. graminearum* inoculation experiments were also undertaken on CS and CS-7EL addition lines to understand the host-pathogen interaction in wheat (Miller et al., 2011). Results demonstrated that the progression of infection in both lines was quite different. In the parental CS line, the fungus spread extensively from the inoculated spikelet into the node and adjacent spikelets; however the fungal spread was greatly reduced in the CS-7EL line (Miller et al., 2011). The exact reason for this is still unknown, although it was suggested that longer internode segments in CS-7EL as compared to the CS could contribute to limiting the fungal spread.

Dr. George Fedak (Agriculture and Agri-Food Canada, Ottawa, Canada) a collaborator with Dr. Ouellet's lab, crossed the substitution line CS-7E(7D), containing two 7E chromosomes and no

7D chromosomes with CS-*ph1b*, containing two 7D chromosomes but no 7E chromosomes (Figure 7).

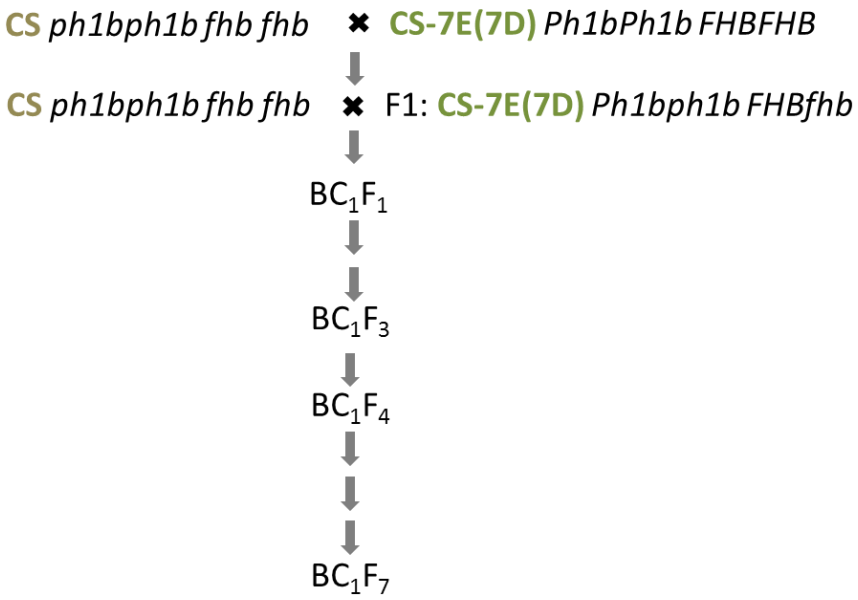


Figure 7: Schema summarizing crossing events in developing families with fragments of 7E from *Th. elongatum* introgressed into wheat chromosome 7D.

The F1 population was then backcrossed (BC) with CS-*ph1b* to develop homozygous lines for *ph1b* (Fig 7). The DNA from BC_1F_1 progeny were tested with *ph1b* specific markers and it was determined that 43% (239 individuals) were homozygous recessive (Fedak et al., 2016). The second generation BC_1F_2 progeny that were *ph1b* homozygous recessive were evaluated for FHB resistance and for evidence of crossover between chromosome 7E and 7D. BC_1F_3 progeny were also evaluated for FHB resistance and characterized using 7E specific markers (Chen et al., 2013; Gou et al., 2016) to identify plants with a small region of 7E introgression while still being resistant to FHB. The fourth generation (BC_1F_4) of these plants were followed up by F. Tekieh (Tekieh, 2016) and F. Calabrian. Subsequent fifth and sixth generation (BC_1F_{5-6}) of one of the

families (64-8-27) was followed up by Margaret Balcerzak (AAFC, Ottawa, Canada); results from PCR screen of that BC_1F_6 family identified three progeny of interest (Figure 8).

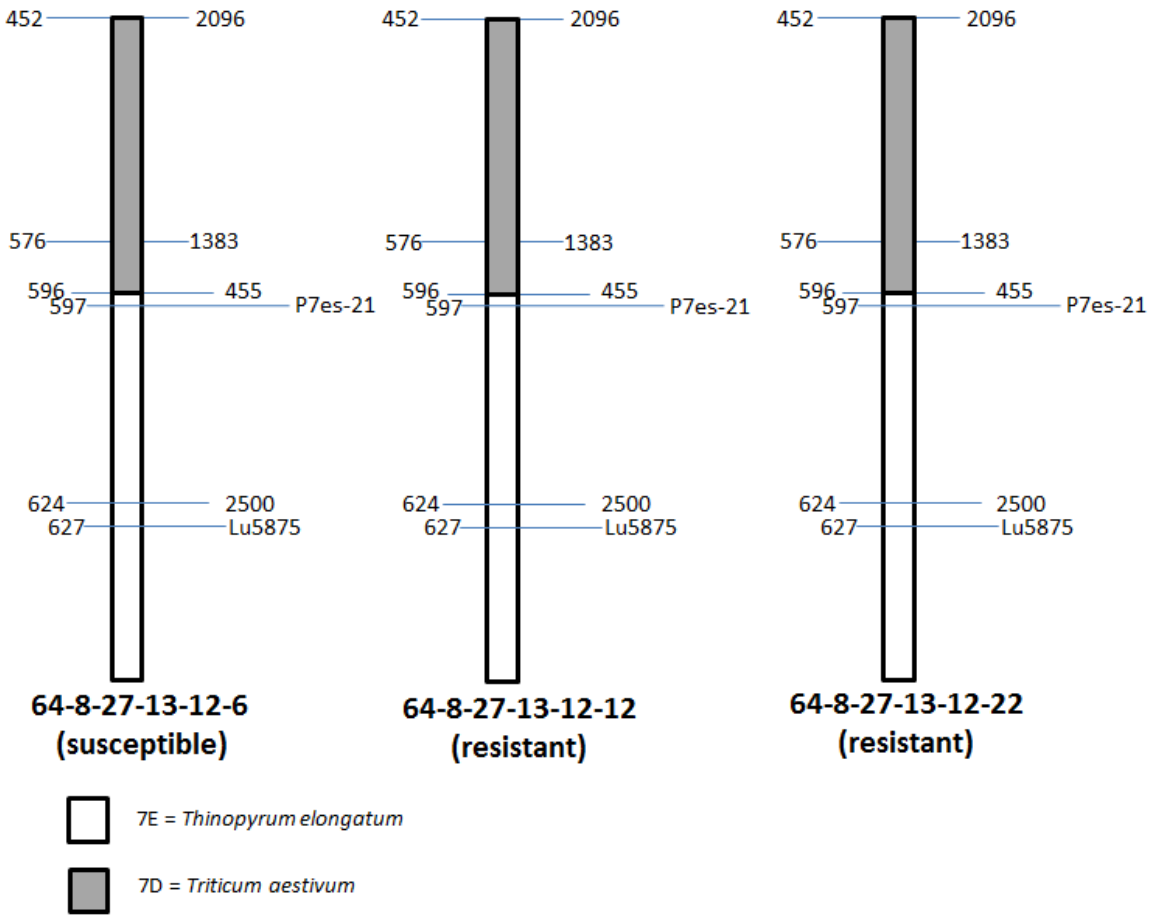


Figure 8: 7EL introgression present in three progeny from BC_1F_6 family.
Marker positions are listed in millions of base pairs (Mbp) on the left, and corresponding marker names are listed on the right. The grey region represents *Triticum aestivum* while the white region represents *Thinopyrum elongatum*.

The region of introgression for all three progeny appeared to be the same, however there were phenotypic differences. Both the 64-8-21-13-12-22 and the 64-8-27-13-12-12 progeny were resistant to FHB while the 64-8-27-13-12-6 progeny appeared to be susceptible to FHB. This suggested that certain genetic elements present in the region of introgression could be responsible for FHB resistance in these progeny. The seventh generation (BC_1F_7) of these

progeny will be grown and used in this project. In addition, the fourth and fifth generation of another family of interest (32-5) will also be characterised.

1.8 Development of 7EL-specific molecular markers

A comparison of the gene expression profiles between CS and CS-7EL was performed using high throughput gene sequencing (RNA-Seq) of rachis samples from *F. graminearum*-infected and water treated CS and CS-7EL heads. Genes expressed from the 7EL chromosome of *Th. elongatum* were identified and used to develop 7EL-specific genetic markers to characterize recombination events between chromosome 7D of wheat and the 7E chromosome (Gou et al., 2016). A total of 48 expressed molecular markers specific for the 7EL chromosome were developed.

An additional seventeen 7EL specific markers were designed, together with fifteen 7DL-specific markers corresponding to their homoeologous sequences on wheat 7D (Tekieh, 2016). Cross-referencing between genomic sequence databases for wheat chromosomes 7 and *Th. elongatum* 7EL was used to design 7EL specific markers. These were located in neighbouring genomic sequences to markers designed by Gou et al. (2016), with a focus on the part of 7EL containing the FHB resistance trait.

7E chromosome specific molecular markers for *Th. elongatum* were also developed based on SLAF-Seq technology (Chen et al., 2013). Specific Locus Amplified Fragment – sequencing (SLAF-Seq) is a type of high throughput sequencing technology that can be used to screen fragments of a specific length from a SLAF-Seq library (Chen et al., 2013). This technology was used to obtain *Th. elongatum* 7E chromosome specific fragments and to develop many 7E chromosome specific molecular markers. Using this technology a total of 135 primer pairs were designed from 135 randomly selected fragments and 89 chromosome 7E specific molecular

markers for *Th. elongatum* were developed (Chen et al., 2013). Many of the molecular markers generated in these three publications will be used in this project.

1.9 Resistance and region of introgression present in *Th. elongatum* plants

Recent research has examined a major locus for resistance to different *Fusarium* diseases which has been mapped to the distal end of *Th. elongatum* 7EL and pyramided with *Th. ponticum* beneficial genes onto the long arm of chromosome 7D of wheat (Ceoloni et al., 2017). The researchers transferred a *Fhb-7el* locus thought to be responsible for FHB resistance, pyramiding it with *Th. ponticum* $7el_1$ segment into the 7DL arm of wheat line T4. The *Th. ponticum* segment was chosen because it contained QTL that provided resistance to wheat rusts and yield enhancement traits. They tested multiple recombinant crosses of which R69-9 contained the smallest fragment of 7E while still being resistant to FHB (Ceoloni et al., 2017). The 7E region from *Th. elongatum* that was related to FHB resistance included markers mapping to the 631-632Mbp region of the wheat 7D chromosome (Figure 9). The plants containing the *Fhb-7el* fragment showed reduced disease severity and fungal biomass, and also proved to be effective against *F. culmorum* and *F. pseudograminearum* (Ceoloni et al., 2017). However, from this study it is not possible to determine whether the 7E region from *Th. elongatum* was solely responsible for the reduced disease incidence or if there was a combined effect of genes present from *Th. ponticum* and *Th. elongatum* that led to the desired effect.

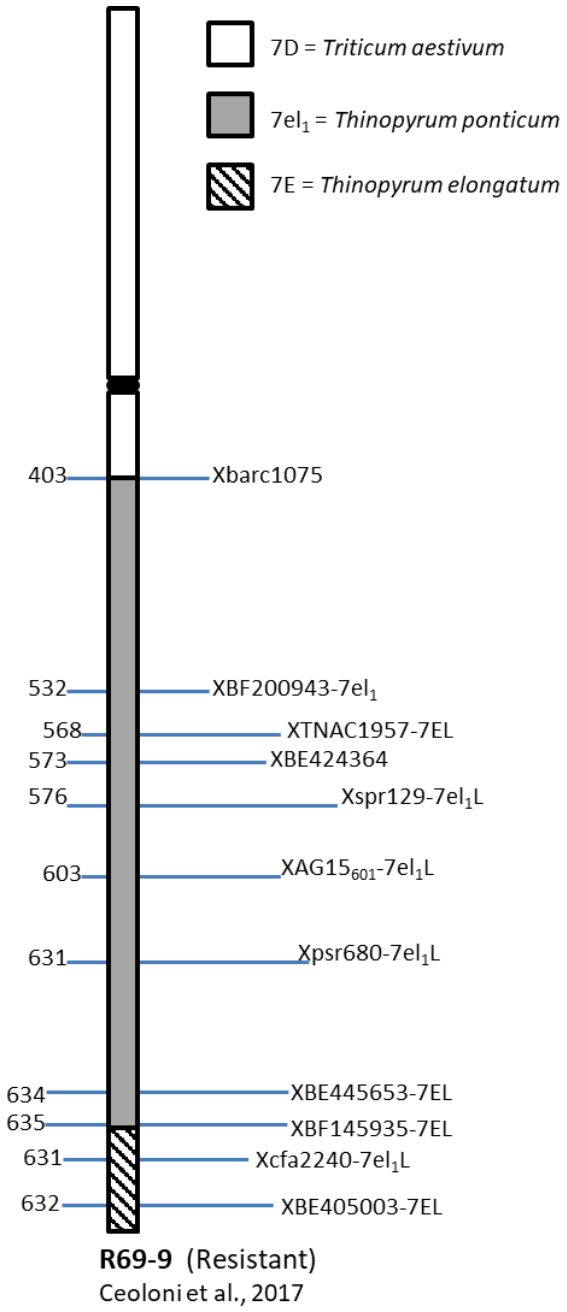


Figure 9: Region of introgression of 7E from *Th. elongatum* and $7el_1$ from *Th. ponticum* into wheat 7D chromosome.
Figure modified from Ceoloni et al., 2017. Marker positions are listed as millions of base pairs (Mbp on 7D) on the left, and their corresponding marker names are listed on the right.

1.10 Statement of research and objectives

The long term goal of this research is to identify any gene(s) responsible for the novel source of resistance to FHB present on the 7EL chromosome of *Th. elongatum*. The specific goal of my research project was to characterize progeny of introgressed wheat lines to determine which progeny contain the smallest fragment of the 7EL chromosome, and subsequently identify gene(s) present in that region that could be associated with the FHB resistance. The specific objectives of my research project included:

I. Propose a genetic order for previously used 7EL-specific markers, based on current wheat pseudomolecule information.

II. Characterize genotype and phenotype of progeny from four novel BC_1F_4 families: 32-5-5, 32-5-7, 32-5-8, and 32-5-9.

III. Select progeny with promising results from the F4 generation (32-5-8-12, 32-5-9-10) and from three families at the BC_1F_7 generation (64-8-27-13-12-6, 64-8-27-13-12-22, 64-8-27-13-12-12) to characterize genotype and phenotype.

IV. Determine expression profiles of specific gene(s) selected from a list of 7EL expressed genes present in the introgressed 7EL fragment associated with FHB resistance (provided by a collaborator), using samples from BC_1F_5 and BC_1F_7 families characterized in III.

V. Determine expression profiles of selected wheat genes present within and outside the 7D region of introgression, from BC_1F_5 and BC_1F_7 samples characterized in III.

Chapter 2: Materials and Methodology

A brief introduction to the required experiments has been provided in Chapter 1. In this chapter detailed materials and methodology are presented.

2.1 Genetic order for previously described 7EL specific markers, based on wheat 7D RefSeq v1.0 information

A genetic order was determined for all 7E molecular markers generated using 7E expressed genes (Gou et al., 2016) and 7E genomic sequences (Tekieh, 2016; Chen et al., 2013). The amplicon sequences used to design these markers were BLASTed against the International Wheat Genome Sequencing Consortium (IWGSC) RefSeq v1.0 sequence (IWGSC, 2018) (https://urgi.versailles.inra.fr/blast_iwgsc/?dbgroup=wheat_iwgsc_refseq_v1_chromosomes&program=blastn, date accessed January - September 2018). Subsequently, the position of the query sequences were used to map the molecular markers onto the Chinese Spring (CS) 7D pseudomolecule.

2.2 Characterizing BC_1F_4, BC_1F_5 and BC_1F_7 plants for genotyping and phenotyping

To characterize progeny from the BC_1F_4, BC_1F_5, and BC_1F_7 families, the plants were genotyped and phenotyped. Figure 10 provides an overview of the schema used to characterize all progeny. Further details from each step are described in the subsections below.

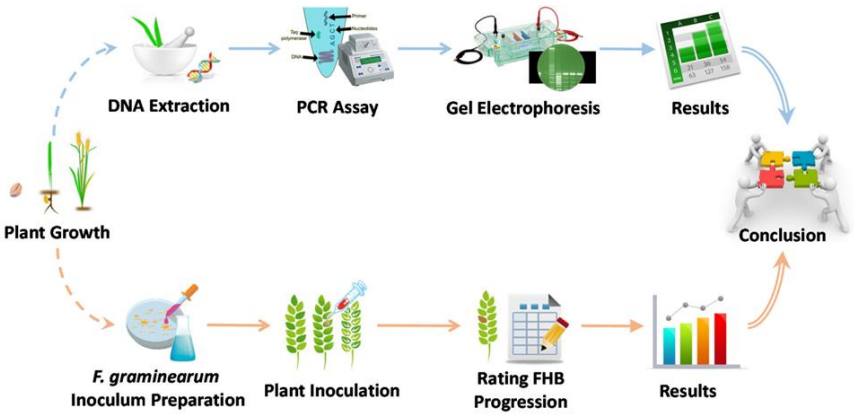


Figure 10: Schema schowing the process used to characterize the 7EL introgression in all families. Figure modified from F. Tekieh thesis (Tekieh, 2016).

2.2.1 Plant material

Seeds for the BC_1F_4 and BC_1F_7 plants were obtained from previous experiments that showed promising results, performed by Firoozeh Chalabian and Margaret Balcerzak (AAFC, Ottawa, Canada). Those plants were derived from BC_1F_3 seeds originally obtained from Dr. George Fedak. Seeds for the BC_1F_5 plants were obtained by growing the F_4 generation and collecting seeds from plants that showed promising results. Additionally, seeds from Chinese Spring (CS) and the addition line CS-7EL were obtained from Dr. George Fedak. Approximately 25-30 seeds from each parent plant and 5-10 seeds from the CS and CS-7EL lines were sterilized and germinated prior to sowing. All seeds were sterilized by washing with 20% bleach solution (v/v) for 5 minutes and then rinsing with distilled water 2 times for 5 minutes each. After sterilizing, the seeds were placed in a petri dish with a wet filter paper and incubated inside a dark box at 24°C for 2 days to germinate. After 2 days, 20 seedlings each from the BC_1F_4, BC_1F_5 and BC_1F_7 plants and 5 seedlings each from the CS and CS-7EL plants were put in individual 5 inch fibre pots and placed in growth cabinets in the greenhouse. The growth cabinets were set to a 16 hour light cycle (20°C) and 8 hour dark cycle (15°C).

2.2.2 Extracting genomic DNA from wheat plants

Fresh young leaves from BC_1F_4, BC_1F_5, BC_1F_7, CS and CS-7EL plants were collected 4 weeks after germination. Immediately after collection, the leaves were flash frozen in liquid nitrogen, then ground to a fine powder using a mortar and pestle. Genomic DNA from leaves were extracted using the "Illustra Nucleon Phytopure Genomic DNA Extraction Kit" (GE Healthcare Life Sciences, U.K.) following the manufacturer's instructions. The optional RNA digestion step was also included at the beginning of the protocol. The DNA pellets were subsequently resuspended in 100µL of 1x Tris-acetate-EDTA (TAE) buffer [40mM Tris (pH 7.6), 20mM acetic acid, 1mM EDTA]. DNA concentration was measured using the QIAxpert® DNA reader (Qiagen, Germany).

2.2.3 PCR assay and gel electrophoresis

PCR reactions were performed on genomic DNA generated from DNA extractions. To perform PCR reactions, the concentrated DNA was diluted to 50ng/µL. Subsequently a working dilution of 14ng/µL was prepared from the concentrated 50ng/µL samples. Amount of reagents used for PCR assays for 1 reaction and 32 reactions is listed in Appendix 1. Molecular markers designed for 7E expressed genes by L. Gou (Gou et al., 2016) and additional 7E genomic DNA markers designed by F. Tekieh (Tekieh, 2016) were used for PCR screening. All markers used for PCR screenings are listed in Appendix 2. The PCR protocol used was as follows: 95°C for 4 minutes, followed by 38 cycles of 94°C for 30 seconds, Tm specific to each primer for 30 seconds, 72°C for 1 minute and primer extension of 72°C for 5 minutes performed at the end of 38 cycles.

2.2.4 *F. graminearum* inoculum preparation

A virulent isolate of *F. graminearum*, DAOM 233423 (Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada), was used to prepare the inoculum. To produce spores, three 3mm plugs of *F. graminearum* mycelium were extracted from SNA (Spezieller-Nährstoffer Agar) plate culture and transferred to 50mL Carboxy-Methyl Cellulose (CMC) medium (Cappellini and Peterson, 1965). The cultures were placed in a shaker inside a dark room for three days at 28°C. The macroconidial spores were separated from the mycelium using sterilised Miracloth and washed three times by resuspending in sterile water. This was followed by centrifugation at 14°C at 4200rpm for 10 minutes to obtain concentrated *F. graminearum* spores free of CMC medium. The spores were counted using a hemocytometer and the concentration was adjusted to $1x10^5$spores/mL.

2.2.5 Inoculating plants and rating disease progression

Inoculation of plants was performed when plant heads reached the mid-anthesis stage. During this stage about half of the spikelets had extruded yellow anthers, and the ideal time for inoculation occurs between the appearance of yellow anthers and before the anthers turn white. Two florets per spikelet around the center of the wheat heads were point inoculated with 10µL of previously prepared *F. graminearum* spore suspension using a micropipette (Figure 11). After inoculation, the plants were transferred to a misting cabinet for 2 days which was set to 18 hour light cycle (25°C) and 8 hour dark cycle (20°C) at 75% humidity and misted for 30seconds every hour. This created a high humidity condition, thus enabling the fungus to infect the wheat heads. After 2 days, the plants were moved to the non-misting side of the same cabinet.

Figure 11: Inoculating wheat heads with *F. graminearum* spores.
Panel on left shows spikelet before inoculation with *F. graminearum* spores. Panel on right shows same spikelet after inoculation. The black arrow indicates the spikelet that will be inoculated.

Phenotyping was done at 7 and 14 days post inoculation (dpi) with *F. graminearum* spores. Phenotyping was performed by examining browning symptoms on the spikelets of the inoculated heads. Browning symptoms occurring along the rachis (up and down) starting from the inoculated floret were also noted. In addition, bleaching of the inoculated florets and above and below the inoculated florets was noted. Disease ratings were calculated at 7 and 14 dpi, as a percentage of infected spikelets over total spikelets present. Spikelets were considered infected when brown lesions covered at least 50% of the spikelet. The disease ratings and observations were all taken into account to classify the plants as resistant, partially resistant or susceptible to *F. graminearum*.

2.3 RT-qPCR and normalizing data with housekeeping genes

Wheat heads from selected BC_1F_5 and BC_1F_7 plants inoculated with *F. graminearum* were used for RNA extraction and RT-qPCR analysis. Specific details regarding each of the steps are provided in the subsections below.

2.3.1 Inoculation of wheat heads with *F. graminearum*

F. graminearum spore suspension ($1x10^5$ spores/mL) was prepared as described in section 2.2.4 and used to inoculate one wheat head per plant which was later collected for RNA sampling. All flowering spikelets on that wheat head were inoculated as described in section 2.2.5. Three days after inoculation, the inoculated wheat heads were collected and flash frozen in liquid nitrogen. They were subsequently transferred to a -80°C freezer.

2.3.2 RNA extractions and cDNA synthesis

Wheat heads that were frozen were ground to a fine powder in liquid nitrogen using a mortar and pestle. RNA extraction from individual wheat heads was performed using Tri Reagent (Sigma-Aldrich, Canada) followed by RNA cleanup using RNeasy Mini Kit (Qiagen, Canada), as per the manufacturer's instructions. cDNA was synthesized from the cleaned RNA with the RETROscript ® Reverse Transcription Kit (Invitrogen, Canada), using 2µg of RNA template per sample. The cDNA was diluted 25 times for RT-qPCR assays which used SensiFAST™ SYBR No-ROX mix (Bioline, USA). The reactions were performed in a PTC-200 Peltier Thermal Cycler (MJ research, Canada) as follows: 95°C for 2 minutes followed by 40 cycles of 95°C for 5 seconds, 60°C for 10 seconds and 72°C for 20 seconds. The results were analysed using the Opticon Monitor 3 software (Bio-Rad, CA, USA).

2.3.3 Normalizing data with housekeeping genes and use of other reference genes

Housekeeping genes were used to normalise expression levels. The wheat housekeeping genes that were used to normalize the data include hn-RNP-Q (*TraesCS2A01G390200*) and IAAOX (*TraesCS2A01G246300*). The primer sequences for the housekeeping genes and other reference genes FgGAPDH (*FGSG_06257*) and PR1 gene family members are listed in Appendix 3. The Opticon Monitor 3 software was used for visualising and analysing the data, including the

quantification cycle (Cq) values and correlation coefficients (R^2) for the standard curve. The relative expression levels were normalized using the obtained Cq values of two wheat reference genes (Appendix 4). The relative fold change of expression was calculated as per (Vandesompele et al., 2002).

2.4 Validating expression profiles of genes of interest present in the introgressed 7EL fragment having FHB resistance

A RNA-Seq dataset was used to identify 7E genes of interest present in the region of introgression. After selecting specific genes, RT-qPCR was used to validate their expression profiles in all families. Primers were designed to amplify the specific genes. Further details regarding each step are provided in the subsections below.

2.4.1 Selecting differentially expressed genes and primer design

Differentially expressed 7EL genes present in the RNA-Seq dataset were chosen based on the following criteria. Firstly, genes with raw sequence counts less than 200 were removed because their expression was too low to be detected using RT-qPCR, based on previous experiences in the laboratory. Secondly, genes showing a $\log_2$fold change of 1.25 or lower were also removed from the list; $\log_2$=1.25 was the cutoff for statistically significant p values ($p \geq 0.05$). Finally the function of the genes was determined by BLASTing the 7EL sequence using blastn, searching the nucleotide collection (nr/nt) database and looking at the best hits. Table 1 lists the six genes that were chosen to be validated using RT-qPCR.

Table 1: Subset of 7EL genes present in region of introgression validated with RT-qPCR. Log_2fold change was generated by computing the fold change between 7EL-Fg treatments and 7EL-H_2O treatments, across three replicates.

