TOWARDS MAP-BASED CLONING OF FUSARIUM HEAD BLIGHT RESISTANCE QTL *Fhb1* AND NON-ADDITIVE EXPRESSION OF HOMOEOLOGOUS GENES IN ALLOHEXAPLOID WHEAT

by

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B.S., Oklahoma State University, 2000
M.S., University of Minnesota, 2003

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Wheat is the most widely grown and consumed grain crop in the world. In order to meet future agricultural production requirements of a growing population, it is essential that we achieve an increased understanding of the basic components and mechanisms shaping growth and productivity of the polyploid wheat plant. *Fusarium* head blight (FHB) (syn. "scab") poses a serious threat to the quantity and safety of the world's food supply. The resistance locus *Fhb1* has provided partial resistance to FHB of wheat for nearly four decades. Map-based cloning of *Fhb1* is justified by its significant and consistent effects on reducing disease levels, the importance of FHB in global wheat production and food safety, and because this gene confers partial resistance to this disease and does not appear to behave in a gene-for-gene manner. A bacterial artificial chromosome (BAC) contig spanning the *Fhb1* region was developed from the cultivar 'Chinese Spring', sequenced and seven candidate genes were identified in an ~250 kb region. Cosmid clones for each of the seven candidate genes were isolated from a line containing *Fhb1* and used for genetic transformation by biolistic bombardment. Transgenic lines were recovered for five candidate genes and evaluated for FHB resistance. All failed to complement the *Fhb1* phenotype. *Fhb1* is possibly one of the two remaining candidate genes, an unknown regulatory element in this region, or is not present in Chinese Spring.

Traditional views on the effects of polyploidy in allohexaploid wheat have primarily emphasized aspects of coding sequence variation and the enhanced potential to acquire new gene functions through mutation of redundant loci. At the same time, the extent and significance of regulatory variation has been relatively unexplored. Recent investigations have suggested that differential expression of homoeologous transcripts, or subfunctionalization, is common in natural bread wheat. In order to establish a timeline for such regulatory changes and estimate the frequency of non-additive expression of homoeologous transcripts in newly formed *T. aestivum*, gene expression was characterized in a synthetic *T. aestivum* line and its *T. turgidum* and *Aegilops tauschii* parents by cDNA-SSCP and microarray expression experiments. The cDNA-SSCP analysis of 30 arbitrarily selected homoeologous transcripts revealed that four (~13%) showed differential expression of homoeoalleles in seedling leaf tissue of synthetic *T. aestivum*. In microarray expression experiments, synthetic *T. aestivum* gene expression was compared to mid-parent expression estimates calculated from parental expression levels. Approximately 16% of genes were inferred to display non-additive expression in synthetic *T. aestivum*. Six homoeologous transcripts classified as non-additively expressed in microarray experiments were characterized by cDNA-SSCP. Expression patterns of these six transcripts suggest that *cis*-acting regulatory variation is often responsible for non-additive gene expression levels. These results demonstrate that allopolyploidization, *per se*, results in rapid initiation of differential expression of homoeologous loci and non-additive gene expression in synthetic *T. aestivum.*

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Table of Contents

List of Figures

List of Tables

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Preface

The origin and domestication of polyploid wheat played a central role in the development of modern civilization by providing a stable and readily available source of nutrition as mankind shifted from migratory clans to stationary societies. *Triticum aestivum*, known as common or bread wheat, is a globally important cereal crop that provides a substantial portion of essential energy and protein to the daily diets of people around the world. Considering the historic and current importance of wheat, while emphasizing the need to meet future agricultural production requirements of a growing world population, it is essential to seek an increased understanding of the basic components and mechanisms shaping growth and productivity of the polyploid wheat plant. By characterizing these underlying factors, we will gradually gain the ability to achieve more rapid genetic improvement.

Maintaining yield potential by limiting loss due to biotic stresses is a major focus in plant improvement. *Fusarium* head blight, or scab, is a major fungal disease of wheat throughout many regions of the world. A portion of this thesis research was focused on identifying a gene that has provided partial resistance to scab for nearly four decades. By eventually identifying the gene and its underlying molecular function, we hope to gain a better understanding of how to combat this devastating disease.

Using the words of Stebbins (1950), "If, therefore, we are to understand the significance of polyploidy in nature, we must first examine the effects of artificially induced polyploidy on pure species of various types". Bread wheat represents a unique allopolyploid system for studying the effects of polyploidization. Although Triticeae species have long been used as polyploid models, relatively few investigations have sought to understand the molecular

dynamics following new polyploidization events. It has become increasingly clear that expression of homoeologous genes may vary dramatically in polyploid organisms. With recent advances in molecular genetics and molecular biology techniques and resources, it is necessary to continue asking how the expression of genes is altered in newly formed polyploid wheat. Without complete genome sequence information and the tools that may be derived from such sequence (i.e. whole-genome tiling arrays) it is difficult to get an exact picture of the extent of expression changes in polyploid wheat. However, it is possible with current resources to begin to reasonably estimate when, how often, and the degree to which expression of homoeologous genes is altered.

CHAPTER 1 - LITERATURE REVIEW

Bread Wheat Phylogeny and Evolution

Triticum aestivum L. is an allohexaploid species (2n=6x=42) with a genome consisting of seven homoeologous groups with three chromosomes in each group (AABBDD). *T. aestivum* arose by two instances of genome doubling via polyploidization. The diploid progenitors of bread wheat belong to two genera (van Slageren, 1994), *Triticum* and *Aegilops*, in the Triticeae tribe. These *Triticum* / *Aegilops* progenitor species diverged from a common ancestor approximately 2.5–4.5 million years ago (MYA) (Huang et al., 2002). As a result of their relatively recent origin, genomes of *Triticum* / *Aegilops* species are highly conserved. Although most *Triticum* / *Aegilops* species are of importance with respect to both their ecological niches and value as source for novel genes in wheat improvement, only those species implicated in the origin and domestication of polyploid wheat will be discussed herein.

The *Triticum* genus is composed of six species. *T. urartu* Tumanian ex Gandilyan and *T. monococcum* L. are both A genome diploids. *T. monococcum* exists as both wild [ssp. *aegilopoides* (Link) Thell.; wild einkorn] and cultivated forms (einkorn wheat). Archeological discoveries and molecular genetic evidence have indicated that *T. monococcum* was domesticated as early as 10,000 years ago, most likely in the area that is now southern Turkey near the Syrian border (Heun et al., 1997). *T. urartu* is a wild species found throughout the Middle East (Johnson, 1975). Although early work pointed to *T. monococcum* as the A genome donor of *T. aestivum* (Sax, 1922; Kihara, 1924), more recent molecular genetic evidence has identified *T. urartu* as the A genome donor (Dvorak et al., 1988, 1993). Two tetraploid *Triticum* species, *T. turgidum* L. (AABB) and *T. timopheevii* (Zhuk.) Zhuk. (AAGG) exist with both wild

and cultivated forms. *T. turgidum* ssp. *dicoccoides* [(Korn. ex Asch. & Graebn.) Thell; wild emmer) and *T. timopheevii* ssp. *armeniacum* [(Jakubz.) van Slageren; wild form] are found throughout the Fertile Crescent. Domestication of emmer wheat [*T. turgidum* ssp. *dicoccon* (Shrank.) Thell] occurred at least 10,000 years ago in Southeast Turkey (Ozkan et al., 2002 and references cited therein). The two hexaploid *Triticum* species, *T. aestivum* (AABBDD) and *T. zhukovskyi* Menabde & Ericz (A^mA^mAAGG), are found as cultivated forms.

The *Aegilops* genus consists of 11 diploid and 12 polyploid species. *Ae. tauschii* Coss. (goat grass) is a D genome diploid that has long been recognized as the D genome donor of *T. aestivum* (Kihara, 1944; McFadden and Sears, 1944, 1946). *Ae. speltoides* Tausch is another diploid implicated in the evolution of cultivated polyploid wheats. Although the exact origin of the B and G genomes of polyploid wheats is still uncertain, convincing evidence indicates that both are most closely related to the S genome of *Ae. speltoides* (Dvorak and Zhang, 1990; Talbert et al., 1991; Daud and Gustafson, 1996; Friebe and Gill, 1996; Wang et al., 1997;), but likely arose through different lineages (Jiang and Gill, 1994; Maestra and Naranjo, 1999; Kilian et al., 2007).

Less than one MYA, diploid species *T. urartu* and a species closely related to *Ae. speltoides* hybridized to form the allotetraploid species *T. turgidum* (Huang et al., 2002). One or more hybridization events occurred approximately 8000 years ago between allotetraploid *T. turgidum* and diploid *Ae. tauschii* to form allohexaploid *T. aestivum* (Nesbitt and Samuel, 1996; Dvorak et al., 1998). Hybridization between *T. turgidum* and *Ae. tauschii* can be recreated (McFadden and Sears, 1944) and is a routine practice to produce synthetic *T. aestivum* lines, though the generation of viable plants most often requires embryo rescue and tissue culture.

Rescued F_1 hybrid progeny are haploid and genome doubling via colchicine treatment (Blakeslee and Avery, 1937) or spontaneous doubling is necessary for production of viable seeds.

Wheat Genome and Genetic System

Chromosome pairing in *T. aestivum* is restricted to strict homologues by genetic control, despite the presence of homoeologous chromosomes in the allohexaploid nucleus. This control maintains the stability of the polyploid genome, resulting in diploid-like pairing of 21 bivalents in metaphase I of meiosis. Pairing homoeologous loci *Ph1* and *Ph2* (Riley and Chapman, 1958; Mello-Sampayo, 1971), located on the long arm of chromosome 5B and the short arm of chromosome 3D, respectively, are primarily responsible for maintaining this genetic control. Tight genetic control of homoeologous chromosome pairing distinguishes wheat from other allopolyploid species; pairing of homoeologous chromosomes in many allopolyploids results in chromosome rearrangements and genomic instability.

Bread wheat has the largest genome among all field crops, with approximately 16,000 Megabases (Mb) of DNA (Arumuganathan and Earle, 1991). In addition to polyploidy, the inflation of the wheat genome, and of most members of the Triticeae tribe, compared to other cereal species such as rice (~430 Mb) and maize (~2500 Mb) is believed to be due to amplification of transposable elements (TE's), higher levels of expression of TE's in Triticeae genomes, and mechanistic differences in retrotransposon activity in Triticeae genomes compared to other grasses (Li et al., 2004). Early studies on the composition of the wheat genome indicated that at least 80% of the wheat genome was repetitive DNA based on $C_o t$ renaturation experiments (Smith and Flavell, 1975). More recent data based on sample sequencing of the D-genome donor *Ae. tauschii* has revealed that 90% or more of the wheat genome may be repetitive DNA (Li et al., 2004).

The vast expanse of repetitive DNA in the bread wheat genome poses a significant barrier to both directed and genomewide sequence-based investigations. However, increasing evidence indicates that genes are not randomly dispersed in repetitive DNA throughout chromosomes, but tend to be clustered in gene-rich regions (reviewed by Faris et al., 2002; Gill et al., 2004). Map-based cloning projects are increasingly common in wheat. Agronomically important genes have been cloned in wheat using a map-based cloning approach, including the leaf rust resistance genes *Lr10* (Feuillet et al., 2003) and *Lr21* (Huang et al., 2003); the vernalization genes *Vrn1* (Yan et al., 2003) and *Vrn2* (Yan et al., 2004); powdery mildew resistance gene *Pm3* (Yahiaoui et al., 2004; Srichumpa et al., 2005); and the domestication gene *Q* (Simons et al., 2006). Map-based cloning of QTL in plants is also feasible and several plant QTL have been cloned (reviewed by Paran and Zamir, 2003), including one in wheat that increases grain protein, iron, and zinc content (Uauy et al., 2006).

Fusarium Head Blight of Wheat

Pathosystem

Fusarium graminearum (Schwabe) [teleomorph= *Gibberella zeae* (Schwein.) Petch] is the primary causal organism responsible for *Fusarium* head blight (FHB) epidemics of wheat in the United States. *Fusarium* head blight causes both severe yield reduction and decreases grain quality (Bai and Shaner, 1994). In addition, contamination of grain with high levels of *Fusarium* mycotoxins may result in serious adverse health effects in mammals (Marasas et al., 1984; Pomeranz et al., 1990), thus contaminated grain is of low or no value. Trichothecene group mycotoxins that are frequently produced by *Fusarium* spp. and are detectable in food or feed include deoxynivalenol (DON), T-2 toxin, diacetoxyscirpenol, and nivalenol (Mirocha et al., 1980).

Ascospores, released from perithecia, and macroconidia, released from sporodochia, are the principal pathogenic propagules of *F. graminearum* in head blight infections (Sutton, 1982). Inoculum production is greatest during warm and moist conditions (Sutton, 1982). Ascospores are abundantly produced from saprophytic growth on plant debris and are the primary cause of epidemics (Bai and Shaner, 2004). Dispersal of macroconidia occurs primarily due to wind or rain-splash events (Sutton, 1982; Parry et al., 1995).

Fusarium graminearum is most able to infect wheat spikes from anthesis through the soft-dough stages of kernel development (Andersen, 1948). Fungal hyphae are able to penetrate directly through stomata present on the glumes, lemma, or palea (Pritsch et al., 2000). Other possible modes of entry into susceptible tissues include the floret apex and the gap between lemma and palea (Bushnell, 2001). Once established, the fungus can colonize the glumes, lemma, palea, and rachis by spreading both intra- and intercellularly (Pugh et al., 1933). Infection is first visible by the appearance of darkened, water-soaked lesions on these tissues. If the rachis is colonized, vascular impairment and cell death may lead to bleaching of parts of the spike or the entire spike in highly susceptible cultivars (Schroeder and Christensen, 1963). Under favorable conditions macroconidia may be produced on infected spikes and provide sources of additional inoculum (Mathre, 1997).

Initial establishment of infection and subsequent disease progression vary considerably depending on temperature and available moisture (Pugh et al., 1933; Andersen, 1948). The optimum temperature for the development of FHB in controlled conditions is approximately 25˚C (Andersen, 1948). Based on several investigations performed under controlled conditions, *F. graminearum* infection is greatest at a temperature of 25˚C and relative humidity approaching 100% for a period greater than 24 hours (Parry et al., 1995).

FHB Resistance

Resistance to FHB is quantitatively inherited in wheat (Bai and Shaner, 1994; Waldron et al., 1999; Anderson et al., 2001). The ability of the pathogen to cause significant damage when appropriate climatic conditions are present makes rapid incorporation of durable resistance into adapted genotypes a priority for wheat breeders. The most commonly investigated mechanisms of FHB resistance are resistance to initial infection and resistance to spread within the spike (Schroeder and Christensen, 1963). Screening for both types of resistance can be done under field conditions, but results are often confounded by environmental factors, and needs to be repeated over environments (Campbell and Lipps, 1998; Groth et al., 1999; Fuentes-Granado et al., 2003). Screening for resistance to spread within the spike can be effectively accomplished using macroconidia under greenhouse conditions (Stack, 1989). Both screening methods are resource intensive and problematic for testing the large numbers of progeny routinely evaluated in most breeding programs.

Because of the difficulties in breeding wheat for resistance to FHB using conventional methods, the identification of DNA markers associated with resistance has been a high priority for wheat breeders and geneticists. To date, most mapping studies have concentrated on resistance to spread of infection within the spike from the Chinese cultivar 'Sumai 3' and its derivatives, which are widely used as FHB resistance sources. A major quantitative trait locus (QTL), now designated as *Fhb1* (syn. *Qfhs.ndsu-3BS*), derived from Sumai 3 was identified in a Sumai 3/'Stoa' recombinant inbred population (Waldron et al., 1999) and verified in a second mapping population ['ND2603'(Sumai 3/'Wheaton')/'Butte 86'] (Anderson et al., 2001). The best simple sequence repeat (SSR) markers explained 41.6 and 24.8% of the variation in FHB resistance in the two mapping populations, respectively (Anderson et al., 2001). The large effect of *Fhb1* has been confirmed in other populations by other researchers (Buerstmayr et al., 2002;

Zhou et al., 2002; Miedaner et al., 2006). Nineteen pairs of near-isogenic lines (NILs) for *Fhb1* were developed in diverse genetic backgrounds and a ~25% reduction in FHB severity and infected kernels was found in field experiments (Pumphrey et al., 2007). This is the largest, most consistent gene effect discovered to date for FHB of wheat.

The consistency and magnitude of phenotypic effects associated with *Fhb1* prompted further efforts to develop tightly linked DNA markers and advance toward map-based cloning. Synteny between rice chromosome 1 and wheat chromosome 3 (Moore et al., 1995) was exploited for positioning wheat ESTs based on rice genomic sequence data to develop additional sequence tagged site (STS) markers (Liu and Anderson, 2003). Eight new STS markers were mapped in the *Fhb1* region in the Sumai 3/Stoa mapping population.

Due to limited progeny numbers and quantitative segregation of FHB resistance observed in available mapping populations, fine-scale mapping or cloning of *Fhb1* would require a suitable high-resolution mapping population. The 19 pairs of *Fhb1* NILs developed by Pumphrey et al. (2007) were considered as parents to develop a fine mapping population. One pair of NILs was chosen based on the consistent and significant differences in FHB resistance between isolines (Figure 1-1). Genetic mapping in the resulting NIL population of over 3000 F_2 progeny resulted in a fine scale map with *Fhb1* located within a 1.2 cM interval (Liu et al., 2006). Furthermore, the ability to unequivocally identify recombinant NIL progeny as either resistant or susceptible demonstrated that this resistance QTL could be mapped as a Mendelian locus. A similar effort to fine map *Fhb1* by another research group using two different advanced backcross populations verified the approximate map location (Cuthbert et al., 2006).

Wheat-Fusarium Interactions

There are conflicting reports regarding the specificity of host-pathogen interactions in the wheat-FHB pathosystem. A significant interaction was reported between 17 winter wheat cultivars and four strains of *F. culmorum*, though the relationships were not completely consistent (Snijders and van Eeuwijk, 1991). However, it is now widely accepted that differences in pathogenicity between and within *Fusarium* spp. isolates are the primary cause of variation (van Eeuwijk et al., 1995; Bai and Shaner, 1996; Mesterhazy et al., 1999). These studies concluded that resistance was horizontal (i.e. uniformly effective against various pathogen isolates) and screening for resistance to FHB may be carried out reliably using reasonably aggressive *F. graminearum* isolates.

Trichothecene toxins serve as aggressiveness factors that affect disease progression, but are not required for infection. Trichothecenes are potent translation inhibitors of eukaryotic ribosomes (McLaughlin et al., 1977). Mutant strains of *F. graminearum* that do not produce the trichothecene toxin DON have reduced aggressiveness on wheat, though the strains do incite infection (Desjardins et al., 1996; Bai et al., 2002; Dyer et al., 2005). Elegant confocal microscopy research using GFP-expressing wild type and trichothecene mutant *F. graminearum* strains revealed that without trichothecene production, the fungus is blocked from entry into the rachis (Jansen et al., 2005). Thus, infections of trichothecene knockout mutants are limited to the initial site of infection, including developing caryopses and floret tissues. Cell wall fortifications at the rachis node were responsible for blocking *Fusarium* spread in the absence of trichothecene production; wild type *F. graminearum* readily passes into the rachis within one week after infection.

The role of DON in *Fusarium* aggressiveness has been exploited to develop enhanced resistance in wheat. Transgenic wheat expressing a *Fusarium* 3-*O*-acetyltransferase, which

acetylates trichothecene toxins and reduces their activity, showed enhanced resistance to FHB (Okubara et al., 2002). Expression of the same gene in transgenic rice plants was also shown to reduce phytotoxic effects of DON (Ohsato et al., 2007). Interestingly, after analyzing the modification of DON following application of purified toxin to a doubled haploid mapping population segregating for Sumai 3-derived resistance, detoxification of DON by glucosylation, resulting in DON-3-*O*-glucoside, was shown to co-segregate with *Fhb1* (Lemmens et al., 2005). This research led Lemmens et al. (2005) to speculate that *Fhb1* is either a glucosyltransferase or regulator of glucosyltransferase activity. Supporting evidence for this hypothesis comes from a functional screen of Arabidopsis cDNAs in a DON-sensitive yeast (*Saccharomyces cerevisiae*) heterologous system. The Arabidopsis *DOGT1* gene, encoding a glucosyltransferase with DON detoxification activity, was isolated and overexpression experiments confirmed its role in enhancing DON tolerance (Poppenberger et al., 2003).

A number of *F. graminearum* genes not directly related to trichothecene toxin production are required for full agressiveness on wheat. An extracellular lipase produced by *F. graminearum* is required for wild type agressiveness on wheat (Voigt et al., 2005). Deletion of nonribosomal peptide synthetase gene, *NPS6*, of *F. graminearum* also resulted in reduced pathogenicity (Oide et al., 2006). Gene replacement of a putative response regulator, *Rrr1*, revealed its role in enhancing fungal sporulation and pathogenicity on wheat (Goswami et al., 2006). Several additional genes involved in pathogenicity were identified by random mutagenesis of *F. graminearum* (Seong et al., 2005, 2006). The completion of a draft sequence of *F. graminearum* (*Fusarium graminearum* Sequencing Project. Broad Institute of MIT and Harvard (http://www.broad.mit.edu), combined with routine mutagenesis techniques, is expected to significantly accelerate advances in knowledge of *Fusarium* pathogenicity.

Multiple studies have focused on expression responses of resistant and susceptible wheat lines following infection of *F. graminearum* (Kruger et al., 2002; Zhou et al., 2006; Bernardo et al., 2007; Golkari et al., 2007; Hill-Ambroz et al., 2007) and hundreds of genes and proteins differentially expressed after *F. graminearum* infection have been identified. Not surprisingly, these studies consistently found expression of classical defense response genes, many genes involved in cell wall metabolism and protein synthesis, and a large number of hypothetical or unclassified genes. In short, these studies fail to provide any mechanistic clues related to enhanced FHB resistance.

Host defense responses have been manipulated to enhance FHB resistance. Overexpression of pathogenesis related (PR) proteins, including α-1-purothionin, chitinase, thaumatin-like protein, and β-1,3-glucanase, in transgenic wheat lines was shown to moderately reduce infection severity (Anand et al., 2003; Mackintosh et al., 2006). Transgenic wheat expressing the *Arabidopsis NPR1* gene, a regulator of defense responses, displayed enhanced FHB resistance in greenhouse evaluations (Makandar et al., 2006).

Virus-Induced Gene Silencing

Virus induced gene silencing (VIGS) is a gene transcript suppression technique that may allow rapid characterization of gene function. VIGS is a type of RNA-mediated gene silencing in which the abundant double stranded RNAs produced during viral replication activate a sequence–specific RNA degradation mechanism in the host cell. VIGS is rapid and does not depend on transformation and plant regeneration, which although feasible and powerful, is comparatively slow and tedious in wheat. Holzberg et al. (2002) demonstrated the feasibility of VIGS-based silencing in monocots by utilizing barley stripe mosaic virus (BSMV) in barley.

BSMV is a single stranded RNA virus with a genome consisting of three RNA transcripts, α, β, and γ (Petty et al., 1989).

Protocols for use of BSMV in hexaploid wheat were established in experiments targeting the *phytoene desaturase* gene (*PDS*) for silencing, which is a convenient visual marker for gene silencing as reduction of PDS blocks carotenoid pigment production, resulting in photobleaching of chlorophyll in the affected tissue (Scofield et al., 2005). A fragment of the *PDS* gene was cloned into a DNA plasmid which encodes for the γ RNA after synthesis by in vitro transcription. After in vitro transcription of α, β, and γ-*PDS* RNA transcripts, combining them in an equal ratio, and rub-inoculation of seedling wheat leaves, evidence of photobleaching was observable by 10 days after virus inoculation. Quantitative RT-PCR measurements indicate that *PDS* expression is typically suppressed to ~15% of normal expression in the photobleached tissue and remains suppressed for at least 21 days after inoculation with BSMV (Scofield et al., 2005).

VIGS has proven to be a very useful tool for the identification of genes encoding essential components of disease resistance pathways in tobacco and tomato (Liu et al., 2002: Brigneti et al., 2004; Rowland et al., 2005). In collaboration with the laboratory of Dr. Steve Scofield, the Gill lab at KSU has established a BSMV-VIGS experimental system in wheat and has started dissecting the *Lr21* disease resistance pathway (Scofield et al., 2005).

Polyploidy in Plants

Polyploidization events have shaped the evolution and domestication of all major crop species. Polyploids are classically designated as one of two basic types: autopolyploids and allopolyploids. Autopolyploids have multiple sets of chromosomes of the same origin, likely arising from the rare union of unreduced gametes (Harlan and deWet, 1975). Allopolyploids

form from the hybridization of two or more distantly related species, also dependent on spontaneous chromosome doubling events. Allopolyploids represent a majority of all polyploid plant species (Levin, 2002; Wendel and Doyle, 2005), including wheat, despite estimates that autopolyploids are formed at higher rates than allopolyploids (Ramsey and Schemske, 1998).

Advances in molecular genetic techniques have largely brought about a paradigm shift regarding polyploid prevalence. Although earlier estimates concluded that polyploidization events have occurred in ~70% of angiosperm lineages (Averett, 1980; Masterson, 1994), it is now proposed that all flowering plants have polyploid ancestry (Wolfe, 2001; Udall and Wendel, 2006). Identification of polyploid species was classically based upon observation of chromosome numbers, cytogenetic landmarks, and meiotic chromosome pairing in F1 hybrids (Stebbins, 1950). This approach worked very well to identify constituent chromosomes/genomes for many existing polyploids. For those species that have undergone substantial genome differentiation and/or have evolved diploid genome constitutions, a more detailed look into their history of polyploidization was made possible by the advent of molecular mapping, DNA sequencing, and advanced cytogenetic techniques.

The prevalence of polyploidization events in angiosperm evolution has led to varied theories that polyploidy can confer lasting benefits, and has raised many questions about the long-term ecological and genetic consequences of genome doubling. In many genera, the distribution of polyploid species exceeds that of their diploid counterparts (Stebbins, 1950). This observation, combined with often-visible increases in polyploid hybrid vigor, has suggested that polyploidy may enhance fitness over diploid counterparts. Speculations on possible genetic mechanisms that may enhance polyploid fitness have abounded for over one-half a century. Convincing arguments include: 1) greater gene and gene expression diversity (heterosis), 2)

genome buffering (reduced vulnerability to detrimental mutation events), and 3) increased potential for redundant genes to evolve novel functions (sub/neo-functionalization) (reviewed by Udall and Wendel, 2006). However, other theoretical explanations for the abundance of polyploidy in flowering plant evolution suggest that polyploids do not necessarily possess enhanced fitness, but that the number of polyploids in a lineage increases simply because polyploidization is largely irreversible (Meyers and Levin, 2006).

The combination of genomes in a single nucleus of a new allopolyploid is expected to result in instantly fixed heterosis, where preferential pairing of homologous chromosomes prohibits homoeologous recombination and therefore ensures maintenance of the initial levels of heterozygosity captured from the parental species. A new allopolyploid containing a combination of genomes, each with unique adaptation to their respective environments, may achieve even broader adaptability (Stebbins, 1950). Hexaploid wheat is considered an exemplary model of this polyploid adaptation concept. Broader global adaptability of *T. aestivum* compared to *T. turgidum*, including adaptability to a range of abiotic and biotic stresses and environmental conditions, is credited to a polyploid advantage (reviewed by Dubcovsky and Dvorak, 2007).

The initial polyploidization process may induce a genomic shock (McClintock, 1984) where rapid changes in genome structure, composition and gene expression are essential for survival of the new species (reviewed by Wendel, 2000; Chen and Ni, 2006). Increasing evidence of novel gene expression patterns in several genera of newly-formed polyploids suggests that differential regulation of genes is indeed a common feature following polyploidization (Adams et al., 2004; Comai et al., 2000; He et al., 2003; Kashkush et al., 2002; Wang et al., 2004, 2006). As such, differential gene regulation, or deviations from additivity or

mid-parent expression levels, may form the molecular basis of allopolyploid heterosis and appearance of novel phenotypic traits (reviewed by Birchler et al., 2003; Chen, 2007). The expression of homoeologous genes in a new allopolyploid nucleus may be altered from that of the parents by epigenetic mechanisms, interactions between diverged regulatory networks present in each progenitor species, deletion events, chromosomal rearrangments, transposon activation, or novel epistatic interactions (reviewed by Chen and Ni, 2006). In short, novel gene expression levels and patterns may be both qualitative, namely activation and silencing, and quantitative. The magnitude of higher-order regulatory interactions that may modulate gene expression in a new allohexaploid nucleus, such as wheat, are difficult to grasp at present.