GeneID	Log_2FoldChange	Average Raw Sequence Counts		Predicted Function
		7EL-Fg	7EL-H_2O	
7EL_scaffold_395	3.47	565	84	NFXL1 zinc finger protein
7EL_scaffold_587	5.19	1471	66	Anthocyanin reductase
7EL_scaffold_649	2.32	488	161	Unknown protein
7EL_scaffold_1517	2.02	3638	1455	Cytochrome P450 (CYP72a)
7EL_scaffold_2197	4.59	2063	139	Indole 3- phosphate glycerol synthase like
7EL_scaffold_2937	2.46	479	141	NEP-1 interacting protein-like

Gene-specific primers for six genes of interest were designed. The sequences corresponding to those genes were extracted from the 7EL genomic scaffold sequence database (collaborator D. Konkin, National Research Council, Saskatoon, Canada). The extracted sequence was then BLASTed against all 7E scaffolds to identify and exclude regions of homology to other genes from primer design. The 7EL scaffold sequence was BLASTed against the entire A, B and D wheat genomes to identify highly similar sequences. All sequences from the A, B and D genomes were aligned with the 7E sequence using ClustalW (Geneious, USA) (Figure 12). After sequence alignment, homologous regions were identified and primers were designed in the non-homologous region to amplify only the gene of interest.

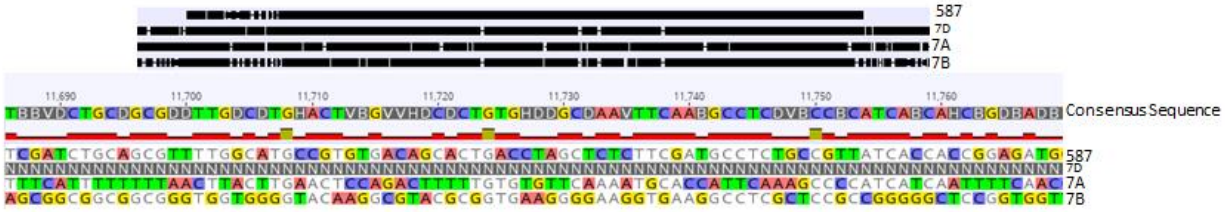


Figure 12: Alignment of the sequence of interest against homoeologous sequences from 7D, 7A and 7B genomes.
Top panel shows alignment of all sequences using ClustalW. Bottom panel shows zoomed in version of top panel that shows the sequence used to design primers. N refers to unknown bases.

The following criteria were used to design gene-specific primers: the melting temperature was ensured to be between 55°C – 65°C, the primer did not form hairpins nor self-dimerized, finally no hetero-dimers were formed between the forward and reverse primers. The primers were checked to meet the above criteria using the OligoAnalyzer 3.1 program from IDT (https://www.idtdna.com/calc/analyzer, date accessed August 2018). Table 2 lists the primer sequences for the 7EL genes selected.

Table 2: Sequences of primers for selected differentially expressed genes from 7EL

7EL genes	Sequences (5'-3')
7EL_scaffold_395 (NFXL1 zinc finger protein)	Forward: GTGCCATCCAGTTGTGAC
	Reverse: GACATTGCGTGCCTACTC
7EL_scaffold_587 (anthocyanin reductase)	Forward: AGTGGCAACATCATCATAG
	Reverse: GGCTCTTCCTTGGTTAC
7EL_scaffold_649 (unknown protein)	Forward: TCGTCCAACTCCAACTCC
	Reverse: TCGAGAACACGCATCCCA
7EL_scaffold_1517 (cytochrome P450, CYP72a)	Forward: GGTAAAGTCGCTCTGAC
	Reverse: CAGCCCGTTGGACA
7EL_scaffold_2197 (indole 3-phosphate glycerol synthase)	Forward: AAGCAGGAGTGAAGGTAATG
	Reverse: CATCGCACGGGCATAATA
7EL_scaffold_2937 (NEP1 interacting protein)	Forward: AGTTTCTGCTCATTCTG
	Reverse: CCACCACCAACATGA

2.5 Validating expression profiles of wheat genes showing differential gene expression in CS vs. CS-7EL

A different set of analysis was performed on the RNA-Seq dataset that was previously generated to compare differential gene expression between CS and CS-7EL. The aim of this analysis was to identify wheat genes present in the region of introgression and outside of the region of introgression. Further details regarding specific steps in the process are outlined in the subsections below.

2.5.1 Selecting differentially expressed genes and primer design

Differentially expressed wheat genes were chosen by examining *F. graminearum* treatment effects between CS and CS-7EL plants. The genes were chosen based on criteria previously described in section 2.4.1. Table 3 lists all five wheat genes that were chosen to be validated using RT-qPCR. Of these five genes, two genes are present within the region of introgression while three genes are present outside the region of introgression.

Table 3: Average raw sequence counts of wheat genes upon infection with *F. graminearum*. Log_2fold change was generated by calculating fold change between 7EL-Fg and CS-Fg treatments across three replicates per treatment.

GeneID	Log_2Fold Change	Average Raw Sequence Counts		Predicted Function
		7EL-Fg	CS-Fg	
7DL-sc402	1.72	2560.33	777.33	Thaumatin-like protein
7DL-sc539	1.98	1023.67	116.67	FTSH-1 metalloprotease
7DL-sc243	3.06	1478	80.23	Thiamine thiazole synthase
7DL-sc2291	1.68	644.67	90	Frucktokinase-2
6DS-sc1131	3.72	729.33	25	Ferredoxin NADP(H) oxidoreductase

Gene specific primers were designed for all genes of interest. The sequence corresponding to each of the five genes was obtained by searching for the GeneID using the wheat IWGSC survey sequence v3 (https://urgi.versailles.inra.fr/gb2/gbrowse/wheat_iwgsc_survey_sequence_v3/, date accessed August 2018). The extracted sequence was subsequently BLASTed against the IWGSC RefSeq v1.0 database to find similar sequences present in the A and B genome. Once the homoeologous sequences from all three wheat genomes and 7EL region of *Th. elongatum* were identified, all the sequences were aligned using ClustalW to find regions of homology. Primers were then designed in non-homologous regions to amplify the specific gene of interest. The primers were designed in the same manner as described in section 2.4.1. Table 4 lists the five primer pairs that were designed to amplify differentially expressed wheat genes.

Table 4: Primer sequences for selected wheat genes.

Wheat genes	Sequences (5'-3')
7DL-sc402 (Thaumatin-like protein)	Forward: AACTACCAGATCACCTTCT
	Reverse: CAAATATCAATGAAACGCTCA
7DL-sc539 (FTSH-1 metalloprotease)	Forward: TTTTCGCAGAGTTTTATGAT
	Reverse: AATGAGTTATCTTCTTCTAC
7DL-sc243 (Thiamine thiazole synthase)	Forward: GGTTTATAGGGCTCAAT
	Reverse: ATTTGATTGTTCCCCATCAT
7DL-sc2291 (Fructokinase-2)	Forward: CCTGTAGGCTTCCCATAGTT
	Reverse: TGGCGGGATTACCTTGATTA
6DS-sc1131 (Ferredoxin NADP(H) oxidoreductase)	Forward: GACGGCGATGATGATGAT
	Reverse: CATCTTGCTCTTTGAAGTTC

Chapter 3: Results

A detailed methodology of the objectives that are part of this project has been introduced in chapter 2. This chapter presents the compiled results from all objectives along with preliminary analysis.

3.1 Objective 1- Determining genetic order for previously described 7EL markers, based on current wheat 7D RefSeq v1.0

The genetic order of all previously generated 7EL and 7D markers from the Ouellet laboratory, as well as selected markers from Chen et al., (2013) was determined by BLASTing against recently published RefSeq v1.0 (IWGSC, 2018). Figure 13 shows the position of the 7D markers on the 7D pseudomolecule and the position of the 7D homoeolog sequences to the 7EL markers. For the purpose of this work, we have assumed that the position of the homoeolog sequences was similar between 7D and 7E. There is a higher density of markers present towards the end of the chromosome, partly due to the increased number of genes present at the chromosome end and partly due to experimental needs. Additionally knowing the position of these markers has been useful in characterizing the region of introgression in all nine families.

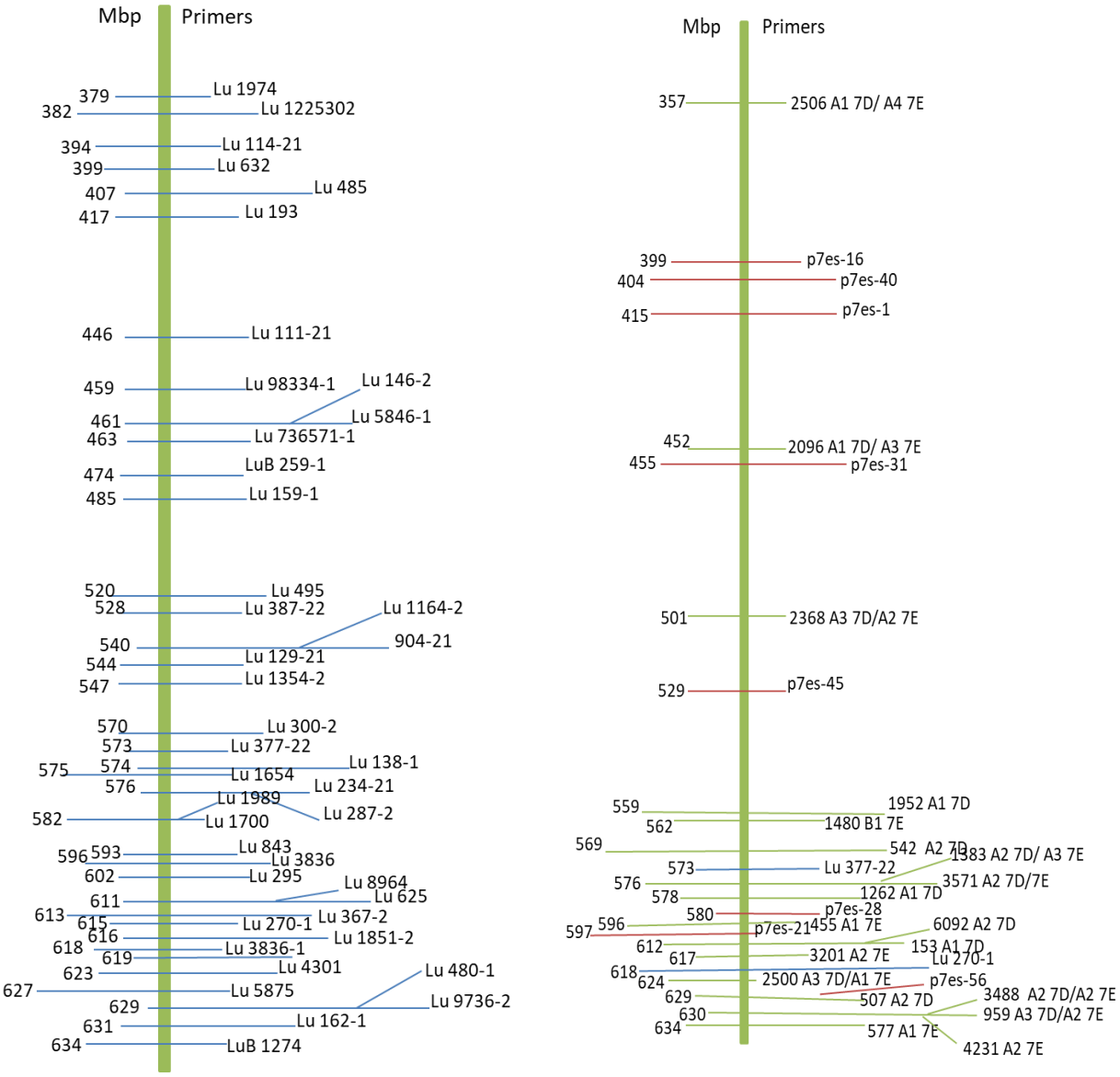


Figure 13: Genetic order of 7EL and 7D markers based on RefSeq v1.0 7D pseudomolecule. Colours represent markers designed by Gou et al., (2016) (blue), F. Tekieh and K. Joustra Tekieh, (2016), (green) or by Chen et al., (2013) (red). Position of markers on left (Mbp) and primer names on right.

3.2 Objective 2- Characterizing BC_1F_4, BC_1F_5 and BC_1F_7 families through genotyping and phenotyping

The aim of this objective was to genotype and phenotype progeny from nine families to determine their resistance or susceptibility to *F. graminearum* and to find the smallest introgressed region from 7EL. All of the BC_1F_4 and BC_1F_5 families have been derived from the plant 32-5 while the BC_1F_7 families have been derived from the plant 64-8-27-13-12. Families were obtained by selfing one plant and all progeny from that backcross formed one generation. All progeny were genotyped with 7EL markers while only a subset were genotyped with 7D markers. Only progeny used for the RNA-Seq experiment and kept for seed were genotyped with the 7D markers.

3.2.1 Evaluating disease progression in BC_1F_4 families

Disease progression was measured at 7 and 14 dpi following inoculation with *F. graminearum* spores (Appendix 5). Figure 14 shows percentage of infected spikelets at 14 dpi in four BC_1F_4 families derived from plant 32-5.

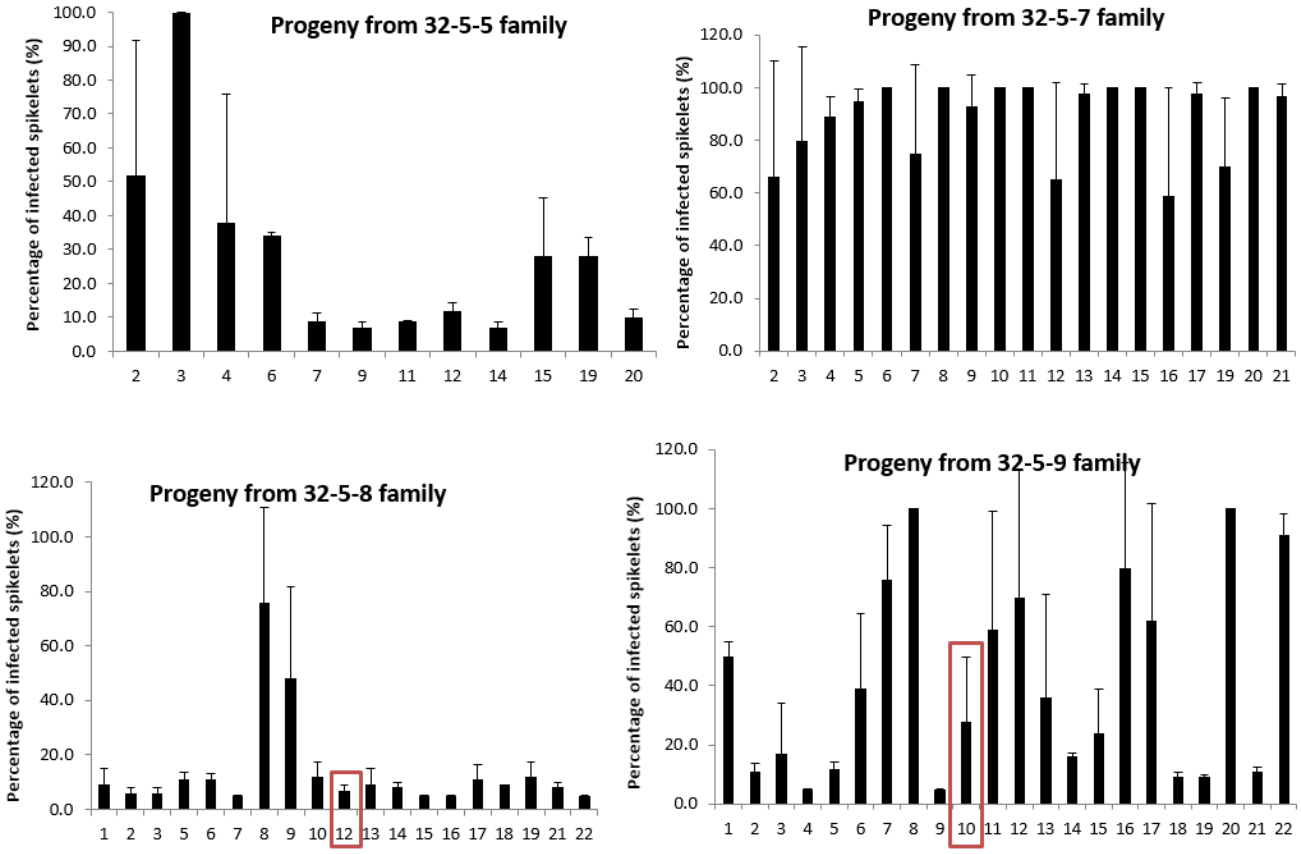


Figure 14: Average percentage of infected spikelets observed in progeny of all four BC_1F_4 families at 14 dpi.
Sample size of each progeny is approximately 4 heads. The numbers on the X-axis indicate the name of individual progeny in each BC_1F_4 family. The two progeny boxed in red were chosen for further experiments.

Progeny in each of the BC_1F_4 families were classified as resistant, partially resistant or susceptible. The rating scale was based on the percentage of infected spikelets in the 7EL and CS plants (Appendix 5). Progeny were classified as resistant if 10% or less of the spikelets were infected, partially resistant if 11-30% of the spikelets were infected, and susceptible if 35% or more of the spikelets were infected. All progeny from the BC_1F_4 families were also genotyped and detailed results can be found in Appendix 6. Results from the 32-5-5 family show that progeny 2, 3, 4 and 15 were susceptible to the infection while the remaining progeny were

resistant or partially resistant (Figure 14). The resistant progeny from this family contained the full complement of the *Th. elongatum* chromosome while the partially resistant progeny did not produce seed, and were removed from further testing. All progeny from the 32-5-7 family were susceptible to *F. graminearum* infection and were removed from further testing (Figure 14). Two progeny from the 32-5-8 and 32-5-9 family were chosen for further testing based on their genotype and phenotype. Figure 15 shows the genotype of 32-5-8-12 and 32-5-9-10. These plants were chosen because they contained the smallest fragment of introgression among the BC_1F_4 progeny while still being resistant or partially resistant to FHB.

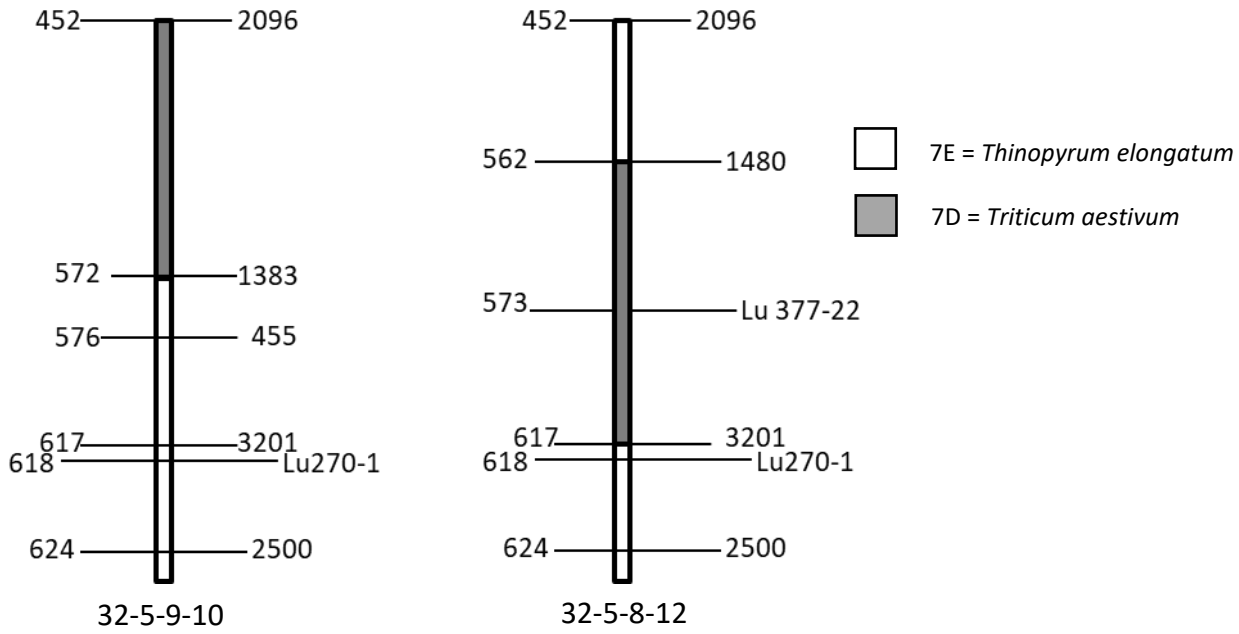


Figure 15: 7EL introgression present in the progeny 32-5-9-10 and 32-5-8-12.
7EL marker positions based on homoeologous sequences in 7DL, in millions of base pairs (Mbp) are listed on the left, and corresponding marker names are listed on the right. The region in white represents *Th. elongatum* DNA while the region in grey represents *T. aestivum* DNA.

3.2.2 Evaluating disease progression and genotype in BC_1F_5 and BC_1F_7 families

Disease progression of two BC_1F_5 families derived from 32-5 plant and three BC_1F_7 families derived from 64-8-27-13-12 plant were performed at 7 and 14 dpi. Additionally the genotype of each progeny in all five families was determined using PCR with 7E and 7D specific markers as

described in section 2.2.3. Figure 16 shows an example of PCR results using a 7EL primer Lu 625. Complete PCR results from all progeny from the five families are listed in Appendix 8.

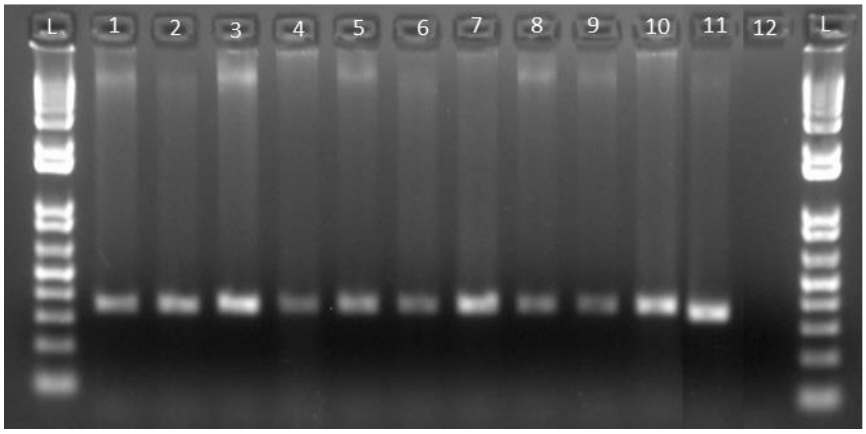


Figure 16: Example of PCR results for family 64-8-27-13-12-6 using 7EL primer Lu 625. Lanes 1-10 contain DNA from family 64-8-27-13-12-6 progeny 1-10 respectively. Lane 11 contains DNA from positive control CS-7EL and lane 12 contains DNA from the negative control CS. L: 1kb plus DNA ladder; 1.5% agarose gel.

Figure 17 and Figure 18 show the percentage of infected spikelets at 14 dpi and the corresponding genotype of the resistant progeny in those families in two BC_1F_5 families and in three BC_1F_7 families. A revised rating scale was used to classify all progeny in this experiment based on percentage of infected spikelets in the 7EL and CS plants (Appendix 7). Progeny were marked as resistant if the percentage of infected spikelets was 20% or lower, partially resistant if percent of infected spikelets was between 21-40% and susceptible if percentage of infected spikelets was 41% or higher. Appendix 7 shows the phenotype of all progeny from the two BC_1F_5 and three BC_1F_7 families.

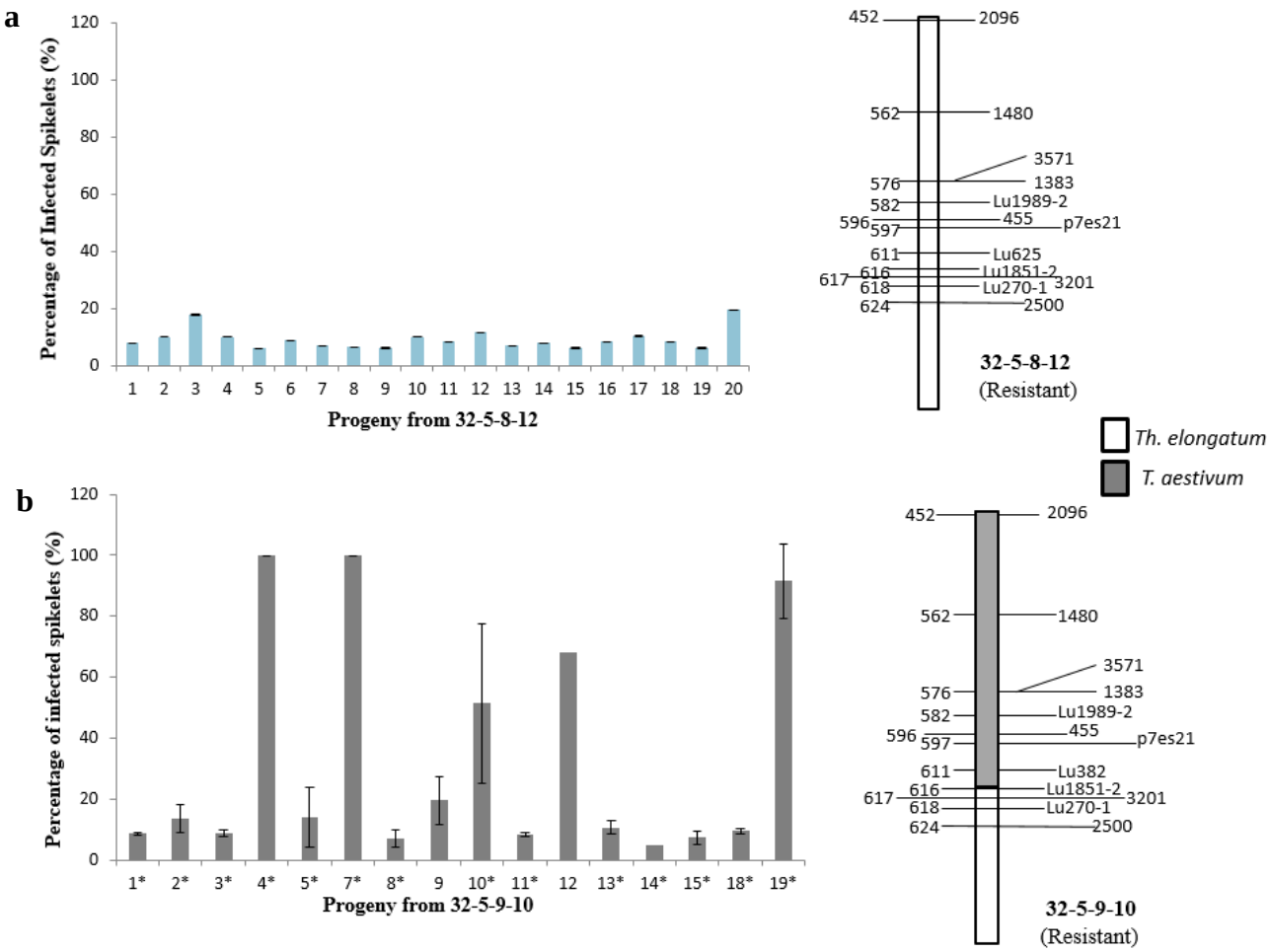
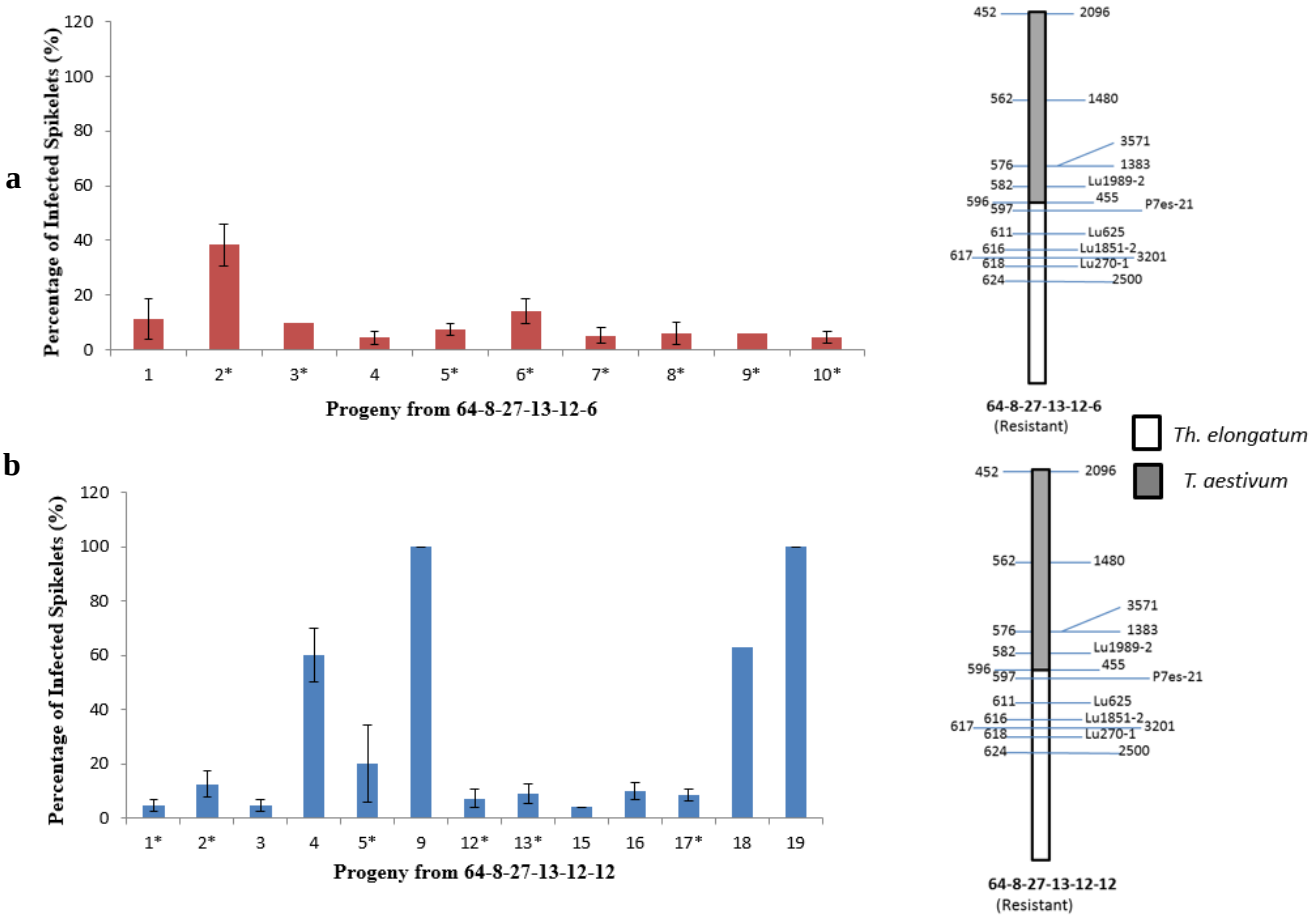


Figure 17 a,b: Infected spikelets (%) at 14 dpi for two BC_1F_5 families derived from 32-5 and corresponding genotype of resistant progeny from those families.
Graphs on the left show the average percentage of infected spikelets in each progeny from the two families. Sample size of each progeny is approximately 4 heads and error bars represent standard deviation. Asterisk (*) indicates progeny from which a single head was collected, RNA extracted and used in subsequent experiments. Ilustrations on the right show the genotype of resistant individuals for each family. Marker positions are listed on the left of the illustration in Mbp and primer names are listed on the right. The white area represents *Th. elongatum* DNA and the area in grey represents *T. aestivum* DNA.

Figure 17 shows the results from the two BC_1F_5 families derived from 32-5 plant. All progeny in the 32-5-8-12 family were classified as resistant because all progeny had less than 20% of infected spikelets and they also contained the full length of the 7EL fragment from *Th. elongatum* (Figure 17a). These genotyping results were not consistent with the results for plant

32-5-8-12 (Figure 15), which showed a smaller region of introgression. Further investigation indicated that absence of bands for five 7EL markers in plant 32-5-8-12, possibly due to poor DNA quality, was wrongly interpreted as negative PCR results. Therefore, this family was excluded from further experiments involving RNA analysis. In the 32-5-9-10 family progeny 4, 7, 10, 12 and 19 were classified as susceptible to *F. graminearum*, progeny 9 was classified as partially resistant and the rest were classified as resistant (Figure 17b).



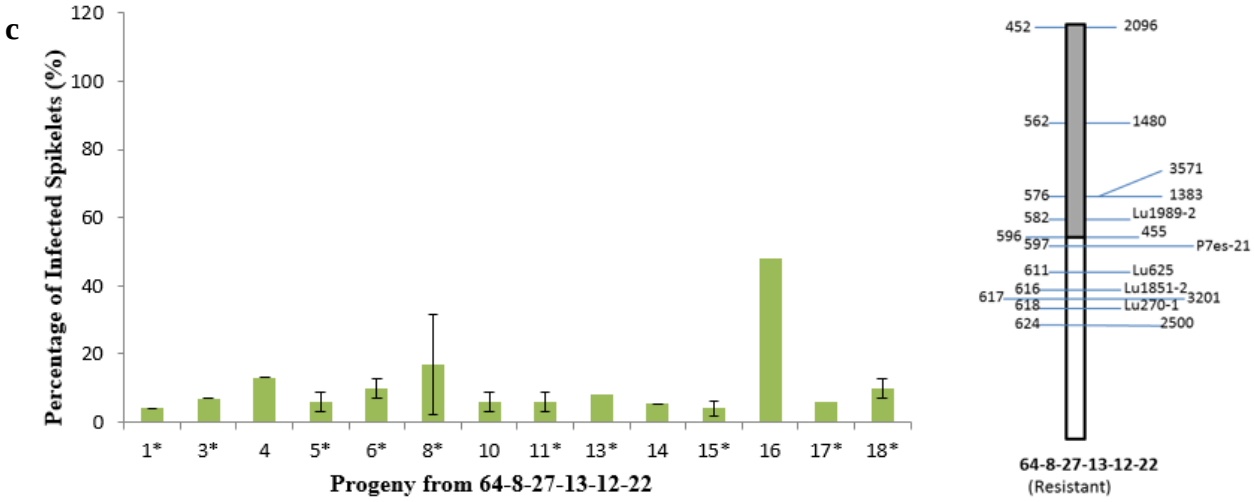


Figure 18 a,b,c: Infected spikelets (%) at 14 dpi for three BC_1F_7 families derived from 64-8-27-13-12 and corresponding genotype of resistant progeny from those families.
Graphs on the left show the average percentage of infected spikelets in each progeny from the two families. Sample size of each progeny is approximately 4 heads and error bars represent standard deviation. Asterisk (*) indicates progeny from which RNA was collected and were used in subsequent experiments. Ilustrations on the right showsthe genotype of resistant individuals for each family. Marker positions are listed on the left of the illustration in Mbp and primer names are listed on the right. The white area represents *Th. elongatum* DNA and the area in grey represents *T. aestivum* DNA.

Based on the results it was observed that in the 64-8-27-13-12-6 family, all progeny were resistant to *F. graminearum* except for progeny 2 which was classified as partially resistant (Figure 18a). In the 64-8-27-13-12-12 family progeny 4, 9, 18 and 19 were susceptible to *F. graminearum* infection; progeny 5 was partially resistant, while the rest were resistant (Figure 18b). In the 64-8-27-13-12-22 family all progeny were resistant to *F. graminearum*, except for progeny 16 which was susceptible to the infection (Figure 18c).

The position of the last positive 7DL marker and the position of the first positive 7EL marker within the introgressed fragments were used to estimate the size of the region of introgression present in each of the families, based on physical distances in the 7D pseudomolecule. The region of introgression in the 32-5-9-10 family was estimated to be at least 22 and less than 25

Mbp long (Figure 17b) and the region of introgression in the 64-8-27-13-12-6, -12 and -22 families was at least 42 and less than 62 Mbp long (Figure 18). Genotyping results were also used to determine whether the region of introgression was homozygous or heterozygous for the 7EL/7D chromosome. The region of introgression was determined to be homozygous if the PCR results showed an absence of bands for 7D markers present in that region. In the 32-5-9-10 family, progeny 8 and 11 were shown to be homozygous (Appendix 8). In the 64-8-27-13-12-22 family, progeny 1 and 6 also appeared to be homozygous for the 7EL/7D chromosome. Finally, all progeny in the 64-8-27-13-12-6 family were shown to be homozygous for the region of introgression (Appendix 8).

3.3 Objective 3 – Determining level of expression of two reference genes

Additional characterization of the 7EL introgressed material was focused on gene expression analysis. RNA was extracted from samples collected at 3 dpi, using only inoculated head samples from resistant progeny (based on phenotyping and genotyping data) for families derived from 64-8-27-13-12, and samples from both resistant and susceptible progeny for the 32-5-9-10 family. Initial gene expression analyses used two reference genes previously used in the Ouellet laboratory. The level of *F. graminearum* infection at 3 dpi was estimated by measuring the gene expression level of FgGAPDH in relation to the wheat housekeeping genes used for normalisation (Figure 19a).

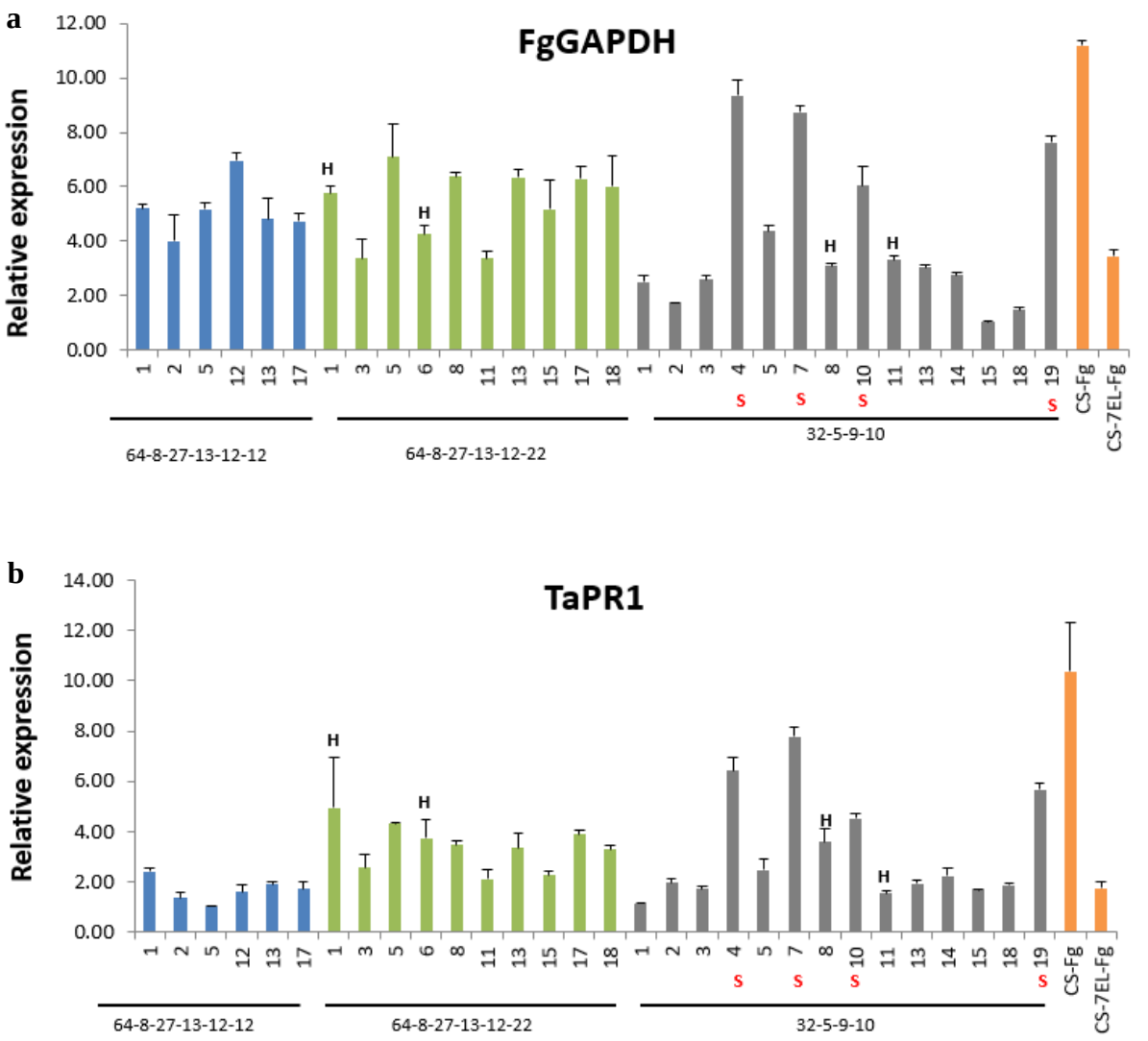


Figure 19a,b: Relative expression of FgGAPDH and TaPR1 in two BC_1F_7 families and one BC_1F_5 family at 3 dpi.
Values are mean of two technical replicates; error bars represent standard deviation. "S" indicates progeny that were susceptible to *F. graminearum* infection. "H" indicates progeny that are homozygous for the 7EL introgression. Orange bars represent the *F. graminearum* inoculated controls CS-Fg and addition line CS-7EL-Fg.

Increased levels of FgGAPDH was observed in CS-Fg plants as compared to the 7EL-Fg plants (Figure 19a). High expression of FgGAPDH is associated with increased *F. graminearum* infection in wheat heads. Therefore, CS-Fg plants were expected to have higher FgGAPDH

expression levels since they are more susceptible to *F. graminearum* infection. The results indicated that all resistant progeny from the 64-8-27-13-12-12 and -22 families had four fold lower level of expression compared to CS-Fg, as expected from their phenotype (Figure 18, Figure 19a). However, progeny from both families showed higher expression level of FgGAPDH than CS-7EL-Fg plants (Figure 19a). In the 32-5-9-10 family the resistant progeny had reduced expression levels compared to the susceptible progeny (Figure 19a). It was also observed that the susceptible progeny in the 32-5-9-10 family had slightly lower expression levels than CS-Fg plants. Although, these results were consistent with the disease ratings previously generated for progeny in each family (Figure 17b, Figure 18).

Additionally, expression level of TaPR1 was also measured. PR1 is a known indicator of defense response in plant/pathogen interactions. In the wheat/ *F. graminearum* interaction, TaPR1 gene is induced in response to the pathogen and increased gene expression indicates increased *F. graminearum* levels. In this experiment, increased levels of PR1 gene expression were quite consistent with the levels of FgGAPDH (Figure 19b).

3.4 Objective 4 – Determining expression profiles of differentially expressed 7EL genes present in the region of introgression using RT-qPCR

The goal of this objective was to examine relative gene expression of six selected 7EL genes present in the region of introgression in three BC_1F_7 families derived from 64-8-27-13-12 and one BC_1F_5 family derived from 32-5-9-10.

3.4.1 Identification of expressed 7EL genes in the region of introgression

A previously generated RNA-Seq dataset (Gou et al., 2016) was used to compare expression differences between the spring wheat variety CS and the addition line CS-7EL, after inoculation with *F. graminearum* or mock inoculation with water. *Th. elongatum* and *T. aestivum* genes were

mapped to their respective genome sequences by collaborators, E. Hsueh and D. Konkin. The dataset was used to identify all 7EL expressed genes present within the region of introgression. Appendix 9 shows the log_2fold change and raw transcript counts between *Fusarium* and water treatments in the CS-7EL addition line, across three replicates, for the identified genes. A total of 426 expressed 7EL genes (Appendix 9) were identified as having a 7D homoeolog located within the 42Mbp region of introgression observed in the progeny derived from 64-8-27-13-12 plant, while 221 expressed 7EL genes were identified in the 22 Mbp region of introgression in progeny from the 32-5-9-10 family. To characterize gene expression in the 7EL-introgressed material, six expressed genes were selected from Appendix 9 as described in section 2.4.1. The primer sequences for the six genes selected are listed in Table 3, section 2.4.1. Additionally these six genes were mapped onto the 7D wheat chromosome to determine the relative position of their homoeologous sequences (Figure 20).

Gene Annotation	Gene ID
Anthocyanidin Reductase	7EL_587
Indole 3 glycerol phosphate (IGPS)	7EL_2197
NFXL-1	7EL_395
CYP450 (CYP72a)	7EL_1517
Unknown Protein	7EL_649
NEP-1	7EL_2937

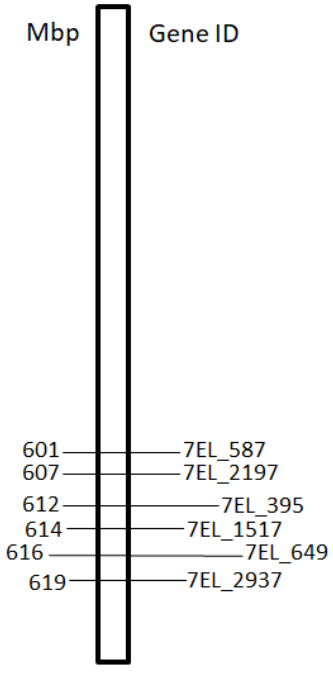


Figure 20: Position of homoeologous sequences to six 7EL genes on the wheat 7D chromosome. Figure shows only the long arm of chromosome 7D from *T. aestivum*.

RT-qPCR was performed to determine the expression profiles of all six genes of interest in three BC_1F_7 families (64-8-27-13-12-12, 64-8-27-13-12-22 and 64-8-27-13-12-6) and one BC_1F_5 family (32-5-9-10) (Figure 21, Figure 22, Figure 23). The results for the 64-8-27-13-12-6 family will be presented separately.

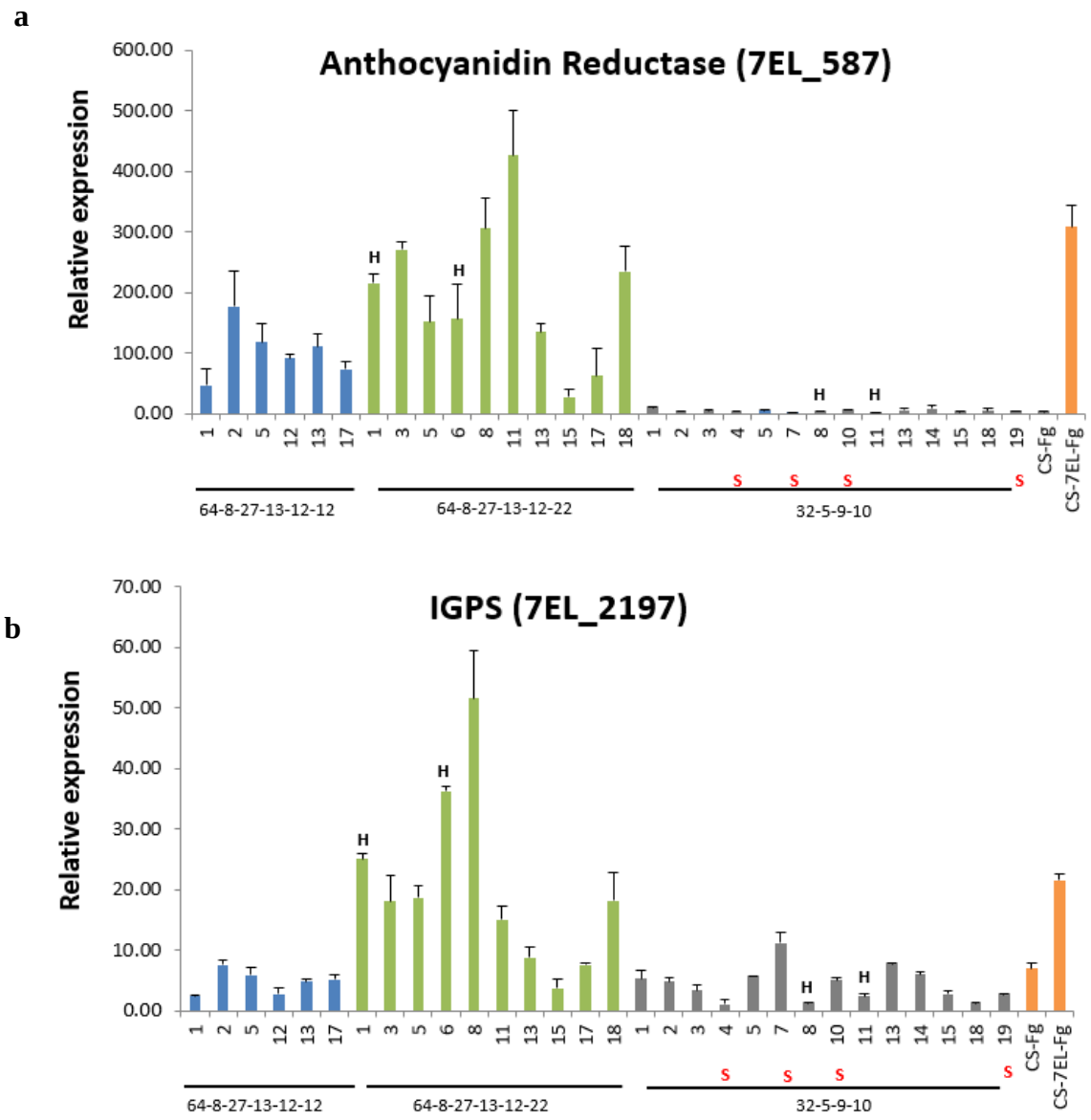


Figure 21a,b: Relative expression of 7EL genes present in the region of introgression in two BC_1F_7 families and one BC_1F_5 family.
Values are mean of two technical replicates; error bars represent standard deviation. "S" indicates progeny that were susceptible to *F. graminearum* infection. "H" indicates progeny that are homozygous for the 7EL introgression. Orange bars represent the controls CS-Fg and addition line CS-7EL-Fg.

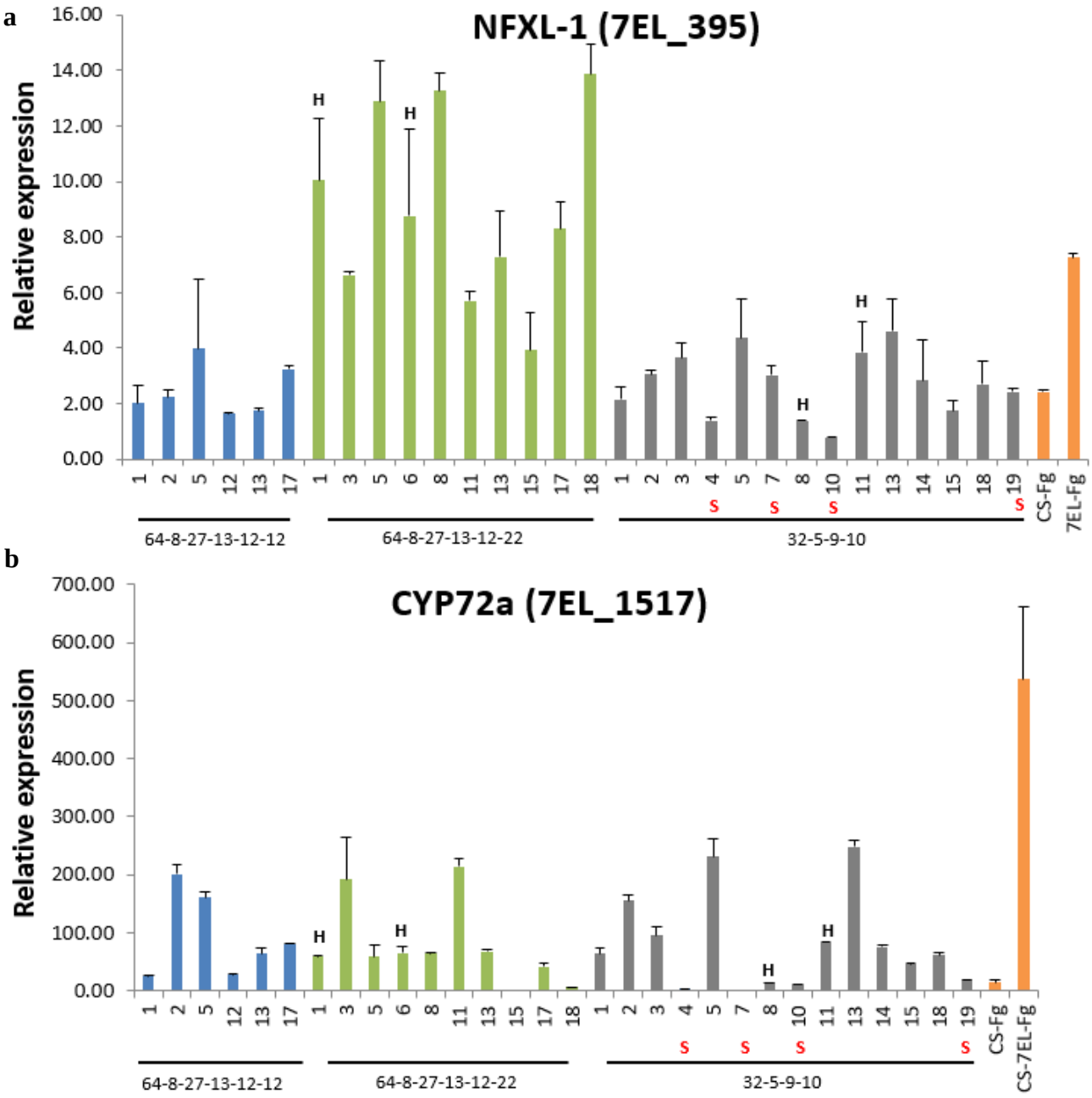


Figure 22a,b: Relative expression of 7EL genes present in the region of introgression in two BC_1F_7 families and one BC_1F_5 family.
Values are mean of two technical replicates; error bars represent standard deviation. "S" indicates progeny that were susceptible to *F. graminearum* infection. "H" indicates progeny that are homozygous for the 7EL introgression. Orange bars represent the controls CS-Fg and addition line CS-7EL-Fg.