Gene expression changes observed to date in neo-allopolyploids of various genera are varied and rather unpredictable. In allotetraploid cotton, unequal expression of homoeologs was frequent, and often organ-specific, though no substantial genomic changes are observed (Liu et al., 2001; Adams et al., 2003, 2004). Some changes are stochastic, and may be different in subsequent generations of the same neo-polyploids as reported in allotetraploids of Arabidopsis and wild wheat (Feldman et al., 1997; Shaked et al., 2001; Wang et al., 2004), while other changes seem to be conserved in both natural and synthetic allopolyploids (He et al., 2003; Adams et al., 2004). Analysis of global expression levels in Arabidopsis synthetic allotetraploids revealed that approximately 6% of all transcripts are expressed in a non-additive manner, relative to mid-parent expression levels (Wang et al., 2006).

Response to Allopolyploidy in Hexaploid Wheat

Gene expression in newly-formed allohexaploid wheat has been the subject of only one investigation, despite the global importance of this species. He et al. (2003) applied a cDNA-AFLP technique (Bachem et al., 1996) to analyze transcriptional changes in stable synthetic

hexaploid wheat (TA4152L3) compared to the *T. turgidum* (cultivar 'Altar 84') and *Ae. tauschii* (accession TA1651) parents. Although no evidence of gene deletion or rearrangement events was observed, non-additive transcriptional changes were observed for 7.7% (168 out of 2200) of measured transcripts. Interestingly, the vast majority of differentially expressed transcripts (160 out of 168) were reduced or absent in the hexaploid, and only a few transcripts were induced. Characterization of nine transcripts by Northern blot and RT-PCR, including analysis on an additional synthetic and natural hexaploid wheat, hinted that some expression changes were non-random and were conserved in natural hexaploid wheat. Due to the nature of the cDNA-AFLP technique used in this study, novel gene expression patterns observed are largely qualitative, and likely fail to capture the complete scope of expression differences. This may be particularly true for genes with moderately higher expression levels in the new allohexaploid.

Expression of homoeologous genes in natural hexploid wheat has been under-investigated. Characterization of homoeologous transcription of a glutathione *S*-transferase, five benzoxazinone biosynthesis genes, the *Mre11* gene, and a MADS box gene provided examples of preferential expression or silencing of homoeologous transcripts, frequently with tissue or developmental specificity (Xu et al., 2002; Nomura et al., 2005; de Bustos et al., 2007; Shitsukawa et al., 2007). These gene-specific investigations do not allow for estimation of the magnitude of differential expression of homoeoloci that may occur in allohexaploid wheat. By mining a database of over 100,000 expressed sequence tags (ESTs), Mochida et al. (2003) identified a set of 90 homoeologous loci with informative single nucleotide polymorphisms (SNPs) that allowed assignment of each homoelogous transcript to a particular genome. After analyzing the expression of each transcript in various plant tissues, they concluded that 12% of homoeoloci (11 triplicated genes) showed complete silencing of one or more genomes in all

tissues. Of the remaining 79 homoeoloci where expression could be detected from each of the three genomes, on average ~28% showed preferential transcription from a certain genome in one or more tissues. Unequal homoeologous transcription varied considerably among tissue types, ranging from 57% of the genes studied in pistils to only 14% in spikes at bolting stage. These estimates suggest that polyploid wheat has harnessed an elaborate system of interaction among diverged regulatory networks and subfunctionalization, paralleling the observations in allotetraploid cotton (Adams et al., 2003). The timeline and mechanisms associated with such expression changes is unclear and studies on newly-synthesized hexaploid wheat are necessary.

A higher estimate of homoeologous gene silencing was found when expression of 236 random single-copy (per genome) loci was assayed by cDNA-SSCP (Bottley et al., 2006). In this study about 27% of genes expressed in leaf tissue in culitvar 'Chinese Spring', and 26% of genes expressed in root tissue showed silencing of one or more homoeologous transcripts. However, it is not clear whether the results of Bottley et al. (2006) represent regulatory differentiation (subfunctionalization) or if genetic changes (diploidization) during the evolution of hexaploid wheat are responsible for such expression patterns [e.g. deletion or mutation in promoter or enhancer regions, after a homoeolog is freed from selective constraints by genome duplication (reviewed by Kellogg, 2003)].

Investigations on genes underlying important traits in wheat have also revealed the often-unequal contribution of homoeoloci to phenotypes. Even though the presence of triplicate homoeologs is expected to buffer the effect of mutation events at the phenotypic level, several major domestication and agronomically important genes are substantially diploidized in natural hexaploids (Gill et al., 2004). A primary example is the recently cloned *Q* gene on chromosome

arm 5AL (Faris et al., 2003; Simons et al., 2006) that controls spike shape, threshability, and may have other pleiotropic effects (Faris, personal communication).

Genome buffering in allohexaploid wheat is evident by its ability to tolerate drastic changes in chromosome structure and composition. In fact, the ability of hexaploid wheat to tolerate aneuploid conditions allowed assignment of each of the 21 chromosomes of wheat to seven homoeologous groups with three chromosomes (from A, B, and D genomes) in each group (Sears, 1966). An extensive collection of aneuploid stocks, including monosomic, nullisomic-tetrasomic, ditelosomic, chromosome deletion lines, and a multitude of alien chromosome substitution and translocation lines demonstrates the genome buffering capacity afforded by polyploidy in *T. aestivum* (Sears, 1954; Sears and Sears, 1978; Endo and Gill, 1996; Gill et al., 2006). Redundancy of homoeoloci controlling some phenotypic traits gives further evidence of genome buffering in hexaploid wheat. As an example, red seed color in hexaploid wheat is under the control of three dominant genes, *R-A1*, *R-B1*, and *R-D1*, which most likely represent a homoeoallelic series on group 3 chromosomes (Metzger and Silbaugh, 1970). One *R* gene is sufficient to maintain red seed color, though combinations of *R* homoeologs show additive effects on color intensity (Nelson et al., 1995).

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Figure 1-1. Wheat spikes of *Fhb1* near-isogenic lines 21 days after inoculation with Fusarium graminearum. The blighted spike on the left does not contain *Fhb1*, a QTL that enhances resistance to Fusarium head blight. Its near-isoline, on the right, contains this QTL, resulting in fewer blighted spikelets.

CHAPTER 2 - TOWARDS MAP-BASED CLONING OF *Fhb1*

Abstract

Fusarium head blight (FHB) resistance loci from cultivar 'Sumai 3' and related lines are widely deployed in wheat varieties throughout the world. Quantitative trait locus (QTL) mapping projects undertaken by several research groups identified a major QTL (designated *Qfhs.ndsu-3BS*) on the short arm of chromosome 3B in Sumai 3. Phenotypic evaluation of a population of near-isogenic lines recombinant for this QTL region demonstrated that progeny with or without *Qfhs.ndsu-3BS* could be classified in a Mendelian manner, leading to re-designation of this locus as *Fhb1* and indicating that map-based cloning was feasible. Map-based cloning of *Fhb1* is justified by its significant and consistent effects on reducing disease levels, the importance of FHB in global wheat production and food safety, and because this gene confers partial resistance to this disease and does not appear to behave in a gene-for-gene manner. Furthermore, although *Fhb1* has been widely deployed for many years in areas with recurrent FHB epidemics and remains effective, synonymous to 'durable resistance', global dependence on *Fhb1* adds impetus to the need for identifying its underlying molecular function. A bacterial artificial chromosome (BAC) contig spanning the *Fhb1* region was developed from cultivar 'Chinese Spring', sequenced, and seven candidate genes were identified. Cosmid clones for each of the seven candidate genes were isolated from a line with *Fhb1* and used for genetic complementation by biolistic bombardment. Transgenic lines for five candidate genes were recovered and evaluated for FHB resistance and all failed to confer the *Fhb1* phenotype. *Fhb1* is possibly one of the two remaining candidate genes, an unknown regulatory element in this region, or not present in Chinese Spring. The long-term goals of this project are to elucidate the molecular basis of resistance to FHB in wheat, and to develop effective and efficient means to combat this disease.

Introduction

Fusarium head blight (FHB) (syn. "scab") poses a serious threat to the quantity and safety of the world's food supply. In the most extreme incident reported, in 1987 as many as 50,000 people in the Kashmir region of India suffered symptoms of mycotoxin poisoning after eating grain products derived from wheat (*Triticum aestivum* L.) heavily infested by *Fusarium* spp. (Pomeranz et al., 1990). Trichothecene toxin deoxynivalenol [DON, syn. vomitoxin] - producing chemotypes of *Fusarium graminearum* (Schwabe) [teleomorph= *Gibberella zeae* (Schwein.) Petch] are primarily responsible for infection of wheat in North America (Mirocha et al., 1994). Recurrent FHB epidemics over the past 15 years have resulted in devastating economic losses to the North American wheat industry, with 1993 loss estimates alone surpassing $1 billion in the Upper Midwest region of the United States (McMullen et al., 1997). Considering the combined arsenal of deleterious effects on yield, grain quality and food safety, FHB truly stands out among crop diseases.

Resistance to FHB is only partial and is quantitatively inherited in wheat (Bai and Shaner, 1994; Waldron et al., 1999). Chinese cultivar 'Sumai 3' and its derivatives are by far the most effective and widely used sources of FHB resistance worldwide, with millions of hectares planted annually to cultivars with Sumai 3 derived resistance. Sumai 3 resistance is generally characterized as a reduction in spread of infection within the wheat inflorescence (spike); ascospores or macrocondia may successfully infect spike tissues and create local blight symptoms, but subsequent colonization of neighboring tissues is significantly reduced. Because of the difficulties associated with breeding for resistance to FHB using conventional methods, the identification of DNA markers associated with resistance has been a high priority for wheat breeders and geneticists. A major quantitative trait locus (QTL) derived from Sumai 3, *Qfhs.ndsu-3BS*, was repeatedly identified in mapping populations with Sumai 3 or related lines

as a resistance source (Waldron et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2002, 2003; Zhou et al., 2002). The large effect of this QTL was verified in additional segregating populations (Yang et al., 2003; Zhou et al., 2003; Miedaner et al., 2006) and in 19 pairs of near-isogenic lines (NILs) derived from 13 different genetic backgrounds (Pumphrey et al., 2007). This is the largest, most consistent gene effect discovered to date for FHB resistance in wheat.

The consistency and magnitude of phenotypic effects associated with *Qfhs.ndsu-3BS* prompted further efforts to develop tightly linked DNA markers. Synteny between rice chromosome 1 and the short arm of wheat chromosome 3B was exploited to develop additional sequence tagged site (STS) markers (Liu and Anderson, 2003). Due to limited progeny numbers and quantitative segregation of FHB resistance observed in available mapping populations, high-resolution mapping and eventual cloning of *Qfhs.ndsu-3BS* required a suitable high-resolution mapping population. Nineteen pairs of *Qfhs.ndsu-3BS* NILs (Pumphrey et al., 2007) were considered as parents to develop a high-resolution mapping population and one pair of NILs was chosen based on the consistent and highly significant differences in FHB resistance between the NILs. Genetic mapping in the resulting NIL population of over 3000 F_2 progeny resulted in a high-resolution map with *Qfhs.ndsu-3BS* placed within a 1.2 cM interval (Liu et al., 2006). Furthermore, the ability to unequivocally identify recombinant NIL progeny as either resistant or susceptible demonstrated that this resistance locus could be mapped as a Mendelian factor, and *Qfhs.ndsu-3BS* was re-designated *Fhb1*. A similar effort to map *Fhb1* using two different advanced backcross populations verified the approximate map location (Cuthbert et al., 2006).

Trichothecene toxins are potent translation inhibitors of eukaryotic ribosomes (Cundliffe and Davies, 1977; McLaughlin et al., 1977). Deoxynivalenol serves as an aggressiveness factor to promote disease progression, but DON is not required for infection (Desjardins et al., 1996;

Bai et al., 2002; Dyer et al., 2005; Jansen et al., 2005). Interestingly, after analyzing the modification of DON following application of purified toxin to a doubled-haploid mapping population segregating for Sumai 3-derived resistance, detoxification of DON by glucosylation, resulting in DON-3-*O*-glucoside, was shown to co-segregate with *Fhb1* (Lemmens et al., 2005). This research led Lemmens et al. (2005) to speculate that *Fhb1* is either a glucosyltransferase or regulator of glucosyltransferase activity. Supporting evidence for this hypothesis comes from a functional screen of Arabidopsis cDNAs in a DON-sensitive yeast (*Saccharomyces cerevisiae*) heterologous system. The *Arabidopsis thaliana* gene *DOGT1*, encoding a glucosyltransferase with DON detoxification activity, was isolated and overexpression experiments confirmed it role in enhancing DON tolerance (Poppenberger et al., 2003).

The ability to classify progeny in a qualitative manner and placement of *Fhb1* on a high-resolution genetic map supported continued efforts toward map-based cloning of *Fhb1*. In collaboration with the laboratory of Dr. James Anderson at the University of Minnesota, the following research was conducted in an effort to map-based clone *Fhb1*. Some results presented, when indicated, are substantially from work in the Anderson lab and were included only for the purpose of continuity; otherwise, the following results are from investigations designed and conducted by the author.

Materials and Methods

Physical Mapping of Fhb1

BAC library screening

Amplicons of STS markers that flank *Fhb1* [3B-32, 3B -80, 3B -142, 3B –189, and 3B-206 (Liu et al., 2006)] were prepared by PCR on genomic DNA of cultivar Chinese Spring to produce probes for BAC library screening. PCR reactions were performed in a total volume of

50 µL containing 100 nM of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM $MgCl_2$, 1.0 unit AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City CA) and 60 ng template with 35 cycles of 94ºC for 60 s, 55ºC-60ºC for 60 s, and 72ºC for 90 s. PCR products were purified by QIAquick PCR Purification columns (Qiagen, Valencia CA) prior to probe labeling. The wheat chromosome 3B-specific BAC library of cultivar Chinese Spring (Safar et al., 2004) was screened by colony hybridization. BAC library filters were pre-hybridized for ~15 h at 65°C in 50 mL of solution containing 6× SSPE (0.9 M NaCl, 0.6 M NaH_2PO_4), 5× Denhardt's solution (0.1% Ficoll, 1 mg mL^{-1} BSA, 1 mg mL^{-1} polyvinylpyrrolidone), 0.05 mg mL^{-1} denatured salmon sperm DNA, and 0.5% sodium dodecyl sulfate (SDS). The pre-hybridization solution was discarded and 10 mL hybridization solution was then added (6× SSPE, 5× Denhardt's solution, 0.05 mg mL^{-1} salmon sperm DNA, 0.5% SDS, and 20% dextran sulfate). Twenty-five ng of probe DNA were labeled using random hexamers with [^{32}P] dCTP (Feinberg and Vogelstein, 1983), purified with Sephadex G50 columns, denatured by boiling for 5 min, and then transferred to membranes for 15–20 hr at 65°C. Membranes were then washed in 2× SSPE, 1× SSPE, and 0.5× SSPE plus 0.5% SDS for 30 min at 65°C each.

BAC clone verification, end-sequencing, sizing and contig assembly

Two DNA isolation protocols were utilized for BAC clone template preparation. Preferably, a 3 mL culture was grown ~16 h at 37°C in LB medium containing 12.5 µg mL^{-1} chloramphenicol, followed by standard alkaline lysis purification (Sambrook et al., 1989). For some clones, this method did not yield DNA of quality suitable for DNA sequencing reactions and pulse-field gel electrophoresis. Alternatively, a Large Construct Kit (Qiagen, Valencia CA) was used for BAC purification per the manufacturer's instructions.

All BAC clones identified by hybridization to chromosome 3B BAC library filters were first verified by PCR amplification with the corresponding STS markers. Two µg of BAC DNA from PCR-positive clones were then double-digested with *Hind*III and *Eco*RI and restriction fragments were separated on denaturing polyacrylamide gels (Litt et al., 1993) and visualized by silver-staining (Bassam et al., 1991). To determine the sizes of BAC inserts, BAC DNAs were digested to completion with *Not*I and fragments were separated by pulse-field gel electrophoresis (PFGE) using 1% Gold SeaKem agarose (GTG) gels in 0.5× TBE buffer at six V cm^{-1}, with 5–15 s initial-final pulse times, for 16 h at 12°C. Fragment sizes were determined by comparison to a Low Range PFG Marker (New England BioLabs, Ipswich MA). Standard vector primers T7 and Sp6 were used for BAC-end sequencing by the Kansas State University DNA Sequencing Facility. Primers were designed based on BAC-end sequences using the Primer 3 program (Rozen and Skaletsky, 2000). The combination of STS marker amplification, restriction fragment overlap, BAC insert sizes, and BAC-end primer amplification results were used to develop BAC contigs.

Sequencing and Annotation

Two BAC clones spanning the *Fhb1* resistance locus were sequenced (~8× coverage), assembled, and initially annotated in collaboration with Dr. Robin Buell at The Institute for Genomic Research (TIGR). The TIGR annotation pipeline used a combination of wheat and other monocot cDNA sequences and multiple gene prediction programs (such as FGENESH) to predict coding regions in the *Fhb1* region.

RFLP Analysis of Candidate Genes

Leaf tissue was collected (~3 g) from four-week-old plants of Chinese Spring (TA3008), Chinese Spring ditelosomic stock 3BL (TA3116), Sumai 3 (TA2966), and homozygous

recombinant NILs [HR97, HR106, HR111, HR112, HR113, HR127, HR134-3, HR139, HR140, HR141, HR143, HR145, HR123-1, HR123-2 (Liu et al., 2006)]. DNA isolation was performed as described by Faris et al. (2000). Twenty-five µg DNA was digested with *Bam*HI, *Dra*I, *Eco*RI, or *Hind*III restriction endonucleases. Electrophoresis and membrane transfer were performed as described by Faris et al. (2000). Probe preparation and labeling, hybridization, and wash steps were performed as described above.

Cosmid Library Construction and Screening

A cosmid library of the *Fhb1* NIL parent 260-2 was constructed using the pHC79 vector (Hohn and Collins, 1980). Genomic DNA of 260-2 was isolated as referenced above and then partially digested with *Sau*3A. Thirty partial digestion reactions were prepared on ice in 20 µL total volumes, with each containing 2 µg DNA, 1× *Sau*3A buffer, 1× bovine serum albumin, and 1 unit *Sau*3A enzyme. Partial digestions were incubated at 37°C for 3 min, heat-inactivated for 10 min at 70°C, and then stored at 4°C prior to PFGE. PFGE was performed using 1% Ultra Pure agarose (Invitrogen, Carlsbad CA) in 0.5× TBE buffer at six V cm^{-1}, with 0.1–40 s initial-final pulse times, for 16 h at 12°C. Digested fragments of ~35-45 kb were excised based on comparison to size standards and electro-eluted in 0.5× TBE buffer at 6 V cm^{-1}, with 30 s pulse time, for 4 h at 12°C. Size selected DNA was then precipitated with 1/10 volume 3M sodium acetate, 0.6 volumes isopropanol and re-suspended at 400 ng μL^{-1}. Ligation reactions were done in 20 µL volumes with a ratio of 1:1.5 *Bam*HI-digested vector to partially digested 260-2 DNA, with 1× T4 ligase buffer and 1µL T4 ligase (Invitrogen, Carlsbad CA) for 8 h at 16°C, followed heat inactivation for 15 min at 65°C. Lambda packaging of ligated cosmids was performed with the MaxPlax Lambda packaging extract (Epicentre, Madison WI) following the manufacturer's instructions. The number of colony forming units (cfu) per ligation/packaging reaction was

tested by counting the number of bacterial colonies from 20 µL of a 1:100 dilution grown on LB agar. After quantification, packaged lambda reactions were diluted 1:50 in phage dilution buffer (Epicentre, Madison WI) and then combined equally with a culture of *E. coli* strain XL1 Blue previously grown to an optical density of 0.8. Bacteria containing cosmids were aliquoted into 80 96-well plates containing 200 µL of LB freezer storage media and ~400 cfu per well. The 80 plates of cosmid primary pools were then manually super-pooled into six 96-well plates using a pin replicator, yielding ~6000 cosmid clones per well in the super-pools. Super-pools were grown overnight in LB media and DNA purified using QIAprep 96 Turbo Minipreps on a BioRobot 3000 (Qiagen, Valencia CA). Cosmid DNA was diluted 1:4 in sterile deionized water for PCR-based library screening with candidate gene primers. PCR reactions were done in 15 µL volumes (with reagents as described above) and 3 µL pooled cosmid DNA (~15-30 ng). After identifying positive super-pools, primary pools were then identified by another round of PCR-based screening of bacterial cultures. Finally, desired cosmid clones were isolated from primary pools by colony hybridization as described by Huang et al. (2003). Clones were verified by a combination of B-genome specific primers, end sequencing, and restriction fragment analysis.

Plant Transformation

In collaboration with Dr. Harold Trick's Plant Transformation Facility, Kansas State University, a modified particle bombardment protocol (Anand et al., 2003; Huang et al., 2003; Simons et al., 2006) was used to transform FHB-susceptible wheat lines Bobwhite, Fielder, and 260-4 (susceptible NIL parent) with cosmid clones containing *Fhb1* candidate genes. The pAHC20 plasmid (Christensen and Quail, 1996) containing the *bar* gene for selection with the herbicide bialophos was co-bombarded into immature embryos. Tissue culture and plant

regeneration were conducted following standard procedures under bialophos selection as described in Huang et al. (2003). Leaves were painted with 0.2% Liberty^{TM} herbicide to identify putative transgenic plants. PCR with candidate-gene-specific primers was used to identify positive T_0 plants. All progeny (T_1) of candidate-gene-positive T_0 plants were tested for the presence of candidate genes by PCR before being evaluated for their reaction to FHB in artificial greenhouse inoculations. Genomic DNA extraction on putative transgenic plants was performed as described by Liu et al. (2006) with modifications. Five hundred µL of extraction buffer (Riede and Anderson, 1996) was added to ground tissue (lypholized) and samples were placed into a 65ºC water bath for 20 min. Then, 500 µL chloroform/isoamyl (24:1, v/v) solution was added and tubes were mixed vigorously before centrifugation (10,000 × g) for 10 min. The resulting aqueous phase (500 µL) was transferred to a new 1.5 mL tube and precipitated with 1 mL 95% ethanol. Tubes were centrifuged (10,000 × g) for 10 min to pellet the DNA and then rinsed with 1 mL 70% ethanol and dried before adding 150 µL TE buffer. All DNA samples were diluted to 20 ng μL^{-1} in sterile deionized water for PCR.

Virus-Induced Gene Silencing

Barley stripe mosaic virus (BSMV)-based virus-induced gene silencing (VIGS) plasmid vectors (pα, pβ, pγ) were obtained from Large Scale Biology Corp (Vacaville, CA). The γRNA vector (pγ) was modified for direct cloning of PCR products (Huang and Gill, unpublished results), and is now designated as pγ-PCR. BSMV γ constructs carrying 150-300 bp long sequences of *Fhb1* candidate genes were assembled by ligation of PCR amplicons into the pγ-PCR vector and transformation of *E. coli* strain DH10B. Approximately 10-20 colonies resulting from transformation were screened by PCR to identify clones containing candidate gene fragments. Plasmid DNA was isolated from multiple PCR-positive clones using Qiagen

Miniprep kits (Qiagen, Valencia CA). Ligated clones were then sequenced for verification of candidate gene fragment inserts and determination of strand orientation. Infectious BSMV RNAs were prepared by *in vitro* transcription of linearized plasmids and rub-inoculation as previously described (Scofield et al., 2005). The BSMV-VIGS protocol was previously applied to Feekes stages (Large, 1954) 1-2 of wheat (Scofield et al., 2005). In order to assess the feasibility of applying the BSMV-VIGS system to silence genes in wheat spike tissue (the site of FHB attack), VIGS experiments were first conducted with the phytoene desaturase γ construct, pγ-*PDS*, as a visual indicator of post-transcriptional repression. Resistant NIL parent 260-2 of the *Fhb1* mapping population was planted with six plants each per BSMV inoculation date with three inoculation dates. In treatment one, 260-2 was inoculated with VIGS construct BSMV:00 (α, β, and γ), as a negative control. In treatment two, 260-2 plants were infected with BSMV:*PDS* (α, β, γ-*PDS*).

Candidate gene VIGS experiments were conducted by inoculation of 260-2 and 260-4 NILs with either BSMV:00 (virus only control) or BSMV:candidate gene RNAs. At least 15 plants of each genotype were treated with each construct. After ~14 days post-BSMV infection, single-florets per spike (only from tillers emerging after the primary tiller) were inoculated with macrocondia of *F. graminearum*, using routine artificial inoculation conditions (see FHB Resistance Screening section below).

FHB Resistance Screening

Inoculum Preparation

F. graminearum isolate of Z-3639 [NRRL accession 29169; USDA/ARS Culture Collection, Peoria, IL; (Bowden and Leslie, 1992)] was used for all artificial inoculation experiments. Glycerol stock cultures of Z-3639 were kindly provided by Dr. John Leslie.

Cultures were maintained on one-fourth-strength potato dextrose agar (¼ -PDA) plates in the dark at room temperature. Three days prior to inoculation of wheat, two pieces (5 × 5 mm) of the ¼ -PDA culture were transferred to 100 ml of sterile mung bean broth (40 g mung beans in 1 L boiling water for 8 min) and incubated on a shaker (~130 RPM, room temperature) to produce macroconidia. Macrocondia concentration was quantified with a hemacytometer and adjusted, if necessary, to 1×10^5 mL^{-1} prior to inoculation.

Plant Growth and Inoculation

For transformation experiments, all T_1 progeny of candidate-gene-positive T_0 plants and control lines 260-2, 260-4, Bobwhite and Fielder were planted with two plants per pot in 3.7 L round plastic pots. Plants were grown in a greenhouse with lighting systems set for 16 h day lengths and temperature set at 20°C daytime and 18°C nighttime (range 16-27°C). At anthesis, a central floret of each wheat spike was inoculated with ~1000 macrocondia of *F. graminearum*. The inoculated heads were covered with a plastic bag for 48 h, and disease spread was recorded at 21 days post-inoculation. For VIGS experiments, two plants per pot were grown in approximately 600 g of Metro-Mix 200 potting media (Sun Gro Horticulture, Bellevue WA) in 3.5 L square plastic pots. Six g of Osmocote 14-14-14 (Scotts Company, Marysville, OH) fertilizer was incorporated with potting media prior to planting. Plants were grown in a growth chamber with 16-hour day lengths at 20°C daytime and 18°C nighttime temperatures.

Results

A Physical Map of the Fhb1 Locus

BAC library screening with probes derived from STS markers STS3B-32, -80, -142, -189, and -206 that flank *Fhb1* on the high-resolution genetic map (Liu et al., 2006), and additional markers developed by the Anderson lab following publication of the high-resolution

map, initially resulted in four distinct BAC contigs with no obvious overlap. BAC-end sequencing provided additional low-copy probes for "chromosome walking" and in one case provided a new polymorphic marker (STS3B-270) in the STS3B-80 contig that further reduced the *Fhb1* interval and provided a distal boundary anchored by BAC clones. Following identification of BAC clones for each marker locus, BACs were then screened by the Anderson lab with a number of previously developed monomorphic STS-markers that were based on wheat ESTs with synteny in the corresponding rice chromosomal region (Liu et al., 2006). Positive PCR-amplification of one monomorphic primer pair on the STS3B-32 BAC contig prompted efforts to develop additional primer pairs towards obtaining a polymorphic marker. As a result, STS3B-242 was developed that provided a proximal boundary and narrowed the *Fhb1* region to less than 0.5 cM.

With a smaller interval delineating the *Fhb1* locus flanked by STS3B-242 and STS3B-270 in two BAC contigs, extensive BAC end-sequencing and primer development efforts were undertaken to span the gap between these contigs. Surprsingly, one end sequence of BAC 1 (contig STS3B-32/STS3B-242) was used to develop a primer pair that was PCR-positive for only BAC 1 and BAC 33 in the STS3B-80/STS3B-270 contig, suggesting that these clones formed a single contig spanning the *Fhb1* locus. At the same time, another round of BAC library screening with a low copy probe derived from an end sequence of BAC 7 in the STS3B-32 contig resulted in identification of one additional BAC clone (BAC 40) that extended into the gap between the two contigs. Restriction fingerprinting, STS primer amplification, and DNA gel blot hybridization confirmed that BAC clones 1, 33, and 40 formed a contig spanning *Fhb1* (Figures 2-1 and 2-2). The sizes of BAC clones 1 and 33 were determined to be ~190 kb each based on PFGE. Subsequent sequencing of these BACs resulted in ~350 kb of assembled

sequence in a single linear scaffold consisting of 17 contigs and no physical gaps. Annotation was initially performed by TIGR, with further analysis by the Anderson lab, and resulted in identification of seven candidate genes (Table 2-1; Figure 2-3).