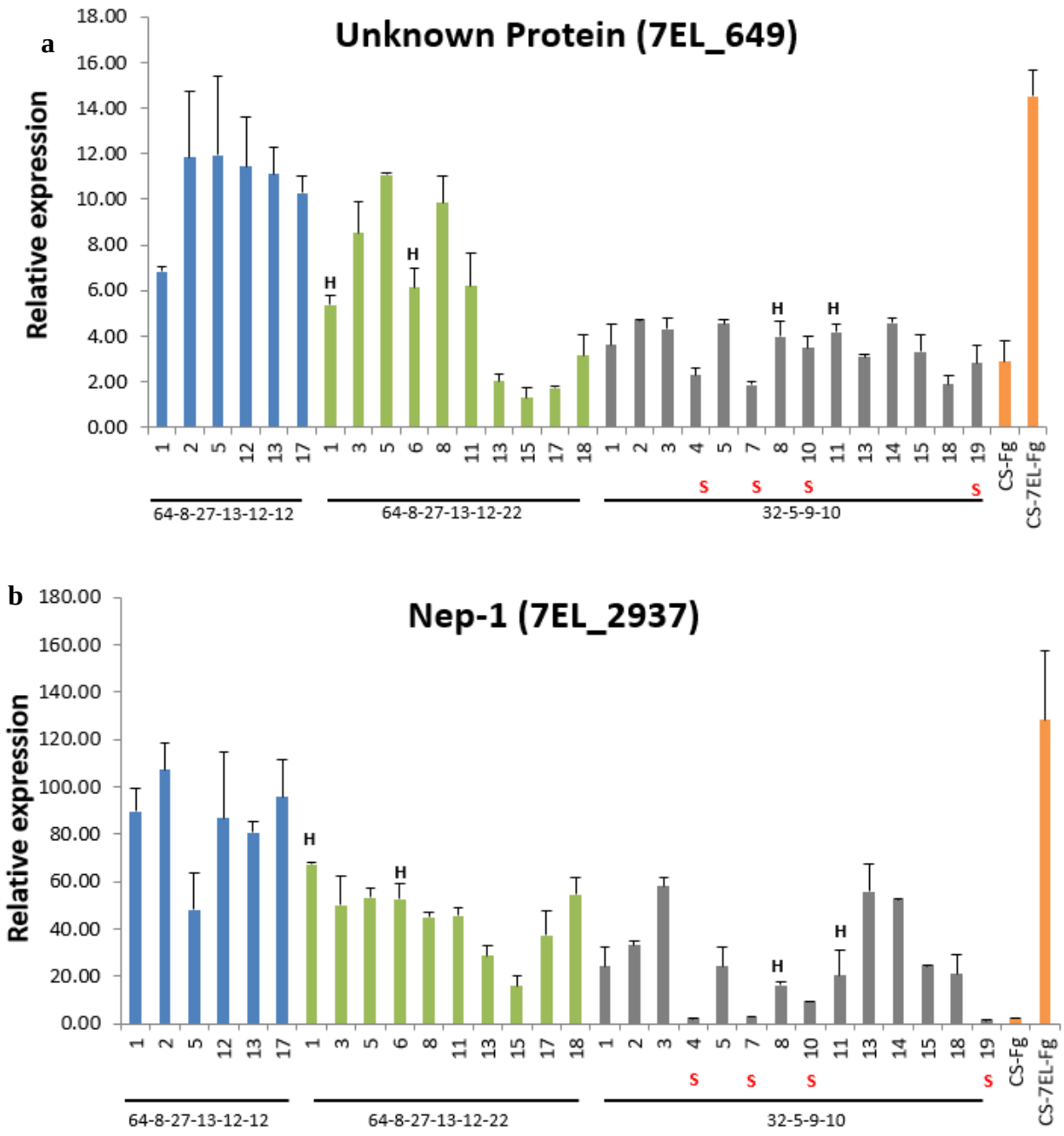


Figure 23a,b: Relative expression of 7EL genes present in the region of introgression in two BC_1F_7 families and one BC_1F_5 family.
Values are mean of two technical replicates; error bars represent standard deviation. "S" indicates progeny that were susceptible to *F. graminearum* infection. "H" indicates progeny that are homozygous for the 7EL introgression. Orange bars represent the controls CS-Fg and addition line CS-7EL-Fg.

Based on the results, three main trends were observed from the gene expression analysis: 1) there was variation in expression level between progeny of a given family, 2) expression level also differed between families, including the two sister families 64-8-27-13-12-12 and 64-8-27-13-12-22, 3) there were variable expression level for some genes when comparing the 7EL introgressed progeny with the addition line CS-7EL.

In the 64-8-27-13-12-22 family, a variation in expression level between progeny can be observed. Examining gene expression for the anthocyanidin reductase gene (Figure 21a) it was observed that progeny 5, 6 and 13 had similar expression levels, while progeny 11 had much higher expression levels and progeny 15 and 17 had much lower expression levels. However, this expression pattern between progeny is not consistent for the other genes. Additional variation in expression levels within this family can also be observed by examining the IGPS (Figure 21b) and CYP72a (Figure 22b) genes. In contrast, progeny 1 and 6, which were both homozygous for the 7EL introgression, appeared to have similar expression levels across all six genes that were tested (Figure 21, Figure 22, Figure 23).

In the 64-8-27-13-12-12 family, there was also variation of expression level within this family. This can be seen particularly for the CYP72a gene where progeny 1 and 12 appear to be lower expressed compared to the rest of the family (Figure 22b). Variation in expression level between this family and family 64-8-27-13-12-22 was observed. Gene expression level tended to be higher among the progeny of 64-8-27-13-12-12 for both the unknown protein and the Nep-1 protein (Figure 23), while they were lower for the other four genes. Furthermore the expression of the IGPS and the NFXL-1 genes appeared to be silenced in this family as the levels were similar to the level observed in CS (Figure 21b). This was surprising since progeny from both

families appeared to be genotypically and phenotypically similar and contained the same region of introgression.

Gene expression results for the 32-5-9-10 family were consistent with the genotype results which showed a smaller region of introgression in this family of approximately 22Mbp (Figure 17b). As expected, only background level of expression was observed in this family for the anthocyanidin reductase and IGPS genes (Figure 21) since they are expected to be outside the region of introgression. NFXL-1 was also expressed at low level, with values for progeny containing the 7EL fragment being similar or close to values for progeny without introgression and for CS; it is unclear whether there is any expression of 7EL NFXL-1 in that family (Figure 22). CYP72a showed higher than background level of expression in most of the 32-5-9-10 progeny carrying the 7EL fragment (Figure 22). Interestingly, the expression of the unknown protein gene, which is located near the first positive 7EL marker in the 32-5-9-10 family, was also at background level while Nep-1 was clearly expressed in the progeny carrying the 7EL fragment (Figure 23a).

Finally, variable expression level was observed for many genes when comparing the 7EL introgressed progeny with the addition line CS-7EL. This is particularly evident for CYP72a (Figure 22b) and NEP-1 (Figure 23b) where expression in the addition line is much higher than the 7EL introgressed progeny.

The expression level of all six 7EL genes was also determined for resistant, homozygous progeny of the 64-8-27-13-12-6 family (Appendix 10). Due to the large differences (3-4 times) in Cq values for all progeny from this family compared to the other families, the data could not be normalized for comparison of gene expression among all families (Appendix 4). A complete failure of the -80°C freezer that contained the RNA and cDNA samples for that family led to a

loss of the samples and made it impossible to repeat the experiments in the timeframe available. The gene expression analysis results demonstrated variation within the family across all six genes (Appendix 10). This was particularly evident when looking at the results for the NFXL-1 and unknown protein genes. These results support the previously made observation that variation exists in the 7EL introgressed progeny within a family.

3.5 Objective 5 – Determining expression levels of wheat genes present within and outside the region of introgression using RT-qPCR

The goal of this objective was to examine the expression profiles of wheat genes differentially expressed between the two treatments and two lines, and present within and outside the region of introgression in two BC_1F_7 families and one BC_1F_5 family. The gene expression was validated using RT-qPCR.

3.5.1 Differentially expressed wheat genes located outside the region of introgression

Three wheat genes located outside of the region of introgression (596 Mbp to 638 Mbp) were selected, as described in section 2.5.1. Figure 24 shows the position of those genes onto wheat chromosomes.

Gene Annotation	Gene ID
Fructokinase -2	7DL-sc2291
Thiamine Thiazole synthase	7DL-sc243
Ferredoxin NADP(H) oxidoreductase	6DS-sc1131

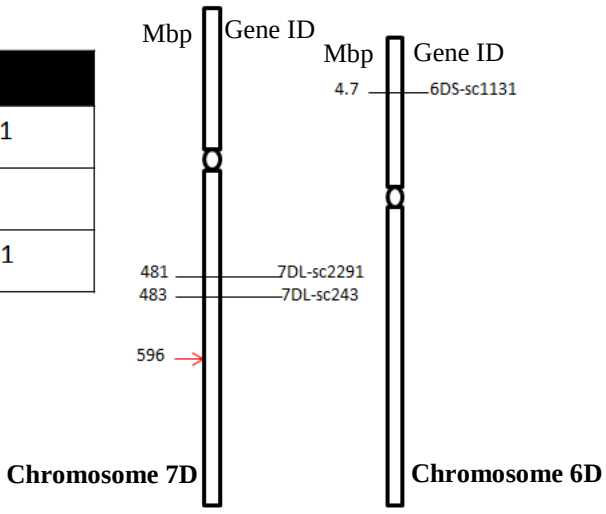
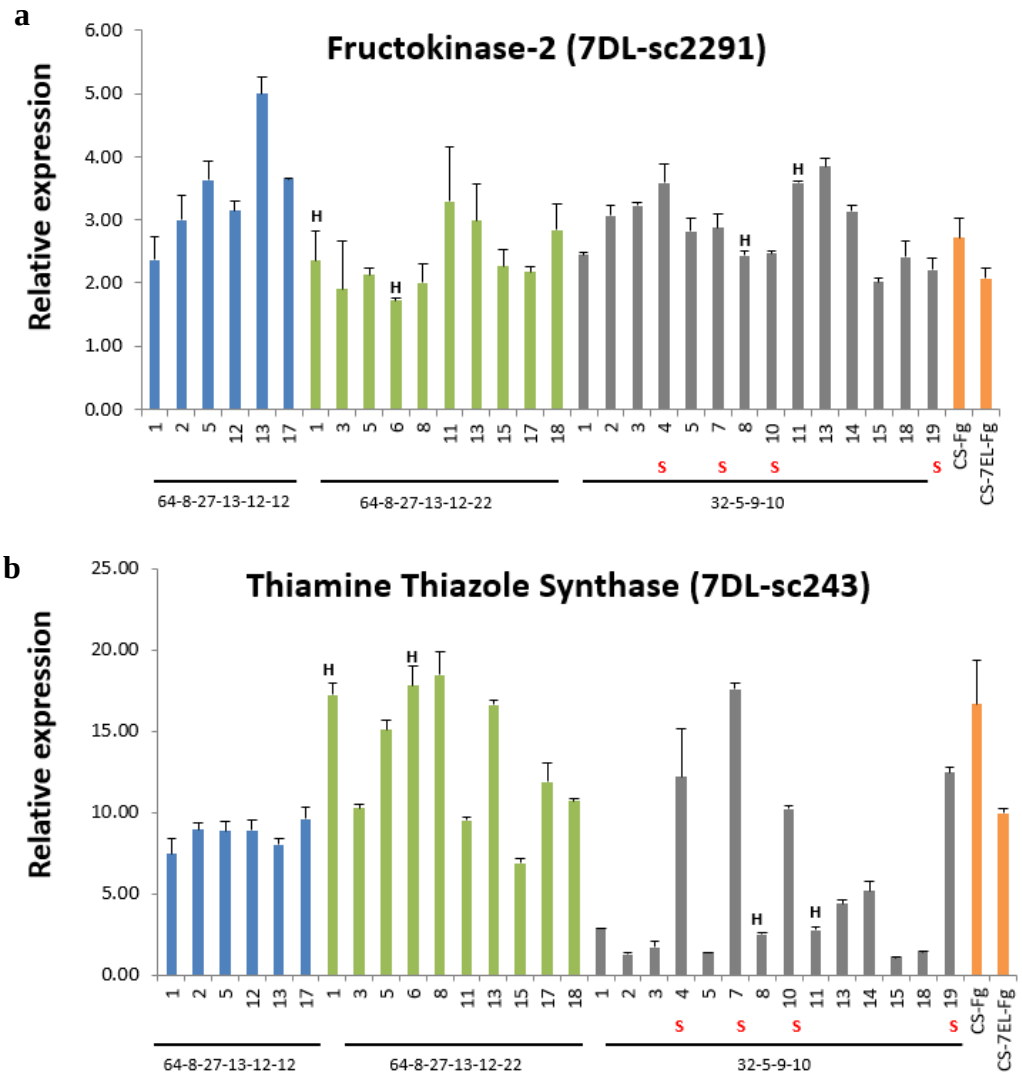


Figure 24: Position of wheat genes located outside of the region of introgression on wheat chromosomes 6D and 7D.
Figure not drawn to scale. Red arrow indicates region of introgression starting at 596 Mbp.

RT-qPCR was performed to determine expression levels of the three wheat genes in two BC_1F_7 families (64-8-27-13-12-12 and 64-8-27-13-12-22) and one BC_1F_5 family (32-5-9-10) (Figure 25).



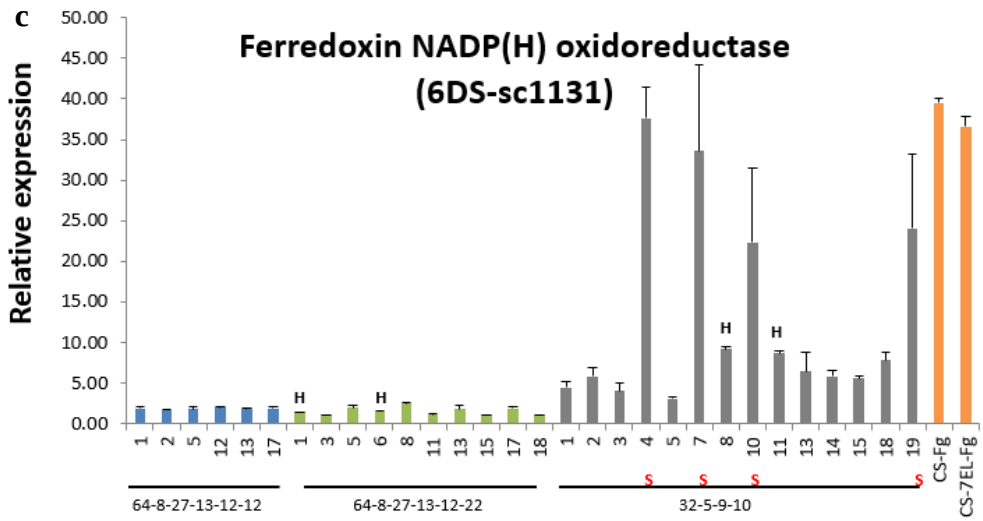


Figure 25 a,b,c: Expression of wheat genes located outside of the region of introgression in two BC_1F_7 families and one BC_1F_5 family.
Values are mean of two technical replicates; error bars represent standard deviation. "S" indicates progeny that were susceptible to *F. graminearum* infection. "H" indicates progeny that were homozygous for the 7EL introgression. Orange bars represent the controls CS-Fg and addition line CS-7EL-Fg.

Based on these results, a few trends were seen. Firstly there was variable expression level for those three wheat genes when comparing the 7EL introgressed progeny with the controls CS-Fg and the addition line CS-7EL-Fg. This was particularly obvious when looking at the expression level of the ferredoxin NADP(H) oxidoreductase gene (Figure 25c). Resistant progeny of all three families had reduced expression level compared to the controls while the susceptible progeny (without introgression) of the 32-5-9-10 family were expressing that gene at a much higher level. It is also interesting to note that the resistant progeny of the 32-5-9-10 family, containing the smaller region of introgression (22 Mbp) had a higher expression level for ferredoxin NADP(H) oxidoreductase than the two sister families containing a larger region of introgression (42 Mbp). These results suggest that this gene was silenced or downregulated within the three families in association with the introgression events.

Another trend that was observed was that the sister families 64-8-27-13-12-12 and 64-8-27-13-12-22 had variable expression levels for the genes tested, even though they had the same genotype. Progeny from the 64-8-27-13-12-12 family had slightly higher expression level in the fructokinase-2 gene when compared with the sister family 64-8-27-13-12-22 (Figure 25a), however when looking at the thiamine thiazole synthase gene, the 64-8-27-13-12-12 family had lower expression level than many progeny of its sister family (Figure 25b).

Additionally, some variation within the family was also observed. This can be seen when looking at the thiamine thiazole synthase gene in the 64-8-27-13-12-22 family (Figure 25b), where some progeny within the family have higher expression level than others.

In summary, the results suggested that introgression events have affected expression of wheat genes outside the region of introgression.

3.5.2 Differentially expressed wheat genes present within the region of introgression

Two wheat genes differentially expressed between CS and CS-7EL and located within the region of introgression were selected as described in section 2.5.1. Figure 26 shows the position of those genes onto wheat chromosome 7D.

Gene Annotation	Gene ID
FTSH-1 Metalloprotease	7DL-sc539
Thaumatin-like protein	7DL-sc402

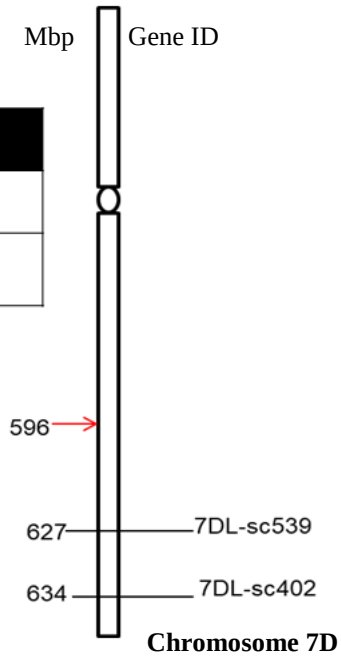


Figure 26: Position of wheat genes located inside the region of introgression on wheat chromosome 7D.
Figure not drawn to scale. Red arrow indicates region of introgression starting at 596 Mbp.

The expression levels of these two genes were determined using RT-qPCR in three families.

Figure 27 shows the relative expression of these two genes.

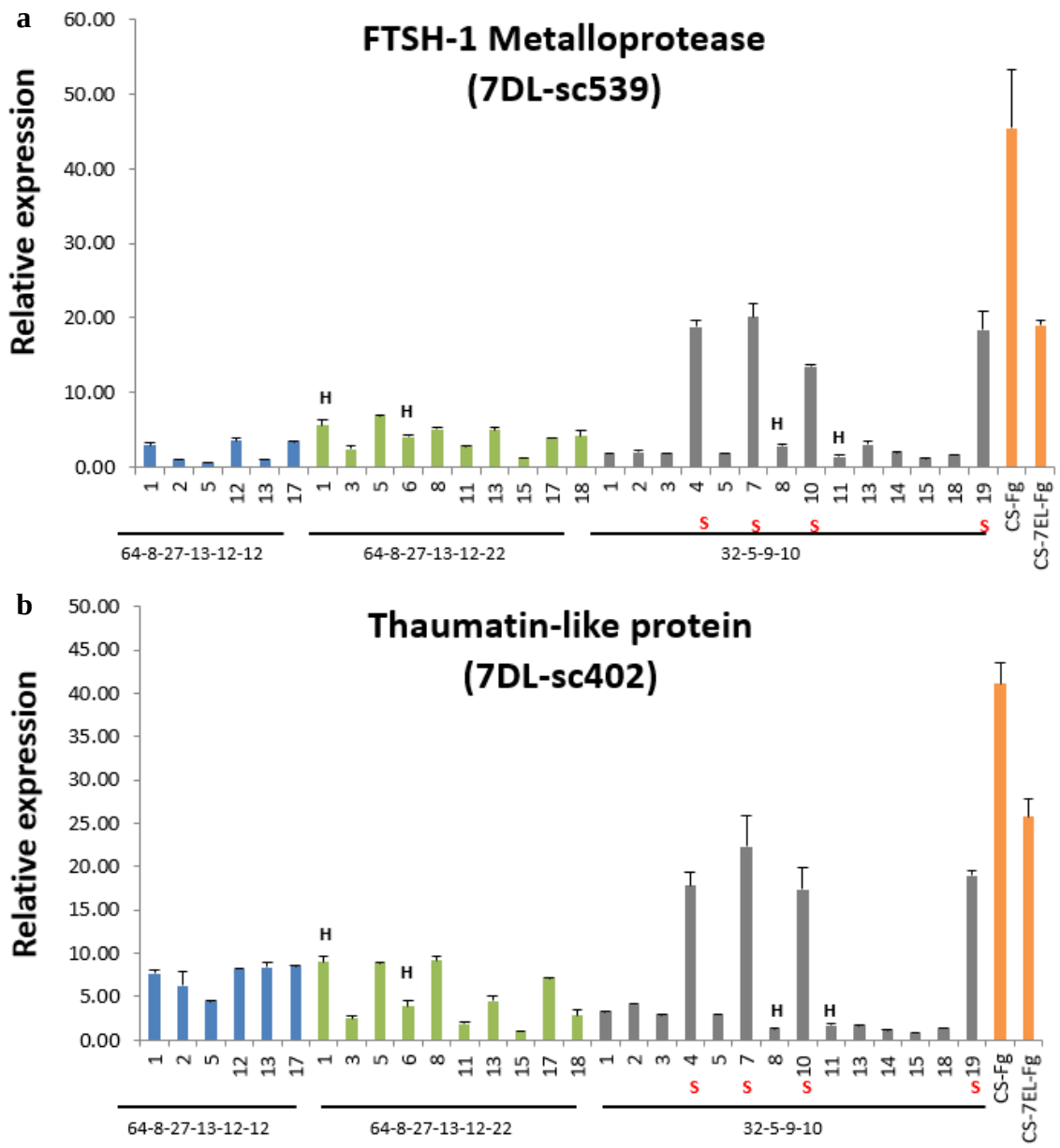


Figure 27 a,b: Expression of wheat genes located inside the region of introgression in two BC_1F_7 families and one BC_1F_5 family.
Values are mean of two technical replicates; error bars represent standard deviation. "S" indicates progeny that were susceptible to *F. graminearum* infection. "H" indicates progeny that were homozygous for the 7EL introgression. Orange bars represent the controls CS-Fg and addition line CS-7EL-Fg.

For both genes, CS-Fg had a higher expression level than the addition line CS-7EL-Fg (Figure

27). In addition, only the susceptible progeny, of the 32-5-9-10 family, showed expression in the

same range as the control samples. The level of expression detected in homozygous resistant progeny, with no 7D DNA in the region of introgression, was considered background level and could be due to the difficulty in designing gene- and sub-genome specific primers given the redundancy in the wheat genome. Surprisingly, resistant progeny heterozygous for the region of introgression had expression level in the same range as resistant progeny homozygous for that region; this is particularly obvious for the FTSH-1 metalloprotease gene (Figure 27a). These results suggest that the expression of at least some of the 7DL genes located in the region overlapping with the 7EL introgression is repressed.

As mentioned in section 3.4.1 the gene expression levels for all five wheat genes were compared separately within resistant, homozygous progeny of the 64-8-27-13-12-6 family (Appendix 11). These results indicate that there is less variation in level of gene expression for the 7D wheat genes (Appendix 11) than was observed with the 7EL genes (Appendix 10). An examination of the Cq values strongly suggested that the expression observed for the genes ferredoxin NADP(H) oxidoreductase, FTSH-1 metalloprotease and thaumatin-like was only at background level, suggesting that those genes were not really expressed in those progeny (data not shown). This would be consistent with the results obtained with the two other sister families.

Chapter 4: Discussion

The long term goal of this research project is to identify and characterize genes present in the 7EL region of *Th. elongatum* that are responsible for FHB resistance in *T. aestivum*. To contribute to this goal, this thesis focused on identification and characterization of the smallest region of introgression responsible for FHB resistance in BC_1F_4, BC_1F_5 and BC_1F_7 families. Secondly, expression patterns of a few 7EL genes present in the region of introgression were also characterized in progeny from the same BC_1F_5 and BC_1F_7 families. Additionally, a few wheat genes present within and outside the region of introgression were tested to examine their expression patterns. The key findings and learning are summarised below.

4.1 Introgressions in all families

Introduction of novel genetic material from *Th. elongatum* also known as *Lophopyrum elongatum*, into wheat is not a new concept and has been used to improve pest resistance and yield (Monneveux et al., 2003; Singh et al., 1998). FHB resistance was mapped onto the 7E chromosome from *Th. ponticum* using wheat substitution lines (Shen and Ohm, 2007). Subsequent research by Wang et al., (2010) looked at addition lines CS-7E and CS-7EL from *Th. elongatum* and identified a potential source of FHB resistance present on the long arm of chromosome 7E. In this study, previously designed 7EL and 7D specific markers from Chen et al., (2013); Gou et al., (2016); Tekieh, (2016) were used to approximate the end points of introgressed 7E fragments. The smallest region of introgression was estimated to be at least 22 Mbp in the 32-5-9-10 family and at least 42 Mbp in the three sister families 64-8-27-13-12-6, -12 and -22. Based on the size of the introgression fragments, the number of genes with a homoeolog

in wheat that were present in those fragments were 427 in the 42 Mbp region and 210 in the 22 Mbp region.

The disease ratings from progeny in the BC_1F_5 and BC_1F_7 families (Appendix 7) were compared with the gene expression results from FgGAPDH (Figure 19a). FgGAPDH was used as a proxy for the amount of *F. graminearum* present in the samples at 3 dpi. The results showed that the difference in infection level between resistant and susceptible progeny was much more obvious with the disease ratings than with the FgGAPDH levels. As the heads were collected for RNA extraction at 3 dpi while the disease rating results were taken at 14 dpi, this suggests that the F. graminearum infection continued to progress over time, increasing the observed difference between resistant and susceptible progeny.

Differences in 7EL gene expression

The main trends observed when examining 7EL gene expression in progeny from the BC_1F_5 and BC_1F_7 families were that there was increased variation in gene expression level within families and among families, including the two sister families 64-8-27-13-12-12 and -22. That differential gene expression could be due to DNA methylation, gene silencing or alterations in repetitive sequences, including transposable elements.

DNA methylation is a heritable epigenetic mark that control gene expression and is responsive to environmental stresses. Research by Chodavarapu et al., (2012) looked at the degree to which DNA methylation is inherited in rice and how it influences transcription in two inbred parental strains (Nipponbare and *indica*) and their hybrid offsprings. Their results showed that epigenetic

heritability is quite variable. They found that cytosines were differentially methylated at a rate of 7.48% between the inbred parental strains while in the hybrid offspring only 0.79% of cytosines were differentially methylated. They also found that the differentially methylated cytosines were clustered on chromosomes in regions where siRNA production differed (Chodavarapu et al., 2012). This study demonstrated the effect of epigenetic plasticity in inbred parental strains and in their hybrid offspring and suggested that it could play an important role in environmental adaptation or hybrid vigour (Chodavarapu et al., 2012). DNA methylation as a mechanism of epigenetic gene expression control has also been studied in wheat. The study by Gardiner et al., (2015) examined genome wide methylation patterns between the three sub-genomes of bread wheat. They demonstrated that differential methylation existed between the three sub-genomes, and this underlying methylation correlated with sub-genome specific changes in gene expression. Methylation was further classified into uni-genome (methylation of a single sub-genome at a site where the other two were non-methylated), bi-genome (methylation of two sub-genomes at a site where the other sub-genome was non-methylated) or tri-genome (methylation of all three sub-genomes) (Gardiner et al., 2015). The results showed that uni-genome methylation led to reduced expression in that specific genome compared with tri-genome methylated sites in promoter regions. They were also able to show that conserved methylation across all three sub-genomes predominated but genome-specific methylation was still significant (Gardiner et al., 2015). Both of these studies showed that methylation patterns play a role in gene expression.

Gene silencing occurring during or after introgression of the 7EL fragment could also play a role in the variation observed. Research by Liu et al., (2015) examined whether somatic hybridizations induced epigenetic variations that affected gene expression or transposon

activation. This was done through DNA profiling techniques to characterize genetic or epigenetic alterations in six derivatives of bread wheat/tall wheatgrass somatic hybrids with different phenotypes. The somatic hybrid introgression lines were generated by fusing bread wheat and tall wheatgrass protoplasts together. The protoplasts were cultured and four regenerated plants were derived through segregating progeny obtained by self-fertilization. Cytogenetic analysis of introgressed lines derived from that material was performed through karyotyping and frequency of sequence variation was estimated using Amplified Fragment Length Polymorphism (AFLP) profiles. The results showed that there was sequence loss following somatic hybridization but only at a rate of 4.7%. A much larger effect was seen through epigenetic alterations, where approximately 24% of cytosine-methylation changes were observed in asymmetric somatic hybrids. This showed that somatic hybridization induced a broad spectrum of cytosine-methylation changes that affected gene expression (Liu et al., 2015). Their results also suggested repetitive sequences were a driving force behind the de novo genetic variation generated in introgression lines. It was proposed that repetitive sequences drive the formation of double strand breaks or other genomic alterations that occur in association with introgression (Liu et al., 2015). This paper identified that the somatic hybridization process lead to many genetic alterations similar to those induced by polyploidization or sexual wide hybridization in a short time frame and with greater extent.

Transposable elements are mobile genetic elements present in most animal and plant genomes. The most abundant transposable elements in plant genomes include long terminal repeat (LTR) retrotransposons and miniature inverted transposable elements (MITEs) (Casacuberta and Santiago, 2003). Transposable elements can perform epigenetic silencing and reduce expression

of adjacent genes and these changes can be heritable. After polyploidization transposable elements can become activated and modify genes located nearby. Additionally, even if silencing of transposable elements is transitory, they can participate in reorganizing of the functional genome after polyploidization (Kashkush et al., 2003). It was also observed that in the polyploidization process, the parent with greater transposable element content will become the recessive sub-genome. This led to the hypothesis that the parental genome with the lowest transposable element may become the dominant genome (Cheng et al., 2016). The silencing of genes is dependent on whichever gene is less transcribed, meaning that those genes can be altered more easily without phenotypic consequences (Cheng et al., 2016). These effects are more important the more divergent the parental species. Presence of transposable elements is a likely explanation leading to the variability in expression. This should be tested first since expression of some transposons were observed in the RNA-Seq dataset indicating that there were active transposons present in the region of introgression.

Examining gene expression results between the two sister families and the 32-5-9-10 family, variation in gene expression were observed. Based on the gene expression results it was seen that the 32-5-9-10 family had lower expression level for the 7E genes than the two sister families, suggesting that the size of the introgression may play a role in gene expression. The size of the introgression in this family was approximately 22Mbp, and the first positive 7EL marker in this family was Lu1851-2 (616 Mbp). Gene expression results for many of the progeny in the 32-5-9-10 family showed higher than background expression level for the CYP72a gene, mapped at 614 Mbp, supporting its presence in the introgressed 7EL fragment. In contrast, the unknown protein gene, at 616 Mbp, and possibly the NFXL-1 gene, at 612 Mbp showed background level of

expression in all progeny of that family, suggesting that these two genes are either located outside of the 7EL fragment integrated in the 32-5-9-10 family or that their expression is silenced as observed for IGPS and NFXL-1 in family 64-8-27-13-12-12 and previously observed by Liu et al., (2015). Research by Hu et al., (2012) designed and generated 43 markers that could distinguish *Th. elongatum* chromatin from wheat genomes. Mapping of those markers by GISH showed closely related profiles for *Th. elongatum* and wheat genome D, yet genomic rearrangements and duplications in each of the two genomes. Their results also suggested that chromosomal structural changes could have occurred during the process of producing *Th. elongatum* chromosome addition lines in wheat (Hu et al., 2012).

4.2 Differences in 7D gene expression

When examining wheat genes located outside the region of introgression, it was observed that there was variation in expression levels among the families and between 7EL introgressed progeny and the control samples (CS-Fg and CS-7EL-Fg). This variation was particularly evident when comparing the expression levels of the introgressed progeny with CS-Fg for the thiamine thiazole synthase gene (Figure 25b) and the Ferredoxin NADP(H) oxidoreductase gene (Figure 25c). Those variation in expression suggest that gene expression levels can be altered by introgression events for genes on the introgressed chromosome as well as a gene on a different chromosome. Although Liu et al., (2015) showed changes in expression of wheat genes in introgressed lines, those changes were not mapped to specific wheat chromosomes.

Although the genes FTSH metalloprotease and thaumatin-like were absent in 7EL fragment homozygous progeny, amplification signals were still detected for those genes. Those signals could be due to cross amplification of homoeologous genes in genomes A, B and possibly E. Although efforts were made to design gene specific amplification primers, the high homology

between the three wheat genomes and the lack of a complete reference sequence for *Th. elongatum* made it hard to eliminate the off-target amplification.

4.3 Future Work

Further work to expand the results of this study and to address long term goals are proposed in this section.

The first proposed experiment would be to determine whether the expression patterns are inherited from parents to the progeny. To test this hypothesis a few progeny from each of the three families 64-8-27-13-12-12, 64-8-27-13-12-22 and 32-5-9-10 would be grown and subsequently genotyped and phenotyped. All homozygous progeny in each family would be used, and if there are none, progeny having the most seed would be used. The genotyping and phenotyping would not only help confirm whether the progeny remain resistant to *F. graminearum* but would also help determine the size of the introgression in the next generation. Assuming the size of the introgression is stable in the next generation of the progeny, and the progeny remain resistant to *F. graminearum*, the next step would be to check the expression patterns of the progeny to see whether they are similar to the parents. Expression patterns similar between parents and progeny would indicate that the expression patterns are heritable. However, if the expression patterns differ between parents and progeny then it would be proposed that there is another factor affecting the inheritance of these patterns.

Secondly, another experiment would be performed to determine the methylation pattern in the parents and progeny in the three families. The goal of this experiment would be to determine whether the methylation patterns are responsible for the variability in gene expression that was observed among the parents (and possible progeny). As shown in other studies, differential methylation patterns play a role in gene expression (Liu et al., 2015; Chodavarapu et al., 2012).

To perform this experiment the methylation patterns can be determined first through genomic enrichment followed by bisulfite treatment as shown by Gardiner et al., (2015).

To achieve the long term goal of identifying candidate genes responsible for FHB resistance from the 7EL region of *Th. elongatum*, the region of introgression would need to be reduced. This can be done by using the radiation hybrid mapping technique as demonstrated by Buerstmayr et al.,(2018). This process uses ionizing radiation to generate double strand breaks which are then repaired through nonhomologous end-joining. This repair pathway can cause various genomic rearrangements and even large chromosomal deletions. Using this technique, it is possible to delete large chunks of the introgressed region to achieve a smaller region of introgression (Buerstmayr et al., 2018). Once the introgressed region is reduced and shown to still carry FHB resistance, it is assumed that a fewer number of candidate genes would be present within the region. Ideally the introgressed region would contain 5-10 candidate genes, where each of them can be tested individually for loss of FHB resistance using CRISPR. The CRISPR procedure would create knock-outs of the specific genes and then the mutant plants can be phenotyped to determine whether they remain resistant to *F. graminearum* infection.

4.4 Conclusion

In conclusion the main goal of this study was to identify the smallest region of introgression from the 7EL chromosome arm of *Th. elongatum* and to subsequently test expression level of a few 7EL genes present in this region. The smallest region of introgression was estimated to be at least 22 Mbp in the 32-5-9-10 family while it was estimated to be at least 42 Mbp in the families derived from the 64-8-27-13-12 plant. Gene expression of 7EL and some wheat genes using RT-qPCR showed variable expression between all three families. Subsequent experiments should

follow up on determining whether these expression patterns are inherited by the progeny and to try to reduce the introgression through other methods besides recombination.

References

Brown, N.A., Urban, M., van de Meene, A.M.L., and Hammond-Kosack, K.E. (2010). The infection biology of *Fusarium graminearum*: Defining the pathways of spikelet to spikelet colonisation in wheat ears. Fungal Biol. **114**: 555–571.

Buerstmayr, H., Ban, T., and Anderson, J.A. (2009). QTL mapping and marker-assisted selection for *Fusarium* head blight resistance in wheat: a review. Plant Breed. **128**: 1–26.

Buerstmayr, M., Steiner, B., Wagner, C., Schwarz, P., Brugger, K., Barabaschi, D., Volante, A., Valè, G., Cattivelli, L., and Buerstmayr, H. (2018). High-resolution mapping of the pericentromeric region on wheat chromosome arm 5AS harbouring the *Fusarium* head blight resistance QTL Qfhs.ifa-5A. Plant Biotechnol. J. **16**: 1046–1056.

Cappellini, R.A. and Peterson, J.L. (1965). Macroconidium formation in submerged cultures by a non-sporulating strain of *Gibberella zeae*. Mycologia **57**: 962.

Casacuberta, J.M. and Santiago, N. (2003). Plant LTR-retrotransposons and MITEs: control of transposition and impact on the evolution of plant genes and genomes. Gene **311**: 1–11.

Ceoloni, C., Forte, P., Kuzmanović, L., Tundo, S., Moscetti, I., De Vita, P., Virili, M.E., and D'Ovidio, R. (2017). Cytogenetic mapping of a major locus for resistance to Fusarium head blight and crown rot of wheat on *Thinopyrum elongatum* 7EL and its pyramiding with valuable genes from a *Th. ponticum* homoeologous arm onto bread wheat 7DL. Theor. Appl. Genet. **130**: 2005–2024.

Chen, S., Huang, Z., Dai, Y., Qin, S., Gao, Y., Zhang, L., Gao, Y., and Chen, J. (2013). The development of 7E chromosome-specific molecular markers for *Thinopyrum elongatum* based on SLAF-seq technology. PLoS One **8**: e65122.

Cheng, F., Sun, C., Wu, J., Schnable, J., Woodhouse, M.R., Liang, J., Cai, C., Freeling, M., and Wang, X. (2016). Epigenetic regulation of subgenome dominance following whole genome triplication in *Brassica rapa*. New Phytol. **211**: 288–99.

Chodavarapu, R.K., Feng, S., Ding, B., Simon, S.A., Lopez, D., Jia, Y., Wang, G.-L., Meyers, B.C., Jacobsen, S.E., and Pellegrini, M. (2012). Transcriptome and methylome interactions in rice hybrids. Proc. Natl. Acad. Sci. U. S. A. **109**: 12040–5.

Colorado Wheat (2018). Why is the wheat genome so complicated?, from: https://coloradowheat.org/2013/11/why-is-the-wheat-genome-so-complicated/

Desjardins, A.E. (2006). Fusarium Mycotoxins: Chemistry, Genetics and Biology (American Phytopathological Society).

Dvořák, J. (1980). Homoeology between *Agropyron elongatum* chromosomes and *Triticum aestivum* chromosomes. Can. J. Genet. Cytol. **22**: 237–259.

Dvořák, J. (1979). Metaphase I pairing frequencies of individual *Agropyron elongatum* chromosome arms with *Triticum* chromosomes. Can. J. Genet. Cytol. **21**: 243–254.

Dvorák, J., Edge, M., and Ross, K. (1988). On the evolution of the adaptation of *Lophopyrum elongatum* to growth in saline environments. Proc. Natl. Acad. Sci. U. S. A. **85**: 3805–9.

Dvorak, J. and Knott, D.R. (1974). Disomic and ditelosomic additions of diploid *Agropyron elongatum* chromosomes to *Triticum aestivum*. Can. J. Genet. Cytol. **16**: 399–417.

FAO of the UN (2018). FAO Cereal Supply and Demand Brief | World Food Situation | Food and Agriculture Organization of the United Nations, from: http://www.fao.org/worldfoodsituation/csdb/en/

Fedak, G., Wolfe, D., Chi, D., Ouellet, T., Cao, W., and Xue, A. (2016). Transfer of FHB Resistance from *Thinopyrum elongatum* to Spring Wheat. In (Florianopolis, Brazil), p. P 44.

Gardiner, L.-J., Quinton-Tulloch, M., Olohan, L., Price, J., Hall, N., and Hall, A. (2015). A genome-wide survey of DNA methylation in hexaploid wheat. Genome Biol. **16**: 273.

Garg, M., Tanaka, H., Ishikawa, N., Takata, K., Yanaka, M., and Tsujimoto, H. (2009). *Agropyron elongatum* HMW-glutenins have a potential to improve wheat end-product quality through targeted chromosome introgression. J. Cereal Sci. **50**: 358–363.

Goswami, R.S. and Kistler, H.C. (2004). Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Pathol. **5**: 515–525.

Gou, L., Hattori, J., Fedak, G., Balcerzak, M., Sharpe, A., Visendi, P., Edwards, D., Tinker, N., Wei, Y.-M., Chen, G.-Y., and Ouellet, T. (2016). Development and validation of –expressed molecular markers specific for the long arm of chromosome 7E. Crop Sci. **56**: 354.

Greer, E., Martin, A.C., Pendle, A., Colas, I., Jones, A.M.E., Moore, G., and Shaw, P. (2012). The *Ph1* locus suppresses Cdk2-type activity during premeiosis and meiosis in wheat. Plant Cell **24**: 152–162.

He, X., Lillemo, M., Shi, J., Wu, J., Bjørnstad, Å., Belova, T., Dreisigacker, S., Duveiller, E., and Singh, P. (2016). QTL characterization of fusarium head blight resistance in CIMMYT bread wheat line Soru#1. PLoS One **11**: e0158052.

Health Canada (2016). Canadian Standards (Maximum Levels) for Various Chemical Contaminants in Foods, from: http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/contaminants-guidelines-directives-eng.php.

Hu, L.-J., Liu, C., Zeng, Z.-X., Li, G.-R., Song, X.-J., and Yang, Z.-J. (2012). Genomic rearrangement between wheat and *Thinopyrum elongatum* revealed by mapped functional molecular markers. Genes Genomics **34**: 67–75.

IWGSC (2014). A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science (80-.). **345**: 1251788–1251788.

IWGSC (2018). Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science (80-.). **361**: eaar7191.

Kashkush, K., Feldman, M., and Levy, A.A. (2003). Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. Nat. Genet. **33**: 102–6.

Komirenko, Z. (2018). Economic Cost of Fusarium (Alberta), from: https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex92#grading.

Liu, S., Li, F., Kong, L., Sun, Y., Qin, L., Chen, S., Cui, H., Huang, Y., and Xia, G. (2015). Genetic and epigenetic changes in somatic hybrid introgression lines between wheat and tall wheatgrass. Genetics **199**: 1035–45.

Long, X., Balcerzak, M., Gulden, S., Cao, W., Fedak, G., Wei, Y.-M., Zheng, Y.-L., Somers, D., and Ouellet, T. (2015). Expression profiling identifies differentially expressed genes associated with the fusarium head blight resistance QTL 2DL from the wheat variety Wuhan-1. Physiol. Mol. Plant Pathol. **90**: 1–11.

Luo, P.-G., Hu, X.-Y., Chang, Z.-J., Zhang, M., Zhang, H.-Q., and Ren, Z.-L. (2009). A new stripe rust resistance gene transferred from *Thinopyrum intermedium* to hexaploid wheat (*Triticum aestivum*). Phytoprotection **90**: 57.

Martinez, M., Cunado, N., Carcelen, N., and Romero, C. (2001). The *Ph1* and *Ph2* loci play different roles in the synaptic behaviour of hexaploid wheat *Triticum aestivum*. TAG Theor. Appl. Genet. **103**: 398–405.

Miller, S.S., Watson, E.M., Lazebnik, J., Gulden, S., Balcerzak, M., Fedak, G., and Ouellet, T. (2011). Characterization of an alien source of resistance to Fusarium head blight transferred to Chinese Spring wheat [1 1] ECORC contribution #10-151. Botany **89**: 301–311.

Monneveux, P., Reynolds, M.P., Aguilar, J.G., and Singh, R.P. (2003). Effects of the 7DL.7Ag translocation from *Lophopyrum elongatum* on wheat yield and related morphophysiological traits under different environments. Plant Breed. **122**: 379–384.

Okubara, P.A. and Jones, S.S. (2011). Seedling resistance to *Rhizoctonia* and *Pythium* spp. in wheat chromosome group 4 addition lines from *Thinopyrum* spp. Can. J. Plant Pathol. **33**: 416–423.

Pot, M. (2017). Canadian Wheat [Image]. Grain Perspect. Dly. Rep.

Rawat, N., Pumphrey, M.O., Liu, S., Zhang, X., Tiwari, V.K., Ando, K., Trick, H.N., Bockus, W.W., Akhunov, E., Anderson, J.A., and Gill, B.S. (2016). Wheat *Fhb1* encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium head blight. Nat. Genet. **48**: 1576–1580.

Roundy, B.A. (1985). Emergence and establishment of basin wildrye and tall wheatgrass in relation to moisture and salinity. J. Range Manag. **38**: 126.

Shen, X., Kong, L., and Ohm, H. (2004). Fusarium head blight resistance in hexaploid wheat (*Triticum aestivum*)- *Lophopyrum* genetic lines and tagging of the alien chromatin by PCR markers. TAG Theor. Appl. Genet. **108**: 808–813.

Shen, X. and Ohm, H. (2006). Fusarium head blight resistance derived from *Lophopyrum elongatum* chromosome 7E and its augmentation with *Fhb1* in wheat. Plant Breed. **125**: 424–429.

Shen, X. and Ohm, H. (2007). Molecular mapping of *Thinopyrum*-derived Fusarium head blight resistance in common wheat. Mol. Breed. **20**: 131–140.

Singh, R.P., Huerta-Espino, J., Rajaram, S., and Crossa, J. (1998). Agronomic effects from chromosome translocations 7DL.7AG and 1BL.1RS in spring wheat. Crop Sci.

Sutton, T., Whitford, R., Baumann, U., Dong, C., Able, J.A., and Langridge, P. (2003). The *Ph2* pairing homoeologous locus of wheat (*Triticum aestivum*): identification of candidate meiotic genes using a comparative genetics approach. Plant J. **36**: 443–56.

Tekieh, F. (2016). Towards the identification of candidate gene(s) for fusarium head blight resistance on the 7EL chromosome of *Thinopyrum elongatum*: design and use of genetic markers.

USDA (2018). World Agricultural Production, from: https://apps.fas.usda.gov/PSDOnline/app/index.html#/app/home,.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., and Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. **3**: 1–12.

Wang, J.-R., Wang, L., Gulden, S., Rocheleau, H., Balcerzak, M., Hattori, J., Cao, W., Han, F., Zheng, Y.-L., Fedak, G., and Ouellet, T. (2010). RNA profiling of fusarium head blight-resistant wheat addition lines containing the *Thinopyrum elongatum* chromosome 7E. Can. J. Plant Pathol. **32**: 188–214.

Wegulo, S.N. (2012). Factors influencing deoxynivalenol accumulation in small grain cereals. Toxins (Basel). **4**: 1157–80.

Yu, J., Bai, G., Zhou, W., Dong, Y., and Kolb, F.L. (2008). Genetics and resistance quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population of wangshuibai/wheaton.

Appendices

Appendix 1: Amount of reagents used for PCR protocol

	1 Reaction (µL)	32 Reactions (µL)
H_2O	5.575	82.4
10x PCR Buffer (Applied Biosciences)	1	32
$MgCl_2$	1	32
dNTPs (10µM)	0.3	9.6
Taq gold polymerase (Applied Biosciences)	0.125	4
Forward Primer (10 µM)	0.5	16
Reverse Primer (10 µM)	0.5	16
DNA Template (14ng/µL)	4	-

Appendix 2: All 7E and 7D molecular markers used for the PCR assay.

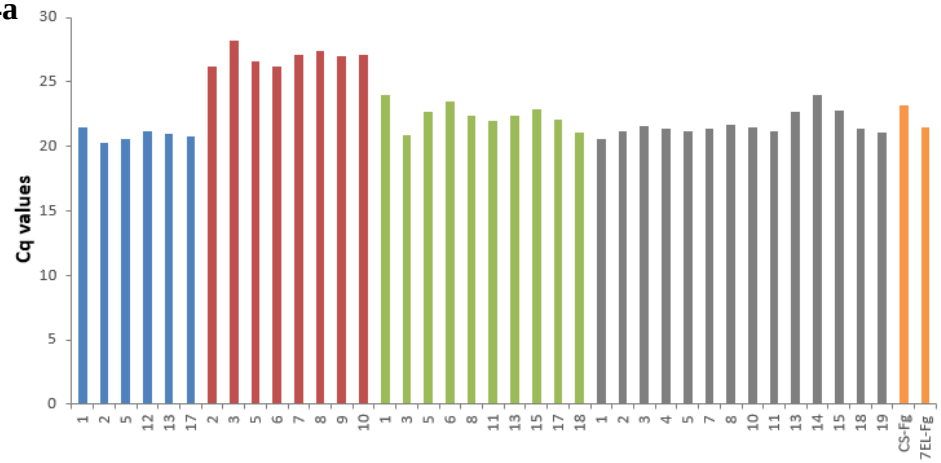
7EL primers	Tm(°C)	Sequence (5'-3')
2096-A3-7E	56	Forward: TAAGGGCTCCTCTGGTTCAA
		Reverse: TCTTGCTTGGTATGTTTCCTCG
1480-B1-7E	56	Forward: TGGACGGAGGTAGTATGAAACA
		Reverse: TGGTTGAATCGGACTGCATTTA
1383-A3-7E	56	Forward: TATGGTTTGGTGTGCTGTGG
		Reverse: AGGTACCGTGTAAACCTGATCT
3571-7E	60	Forward: TACCTTGCTGAAGATAGCCAGA
		Reverse: GCATCATGTAGTTTCAAATGTTAGTCG
Lu 1989-2	50	Forward: AGGGCATGCACTGTCTGG
		Reverse: ATGCCCTAAATGATACCCTGAG
455 7E	60	Forward: CAAACAATCCTCTGTTCGCTCTA
		Reverse: ACTGAACCTTGTTGTCCAATGA
p7es-21	60	Forward: GCACAAAAAGAGCAGAATAT
		Reverse: AAGCTTATTACAGGACCATG
Lu 625	50	Forward: TATATCTAAGAACAAGTAAAA
		Reverse: GTCGCCCCACAGCAGGAA
Lu 382	50	Forward: GCGGCGGCAGCATCAATCA
		Reverse: ACGCATCGAGTGGTGTTCTAT
Lu 1851-2	50	Forward: CTGGCCAGATGAAAGTAGTT
		Reverse: AAGCCAGTGACCCGGACAATAA
3201 7E	60	Forward: CGAGCAGGCCTGGAATATC
		Reverse: ACGCGATGCGCTAGAAAG
Lu-270-1	60	Forward: GCTTGCACACGGCGATTTATTG
		Reverse: CTGGCGGCGATGAGGGAGAAGA
2500-A1-7E	60	Forward: AAGGCTGTGCTACCAATTGAA
		Reverse: AGTCCTGAGTAACTGCGAAGA
4231-A2-7E	60	Forward: AAGCACTAACTAACACACTGAGAG
		Reverse: AGCGGCCACCTTGTTTAC
7D primers	**Tm(°C)**	**Sequence (5'-3')**
2368_A3_7DL	56	Forward: CCCATCTCATTGTTCTAAGAAATAGC
		Reverse: GATTTCCAGACTCTGTGCCA
1262_7DL	56	Forward: GGCGCTCACACAATAGGATAG
		Reverse: TTCTTCGTTGGATCGACCTTG
6092-A2-7D	60	Forward: TTTCATGGGTGATGGCTGAC
		Reverse: CCCAAAGTGACTGTCTCAACTT
2500-A3-7D	60	Forward: AGGGCAGATAACCTGCAAAG
		Reverse: AGATACATCCATTTGAGGGACAAG
3488_A2_7DL	60	Forward: GCCAACACATCCTCACATGAT
		Reverse: GGTAATTTCTAAATGGGTACATGGC

Appendix 3: List of housekeeping and other reference genes and their corresponding primer sequences.