Gene Conservation Between Chinese Spring and Sumai 3

The BAC library used for physical mapping was derived from Chinese Spring, which is moderately susceptible to FHB and most likely does not have an *Fhb1* functional allele. In order to establish that candidate genes identified in Chinese Spring are conserved in *Fhb1*-containing lines, RFLP experiments were conducted for each gene. PCR analyses using STS primers designed for each candidate gene were also performed by the Anderson lab. In short, all candidate genes identified from Chinese Spring BAC sequence were also present in *Fhb1* lines and could be mapped to the *Fhb1* region (examples in Figure 2-4).

Gene Complementation by Plant Transformation

Tentative annotations of the seven genes did not point toward an overwhelming *Fhb1* candidate. Therefore, a cosmid library (~6 genome equivalents) of FHB-resistant NIL 260-2 was constructed and B-genome-specific primers were developed for all seven genes. Cosmid clones were then isolated for the seven candidate genes and the integrity of each candidate gene was verified by sequencing with custom primers. Cosmid clones used for biolistic transformation had full-length coding sequences for each candidate gene. In two cases, a single cosmid clone contained more than one candidate gene (clone B11: genes 3 and 4; clone 6B12: genes 6 and 7; Table 2-1), and the both genes were transformed simultaneously. Multiple T_0 plants were recovered for each of five candidate genes (Table 2-1). Transformation of genes 1 and 5 is ongoing and positive T_0 plants were identified in Summer 2007.

The T_1 progeny of T_0 plants positive for transgenes 2,3,4,6, and 7 were screened by PCR with allele-specific primers to identify all progeny with candidate genes before artificial inoculation with *F. graminearum*. A total of 64 plants containing candidate genes 6 and 7 (from three different T_0 events with cosmid clone 6B12), 113 plants containing candidate genes 3 and 4 (from three different T_0 events with cosmid clone B11), and 66 plants containing candidate gene 2 (from five different T_0 events with cosmid clone 6F6) were evaluated for resistance to FHB, along with the transformation recipient cultivars (Bobwhite, Fielder, and susceptible NIL 260-4) and the resistant NIL 260-2. All T_1 plants were found to be highly susceptible to FHB (>85% disease severity), while the 260-2 was resistant (~6% disease severity) and 260-4, Bobwhite, and Fielder were moderately susceptible to susceptible (disease severity >70%). An identical effort in the Anderson lab with additional T_1 progeny produced the same results. The expression of candidate transgenes was verified in multiple plants from each transformation event by RT-PCR using allele-specific primers in the Anderson lab.

Virus-Induced Gene Silencing

In order to assess the feasibility of applying the BSMV-VIGS system to silence genes in wheat spike tissue and to optimize timing of BSMV inoculation, experiments were first conducted with the phytoene desaturase γ construct, pγ-*PDS*, as a visual indicator of post-transcriptional repression. After testing a range of growth stages (Feeke's growth stages 6-10), BSMV inoculation of newly emerging flag leaves on primary tillers (Feekes 8-9) resulted in photobleaching in spikes of subsequent tillers (Fig 2-5), which appeared to be the best timing for *Fhb1* candidate gene experiments. BSMV constructs containing ~200 bp fragments of *Fhb1* candidate genes 1, 3 and 7 were developed. BSMV-VIGS experiments with candidate genes 3 and 7 were performed prior to screening transgenic progeny for each gene as a more rapid

attempt to identify *Fhb1*. In agreement with FHB resistance screening of transgenic progeny for candidate genes 3 and 7, VIGS experiments provided no evidence that either gene was *Fhb1* (Figure 2-6). Disease severity in *Fhb1*-containing NIL 260-2 was abnormally high in both VIGS experiments, irrespective of the BSMV construct used for virus infection. However, disease severity means of 260-2 and 260-4 NILs were clearly separated in each experiment. For candidate gene 1, both sense and antisense transcripts were tested. No difference was observed between control (BSMV:00) and candidate gene (BSMV:1F/R) treatments in VIGS experiments with candidate gene 1, again providing no evidence that this gene encodes *Fhb1*.

Discussion

The chromosome 3B short arm region spanning the *Fhb1* resistance locus was delineated using a BAC library of cultivar Chinese Spring, sequenced, and seven candidate genes were identified. None of the seven candidate genes showed homology with known disease resistance genes or glucosyltransferase genes that may play an important role in detoxification of DON (Lemmens et al., 2005). Five candidate genes were tested by genetic transformation experiments; unfortunately, these efforts failed to result in identification of the *Fhb1* gene. The inability to identify *Fhb1* may be due to several possibilities. First, silencing of transgenes is a concern in plant transformation experiments. Multiple strategies were employed to address this concern. First, plant transformation was conducted with cosmid clone DNA harboring each candidate gene isolated from the *Fhb1* donor line 260-2. In this situation, the transgene was most likely under the control of its native promoter, where transgene silencing is less likely than if the candidate gene was under the control of a highly expressed constitutive promoter (Huang et al., 2003). Second, multiple T_0 plants (independent transformation events) were recovered for each construct. By recovering multiple events, the likelihood of silencing based on an

unfavorable local chromatin environment is reduced. Finally, the expression of candidate transgenes was verified in T_1 progeny by the Anderson lab using RT-PCR with allele-specific primers. Therefore, it is unlikely that candidate genes 2, 3, 4, 6, or 7 encode *Fhb1*. Ongoing complementation experiments with genes 1 or 5 may result in identification of *Fhb1*.

Candidate gene 1 was putatively annotated as a F-box domain-containing protein. F-box domain proteins often function as part of an E3-ubiquitin ligase complex to regulate protein turnover (reviewed by Dreher and Callis, 2007) and are widespread in eukaryotic genomes with ~700 predicted in the rice genome (Jain et al., 2007). Multiple E3 ligase-associated proteins have been implicated as components of defense responses in plants, presumably based on their role in ubiquitin-mediated protein degradation (Wang et al., 2006; Dreher and Callis, 2007 and references cited therein). Candidate gene 5 was putatively annotated as an oxidoreductase, a very broad category of enzymes that may catalyze redox reactions in diverse cellular processes.

Virus-induced gene silencing experiments with *PDS* indicated that the BSMV-VIGS system might be useful to silence genes in wheat spike tissue. Thus, VIGS experiments were conducted for candidate genes 1, 3 and 7. Results of both VIGS and genetic transformation approaches indicated that candidate genes 3 and 7 do not encode *Fhb1*. Although a VIGS experiment with BSMV RNAs harboring a fragment of candidate gene 1 suggests that this gene is not *Fhb1*, these results are inconclusive. It is possible that the *Fhb1* gene product is accumulated prior to BSMV inoculation and, if stable, transcriptional repression at this stage might have a diminished or no effect. In this case, the ongoing plant transformation experiments will be essential. It is also possible that VIGS-based silencing in spike tissues does not sufficiently eliminate transcript accumulation. Application of the BSMV-VIGS system in wheat seedlings results in sectors of silenced tissues (Scofield et al., 2005), consistent with the mosaic

symptoms of the virus. For qualitative traits such as leaf rust resistance, sectored silencing may be accounted for by the large number of independent infection events assayed on a single leaf. When applied to quantitative FHB resistance, this scenario would most likely result in highly variable phenotypic evaluations and greater replication would be necessary to achieve statistically significant differences. The FHB severities of NIL 260-2 in virus-only control experiments suggest that BSMV infection, *per se*, alters host defense and causes higher variability in phenotypic evaluations. Line 260-2 was previously screened in over 10 replicated experiments and displayed stable FHB resistance (disease severity ratings less than 25%, data not shown). The suitability of the VIGS system to functionally analyze FHB resistance genes may be best assessed when the *Fhb1* gene is identified.

If genetic transformation of all seven candidate genes fails to result in identification of *Fhb1*, additional possibilities exist. It is possible that the transformation-recipient genotypes Bobwhite and Fielder are not suitable for *Fhb1* confirmation. Unlike the effect of *Fhb1* in the NIL mapping population utilized in this research, the effect of *Fhb1* was somewhat variable in other NILs derived from 13 different cross combinations (Pumphrey et al., 2007). FHB resistance evaluation of hybrids between Bobwhite/Fielder and *Fhb1* NIL 260-2 should address this concern. In this case, it may be necessary to backcross transgenes into a suitable background, such as susceptible NIL 260-4. Direct transformation of 260-4 would be preferred, but this genotype responds poorly to tissue culture. Alternatively, candidate genes may also be tested by expression in heterologous systems (Harris and Gleddie, 2001; Poppenberger et al., 2003; Di and Tumer, 2005), if the DON-modification hypothesis (Lemmens et al., 2005) is correct.

Association mapping and positional cloning of the maize (*Zea mays* L.) flowering-time QTL (*Vgt1*) uncovered an interesting circumstance where the QTL is most likely an ~2 kb non-coding regulatory element located ~70 kb upstream of an *Ap2*-like flowering-time gene (Salvi et al., 2007). Lemmens et al. (2005) suggested that *Fhb1* might be either a glucosyltransferase gene or regulator of glucosyltransferase activity. If a non-coding regulatory element confers the *Fhb1* functional allele, gene complementation experiments may not be fruitful, unless the fortunate circumstance occurred where the functional regulatory region was present in one or more cosmid clones.

Of similar concern, comparative sequence analysis has revealed that non-conserved gene content within the same species may be more common than previously expected. Substantial violation of colinearity in maize arises from the movement of genes or gene fragments by helitrons (Brunner et al., 2005, Lai et al., 2005, Morgante et al., 2005). In wheat, two haplotypes were identified in the leaf rust resistance gene *Lr10* region (Scherrer et al., 2002). The isolation of *Lr10* was only possible because the BAC library used in the sub-genome map-based cloning approach was constructed from a genotype belonging to the same haplotype as that of lines carrying *Lr10*. Recently, gene *Sub1A* conferring submergence tolerance in rice was cloned (Xu et al., 2006). A haplotype survey revealed two types of submergence-intolerant lines carrying either a null allele or *Sub2A* allele. It would have been impossible to clone *Sub1A* based on the fully sequenced genome of Nipponbare rice, which is intolerant to submergence and carries a null allele. In summary, it is possible that Sumai 3 has additional genes in this region that are not present in Chinese Spring. Construction of a Sumai 3 BAC library, followed by isolation and sequencing of the *Fhb1* region would shed light on this possibility.

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Figure 2-1. Restriction fingerprint (A) and DNA gel blot hybridization (B) of bacterial artificial chromosome (BAC) clones 1, 6, 32, 33, 34, and 40 from cultivar Chinese Spring that span the *Fhb1* locus. BAC DNAs were digested with *Hind* III, followed by electrophoresis in a 1% agarose gel and ethidium bromide staining. Restriction fragments were then transferred to a nylon membrane and gel blot hybridization was performed using a probe amplified from the end sequence of BAC 1.

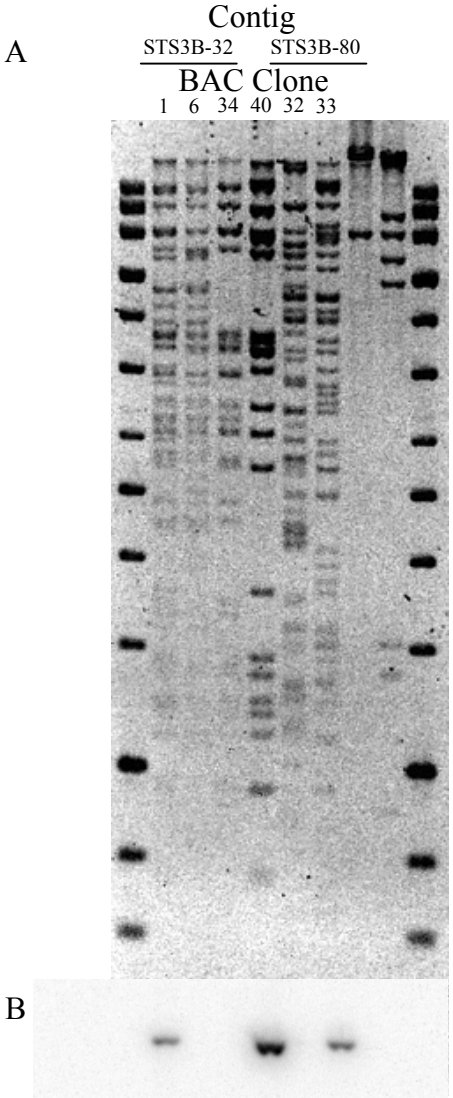

Figure 2-2. Genetic and physical maps of the *Fhb1* region. Top, graphic genotypes of three homozygous recombinant NILs and their FHB phenotypes. The black and open boxes indicated homozygous alleles of Sumai 3 and non-Sumai 3, respectively; Middle, high-resolution genetic map of the *Fhb1* region, *Fhb1* was placed into the interval delimited by DNA markers 3B-334 and 3B-355; Bottom, the physical map of the *Fhb1* region. Dots indicate BAC connections that were validated by the corresponding DNA markers. The genetic map was constructed by the Anderson lab.