Housekeeping genes	Sequence (5'-3')
hn-RNP-Q [1] **(Heterogenous nuclear ribonucleoprotein Q)**	Forward: TCACCTTCGCCAAGCTCAGAACTA
	Reverse: AGTTGAACTTGCCCGAAACATGCC
IAAOX [1] **(Indole – 3 acetaldehyde oxidase)**	Forward: CACAGCAGGATTTAAGCTCTGG
	Reverse: GGGATGGACTAATTTCACAGGC
FgGAPDH [1] **(*Fusarium graminearum* GAPDH)**	Forward: TGACTTGACTGTTCGCCTCGAGAA
	Reverse: ATGGAGGAGTTGGTGTTGCCGTTA
PR1 [1] **(Pathogenesis related protein 1)**	Forward: CGGTACATATATACAGCCGGTCTAA
	Reverse: TTCATCATCTGCAGCTACAACC

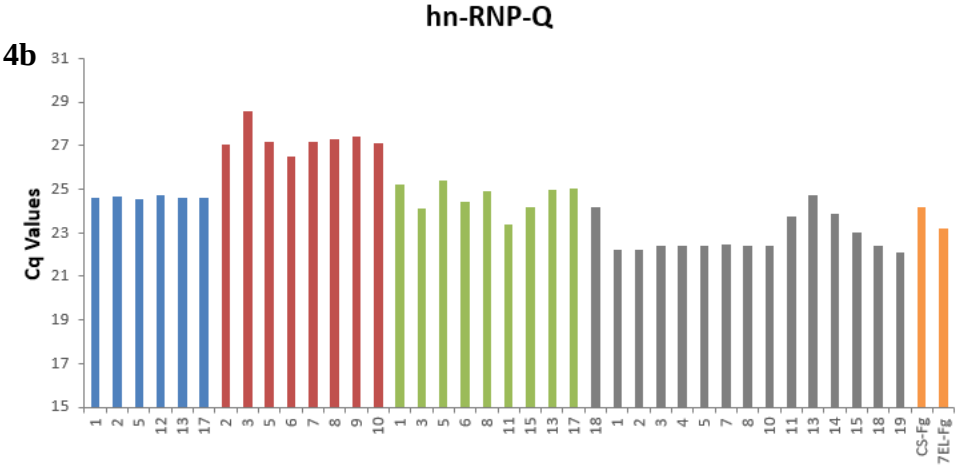
[1] (Long et al., 2015)

4a



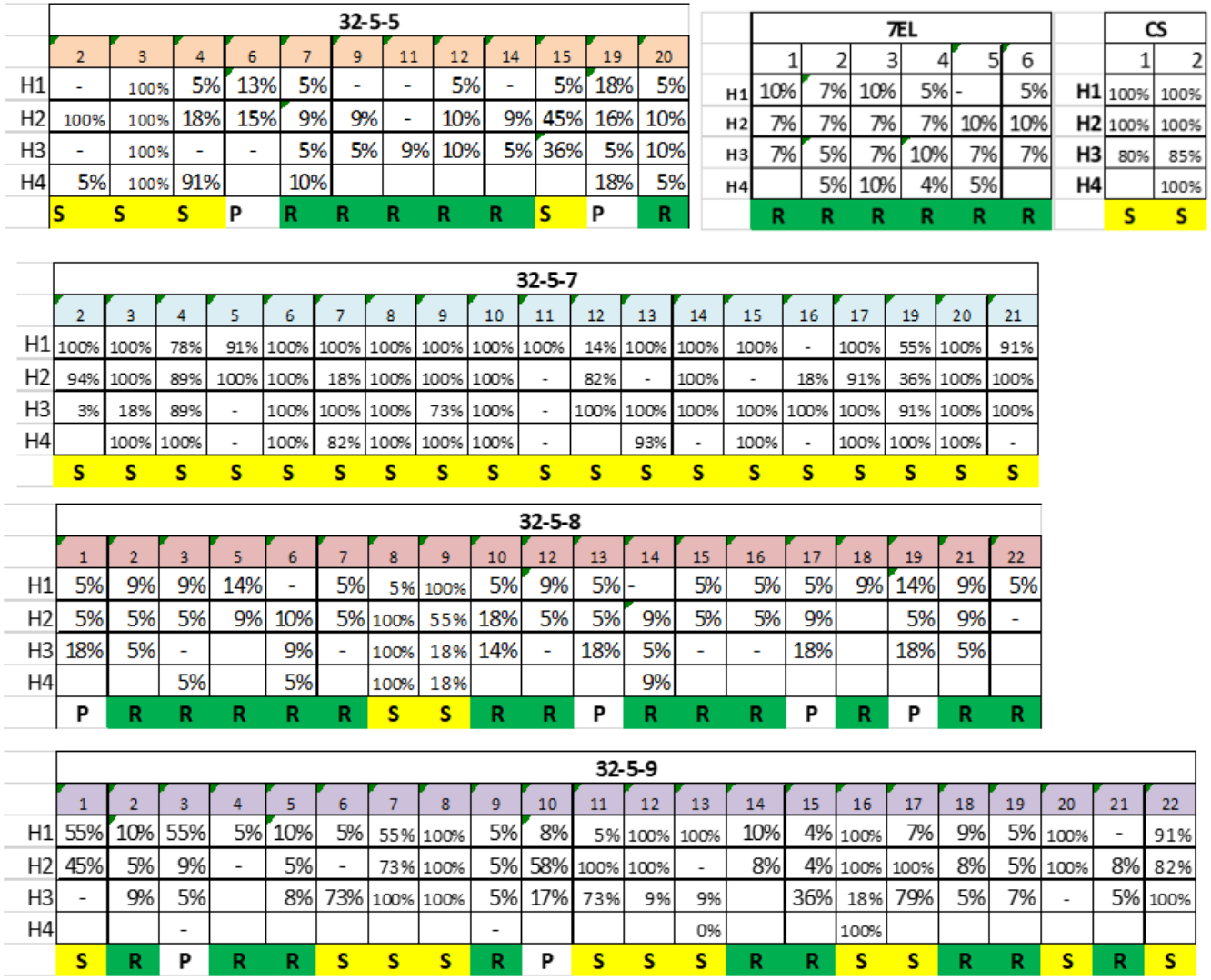
Progeny	Cq Value	
1	21.44	64-8-27-13-12-12
2	20.3	
5	20.57	
12	21.22	
13	21.02	
17	20.77	
2	26.21	64-8-27-13-12-6
3	28.25	
5	26.61	
6	26.18	
7	27.09	
8	27.39	
9	27.01	
10	27.11	
1	23.96	64-8-27-13-12-22
3	20.905	
5	22.64	
6	23.505	
8	22.415	
11	22	
13	22.425	
15	22.91	
17	22.03	
18	21.04	
1	20.62	32-5-9-10
2	21.185	
3	21.605	
4	21.38	
5	21.225	
7	21.42	
8	21.705	
10	21.45	
11	21.22	
13	22.695	
14	24.035	
15	22.76	
18	21.41	
19	21.06	
CS-Fg	23.15	
7EL-Fg	21.52	

4b



Progeny	Cq Value	
1	24.59	64-8-27-13-12-12
2	24.68	
5	24.55	
12	24.71	
13	24.58	
17	24.6	
2	27.02	64-8-27-13-12-6
3	28.58	
5	27.18	
6	26.48	
7	27.14	
8	27.31	
9	27.43	
10	27.12	
1	25.19	64-8-27-13-12-22
3	24.12	
5	25.4	
6	24.4	
8	24.89	
11	23.4	
13	24.94	
15	24.2	
17	25	
18	24.2	
1	22.2	32-5-9-10
2	22.205	
3	22.375	
4	22.395	
5	22.38	
7	22.47	
8	22.375	
10	22.43	
11	23.73	
13	24.725	
14	23.89	
15	23.03	
18	22.375	
19	22.085	
CS-Fg	24.2	
7EL-Fg	23.19	

Appendix 4a,b: Cq values of housekeeping genes IAAOX and hn-RNP-Q derived from BC_1F_7 and BC_1F_5 families.
The blue bars represent progeny from the 64-8-27-13-12-12 family, the red bars represent progeny from the 64-8-27-13-12-6 family, the green bars represent progeny from the 62-8-27-13-12-22 family and the grey bars represent the 32-5-9-10 family. Orange bars represent the controls CS-Fg and 7EL-Fg. The table on the left shows the actual Cq values.



32-5-5												
	2	3	4	6	7	9	11	12	14	15	19	20
H1	-	100%	5%	13%	5%	-	-	5%	-	5%	18%	5%
H2	100%	100%	18%	15%	9%	9%	-	10%	9%	45%	16%	10%
H3	-	100%	-	-	5%	5%	9%	10%	5%	36%	5%	10%
H4	5%	100%	91%		10%						18%	5%
	S	S	S	P	R	R	R	R	R	S	P	R

7EL						
	1	2	3	4	5	6
H1	10%	7%	10%	5%	-	5%
H2	7%	7%	7%	7%	10%	10%
H3	7%	5%	7%	10%	7%	7%
H4		5%	10%	4%	5%	
	R	R	R	R	R	R

CS		
	1	2
H1	100%	100%
H2	100%	100%
H3	80%	85%
H4		100%
	S	S

32-5-7																			
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	19	20	21
H1	100%	100%	78%	91%	100%	100%	100%	100%	100%	100%	14%	100%	100%	100%	-	100%	55%	100%	91%
H2	94%	100%	89%	100%	100%	18%	100%	100%	100%	-	82%	-	100%	-	18%	91%	36%	100%	100%
H3	3%	18%	89%	-	100%	100%	100%	73%	100%	-	100%	100%	100%	100%	100%	100%	91%	100%	100%
H4		100%	100%	-	100%	82%	100%	100%	100%	-		93%	-	100%	-	100%	100%	100%	-
	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

32-5-8																			
	1	2	3	5	6	7	8	9	10	12	13	14	15	16	17	18	19	21	22
H1	5%	9%	9%	14%	-	5%	5%	100%	5%	9%	5%	-	5%	5%	5%	9%	14%	9%	5%
H2	5%	5%	5%	9%	10%	5%	100%	55%	18%	5%	5%	9%	5%	5%	9%		5%	9%	-
H3	18%	5%	-		9%	-	100%	18%	14%	-	18%	5%	-	-	18%		18%	5%	
H4			5%		5%		100%	18%				9%							
	P	R	R	R	R	R	S	S	R	R	P	R	R	R	P	R	P	R	R

32-5-9																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
H1	55%	10%	55%	5%	10%	5%	55%	100%	5%	8%	5%	100%	100%	10%	4%	100%	7%	9%	5%	100%	-	91%
H2	45%	5%	9%	-	5%	-	73%	100%	5%	58%	100%	100%	-	8%	4%	100%	100%	8%	5%	100%	8%	82%
H3	-	9%	5%		8%	73%	100%	100%	5%	17%	73%	9%	9%		36%	18%	79%	5%	7%	-	5%	100%
H4			-						-				0%			100%						
	S	R	P	R	R	S	S	S	R	P	S	S	S	R	R	S	S	R	R	S	R	S

Appendix 5: Phenotype of all progeny from each of the four BC_1F_4 families at 14 dpi.
Plants labelled with R were resistant, S were susceptible, and P were partially resistant. The "-" indicates heads where the initiation of infection failed.

7EL primers	Tm	controls:				32-5-5											
		7EL	CS	7E(D)	H2O	2	3	4	6	7	9	11	12	14	15	19	20
2096-A3-7E	56	1	0	1	0	0	1	1	1	1	1	1	1	1	0	1	1
1480-B1-7E	56	1	0	1	0	0	0	1	1	1	0	1	1	1	0	1	1
Lu-377-22	60	1	0	1	0	0	0	0	1	1	1	1	1	1	0	1	1
1383-A3-7E	56	1	0	1	0	0	0	0	1	1	1	1	1	1	0	1	1
455-A1-7E	60	1	0	1	0	0	0	0	1	1	1	1	1	1	0	1	1
p7es-21	60	1	0	1	0	0	0	0	1	1	1	1	1	1	0	1	1
3201-A2-7E	60	1	0	1	0	0	0	0	1	1	1	1	1	1	0	0	1
Lu-270-1	60	1	0	1	0	0	0	0	1	1	1	1	1	1	0	0	1
2500-A1-7E	60	1	0	1	0	0	0	0	1	1	1	1	1	1	0	0	1
4231-A2-7E	60	1	0	1	0	0	0	0	1	1	1	1	1	1	0	0	1

7D primers	Tm	Controls				32-5-5											
		7EL	CS	7E(D)	H 2O				6				12	14		19	20
2506_A1_7DL	60	1	1	0	0				1				1	1		1	1
2368-A3-7DL	56	1	1	0	0				1				1	1		1	1
542-A2-7DL	60	1	1	0	0				1				1	1		1	1
1383-A2-7DL	56	1	1	0	0				1				1	1		1	1
3571-A2-7DL	56	1	1	0	0				1				1	1		1	1
6092-A2-7D	60	1	1	0	0				1				1	1		1	0
959-A3-7DL	60	1	1	0	0				1				1	1		1	0
507-A2-7DL	60	1	1	0	0				1				1	1		1	1
3488_A2_7DL	60	1	1	0	0				1				1	1		1	1

7EL primers	Tm	controls:				32-5-7																
		7EL	CS	7E(D)	H2O	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	19	20	21
2096-A3-7E	56	1	0	1	0	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1
1480-B1-7E	56	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lu-377-22	60	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1383-A3-7E	56	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
455-A1-7E	60	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
p7es-21	60	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3201-A2-7E	60	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lu-270-1	60	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2500-A1-7E	60	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4231-A2-7E	60	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

7D primers	Tm	Controls				32-5-7																
		7EL	CS	7E(D)	H2O							8					13			16		
2506_A1_7DL	60	1	1	0	0							1					1			1		
2368-A3-7DL	56	1	1	0	0							1					1			1		
542-A2-7DL	60	1	1	0	0							1					1			1		
1383-A2-7DL	56	1	1	0	0							1					1			1		
3571-A2-7DL	56	1	1	0	0							1					1			1		
6092-A2-7D	60	1	1	0	0							1					1			1		
959-A3-7DL	60	1	1	0	0							1					1			1		
507-A2-7DL	60	1	1	0	0							1					1			1		
3488_A2_7DL	60	1	1	0	0							1					1			1		

7EL primers	Tm	controls:				32-5-8																	
		7EL	CS	7E(D)	H2O	1	2	3	5	6	7	8	9	10	12	13	14	15	16	17	18	19	21	22
2096-A3-7E	56	1	0	1	0	0	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1
1480-B1-7E	56	1	0	1	0	1	1	1	1	1	0	0	0	1	1	1	1	0	0	1	1	1	1	1
Lu-377-22	60	1	0	1	0	0	1	1	1	1	0	0	0	1	0	1	1	1	1	0	1	1	0	1
1383-A3-7E	56	1	0	1	0	0	1	1	1	1	0	0	0	1	0	1	1	1	1	1	1	1	1	1
455-A1-7E	60	1	0	1	0	1	1	1	1	1	1	0	0	1	0	1	0	1	1	1	1	1	1	1
p7es-21	60	1	0	1	0	0	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	0	1
3201-A2-7E	60	1	0	1	0	0	1	1	1	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1
Lu-270-1	60	1	0	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1
2500-A1-7E	60	1	0	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	1
4231-A2-7E	60	1	0	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1
7D primers	Tm	Controls				32-5-8																	
		7EL	CS	7E(D)	H2O			3						10	12	13		15	16			19		
2506_A1_7DL	60	1	1	0	0			1						1	1	0		1	1			1		
2368-A3-7DL	56	1	1	0	0			1						1	1	0		1	1			1		
542-A2-7DL	60	1	1	0	0			1						1	1	0		1	1			1		
1383-A2-7DL	56	1	1	0	0			1						1	1	0		1	1			1		
3571-A2-7DL	56	1	1	0	0			1						1	1	0		1	1			1		
6092-A2-7D	60	1	1	0	0			1						1	1	0		1	1			1		
959-A3-7DL	60	1	1	0	0			1						1	1	0		1	1			1		
507-A2-7DL	60	1	1	0	0			1						1	1	0		1	1			1		
3488_A2_7DL	60	1	1	0	0			1						1	1	0		1	1			1		

7EL primers	Tm	controls:				32-5-9																				
		7EL	CS	7E(D)	H2O	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
2096-A3-7E	56	1	0	1	0	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1	0	0	1	1	0	1	1
1480-B1-7E	56	1	0	1	0	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1	0	0	1	1	0	1	0
Lu-377-22	60	1	0	1	0	0	1	0	1	1	0	0	0	1	0	0	0	0	1	1	0	0	1	1	0	0	0
1383-A3-7E	56	1	0	1	0	0	1	1	1	1	0	0	0	1	0	0	0	0	1	1	0	0	1	1	0	1	0
455-A1-7E	60	1	0	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	1	1	0	0	1	1	0	0	0
p7es-21	60	1	0	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	1	1	0	0	1	1	0	0	0
3201-A2-7E	60	1	0	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	1	1	0	0	1	1	0	1	0
Lu-270-1	60	1	0	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	1	1	0	0	1	1	0	1	0
2500-A1-7E	60	1	0	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	1	1	0	0	1	1	0	1	0
4231-A2-7E	60	1	0	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	1	1	0	0	1	1	0	1	0
7D primers	Tm	Controls				32-5-9																				
		7EL	CS	7E(D)	H2O	1				5					10				14	15							
2506_A1_7DL	60	1	1	0	0	1				1					1				1	1							
2368-A3-7DL	56	1	1	0	0	1				1					1				1	1							
542-A2-7DL	60	1	1	0	0	1				1					1				1	1							
1383-A2-7DL	56	1	1	0	0	1				1					1				1	1							
3571-A2-7DL	56	1	1	0	0	1				1					1				1	1							
6092-A2-7D	60	1	1	0	0	1				1					1				0	1							
959-A3-7DL	60	1	1	0	0	1				1					0				0	1							
507-A2-7DL	60	1	1	0	0	1				1					0				0	1							
3488_A2_7DL	60	1	1	0	0	1				1					0				0	1							

Appendix 6: Results of PCR assay for all BC_1F_4 families using 7EL and 7D markers.
Columns highlighted in grey show plants discarded after genotyping and phenotyping; columns highlighted in green show plants kept for seed collection and future work. PCR results were scored as 1 (presence of band) and 0 (absence of band).

	7EL					CS		
	1	2	3	4		**1**	**2**	**3**
H1	6%	7%	13%	8%	H1	100%	100%	100%
H2	6%		19%	12%	H2	80%	100%	100%
H3			13%	16%	H3	75%		90%
	R	R	R	R		**S**	**S**	**S**

	64-8-27-13-12-6									
	1	2	3	4	5	6	7	8	9	10
H1	7%	34%	10%	7%	6%	15%	6%	3%	6%	3%
H2	20%	47%		3%	9%	18%	9%	9%	6%	6%
H3	7%	34%		3%		9%	3%			
H4							3%			
	R	P	R	R	R	R	R	R	R	R

	64-8-27-13-12-12												
	1	2	3	4	5	9	12	13	15	16	17	18	19
H1	6%	9%	6%	53%	10%	100%	6%	7%	4%	13%	10%	63%	100%
H2	3%	16%	3%	67%	30%	100%	10%	7%	4%	7%	7%		100%
H3						4%	10%	13%		10%			
H4							3%						
	R	R	R	s	P	S	R	R	R	R	R	S	S

	64-8-27-13-12-22													
	1	3	4	5	6	8	10	11	13	14	15	16	17	18
H1	4%	7%	13%	4%	8%	36%	4%	8%	8%	8%	4%	48%	4%	10%
H2	4%	7%	13%	8%	12%	21%	8%	-	8%	4%	-		8%	
H3						7%		4%		4%				
H4						4%								
	R	R	R	R	R	R	R	R	R	R	R	S	R	R

	32-5-8-12																			
	1	2	3	4	5	6	7	8	9	10	11	12	12	14	15	16	17	18	19	20
H1	8%	12%	4%	8%	8%	11%	7%	7%	4%	7%	13%	13%	7%	12%	4%	13%	13%	8%	8%	21%
H2	8%	8%	32%	12%	4%	7%		7%	8%	13%	3%	10%	7%	4%	8%	3%	8%		4%	18%
H3																				
H4																				
	R	R	P	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	P

	32-5-9-10															
	1	2	3	4	5	7	8	9	10	11	12	13	14	15	18	19
H1	9%	18%	8%	100%	7%	100%	-	25%	70%	9%	68%	13%	5%	9%	9%	83%
H2	8%	9%	9%	100%	21%	100%	9%	14%	33%	8%	-	9%	-	5%	10%	100%
H3	9%	14%	10%	100%		100%	-				-	10%		8%		
H4			8%				5%									
	R	R	R	S	R	S	R	P	S	R	S	R	R	R	R	S

Appendix 7: Phenotype of all progeny from three BC_1F_7 families and two BC_1F_5 families at 14 dpi.
Plants labelled with R were resistant, S were susceptible and P were partially resistant. The "-"indicates heads where the initiation of infection failed.

		Controls:			32-5-8-12																			
7E Primers	**Tm**	7EL	CS	H2O	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2096-A3-7E	56	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1480-B1-7E	56	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1383-A3-7E	56	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
455-A1-7E	60	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
p7es-21	60	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3201-A2-7E	60	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lu-270-1	60	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2500-A1-7E	60	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4231-A2-7E	60	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

		Controls:				32-5-9-10																
7E Primers	**Tm**	7EL	CS	7E(D)	H2O	1	2	3	4	5	7	8	9	10	11	12	13	14	15	18	19	
2096-A3-7E	56	1	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1480-B1-7E	56	1	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1383-A3-7E	56	1	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3571-7E	60	1	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lu 1989-2	50	1	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
455 7E	60	1	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
p7es-21	60	1	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lu 382	50	1	0	-	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Lu 1851-2	50	1	0	-	0	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	0	
3201 7E	60	1	0	-	0	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	0	
Lu-270-1	60	1	0	-	0	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	0	
2500-A1-7E	60	1	0	-	0	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	0	
4231-A2-7E	60	1	0	-	0	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	0	
		Controls:				**32-5-9-10**																
7D Primers	**Tm**	7EL	CS	7E(D)	H2O	1	2	3	4	5	7	8	9	10	11	12	13	14	15	18	19	
2368_A3_7DL	56	1	1	0	0	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	
1262_7DL	56	1	1	0	0	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	
6092-A2-7D	60	1	1	0	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	
2500-A3-7D	60	1	1	0	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	
3488_A2_7DL	60	1	1	0	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	

		Controls:				64-8-27-13-12-6									
7E Primers	**Tm**	7EL	CS	7E(D)	H2O	1	2	3	4	5	6	7	8	9	10
2096-A3-7E	56	1	0	-	0	0	0	0	0	0	0	0	0	0	0
1480-B1-7E	56	1	0	-	0	0	0	0	0	0	0	0	0	0	0
1383-A3-7E	56	1	0	-	0	0	0	0	0	0	0	0	0	0	0
3571-7E	60	1	0	-	0	0	0	0	0	0	0	0	0	0	0
Lu 1989-2	50	1	0	-	0	0	0	0	0	0	0	0	0	0	0
455 7E	60	1	0	-	0	0	1	1	1	1	1	1	1	1	1
p7es-21	60	1	0	-	0	1	1	1	1	1	1	1	1	1	1
Lu 625	50	1	0	-	0	1	1	1	1	1	1	1	1	1	1
Lu 1851-2	50	1	0	-	0	1	1	1	1	1	1	1	1	1	1
3201 7E	60	1	0	-	0	1	1	1	1	1	1	1	1	1	1
Lu-270-1	60	1	0	-	0	1	1	1	1	1	1	1	1	1	1
2500-A1-7E	60	1	0	-	0	1	1	1	1	1	1	1	1	1	1
4231-A2-7E	60	1	0	-	0	1	1	1	1	1	1	1	1	1	1
		Controls:				**64-8-27-13-12-6**									
7D Primers	**Tm**	7EL	CS	7E(D)	H2O	1	2	3	4	5	6	7	8	9	10
2368_A3_7DL	56	1	1	0	0	1	1	1	1	1	1	1	1	1	1
1262_7DL	56	1	1	0	0	1	1	1	1	1	1	1	1	1	1
6092-A2-7D	60	1	1	0	0	0	0	0	0	0	0	0	0	0	0
2500-A3-7D	60	1	1	0	0	0	0	0	0	0	0	0	0	0	0
3488_A2_7DL	60	1	1	0	0	0	0	0	0	0	0	0	0	0	0

		Controls:				64-8-27-13-12-12												
7E Primers	**Tm**	7EL	CS	7E(D)	H2O	1	2	3	4	5	9	12	13	15	16	17	18	19
2096-A3-7E	56	1	0	-	0	0	0	1	0	0	0	0	0	1	1	0	0	0
1480-B1-7E	56	1	0	-	0	0	0	1	0	0	0	0	0	1	1	0	0	0
1383-A3-7E	56	1	0	-	0	0	0	1	0	0	0	0	0	1	1	0	0	0
455 7E	60	1	0	-	0	1	1	1	0	1	0	1	1	1	1	1	0	0
p7es-21	60	1	0	-	0	1	1	1	0	1	0	1	1	1	1	1	0	0
3201 7E	60	1	0	-	0	1	1	1	0	1	0	1	1	1	1	1	0	0
Lu-270-1	60	1	0	-	0	1	1	1	0	1	0	1	1	1	1	1	0	0
2500-A1-7E	60	1	0	-	0	1	1	1	0	1	0	1	1	1	1	1	0	0
4231-A2-7E	60	1	0	-	0	1	1	1	0	1	0	1	1	1	1	1	0	0
		Controls:				**64-8-27-13-12-12**												
7D Primers	**Tm**	7EL	CS	7E(D)	H2O	1	2			5		12	13			17		
2368_A3_7DL	56	1	1	0	0	1	1			1		1	1			1		
1262_7DL	56	1	1	0	0	1	1			1		1	1			1		
6092-A2-7D	60	1	1	0	0	1	1			1		1	1			1		
2500-A3-7D	60	1	1	0	0	1	1			1		1	1			1		
3488_A2_7DL	60	1	1	0	0	1	1			1		1	1			1		

		Controls:				64-8-27-13-12-22														
7E Primers	**Tm**	7EL	CS	7E(D)	H2O	1	3	4	5	6	8	10	11	13	14	15	16	17	18	
2096-A3-7E	56	1	0	-	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	
1480-B1-7E	56	1	0	-	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	
1383-A3-7E	56	1	0	-	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	
455 7E	60	1	0	-	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
p7es-21	60	1	0	-	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
3201 7E	60	1	0	-	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Lu-270-1	60	1	0	-	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
2500-A1-7E	60	1	0	-	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
4231-A2-7E	60	1	0	-	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
		Controls:				**64-8-27-13-12-22**														
7D Primers	**Tm**	7EL	CS	7E(D)	H2O	1	3		5	6	8	10	11	13		15		17	18	
2368_A3_7DL	56	1	1	0	0	0	1		1	1	1	1	1	1		1		1	1	
1262_7DL	56	1	1	0	0	0	1		1	0	1	1	1	1		1		1	1	
6092-A2-7D	60	1	1	0	0	0	1		1	0	1	1	1	1		1		1	1	
2500-A3-7D	60	1	1	0	0	0	1		1	0	1	0	1	1		1		1	1	
3488_A2_7DL	60	1	1	0	0	0	1		1	0	1	0	1	1		1		1	0	

Appendix 8: Results of PCR assay for all two BC_1F_5 and three BC_1F_7 families using 7EL and 7D markers.
PCR results were scored as 1 (presence of band) and 0 (absence of band).