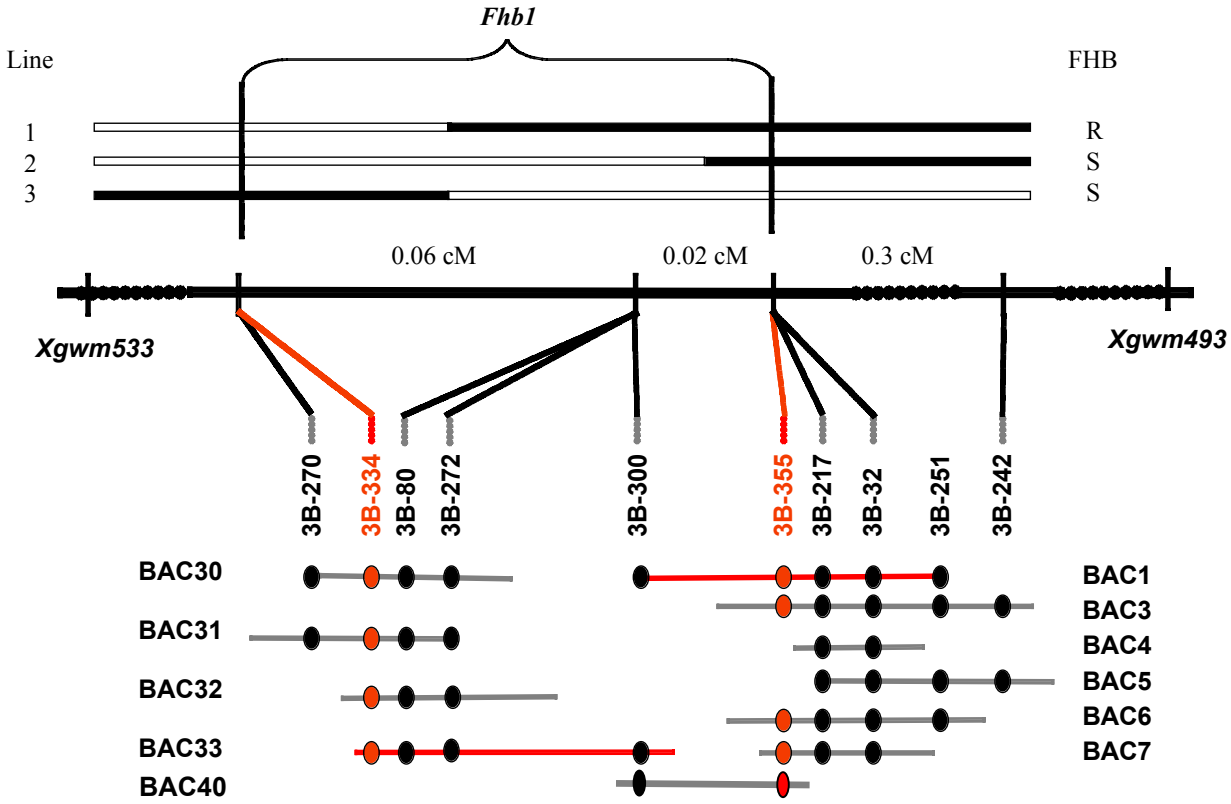

Figure 2-3. Recombination events and physical distance of genes in the *Fhb1* region from the BAC contig sequence of Chinese Spring wheat. Genes are represented as green boxes numbered 1-12. Genes 8-12 were excluded as candidates for *Fhb1* based on the phenotypes of the recombinant lines shown in Figure 2-2. Red arrows indicate recombination boundaries. Genes 1-7 do not show recombination with *Fhb1* and are therefore all candidates.

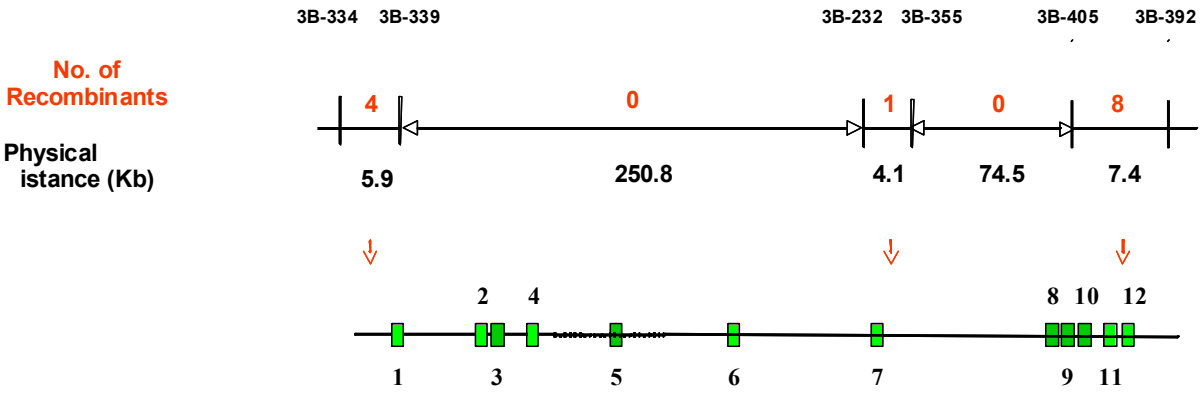

Figure 2-4. Restriction fragment length polymorphism (RFLP) results for candidate genes 1, 5 and 6 on selected recombinant NIL progeny. Probes used for RFLP experiments were amplified from Chinese Spring BAC clones by PCR with custom primers for each gene. Phenotypic classifications of NILs based on FHB resistance-screening experiments are indicated as resistant (R) and susceptible (S).

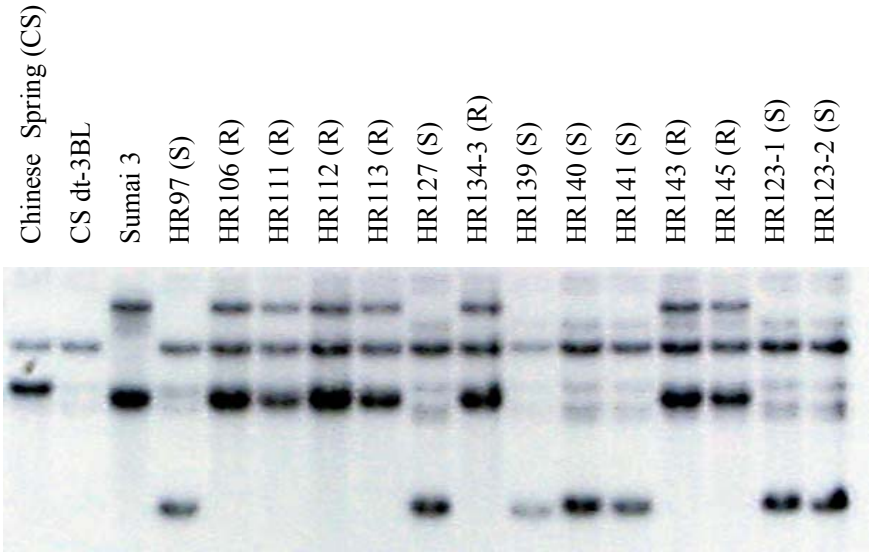

Figure 2-5. Organization and cloning site of Barley Stripe Mosaic Virus (BSMV) γ RNA (top). Silencing of *phytoene desaturase* (PDS) in wheat leaf blades (bottom left, images courtesy of Dr. Li Huang) and spikes (bottom right). For silencing of leaves, wheat plants were inoculated with in vitro transcribed RNAs representing the α, β, and γ RNAs of BSMV:00 or BSMV:PDS at seven days after emergence (Feeke's 1-2). Complete bleaching was observed 12 days later. For spikes, BSMV inoculations were applied to the emerging flag leaf on the main tiller at Feeke's stage 8-9. Spikes of secondary tillers showed bleaching as they emerged from the boot.

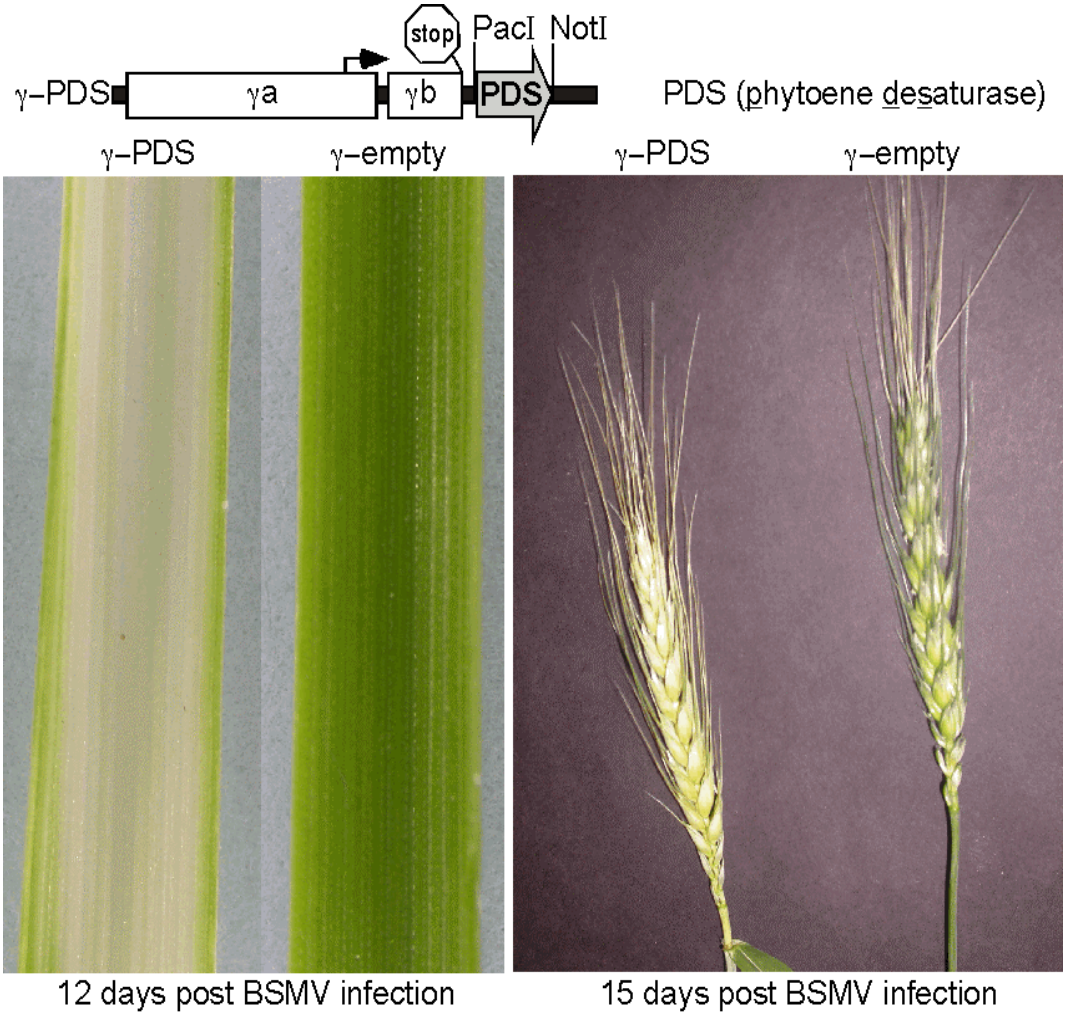

Figure 2-6. Fusarium head blight disease severity of *Fhb1* near-isogenic lines 260-2 (NIL R) and 260-4 (NIL S) in BSMV-based VIGS experiments with candidate genes 1, 3 and 7. Emerging flag leaves were rub-inoculated with BSMV RNAs, followed by *F. graminearum* inoculation ~two weeks after BSMV infection. Disease severity was measured 21 days after inoculation. Gray bars indicate virus-only experimental controls (BSMV:00) and white bars indicate candidate gene constructs (BSMV:1, 3 or 7). For candidate gene 1, both sense (BSMV:1S) and antisense (BSMV:1A) transcripts were used. Error bars indicate the standard error of each assessment.

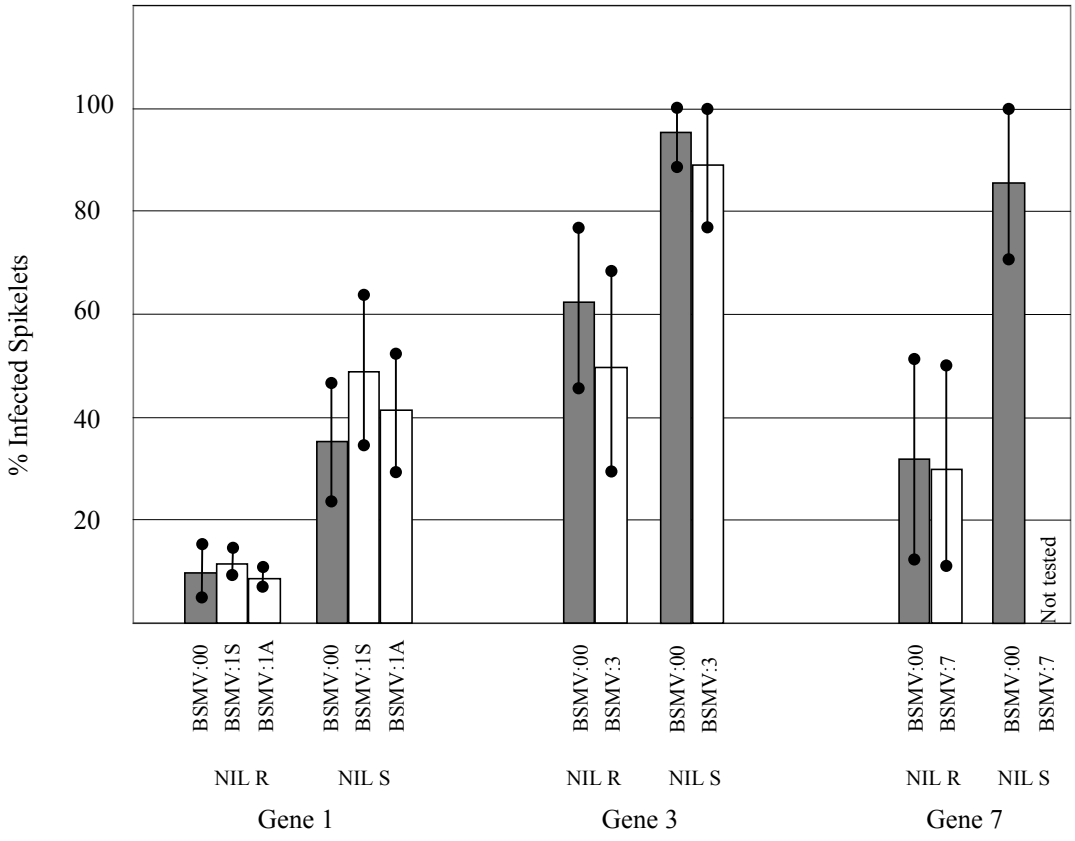

Table 2.1. Putative annotation of candidate genes for *Fhb1*, their expression results based on testing with RT-PCR, cosmid clones isolated from an *Fhb1* donor for each gene, and number of transgenic plants produced with each cosmid.

Gene	Putative Function	RT-PCR[a]	Cosmid Clone[b]
1	F-box domain-containing	+	3E8 (5)
2	N-hydroxycinnamoyl/benzoyltransferase	-	6F6 (9)
3	unknown	+	B11 (3)
4	terpene synthase	-	B11 (3)
5	oxidoreductase	+	4E5 (1)
6	polygalacturonase	-/+	6B12 (3)
7	translation initiation factor	+	6B12 (3)

a) RT-PCR was performed by the Anderson lab with total RNA extracted from spikes of FHB resistant NIL line 260-2. '+' stands for positive and '-' stands for negative. Expression of gene 6 was detected in roots of seedling plants even though it is not expressed in the spikes of the R NIL.

b) In parentheses are the numbers of positive transgenic plants recovered to date for each cosmid clone.

CHAPTER 3 - NON-ADDITIVE EXPRESSION OF HOMOEOLOGOUS GENES IS RAPIDLY ESTABLISHED IN ALLOHEXAPLOID WHEAT

Abstract

Traditional views on the effects of polyploidy in allohexaploid *Triticum aestivum* L (bread wheat) have primarily emphasized aspects of coding sequence variation and the enhanced potential to acquire new gene functions through mutation of redundant loci. At the same time, the extent and significance of regulatory variation has been relatively unexplored. Recent investigations have suggested that differential expression of homoeologous transcripts, or subfunctionalization, is common in natural bread wheat. However, it is unclear if this apparent subfunctionalization is the result of mutation and artificial selection in the brief history of bread wheat, or if a substantial fraction of regulatory changes are dynamically established during initial allopolyploidization events that may immediately contribute to phenotypic plasticity. In order to establish a timeline for such regulatory changes and estimate the frequency of non-additive expression of homoeologous transcripts in newly formed *T. aestivum*, gene expression was characterized in a synthetic *T. aestivum* line and its *T. turgidum* and *Aegilops tauschii* parents by cDNA-single stranded conformational polymorphism (SSCP) and microarray expression experiments. The cDNA-SSCP analysis of 30 arbitrarily selected homoeologous loci revealed that four (~13%) showed differential expression of homoeoalleles in seedling leaf tissue of synthetic *T. aestivum*. In microarray expression experiments, synthetic *T. aestivum* gene expression was compared to mid-parent expression level estimates calculated from parental expression values. Approximately 16% of genes were inferred to display non-additive expression in synthetic *T. aestivum*. The cDNA-SSCP expression profiles of six homoeoloci classified as non-additively expressed in microarray experiments suggest that *cis*-acting regulatory variation is often responsible for non-additive gene expression levels. These results demonstrate that allopolyploidization, *per se*, results in rapid initiation of differential expression of homoeologous loci and non-additive gene expression in synthetic *T. aestivum.*

Introduction

The prevalence of polyploidization events in angiosperm evolution (MASTERSON 1994) has inspired varied theories that polyploidy can instill lasting benefits, and has raised many questions about the ecological and genetic consequences of genome doubling. In many genera, the distribution of polyploid species exceeds that of their diploid counterparts, suggesting that polyploidy may confer enhanced fitness (STEBBINS 1950). *Triticum* species are an exemplary model of this polyploid adaptation concept. Broader global adaptability of *T. aestivum* (allohexaploid, 2n=6x=42, known as common or bread wheat) compared to *T. turgidum* (allotetraploid, 2n=4x=28, known as durum or macaroni wheat), including adaptability to a range of abiotic and biotic stresses and environmental conditions, is credited to a polyploid advantage (reviewed by DUBCOVSKY and DVORAK 2007). Speculations on genetic mechanisms that may contribute to a polyploid advantage have abounded for over one-half a century. Convincing mechanisms include: 1) greater gene and gene expression diversity (heterosis), 2) genome buffering (increased capacity to tolerate mutation events), and 3) increased potential for genes to evolve novel functions (sub/neo-functionalization) (reviewed by UDALL and WENDEL 2006). Of the three broad mechanisms, current evidence supports the contributions of both "buffering" and a more rapid evolution of novel gene function in enhancing the success of *T. aestivum*.

Bread wheat represents a unique allopolyploid system for studying the effects of polyploidization. *T. aestivum* arose by two instances of genome doubling via polyploidization. The progenitors of bread wheat belong to two genera, *Triticum* and *Aegilops*, in the Triticeae tribe (VAN SLAGEREN 1994), which diverged from a common ancestor approximately 2.5–4.5 million years ago (MYA) (HUANG *et al*. 2002). As a result of their relatively recent origin, the genomes of *Triticum* / *Aegilops* species are highly conserved. Less than one MYA (HUANG *et al*. 2002), diploid species *T. urartu* (DVORAK *et al*. 1988, 1993) and a species closely related to

Ae. speltoides (DVORAK and ZHANG 1990; TALBERT *et al*. 1991; DAUD and GUSTAFSON 1996; FRIEBE and GILL 1996) hybridized to form *T. turgidum*. One or more hybridization events occurred approximately 8000 years ago (NESBITT and SAMUEL 1996) between cultivated *T. turgidum* and diploid goatgrass *Ae. tauschii* (KIHARA 1944; MCFADDEN and SEARS 1944, 1946) to form the highly stable allohexaploid *T. aestivum*. Hybridization between *T. turgidum* and *Ae. tauschii* can be recreated (MCFADDEN and SEARS 1944) and is a routine practice to produce synthetic *T. aestivum* lines. Hybrid progeny are haploid and genome doubling via colchicine treatment (BLAKESLEE and AVERY 1937) or spontaneous doubling is necessary for production of viable seeds.

Genome buffering in allohexaploid wheat is evident by its ability to tolerate drastic changes in chromosome structure and composition. Comparison of X-ray induced mutation rates of *T. turgidum* and *T. aestivum* under identical treatments provides classical evidence of greater genome buffering capacity in allohexaploid wheat (STADLER 1929). In fact, the ability of hexaploid wheat to tolerate aneuploid conditions allowed assignment of each of the 21 chromosomes of wheat to seven homoeologous groups with three chromosomes (from A, B, and D genomes) in each group (SEARS 1966). An extensive collection of aneuploid stocks, including monosomic, nullisomic-tetrasomic, ditelosomic, chromosome deletion lines, and a multitude of alien chromosome substitution and translocation lines further demonstrates the genome buffering capacity afforded by polyploidy in *T. aestivum* (SEARS 1954; SEARS and SEARS 1978; ENDO and GILL 1996; GILL *et al*. 2006). In contrast, attempts to produce a similar array of genetic stocks in allotetraploid *T. turgidum* have failed due to diminished buffering capacity (FARIS *et al*. 2002).

Although difficult to directly assess, the role of polyploidy in accelerating evolution of novel gene functions in *T. aestivum* is becoming clearer. In a recent review, DUBCOVSKY and

DVORAK (2007) summarized extensive evidence that documents stunning rates of DNA sequence divergence in polyploid wheat, compared to its diploid relatives. Much of the rapid change observed in the hexaploid wheat genome is credited to the abundance of repetitive elements that appear to accelerate gene deletion/duplication events and may alter expression of neighboring genes, all of which is tolerated by enhanced buffering. The true magnitude and significance of subfunctionalization in hexaploid wheat is unknown at present, though attempts have been made to estimate the frequency of differential expression of homoeologous transcripts. In one study, quantification of homoeologous transcript abundance for 90 homoeoloci revealed that 12% showed silencing from one or more genomes in all tissues investigated, while ~28% showed preferential expression from a particular genome in one or more tissues (MOCHIDA *et al*. 2003). Differential expression of homoeologs varied considerably among tissue types, ranging from 57% of the genes studied in pistils to only 14% in spikes at bolting stage. With three component genomes to "choose" from, this potential level of subfunctionalization represents a massive pool of phenotypic and evolutionary flexibility.

A higher estimate of homoeologous gene silencing was found when expression profiles of 236 loci were assayed by cDNA–single-stranded confirmation polymorphism (cDNA-SSCP) (BOTTLEY *et al*. 2006). In this study ~27% of genes expressed in leaf tissue of cultivar 'Chinese Spring', and 26% of genes expressed in root tissue, showed silencing of one or more homoeologous transcripts. Characterization of homoeologous transcript abundance for a glutathione *S*-transferase, five benzoxazinone biosynthesis genes, *Mre11*, and MADS box gene *WLHS1* provide additional examples of differential expression of homoeologous loci, frequently with tissue or developmental specificity (XU *et al*. 2002; NOMURA *et al*. 2005; DE BUSTOS *et al*. 2007; SHITSUKAWA *et al*. 2007).

Increasing evidence of novel gene expression patterns in several genera of neo-polyploids suggests that differential regulation of genes is also a common feature following polyploidization (ADAMS *et al*. 2004; COMAI *et al*. 2000; HE *et al*. 2003; KASHKUSH *et al*. 2002; WANG *et al*. 2004, 2006). As such, differential gene regulation, or deviations from additivity or mid-parent expression levels, may form the molecular basis of an allopolyploid advantage and appearance of novel phenotypic traits (BIRCHLER *et al*. 2003; CHEN 2007). The expression of homoeologous genes in a new allopolyploid nucleus may be altered from that of the parents by epigenetic mechanisms, interactions between diverged regulatory networks present in each progenitor species, deletion events, chromosomal rearrangements, or novel epistatic interactions (CHEN and NI 2006). In short, novel gene expression levels and patterns may be both qualitative and quantitative. The magnitude of higher-order regulatory interactions that may modulate gene expression in a new allohexaploid nucleus are difficult to grasp at present.

Differential gene expression in newly formed allohexaploid wheat has been the subject of only one investigation, despite the global importance of this species. HE *et al*. (2003) applied a cDNA-AFLP technique (BACHEM *et al*. 1996) to analyze transcriptional changes in a synthetic neo-allohexaploid wheat line compared to its *T. turgidum* and *Ae. tauschii* parents. Although no evidence of gene deletion or rearrangement events was observed, non-additive expression changes were observed for 7.7% of measured transcripts. Interestingly, the vast majority of differentially expressed transcripts (~95%) was reduced or absent in the allohexaploid, and only a few transcripts were induced.

The following research was conducted to gain better insight into the spectrum of gene expression changes that occur during initial stages of polyploidization in allohexaploid *T. aestivum*. The remarkable frequency of differential expression of homoeologous loci measured

in natural allohexaploid wheat (MOCHIDA *et al*. 2003; BOTTLEY *et al*. 2006), which parallels observations in natural allotetraploid cotton (ADAMS *et al*. 2003), prompted a similar investigation on recently synthesized allohexaploid wheat to establish a timeline for such changes. In order to test the hypothesis that differential expression of homoeologous transcripts is rapidly established following polyploidization in *T. aestivum*, expression of 30 arbitrarily selected homoeologous transcripts was monitored in a synthetic wheat and parental *Ae. tauschii* and *T. turgidum* lines by cDNA-SSCP. Furthermore, expression of 825 transcripts was quantified in the same lines by microarray expression experiments. Synthetic allohexaploid expression levels were compared to mid-parent expression values inferred from the parental species to determine the potential role of non-additive gene expression in contributing to a polyploid advantage in allohexaploid wheat.

Materials and Methods

Plant Materials

The newly formed stable synthetic hexaploid wheat line TA4152L3 (AABBDD) and parental genotypes *T. turgidum* (cultivar 'Altar 84', accession TA2970, AABB) and *Ae. tauschii* (accession TA1651 , DD) were used for cDNA-SSCP and microarray expression experiments. The synthetic hexaploid was self-pollinated to S_{5-6} generations after synthesis at the International Maize and Wheat Improvement Center (CIMMYT) and demonstrates disomic meiotic chromosome pairing behavior (HE *et al*. 2003). Both parental species and the derived synthetic hexaploid are almost exclusively self-pollinating, and no evidence of out-crossing/heterozygosity in these materials was discovered when ~2800 fragments were visualized by cDNA-AFLP (HE *et al*. 2003). One plant per pot was grown in approximately 600 g of Metro-Mix 200 potting media (Sun Gro Horticulture, Bellevue WA) in 3.5 L square plastic pots. Six g Osmocote 14-14-14

(Scotts Company, Marysville, OH) slow-release fertilizer were incorporated with potting media prior to planting. Plants were grown in a growth chamber with 16-hour day lengths at 20°C daytime and 18°C nighttime temperatures.

Nucleic Acid Extraction

Leaves of seedling plants grown for 30 days were harvested into liquid nitrogen prior to extraction of total RNA. Total RNA was isolated from each of three plants per genotype using the TRIzol reagent (Invitrogen, Carlsbad CA). Three hundred μg of total RNA was used for mRNA isolation with the polyATract mRNA kit (Promega, Madison WI). First and second strand cDNA was synthesized from mRNA using a SMART cDNA synthesis kit (Clontech, Palo Alto CA). DNase treatment was not performed, however, subsequent amplification with over ten intron-spanning primers revealed that genomic DNA (gDNA) contamination was undetectable in all samples. Second strand cDNA concentration was adjusted to 10 ng μL^{-1} for all samples. For microarray experiments, total RNA was purified from each of three replicates of TA4152L3, Altar 84, and TA1651 by using an RNeasy Plant Mini Kit (Qiagen, Valencia CA). RNA concentration was quantified with an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington DE) and all samples were adjusted to 300 ng μL^{-1}. RNA integrity was confirmed prior to microarray probe synthesis and labeling on an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto CA).

Genomic DNA extraction was performed as described by LIU *et al*. (2006) with modifications. Five hundred μL of extraction buffer (RIEDE and ANDERSON 1996) was added to ground tissue and samples were placed into a 65ºC water bath for 20 min. Then, 500 μL chloroform/isoamyl (24:1, v/v) solution was added and tubes were mixed vigorously before centrifugation (10,000 × g) for 10 min. The resulting aqueous phase (500 μL) was transferred to

a new 1.5 mL tube and precipitated with 1 mL 95% ethanol. Tubes were centrifuged (10,000 × g) for 10 min to pellet the DNA and then rinsed with 1 mL 70% ethanol and dried before adding 150 µL TE buffer. All gDNA samples were diluted to 20 ng μL^{-1} in sterile deionized water for PCR.

Homoeologous Transcript Selection for cDNA-SSCP

Low copy expressed sequence tags (ESTs) were arbitrarily selected by mining results of the National Science Foundation-supported wheat deletion-mapping project (QI *et al*. 2004). Low-copy loci were parsed from the complete wEST-SQL database of deletion-bin mapped ESTs (http://wheat.pw.usda.gov/westsql/index.html) by a custom query requiring the number of bands detected by Southern blot hybridization to equal the same number of deletion bins (e.g. three bands, with one band from each homoeologous locus in a group of chromosomes). The resulting subset of low-copy ESTs was then manually verified by viewing Southern blot images for each EST, and 30 high-confidence homoeoloci were chosen. Conserved primers for the 30 homoeologous loci were designed using the Primer 3 program (ROZEN and SKALETSKY 2000) based on sequence alignments of contigs assembled by The Institute for Genomics Research (Table 3.1).