Appendix 9: All expressed 7EL genes present in the region of introgression.
Table shows the fold change between the treatments, the raw sequence counts and the approximate position of the homoeologous sequences on the 7D chromosome. Table is organized with decreasing values of raw counts for the 7EL-Fg treatment. Genes that were selected for RT-qPCR are highlighted in brown.

GeneID	log2foldchange	7EL-Fg-R1	7EL-H2O-R1	7EL-Fg-R2	7EL-H2O-R2	7EL-Fg-R3	7EL-H2O-R3	start	end
7EL_scaffold1517-61848-64674	**-2.0**	**3544**	**1690**	**4146**	**1345**	**3224**	**1332**	**614277926**	**614276804**
7EL_scaffold3201-16517-22057	-1.2	2471	1838	3150	1688	2440	2087	616510516	616506682
7EL_scaffold2197-28673-32163	**-4.6**	**2056**	**140**	**2331**	**124**	**1804**	**154**	**607541630**	**607539172**
7EL_scaffold587-27673-30880	**-5.2**	**1631**	**85**	**1496**	**55**	**1286**	**59**	**601288508**	**601286096**
7EL_scaffold1725-40996-55157	-1.5	913	0	215	39	29	35	604890877	604895866
7EL_scaffold3181-19450-27623	-1.2	817	616	922	538	781	604	621645641	621643517
7EL_scaffold713-98853-99840	-0.7	690	822	842	697	826	918	615896830	615897835
7EL_scaffold1633-29901-33565	0.4	684	0	545	33	80	39	612321455	612317855
7EL_scaffold395-62699-66599	**-3.5**	**610**	**99**	**632**	**69**	**455**	**84**	**612437860**	**612439920**
7EL_scaffold160-50074-51771	-0.6	549	596	594	542	550	659	597684191	597682525
7EL_scaffold649-18007-24053	**-2.3**	**548**	**183**	**530**	**103**	**387**	**198**	**616038944**	**616041464**
7EL_scaffold4320-1212-7761	-0.2	524	859	679	765	623	874	614541360	614538973
7EL_scaffold404-137223-138822	-3.5	486	0	131	45	6	54	629496291	629497616

7EL_scaffold2937-10531-14336	**-2.5**	**475**	**164**	**513**	**127**	**450**	**134**	**619040555**	**619042746**
7EL_scaffold996-31092-34382	0.6	433	0	323	21	92	35	632009375	632008171
7EL_scaffold2459-4844-18167	-0.3	412	597	472	515	445	639	611252014	611247070
7EL_scaffold2227-28119-34771	-0.8	399	372	441	333	343	439	611352193	611349191
7EL_scaffold3488-6438-15405	-0.1	397	677	448	567	456	700	629835209	629830377
7EL_scaffold2429-325-5221	-0.1	301	555	305	398	327	490	605909285	605912902
7EL_scaffold109-125405-130914	0.0	297	608	342	482	426	601	635022979	635023697
7EL_scaffold476-103894-106480	-1.0	293	294	293	150	248	237	616902269	616903100
7EL_scaffold2046-20673-21931	-0.4	275	356	305	343	337	390	610667050	610665847
7EL_scaffold109-2250-4100	-0.7	242	268	248	215	224	237	634898356	634897733
7EL_scaffold266-94702-96314	-0.5	233	318	274	263	237	303	596897388	596898788
7EL_scaffold2043-19519-24225	0.3	231	678	320	510	359	591	635579150	635583502
7EL_scaffold1945-25476-26360	-8.9	228	2	250	0	195	0	611168735	611168437
7EL_scaffold2263-25201-26005	-0.9	226	330	249	160	290	192	612774694	612775504
7EL_scaffold704-15789-33720	0.1	221	432	204	342	271	411	610814788	610817208
7EL_scaffold3671-5413-6985	-5.7	211	9	322	7	222	8	615947634	615946318
7EL_scaffold2058-37474-44225	-1.6	211	182	210	128	300	79	631427243	631424354

7EL_scaffold564-24436-35994	-0.4	211	274	218	224	190	266	610555383	610556728
7EL_scaffold2847-17900-22167	-0.6	201	231	183	187	205	203	627409014	627407074
7EL_scaffold58-82699-84602	-2.2	200	65	158	46	164	74	616521399	616521356
7EL_scaffold530-118491-121651	-8.7	193	2	251	0	157	0	628605653	628603355
7EL_scaffold1305-7874-16021	0.0	182	341	205	269	177	287	600457853	600454909
7EL_scaffold1446-65486-66315	-9.3	180	0	227	1	137	0	634195857	634195829
7EL_scaffold857-88272-90564	-0.5	180	245	191	202	264	272	626357796	626359310
7EL_scaffold1587-211-14612	-0.1	175	302	222	256	167	328	632322236	632328874
7EL_scaffold5669-3201-4130	-9.1	173	0	120	0	161	1	611441514	611442279
7EL_scaffold368-125340-128842	-0.2	164	265	192	207	171	260	611742439	611741114
7EL_scaffold704-43750-57855	0.1	159	323	176	257	165	304	610844474	610848463
7EL_scaffold2363-34248-35665	-2.3	157	53	176	30	131	76	612978728	612977296
7EL_scaffold2467-19325-30934	-0.8	154	154	138	127	141	116	603359141	603362805
7EL_scaffold836-43603-51129	-0.1	152	286	198	241	189	281	610779933	610776542
7EL_scaffold4317-8153-10210	0.1	152	0	185	1711	3456	1804	629382378	629381253
7EL_scaffold14-24842-25820	-7.4	149	2	117	0	71	1	602073476	602072586

7EL_scaffold726-77873-82334	-1.5	146	0	142	58	30	58	607229735	607229016
7EL_scaffold4200-13005-13851	-7.7	144	3	161	0	100	0	611168735	611168437
7EL_scaffold2500-11839-16054	-0.3	141	207	152	173	141	205	623529933	623527116
7EL_scaffold126-1-4732	0.5	133	388	156	319	167	317	620489783	620491930
7EL_scaffold4280-2392-5815	-0.7	131	153	132	100	139	145	613547802	613549656
7EL_scaffold43-109885-111665	0.2	129	305	151	299	228	329	634412302	634410696
7EL_scaffold3499-21088-23689	-0.1	126	190	130	187	132	198	604294387	604297029
7EL_scaffold109-12437-16557	-1.2	122	99	138	79	126	98	635395336	635397408
7EL_scaffold739-93293-95535	-9.8	118	0	146	0	119	0	602664466	602663642
7EL_scaffold4231-5988-13918	1.4	114	668	156	547	138	595	634692208	634696435
7EL_scaffold1720-1-924	-1.0	114	106	115	79	128	111	600886558	600887075
7EL_scaffold1108-64291-75317	0.5	114	276	135	257	136	343	635566500	635562277
7EL_scaffold1210-63793-67859	-3.3	107	12	86	10	62	21	613636818	613637818
7EL_scaffold4367-12350-13093	0.0	106	251	127	127	92	180	602089831	602090546
7EL_scaffold133-191850-195208	0.2	105	223	130	201	109	208	636852214	636850339
7EL_scaffold1796-35953-39235	-4.1	104	13	128	7	62	8	628212407	628211847
7EL_scaffold133-182135-186003	0.2	102	725	94	169	104	175	636858396	636856907

7EL_scaffold704-34379-40233	0.0	101	193	360	262	357	217	610821304	610825682
7EL_scaffold4206-76-2124	-0.6	99	126	116	118	137	120	634688644	634687508
7EL_scaffold153-127375-134020	-0.6	94	159	146	120	120	120	611713330	611716698
7EL_scaffold482-233-3852	-0.2	93	103	77	108	65	117	613700948	613702627
7EL_scaffold4816-4734-10128	0.0	93	187	100	145	124	174	631796634	631797942
7EL_scaffold1305-6633-7059	0.2	93	0	0	0	1	0	600457429	600457854
7EL_scaffold1341-7328-13899	0.0	90	164	88	102	68	150	628708824	628710195
7EL_scaffold4305-11737-14392	-2.5	89	52	125	27	178	37	602662180	602664633
7EL_scaffold4224-645-3545	-6.0	87	0	52	300	13	365	614275656	614276669
7EL_scaffold1746-54909-55347	-0.1	86	183	118	162	152	200	633257404	633257775
7EL_scaffold6092-2689-5041	-1.2	85	60	81	50	69	61	611590500	611592806
7EL_scaffold296-150495-152343	1.0	84	454	114	309	189	539	595669198	595668313
7EL_scaffold1893-18084-18481	-0.1	84	0	76	3	1	0	632008793	632008419
7EL_scaffold436-81478-84975	-0.4	82	84	75	59	56	132	618032503	618033726
7EL_scaffold2361-25107-32572	0.0	81	146	73	108	82	135	596010987	596005474
7EL_scaffold368-111118-117019	0.5	81	184	474	168	419	193	611750931	611747215
7EL_scaffold737-93875-98836	0.5	78	213	74	152	76	175	615232428	615231862
7EL_scaffold1170-50822-	-1.1	76	80	86	64	117	68	608087276	608085753

56002									
7EL_scaffold350-82358-86242	0.0	75	159	73	111	93	121	636766929	636768088
7EL_scaffold3176-19-5223	0.5	75	213	97	189	93	217	618415560	618415699
7EL_scaffold1783-38323-39485	-1.8	72	37	61	19	55	36	602410305	602409122
7EL_scaffold2576-27756-32641	-0.9	71	61	74	65	83	75	602751494	602750654
7EL_scaffold1767-9144-13480	0.6	70	229	101	147	72	241	614513865	614511433
7EL_scaffold2229-7293-15319	0.0	70	182	332	246	293	179	611822511	611817073
7EL_scaffold428-109953-112875	-1.7	69	30	50	9	22	36	634177900	634176740
7EL_scaffold298-78909-83065	0.6	66	207	60	138	83	174	612360007	612359720
7EL_scaffold3671-18373-19845	-2.5	65	17	61	6	36	23	615943389	615941944
7EL_scaffold3499-12332-14429	-0.4	65	78	67	75	61	87	604866327	604865015
7EL_scaffold4805-129-6140	0.5	64	184	79	151	80	174	613452386	613453635
7EL_scaffold564-106734-112156	-0.1	64	104	65	89	67	107	633053983	633055299
7EL_scaffold993-44365-48591	-4.3	63	4	77	3	28	7	631688933	631684810
7EL_scaffold3341-15920-21278	-0.1	63	127	244	155	199	148	633822535	633825170
7EL_scaffold2216-16695-34121	0.2	62	139	62	112	83	139	601677179	601677426
7EL_scaffold169-94616-96806	-0.3	61	85	57	62	68	96	601492833	601494938
7EL_scaffold1164-15163-	-7.3	59	1	106	0	58	1	597475259	597474054

24004									
7EL_scaffold31-135426-141995	-3.3	59	16	79	10	66	8	632143099	632141124
7EL_scaffold1627-1-4053	0.8	59	213	71	192	86	203	621867820	621869313
7EL_scaffold2754-2049-11542	0.5	59	148	57	118	63	146	601056532	601059682
7EL_scaffold1222-4576-13203	0.6	56	166	71	140	68	173	633151280	633148205
7EL_scaffold3201-8968-13107	0.6	56	188	56	123	66	127	616606187	616605691
7EL_scaffold2439-11034-16967	-8.7	54	0	62	0	57	0	632033311	632034289
7EL_scaffold1270-39192-40644	-0.7	53	57	65	51	53	62	628520045	628521485
7EL_scaffold1431-41668-44103	0.7	52	162	49	142	77	162	634096904	634094658
7EL_scaffold704-63364-64239	-7.5	47	0	53	0	44	1	610860268	610860854
7EL_scaffold265-158179-160804	0.1	47	47	71	52	40	172	620984570	620985640
7EL_scaffold2208-7889-13814	1.1	46	212	55	166	63	206	618895120	618889889
7EL_scaffold441-115775-118473	-0.8	46	69	41	26	71	61	602662113	602664627
7EL_scaffold436-52749-56471	-0.4	46	70	40	54	57	51	617932190	617934213
7EL_scaffold3276-12453-18710	-0.5	46	-	88	113	101	93	629531501	629535163
7EL_scaffold3844-1493-6104	0.2	46	180	29	60	31	50	631858249	631858671
7EL_scaffold2555-28793-36352	0.1	45	113	73	100	63	111	610480443	610482228

7EL_scaffold577-116668-119286	-0.1	45	74	33	52	53	81	633597963	633599626
7EL_scaffold765-9815-12155	-5.2	44	3	54	2	43	1	628214090	628212829
7EL_scaffold58-126267-128000	-4.0	43	3	24	3	51	6	610623442	610621812
7EL_scaffold278-66529-71198	-0.1	42	82	47	62	47	61	599183628	599185987
7EL_scaffold1908-1066-5615	0.3	41	117	58	85	51	107	626133604	626135772
7EL_scaffold1957-47807-50448	-0.3	40	67	52	67	49	52	631858676	631858027
7EL_scaffold2351-38851-42852	0.1	40	84	43	60	41	68	602185482	602187075
7EL_scaffold3671-17238-17754	-2.8	38	9	42	4	13	9	615943765	615943611
7EL_scaffold2500-734-4106	0.2	37	46	29	58	21	62	623536386	623535371
7EL_scaffold1543-22341-23960	-0.2	37	54	34	48	40	52	599498058	599499321
7EL_scaffold286-148730-150151	-0.6	36	42	46	42	38	45	618714891	618715550
7EL_scaffold350-74606-75034	0.9	34	130	34	84	38	103	636716463	636716622
7EL_scaffold1565-43287-48726	0.4	33	71	36	68	26	62	616510412	616506686
7EL_scaffold3361-21572-24091	-0.3	33	42	28	28	25	44	600861182	600858677
7EL_scaffold4799-4001-5317	-7.6	31	0	29	0	22	0	625225244	625226258
7EL_scaffold1446-60039-61127	-5.8	30	0	32	0	17	2	634401911	634401269
7EL_scaffold3361-17635-	0.3	30	46	21	38	12	40	600864934	600863168

19404									
7EL_scaffold59-98814-106372	-0.7	29	33	38	33	38	37	623857687	623850248
7EL_scaffold1134-79564-81718	0.7	29	129	43	103	66	128	596711767	596710434
7EL_scaffold2949-25260-28990	0.0	29	63	125	58	87	61	597513367	597514192
7EL_scaffold1547-1452-5747	0.0	28	62	34	39	34	58	633202692	633205568
7EL_scaffold286-127389-130673	0.3	28	73	35	65	36	63	618870863	618867599
7EL_scaffold204-21540-24753	-0.4	27	39	32	33	29	40	598360847	598357799
7EL_scaffold1209-35038-37897	0.7	26	78	19	43	20	59	610533503	610534747
7EL_scaffold1497-17360-19540	-0.5	26	32	35	38	39	40	601700081	601698231
7EL_scaffold1446-31795-32407	-5.8	25	1	12	0	7	0	634347099	634346598
7EL_scaffold476-61133-69697	1.1	25	141	36	101	40	101	616776186	616778486
7EL_scaffold4912-3854-5846	1.0	25	115	37	115	42	109	633873833	633874564
7EL_scaffold194-27782-31513	0.9	25	51	37	34	23	168	622002809	622004900
7EL_scaffold2645-35693-36279	-0.5	25	23	15	24	14	15	606961305	606961604
7EL_scaffold109-8968-11211	0.1	25	36	18	32	17	37	635389665	635390760
7EL_scaffold1627-8938-13174	0.4	25	34	74	30	65	22	621968828	621970751
7EL_scaffold28-171415-	1.8	24	174	26	135	31	152	633672091	633670029

175545									
7EL_scaffold1767-27898-30377	-0.8	24	23	19	20	21	16	614505520	614503034
7EL_scaffold272-85498-88863	0.3	24	81	43	48	31	66	612357063	612358147
7EL_scaffold43-63820-66223	0.8	23	68	23	77	30	66	634457076	634455259
7EL_scaffold609-44127-46859	-0.1	23	31	30	41	25	45	614104843	614105763
7EL_scaffold462-82329-84419	0.0	23	51	28	36	39	56	625119494	625121580
7EL_scaffold2921-12308-17569	1.1	22	83	22	81	32	95	618890014	618894625
7EL_scaffold14-28179-30222	0.3	22	55	37	54	29	69	602286685	602288559
7EL_scaffold726-71039-75393	0.3	22	35	9	16	13	41	607297995	607296762
7EL_scaffold28-1-3486	0.1	22	44	24	33	23	42	633671650	633669994
7EL_scaffold1533-50932-52984	0.1	22	45	34	51	30	49	601462129	601463638
7EL_scaffold4480-12035-13400	0.0	21	37	19	36	21	26	615461462	615462243
7EL_scaffold1543-11782-12118	-0.5	21	47	47	43	71	67	602908362	602908060
7EL_scaffold637-578-5837	-0.5	21	46	42	32	37	41	610556728	610555841
7EL_scaffold4367-6135-10686	-0.3	21	31	23	35	30	32	602078423	602079237
7EL_scaffold2156-10502-12427	-0.2	21	41	27	25	30	46	618873912	618875799
7EL_scaffold6530-1360-5319	-2.7	20	5	16	0	15	8	613635194	613635725
7EL_scaffold2033-12258-	0.0	20	23	17	32	16	31	629142547	629141531

20597									
7EL_scaffold1599-40324-42604	0.3	20	50	21	33	30	57	626376742	626377980
7EL_scaffold169-136023-138129	0.2	20	40	22	27	16	45	601698314	601699790
7EL_scaffold1985-38122-41064	0.1	20	57	24	32	34	51	600282120	600284077
7EL_scaffold31-118205-123312	-0.1	20	0	4	1	0	2	632087663	632086319
7EL_scaffold2026-5332-6314	-2.3	19	8	18	7	24	5	616496261	616496384
7EL_scaffold2305-41697-43614	1.6	19	110	14	66	20	86	633568048	633566635
7EL_scaffold741-62686-65287	0.3	19	47	13	39	32	46	634659571	634661009
7EL_scaffold1436-18020-19834	-0.2	19	31	18	35	34	35	603833087	603831295
7EL_scaffold602-6682-7851	-1.2	18	11	19	12	21	17	603676333	603677496
7EL_scaffold478-89327-96494	1.2	18	80	17	64	21	66	619517558	619514493
7EL_scaffold2716-22214-26942	0.9	18	41	12	44	17	61	618894234	618890582
7EL_scaffold263-128935-133939	0.6	18	37	14	41	17	39	603362794	603361942
7EL_scaffold3490-18817-22380	0.1	18	41	74	36	65	55	621298638	621296809
7EL_scaffold178-62584-63360	-0.4	17	26	26	24	18	23	629570247	629570203
7EL_scaffold227-160874-163229	0.3	17	40	22	22	11	40	615477192	615478660
7EL_scaffold1971-42666-46104	0.1	17	39	25	37	24	36	623450674	623450259

7EL_scaffold2500-9514-10971	0.4	17	2	10	5	8	5	623533762	623533219
7EL_scaffold9679-1207-2102	-1.2	16	9	21	5	24	30	630502900	630503368
7EL_scaffold1134-53463-55244	-0.4	16	21	23	27	18	20	596877636	596875858
7EL_scaffold321-21743-24992	0.0	16	33	14	25	26	31	595348284	595346662
7EL_scaffold1985-27458-29792	1.0	15	34	14	47	8	40	611015067	611013057
7EL_scaffold3801-5827-8078	-3.8	14	3	15	0	14	2	625225182	625227367
7EL_scaffold936-9239-10424	-2.2	14	6	18	2	10	7	604296687	604296124
7EL_scaffold3273-7358-8863	-1.5	14	15	35	14	20	11	630706530	630707447
7EL_scaffold3499-17575-18597	-1.0	14	9	13	7	7	11	604290984	604291921
7EL_scaffold1399-2940-4433	-1.0	14	16	22	12	24	21	603677922	603676511
7EL_scaffold1856-9772-13683	-0.2	14	33	14	16	23	27	618224966	618222585
7EL_scaffold4799-5600-7153	-7.0	13	0	22	0	18	0	625226263	625227767
7EL_scaffold704-58860-63143	-6.7	13	0	15	0	16	0	610857895	610858941
7EL_scaffold2598-17665-19026	2.6	13	156	14	126	20	171	595510295	595509099
7EL_scaffold3257-12080-15900	-1.4	13	13	14	10	45	22	598626858	598625435
7EL_scaffold2263-35879-37373	0.2	13	19	6	12	7	18	612780204	612781668

7EL_scaffold1767-51122-52810	0.1	13	33	16	25	21	31	613767196	613766303
7EL_scaffold350-81254-82292	-0.1	13	40	21	31	33	29	636765887	636766845
7EL_scaffold996-62287-65650	1.0	13	23	67	21	71	19	632001049	631999861
7EL_scaffold2829-23821-25597	-6.1	12	0	11	0	5	0	620584967	620583163
7EL_scaffold2737-23739-25322	2.1	12	70	6	32	8	82	625854549	625854027
7EL_scaffold1908-45055-46940	-1.7	12	16	21	5	30	10	626259870	626260919
7EL_scaffold5359-110-3413	0.0	12	24	6	11	11	14	604984079	604982206
7EL_scaffold765-12560-14693	0.7	12	46	17	39	15	35	628212439	628211520
7EL_scaffold252-63550-72601	0.4	12	35	17	31	22	45	595086360	595081940
7EL_scaffold2646-5402-8078	-0.1	12	25	11	14	12	15	605680081	605682689
7EL_scaffold1361-55024-66023	0.0	12	42	35	28	24	49	626212275	626213513
7EL_scaffold2214-6437-8273	0.0	12	34	80	32	50	37	606314270	606315025
7EL_scaffold649-89722-90970	3.0	11	220	8	140	19	148	616067970	616068820
7EL_scaffold2732-15708-21270	0.8	11	41	9	30	19	41	617023876	617021627
7EL_scaffold1796-55570-56569	0.4	11	22	9	11	6	25	628212420	628211505
7EL_scaffold3253-24061-24646	-4.1	10	1	5	1	9	0	615469519	615469193
7EL_scaffold874-39497-	-1.7	10	5	17	6	23	13	603155494	603156237

40246									
7EL_scaffold1783-14255-15076	-1.0	10	7	7	7	15	11	602196930	602197735
7EL_scaffold153-187251-188192	0.7	10	11	8	20	5	31	630254289	630255213
7EL_scaffold482-36833-38173	-0.2	10	15	9	11	11	16	613698853	613699521
7EL_scaffold1908-17039-17842	-6.7	9	0	9	0	26	0	626232856	626233669
7EL_scaffold213-37328-38037	-5.5	9	0	7	0	3	0	602750852	602751527
7EL_scaffold174-186957-193538	1.0	9	35	9	36	11	23	634659530	634661009
7EL_scaffold3314-13280-13814	-0.9	9	4	3	7	6	4	634087771	634087238
7EL_scaffold2869-9100-10463	-0.7	9	17	26	16	23	24	595668327	595669025
7EL_scaffold1533-62726-64834	-0.3	9	38	18	13	21	14	601588795	601589834
7EL_scaffold1247-32381-36032	0.6	9	27	79	36	61	29	630505842	630502902
7EL_scaffold1515-50664-51246	-5.8	8	0	9	0	7	0	611316771	611316181
7EL_scaffold2724-7965-8431	-4.5	8	0	1	0	1	0	602770694	602771159
7EL_scaffold1209-62796-63364	2.6	8	31	4	50	2	55	609981380	609980808
7EL_scaffold1893-13948-14339	1.9	8	25	1	2	1	6	632033227	632033072
7EL_scaffold2680-32441-33354	0.0	8	10	3	9	5	7	622542533	622542882
7EL_scaffold1881-3999-	0.0	8	23	9	10	9	10	613578599	613577217

5358									
7EL_scaffold1783-40374-43888	1.1	8	47	13	32	11	32	628181801	628181330
7EL_scaffold2390-22495-24583	-0.8	8	7	2	6	5	1	604876041	604874433
7EL_scaffold315-103906-117321	0.8	8	46	19	34	17	43	604896278	604890877
7EL_scaffold3465-5132-8749	0.7	8	30	13	25	11	28	612048415	612049366
7EL_scaffold649-110728-111099	0.6	8	22	1	6	5	9	616076371	616076637
7EL_scaffold1767-33114-33716	-0.5	8	9	8	13	6	3	614499658	614499058
7EL_scaffold726-78677-79294	-0.1	8	8	5	8	7	14	607228682	607228259
7EL_scaffold621-105108-106541	0.1	8	23	13	20	20	27	618378968	618378038
7EL_scaffold227-163315-165351	0.0	8	58	108	55	67	55	615478747	615480727
7EL_scaffold888-58822-59129	-2.6	7	0	4	0	5	4	609334226	609334534
7EL_scaffold2258-14980-15713	2.3	7	69	11	53	8	82	607546189	607546695
7EL_scaffold2258-14980-15709	1.9	7	40	5	32	8	50	607546189	607546695
7EL_scaffold13349-1-351	-0.6	7	5	6	1	2	10	602042884	602042548
7EL_scaffold1783-46447-57308	0.6	7	24	9	11	11	33	602402951	602399735
7EL_scaffold844-1362-3498	0.4	7	27	11	14	9	20	623453067	623452869
7EL_scaffold1767-26457-27678	-0.4	7	11	9	8	10	13	614506946	614505740
7EL_scaffold504-112072-	0.2	7	25	13	16	9	15	618880896	618881634

114675									
7EL_scaffold632-7564-8483	0.0	7	8	3	4	8	19	613101802	613100925
7EL_scaffold429-15847-19568	-0.2	7	42	74	35	42	36	613724408	613722985
7EL_scaffold10078-1141-1793	-3.8	6	0	5	0	0	1	610623423	610622782
7EL_scaffold210-121447-122719	2.0	6	52	6	41	13	67	595586364	595587382
7EL_scaffold4199-4511-5602	1.4	6	58	11	37	17	48	611840528	611839780
7EL_scaffold2628-7989-8317	-1.0	6	4	5	2	4	6	626300372	626300039
7EL_scaffold2628-8637-9457	-1.0	6	15	7	5	17	5	626299718	626298940
7EL_scaffold225-45038-46937	0.9	6	11	6	21	3	13	616705827	616705483
7EL_scaffold1303-64699-68621	0.9	6	39	25	44	13	45	626372188	626372786
7EL_scaffold3469-6031-6421	-0.8	6	6	5	3	5	6	605734071	605734480
7EL_scaffold765-19473-19857	-0.6	6	5	2	10	14	8	628198981	628198588
7EL_scaffold1210-68190-68906	-4.3	5	1	5	0	6	0	613637823	613638175
7EL_scaffold930-72312-73318	-2.7	5	0	5	1	3	2	628832125	628831393
7EL_scaffold4084-1680-5080	2.7	5	31	2	23	1	32	633672088	633670074
7EL_scaffold1121-18008-18260	-2.2	5	1	4	4	9	1	595090822	595090577
7EL_scaffold741-64408-64755	1.3	5	16	5	14	2	19	634661329	634661592

7EL_scaffold3781-11232-13978	1.2	5	23	9	23	8	38	617823655	617822172
7EL_scaffold649-109929-110297	1.1	5	28	4	4	2	10	616067955	616068300
7EL_scaffold483-26255-38207	1.0	5	34	12	31	9	18	629328848	629330662
7EL_scaffold3202-23234-26911	0.9	5	15	4	19	8	16	631820856	631819330
7EL_scaffold252-29979-30356	-0.8	5	7	4	1	1	2	615459266	615459636
7EL_scaffold3769-2317-6518	0.7	5	22	10	26	15	28	629137211	629137820
7EL_scaffold266-98599-100062	-0.7	5	8	2	2	10	8	598027383	598026719
7EL_scaffold2533-319-2719	0.6	5	13	6	12	7	19	611746060	611745936
7EL_scaffold735-39019-39530	-0.5	5	6	5	7	3	2	621795872	621796248
7EL_scaffold3552-20930-21504	-0.4	5	7	7	5	9	13	601074003	601073482
7EL_scaffold59-109300-109986	0.4	5	9	6	15	2	4	623846662	623845951
7EL_scaffold153-188571-189137	-0.4	5	1	6	6	2	8	630505249	630505820
7EL_scaffold1587-17851-19907	0.3	5	6	2	6	3	9	632337869	632339092
7EL_scaffold1842-90237-90668	0.3	5	14	3	6	6	9	621213263	621212848
7EL_scaffold3361-19442-19806	0.3	5	9	1	7	6	8	600863109	600862743
7EL_scaffold1796-38774-40969	-0.2	5	14	11	7	5	10	628212305	628211524
7EL_scaffold2069-225-4872	0.3	5	12	32	11	24	16	617646390	617649133

7EL_scaffold881-6552-6856	-4.0	4	0	2	0	1	0	625227390	625227680
7EL_scaffold1915-36948-38073	3.5	4	93	0	47	6	52	616055478	616056011
7EL_scaffold180-110707-116174	2.1	4	14	0	22	4	20	635517922	635516909
7EL_scaffold1938-11555-13130	1.9	4	31	5	29	5	24	634767851	634766629
7EL_scaffold160-13195-15723	1.3	4	29	8	21	4	16	617027820	617029535
7EL_scaffold3552-20569-20824	-1.3	4	3	3	1	2	2	601074254	601074054
7EL_scaffold4357-9419-10435	1.2	4	18	6	11	4	23	605755032	605755595
7EL_scaffold179-183313-185157	0.7	4	11	3	8	3	8	634890927	634890881
7EL_scaffold395-69848-70535	0.5	4	7	5	4	1	12	612443907	612444188
7EL_scaffold1957-44449-46083	0.3	4	4	2	7	4	9	631859714	631859401
7EL_scaffold888-56831-60053	-0.1	4	11	19	9	12	10	609652071	609650314
7EL_scaffold22902-124-508	-4.5	3	0	5	0	2	0	604531606	604531220
7EL_scaffold1908-18028-18467	-3.6	3	0	8	2	7	0	626233860	626234037
7EL_scaffold786-100473-100680	-3.4	3	0	2	0	0	0	627139441	627139241
7EL_scaffold160-38614-39396	3.1	3	57	2	19	2	32	616226190	616226633
7EL_scaffold3323-16320-22636	2.6	3	30	0	11	1	3	620251666	620252567
7EL_scaffold2659-3071-6627	-2.4	3	3	3	0	7	1	596942981	596942565

7EL_scaffold1617-27883-28463	-2.1	3	2	5	0	3	2	626660471	626659892
7EL_scaffold1121-18439-20159	-1.5	3	5	4	1	10	4	595090393	595088652
7EL_scaffold2392-22595-24628	0.0	3	13	4	10	10	4	612965336	612964030
7EL_scaffold3035-5446-5725	1.0	3	5	0	5	1	4	628678772	628678494
7EL_scaffold3499-19061-19501	1.0	3	1	1	6	0	6	605211499	605211833
7EL_scaffold1842-88312-90146	0.9	3	7	5	18	7	19	621206394	621205651
7EL_scaffold1627-16777-22692	0.8	3	8	4	7	3	14	631961279	631960335
7EL_scaffold3770-18408-20064	0.7	3	21	6	8	6	13	633610247	633609004
7EL_scaffold2445-874-3713	0.7	3	18	5	19	10	11	614287824	614287911
7EL_scaffold382-51375-52546	-0.7	3	5	9	3	7	11	625472086	625471495
7EL_scaffold624-17937-19920	0.6	3	14	3	8	7	10	616276464	616275061
7EL_scaffold1297-18273-19465	0.4	3	15	7	8	5	10	619218684	619219486
7EL_scaffold1767-15782-16227	-0.2	3	8	4	4	5	5	614509348	614508905
7EL_scaffold3314-11124-11816	-0.1	3	5	4	6	7	10	634089928	634089237
7EL_scaffold43-135948-136203	-4.2	2	0	4	0	2	0	634376622	634376370
7EL_scaffold428-91217-91454	-4.0	2	0	2	0	3	0	634216093	634215857
7EL_scaffold1331-59239-	-2.9	2	2	3	0	10	1	632141124	632141728

61090									
7EL_scaffold936-14010-14790	2.0	2	12	2	16	1	6	604304391	604303776
7EL_scaffold1274-57753-60052	1.9	2	34	7	24	5	28	600703870	600701603
7EL_scaffold1700-42796-43739	1.7	2	3	1	7	0	6	619516672	619517095
7EL_scaffold936-1-307	1.6	2	5	0	2	0	4	604324827	604324563
7EL_scaffold1893-7876-8868	1.5	2	9	0	11	5	12	632038845	632038389
7EL_scaffold1893-10018-10491	1.3	2	24	9	25	6	16	632047348	632046876
7EL_scaffold1893-6415-7626	1.1	2	12	1	2	1	2	632099945	632099448
7EL_scaffold720-100483-101119	0.0	2	6	6	3	3	9	605540232	605540853
7EL_scaffold602-5437-6010	-1.1	2	3	8	5	5	3	603674422	603675003
7EL_scaffold1134-49236-50420	-1.0	2	3	6	4	1	0	596889002	596888135
7EL_scaffold916-76333-77269	0.9	2	5	2	5	2	8	612877804	612877346
7EL_scaffold4599-73-296	-0.8	2	1	4	3	4	5	613608283	613608064
7EL_scaffold3673-17370-17728	-0.7	2	1	1	1	0	1	620085470	620085837
7EL_scaffold180-111223-111765	0.7	2	4	2	9	2	2	635519993	635519664
7EL_scaffold3621-17883-18580	0.4	2	9	1	4	5	5	602292540	602292093
7EL_scaffold1258-34593-36788	0.2	2	15	15	19	10	15	600755530	600756254
7EL_scaffold1280-55800-65769	0.6	2	86	266	126	248	111	618368113	618367274

7EL_scaffold325-126938-129438	0.5	2	19	69	23	36	24	634662126	634660244
7EL_scaffold815-41858-44569	0.1	2	35	12	15	7	15	621968828	621970771
7EL_scaffold1653-47752-48016	-4.0	1	0	1	0	5	0	634411994	634412199
7EL_scaffold7820-1422-2255	2.5	1	15	2	19	5	43	598496989	598496410
7EL_scaffold3994-3757-4474	2.5	1	12	1	5	1	13	624781217	624780541
7EL_scaffold1842-91133-91664	2.4	1	4	1	7	0	8	621159629	621159121
7EL_scaffold127-123317-124542	2.2	1	12	1	9	2	11	602397230	602397398
7EL_scaffold1062-16892-18414	1.9	1	10	1	9	2	7	617661217	617661722
7EL_scaffold828-5988-6463	-1.9	1	0	1	1	1	0	631994109	631994386
7EL_scaffold778-15143-15367	1.8	1	1	0	2	0	4	599254655	599254432
7EL_scaffold2281-20739-22781	1.8	1	2	0	10	2	5	630972541	630973096
7EL_scaffold436-61839-62207	1.5	1	9	4	5	0	10	618031945	618032326
7EL_scaffold2305-40180-41610	1.4	1	12	26	136	33	115	612285219	612285581
7EL_scaffold2043-27131-27939	1.3	1	6	2	3	1	8	635587230	635588038
7EL_scaffold1121-20228-20528	-1.3	1	5	4	0	7	3	595088579	595088283
7EL_scaffold1746-34320-35085	1.3	1	11	2	6	4	11	633407272	633406722
7EL_scaffold1258-8669-	-1.2	1	0	0	0	0	0	600838619	600839020

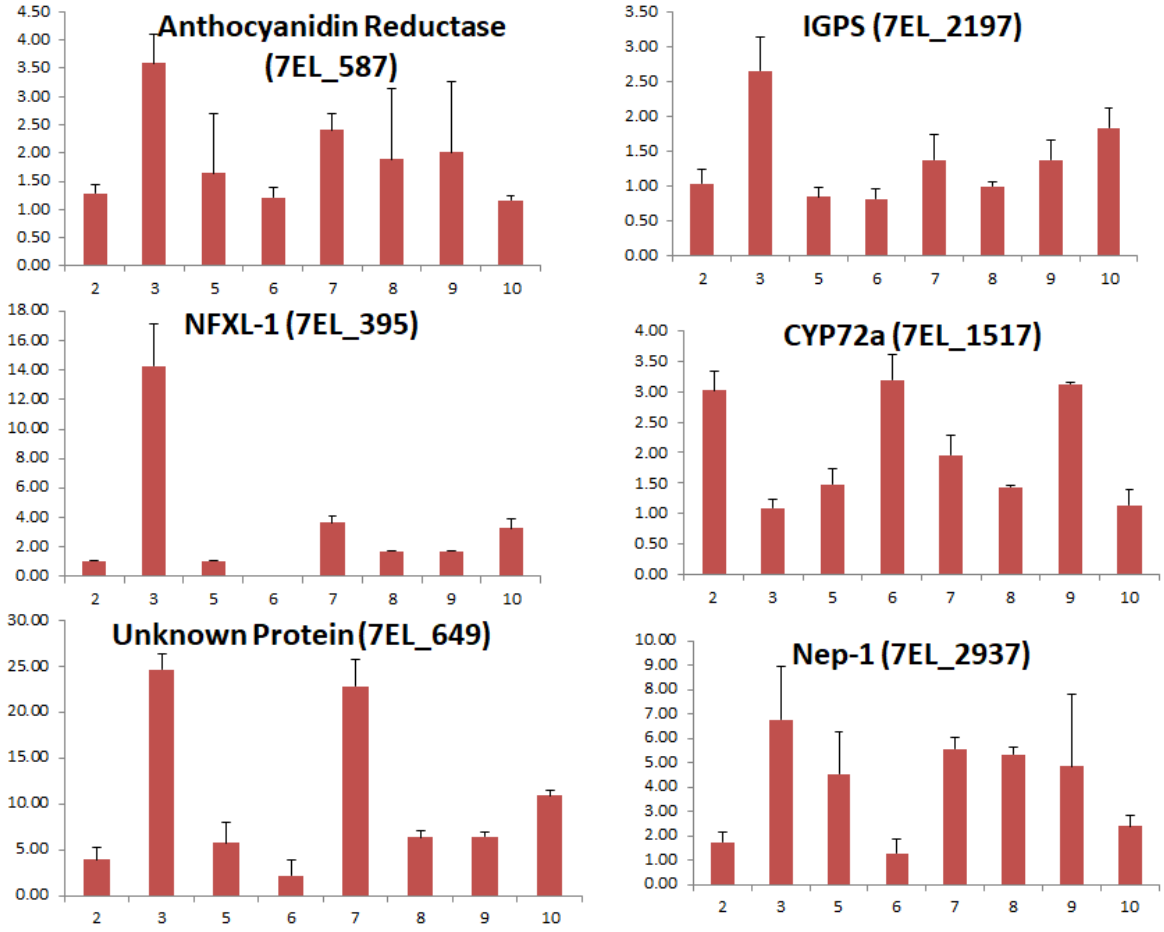
9068									
7EL_scaffold144-32773-33179	1.1	1	6	2	4	0	1	628679569	628679951
7EL_scaffold1349-6675-6946	1.0	1	7	2	7	5	13	629832356	629832195
7EL_scaffold506-73404-74349	0.9	1	10	4	8	4	10	629023103	629023991
7EL_scaffold1587-15985-17064	-0.9	1	0	1	1	3	3	632339014	632338207
7EL_scaffold3469-12905-13140	0.8	1	0	0	3	0	0	605737550	605737742
7EL_scaffold3465-3239-4497	0.8	1	7	2	12	6	5	611877874	611877402
7EL_scaffold144-20099-20329	0.7	1	4	2	4	4	10	628676816	628677045
7EL_scaffold3844-7297-7510	-0.5	1	4	1	3	6	2	633405675	633405888
7EL_scaffold11121-16-1319	0.5	1	2	0	3	2	2	612891739	612892674
7EL_scaffold1433-68497-68817	0.5	1	1	1	5	3	5	633516387	633516699
7EL_scaffold438-62410-62855	0.4	1	0	2	3	0	3	632099104	632098662
7EL_scaffold1842-91993-92232	0.1	1	5	0	0	4	4	621202946	621202746
7EL_scaffold2251-714-1307	0.0	1	9	3	4	6	3	615558517	615558949
7EL_scaffold153-135567-141012	0.7	1	1328	4886	1890	4146	2006	611718743	611721822
7EL_scaffold71-249680-253015	0.0	1	16	26	19	31	23	599184678	599186060
7EL_scaffold1247-41659-42658	NA	0	0	0	0	0	0	601848691	601849078
7EL_scaffold3671-20736-	NA	0	0	0	0	0	0	615943389	615942781

21345									
7EL_scaffold3929-4362-4851	NA	0	0	0	0	0	0	616506824	616507312
7EL_scaffold2156-9118-9992	NA	0	0	0	0	0	0	618874664	618875533
7EL_scaffold1262-620-3037	0.0	0	0	12	30	20	19	613499810	613500332
7EL_scaffold836-58059-58711	4.9	0	19	0	13	0	1	610764528	610763879
7EL_scaffold1209-46892-47467	3.5	0	16	2	20	2	39	609981370	609980798
7EL_scaffold1274-69443-71756	3.4	0	13	2	14	0	11	600703870	600701739
7EL_scaffold881-77682-78413	3.4	0	3	0	7	0	1	624836087	624835350
7EL_scaffold2598-11163-12029	3.3	0	8	1	16	1	10	595514273	595513430
7EL_scaffold235-91320-92259	3.1	0	1	0	4	0	4	620590604	620590549
7EL_scaffold1070-8715-12015	3.0	0	30	4	18	1	21	602914057	602916485
7EL_scaffold2191-36704-42722	-2.9	0	0	45	22	7	36	625612060	625610865
7EL_scaffold3361-770-3017	-2.9	0	0	118	29	82	24	600875696	600874234
7EL_scaffold1543-13444-13883	2.8	0	5	0	2	0	1	599482130	599482561
7EL_scaffold632-4510-4809	2.7	0	6	1	3	0	4	613104753	613104464
7EL_scaffold649-70050-71478	2.7	0	30	3	16	2	8	616104380	616105038
7EL_scaffold2097-44220-44570	2.6	0	1	0	1	0	5	636242879	636242551
7EL_scaffold1820-44607-44842	2.2	0	0	0	3	0	2	625635069	625634862

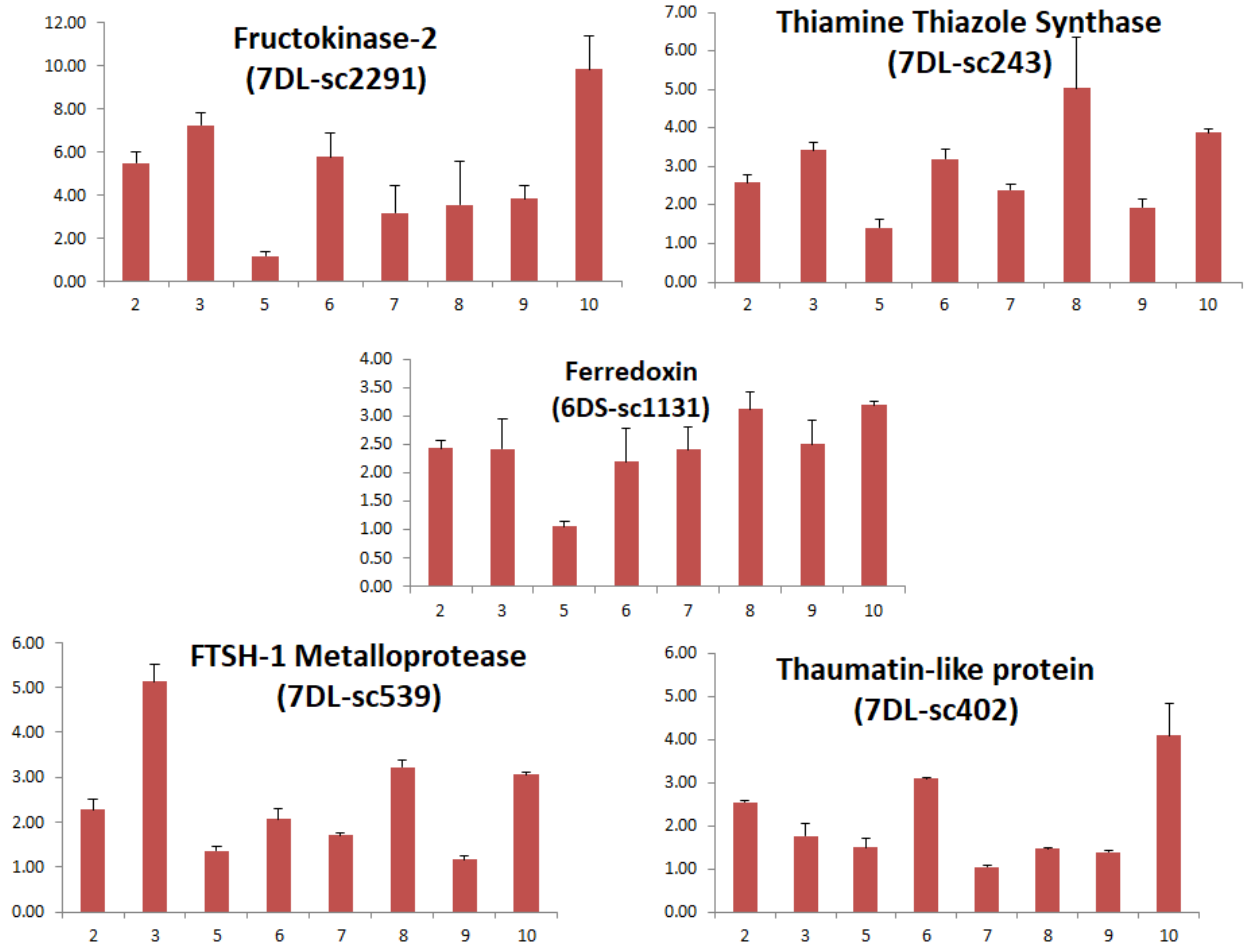
7EL_scaffold1004-20551-22388	2.1	0	1	0	1	0	3	613911067	613911510
7EL_scaffold1641-15197-15431	2.1	0	3	0	2	0	0	629810728	629810898
7EL_scaffold795-25861-28916	2.0	0	11	5	10	1	18	633670690	633671640
7EL_scaffold2291-11029-11234	2.0	0	2	0	1	1	5	605007624	605007794
7EL_scaffold1700-36736-41573	1.9	0	2	0	0	1	6	619510875	619511174
7EL_scaffold2966-29513-29754	1.9	0	0	0	3	0	1	616930279	616930033
7EL_scaffold3454-2221-2485	1.9	0	0	0	4	0	0	611015067	611014895
7EL_scaffold1767-50774-51020	1.6	0	4	1	2	1	5	613767502	613767376
7EL_scaffold1893-9070-9588	1.6	0	6	2	14	3	8	632101415	632100898
7EL_scaffold2156-14499-15442	1.5	0	7	2	5	1	2	618877882	618878750
7EL_scaffold1720-24138-25327	-1.2	0	85	79	59	79	52	600967732	600968272
7EL_scaffold1381-59125-65576	1.2	0	0	-	-	36	127	629532222	629535773
7EL_scaffold58-48165-51569	-1.1	0	0	0	4	1	4	608196955	608199185
7EL_scaffold2097-37617-44939	0.0	0	2	10	28	18	16	636158832	636155284
7EL_scaffold1700-41741-42090	1.1	0	3	2	3	0	1	619571582	619571689
7EL_scaffold560-103418-103642	1.0	0	0	1	0	0	4	619194004	619193824

7EL_scaffold144-45074-52137	1.0	0	8	5	9	2	5	628691080	628693545
7EL_scaffold3201-13193-13442	0.9	0	2	3	3	0	4	601157745	601157935
7EL_scaffold1078-84344-85342	0.7	0	1	1	1	0	1	626379567	626379020
7EL_scaffold438-57106-58042	0.6	0	1	3	4	0	2	632049189	632049007
7EL_scaffold19-91748-95142	0.6	0	0	101	359	150	293	618364080	618363888
7EL_scaffold123-100521-113152	0.4	0	0	149	391	202	351	625526406	625517671
7EL_scaffold59-107951-109230	0.3	0	0	8	11	5	8	623848003	623846718
7EL_scaffold916-66142-67351	0.1	0	9	2	4	7	3	611856892	611857318
7EL_scaffold3883-4647-11403	0.1	0	10	142	316	163	239	611586211	611584271
7EL_scaffold3314-11849-12058	0.0	0	3	1	1	4	4	634089203	634088995
7EL_scaffold923-85800-86380	-8.1	0	574	4	711	5	456	620143317	620142742
7EL_scaffold404-97144-98542	-7.9	0	0	0	39	0	44	629449753	629450908
7EL_scaffold1987-44230-45761	-6.1	0	11	0	8	0	10	604532282	604531018
7EL_scaffold3488-17789-21398	-5.5	0	39	3	51	0	30	629825024	629823683
7EL_scaffold1781-10679-12481	-5.5	0	51	0	38	0	28	604532246	604531040
7EL_scaffold1246-300-1631	-4.1	0	75	0	124	170	17	615468580	615469325
7EL_scaffold2285-23483-	-3.6	0	179	20	153	9	103	604532220	604530789

25386									
7EL_scaffold1908-25789-26112	-3.3	0	2	0	3	1	3	626254802	626255042
7EL_scaffold649-89462-91154	3.3	0	6	209	7	129	16	616067748	616068820
7EL_scaffold1577-54308-58344	-3.3	0	444	65	523	42	286	633804131	633808049
7EL_scaffold428-116081-118123	-1.8	0	15	3	6	4	7	634139053	634137459
7EL_scaffold959-45952-49701	1.6	0	16	119	10	77	28	630527675	630529697
7EL_scaffold696-56994-65566	1.3	0	1	8	3	3	0	605005543	605007932
7EL_scaffold486-59575-59918	1.0	0	0	0	1	0	0	595970226	595970568
7EL_scaffold1499-65524-67067	0.6	0	3	13	8	13	5	621160717	621159186
7EL_scaffold1893-23629-27621	-0.4	0	206	325	249	255	254	632000608	631999270
7EL_scaffold1746-46431-49272	0.1	0	15	0	7	606	0	633251622	633252786



Appendix 10: Expression of all six 7EL genes present in the 64-8-27-13-12-6 family. Values are mean of two technical replicates; error bars represent standard deviation.



Appendix 11: Expression of all six wheat genes present in the 64-8-27-13-12-6 family. Values are mean of two technical replicates; error bars represent standard deviation.