PCR Amplification

PCR reactions were performed in a total volume of 25 µL containing 100 nM of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM $MgCl_2$, and 1.0 unit AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City CA). Template gDNA (60 ng) and cDNA (20 ng) was PCR-amplified with 32 cycles of 94ºC for 60 s, 55ºC-60ºC for 60 s, and 72ºC for 90 s. Primer efficacy was first tested on gDNA of the synthetic wheat and parental genotypes. cDNA-SSCP analyses were only performed with thirty genes (primers) that amplified from both *T.*

turgidum and *Ae. tauschii*. cDNA '*in vitro* synthetic' controls were included by equally mixing cDNA from the parental species to account for amplification bias and mobility shifts in electrophoresis. Initially, cDNA-SSCP analyses were done on each of the three replicates of cDNA synthesized from separate RNA isolations. However, lack of detectable differences between biological replicates supported mixing equal amounts of cDNA (1:1:1) from each replicate to conservatively streamline the experimental procedure.

Electrophoresis

Following PCR amplification, products were diluted 1:5 (v/v) in formamide loading dye (95% formamide, 10 mM EDTA, bromophenol blue, xylene cyanol), denatured for 5 min at 94°C, and chilled on ice. Denatured products were run in MDE polyacrylamide gels (Cambrex, Rockland ME) according to the manufacturer's instructions at room temperature using BioRad Sequi-Gen GT sequencing systems (Bio-Rad Laboratories, Hercules CA). Gels were run at 4-5 W constant power for 15 h and visualized by silver staining using the protocol of BASSAM *et al*. (1991). To validate this modified cDNA-SSCP technique, analysis was first performed on differentially expressed cDNA-AFLP fragment 'AFLP-23' (identified by HE *et al*. 2003) for comparison.

Microarray Procedures

Microarray hybridizations were conducted using RNA from three biological replicates of the *Ae. tauschii*, *T. turgidum*, and synthetic *T. aestivum* lines in each pair-wise combination (Treatment 1: *Ae. tauschii* vs. *T. turgidum*; Treatment 2: *Ae. tauschii* vs. *T. aestivum*; Treatment 3: *T. turgidum* vs. *T. aestivum*; three biological replicates per treatment for a total of nine slides; Figure 3-1). Rather than mixing RNAs from *Ae. tauschii* and *T. turgidum* to create an *in vitro* synthetic for competitive hybridizations against synthetic *T. aestivum*, each parental line was

independently co-hybridized and later averaged to estimate mid-parent expression levels. This was done to reduce potential homoeolog labeling/hybridization bias and because there is no empirical data on suitable ratios of parental RNAs to imitate allohexaploid gene expression (i.e. in an allohexaploid nucleus, two of the three component genomes are from one parent while one genome is from another parent, suggesting a 2:1 ratio of tetraploid to diploid RNAs; however, from a parental/species perspective, a 1:1 ratio may be appropriate). The spotted-oligonucleotide microarray slides used in this work were generously provided by Drs. Olin Anderson and Debbie Laudencia-Chingcuanco (USDA-ARS Genomics and Gene Discovery Research Unit, Albany CA). Oligonucleotide selection and slide design will be described elsewhere by the developers. Oligonucleotides were spotted in duplicate on each slide, meaning two data points constitute the expression value of each transcript per slide.

RNAs were reverse-transcribed (RT) and indirectly labeled with Cy3 or Cy5 fluorophores using the Array 900 system (Genisphere Inc., Hatfield PA). Briefly, 2 µg total RNA was annealed to 2 pico moles of either Cy3 or Cy5 RT primer and cDNA synthesis was carried out in a 20 µL reaction containing 1X Superscript III reaction buffer, 200 units Superscript III (Invitrogen, Carlsbad CA) reverse transcriptase, 1µL dNTP mix, 10 mM dithiothreitol, and 1µL Superase-In Rnase inhibitor. The RT reactions were incubated for 10 min at 22ºC, 2 h at 50ºC, then stopped and denatured to degrade RNA according to manufacturer's instructions. RT-reactions were purified using a CyScribe GFX Purification kit (GE Healthcare, Piscataway NJ) to remove unincorporated primer and then combined equally in hybridization reaction mixtures per manufacturer's instructions. Hybridization mixtures were incubated on microarray slides for 16 h at 50ºC. Slides were washed in a 2X saline-sodium citrate (SSC), 0.1% sodium dodecyl sulfate (w/v) solution for 10 min at 50ºC with gentle

agitation, followed by 0.2X SSC and finally 0.1X SSC washing steps for 10 min each at room temperature. Cy3/Cy5 fluorophores were then hybridized for 4 h at 50ºC, washed as above, and slides scanned using an Axon GenePix 4000B (Molecular Devices Corporation, Union City CA). Channel intensity was balanced with 100% laser power for each wavelength with photomultiplier tube (PMT) gain set at 760-800 for the 635-nm (red) laser, and 680-720 for the 532-nm (green) laser. Each slide was scanned twice at different PMT gain settings so that scans with similar intensity values were obtained for across-treatment comparisons.

Fluorescence intensity data were recorded using GenePix Pro v6.0 (Molecular Devices Corporation, Union City CA). Lower-quality and weakly hybridizing features were flagged for exclusion (by requiring a circularity ratio ≥ 0.8; signal intensity > 75% of background plus one standard deviation; and the sum of median intensity from each channel > 900) to initially reduce local printing, washing and hybridization errors and exclude oligonucleotide probes with poor hybridization characteristics (including blanks). Data normalization was performed to reduce non-biologically significant variation and further equilibrate channel intensities within and between microarray hybridizations. Slides were normalized individually to reduce potential interdependency among replicates. GeneSpring GX v7.3 (Agilent Technologies, Santa Clara CA) was used for analysis of variance (ANOVA), applying the Benjamini and Hochberg multiple testing correction procedure to control the false discovery rate ($\alpha = 0.05$). Mid-parent expression values (MPV) were calculated by averaging across treatments, assuming either 1:1 (*T. turgidum* : *Ae. tauschii*, $MPV_{1:1}$) or 2:1 (*T. turgidum* : *Ae. tauschii*, $MPV_{2:1}$) ratios of RNA abundance in the allohexaploid nucleus. Pearson's correlation coefficients among gene expression levels were calculated between and across genotypes/treatments in Microsoft Excel.

Results

Expression of Homoeologous Transcripts in Neo-Allohexaploid Wheat

Expression levels of individual homoeoloci were assayed by cDNA-SSCP from seedling leaf tissue of *Ae. tauschii* (DD), *T. turgidum* (AABB) and synthetic *T. aestivum* (AABBDD). Because the cDNA-SSCP technique reported by CRONN and ADAMS (2003) was modified in this study, a well-characterized transcript (AFLP-23) that was differentially expressed in the same plant materials (HE *et al*. 2003) was first used for comparison and validation. AFLP-23 provided a particularly useful comparison; HE *et al*. (2003) sequenced each AFLP-23 homoeoallele and conducted competitive RT-PCR experiments to quantify differential homoeologous expression of this transcript. The modified cDNA-SSCP technique used in this study was suitable to confirm differential expression of AFLP-23 homoeologous transcripts (Figure 3-2).

Expression profiles of 30 arbitrarily selected homoeologous transcripts (Table 3-1) were analyzed by cDNA-SSCP. Four of the 30 homoeoloci (~13%) showed altered expression patterns in the synthetic wheat (Figure 3-3). Silencing of *Ae. tauschii* D-genome homoeologs was observed for two differentially expressed homoeologous transcripts, TC270558 and TC273936. TC253445 displayed up-regulation of the *Ae. tauschii* homoeolog and possible concurrent down-regulation of a *T. turgidum* homoeolog based on comparison to the *in vitro* synthetic control. The same general pattern was found for TC264908, where the *Ae. tauschii* homoeolog was clearly more abundant, while a *T. turgidum* transcript appeared to be silenced. Amplification and electrophoresis were repeated for genes classified as differentially expressed and all results were reproduced.

Microarray

Data Analysis

Stringent filtering of low-quality features and normalization were employed to reduce non-biological variation. Due to the balanced channel median intensity maintained through slide scanning and data normalization, channel median intensities were very similar (±10% of experiment-wide mean) between and within slides. Therefore, no median centering calculations were applied. Expression of 825 high-quality features (Appendix A) was analyzed using the GeneSpring ANOVA tool. Putative annotations of the list of 825 transcripts revealed a diverse representation of genes involved in all aspects of plant growth and development, including: biosynthesis, carbohydrate metabolism, cell division and structure, nucleic acid metabolism, photosynthesis, protein synthesis and metabolism, signal transduction, transport, and ~17% unknown or hypothetical. The correlation among expression levels across treatments was sufficiently high for each genotype to indicate that both dye and hybridization bias were minimal in the normalized data set and data may be analyzed across treatments [$r = 0.94$ for *Ae. tauschii* expression levels when competitively hybridized against either *T. turgidum* or *T. aestivum*; $r = 0.97$ for *T. turgidum* when hybridized against either *Ae. tauschii* or *T. aestivum* (*T. turgidum* was labeled alternatively with either Cy3 or Cy5); $r = 0.97$ for *T. aestivum* when hybridized against either *Ae. tauschii* or *T. turgidum* (Table 3-2)].

Expression Divergence and Non-Additive Gene Expression Estimates

Expression divergence between *Ae. tauschii* and *T. turgidum* was substantial, with expression level correlation (r) values ranging from only $r = 0.26$ to 0.37 ($P < .0001$) when compared between individual treatments (Table 3-2). Using experiment-wide mean values, *Ae. tauschii* expression levels accounted for only 11% of the variation in *T. turgidum* expression levels, or vice versa (Table 3-3). Variance analyses indicated that ~78% (641/825) of genes were differentially expressed (threshold of > two-fold higher/lower) between these parental species.

Expression level divergence was significantly lower between either *Ae. tauschii* or *T. turgidum* and the derived synthetic *T. aestivum*. *Ae. tauschii* and *T. turgidum* expression levels explained 49% and 54%, respectively, of the variation in synthetic *T. aestivum* gene expression [individual treatment correlations ranging from $r = 0.65$ to 0.70 for *Ae. tauschii* vs. *T. aestivum* ($P < .0001$); from $r = 0.69$ to 0.75 for *T. turgidum* vs. *T. aestivum* ($P < .0001$)].

Two mid-parent expression models were tested for their ability to predict synthetic allohexaploid gene expression levels: $MPV_{1:1}$, the average of *T. turgidum* and *Ae. tauschii* expression levels (assuming a 1:1 ratio of transcript abundance from each parental genome), and $MPV_{2:1}$, a weighted 2:1 average of *T. turgidum* and *Ae. tauschii* expression levels (assuming 2/3 of total transcription is from A and B genome homoeoalleles of *T. turgidum* and 1/3 is from the D genome homoeoallele of *Ae. tauschii* in a synthetic hexaploid). Linear regression on $MPV_{1:1}$ and $MPV_{2:1}$ expression values accounted for 77% and 74% of the variation in synthetic *T. aestivum* expression levels, respectively, and both models are better predictors of hexaploid gene expression than expression levels observed for either parental species (Table 3-3). Despite the slightly better agreement between observed *T. aestivum* expression levels and $MPV_{1:1}$ model estimates, identification of transcripts with non-additive expression levels was conducted separately using each model for comparison. Non-additive gene expression (threshold of > two-fold higher/lower) was estimated for 16.2% of transcripts (134/825) when compared to $MPV_{1:1}$ additive model values (Table 3-4). A slight majority of genes classified as differentially expressed, 8.5% (70/825), had higher expression levels in synthetic *T. aestivum* than expected, while 7.8% (64/825) were lower and potentially down-regulated. $MPV_{2:1}$ model estimates yielded very similar results, with 15.5% of genes (128/825) estimated to have non-additive expression levels. The estimate of up-regulated genes was similar with the $MPV_{2:1}$ model at

9.2% (76/825) and down-regulation estimates were slightly lower at 6.3% (52/825). Approximately two-thirds of genes classified as down-regulated and three-fourths of genes classified as up-regulated were the same between the two models. Surprised by the consistent fraction of genes classified as up-regulated in synthetic *T. aestivum*, a third "high-parent" gene expression value was used for comparison. Instead of averaging parental expression levels, the higher of the two was compared to observed *T. aestivum* levels, reflective of overdominant expression in heterosis literature. The fraction of genes categorized as having greater than two-fold higher expression over high-parent estimates (overdominant) was still surprising at 1.7% [14/825 (Table 3-5)]. Similarly, 1.2% (10/825) of transcripts had expression levels lower than the "low-parent" (underdominant).

Characterization of Genes classified as Non-Additively Expressed

Expression of six genes with non-additive expression levels based on microarray experiments was characterized by cDNA-SSCP. cDNA-SSCP was chosen because non-additive expression was expected in some cases to be due to differential expression of homoeologous transcripts. cDNA-SSCP assays were performed on three transcripts that were classified as up-regulated (TC252860, TC267682, TC262784) and three transcripts that were classified as down-regulated (TC267455, TC267082, TC23840) in synthetic *T. aestivum* compared to $MPV_{1:1}$ values (Figure 3-4). TC252860 (oligonucleotide feature FGAS.02071; 2.3 fold higher; annotated as a putative respiratory burst oxidase homolog) was specifically selected due to the low, but significant, level(s) of expression observed for this gene based on microarray fluorescence intensity, as an indicator of lower detection limits in hybridization experiments. The low expression levels of TC252860 were confirmed by cDNA-SSCP analysis; despite strong amplification products using gDNA, cDNA amplification products were relatively faint.

Elevated expression of a *T. turgidum* homoeolog(s) and possible suppression of the *Ae. tauschii*-derived transcript accounts for non-additive expression of TC252860 in synthetic hexaploid wheat. The cDNA-SSCP analysis of TC267682 (oligonucleotide feature FGAS.03312; 3.7 fold higher; overdominant; putative sucrose synthase) supported higher expression levels in synthetic *T. aestivum*, which is most likely due to up-regulation of a *T. turgidum* homoeoallele. TC262784 (oligonucleotide feature FGAS.00736; 2.6 fold higher; overdominant; putative transketolase) also displayed an interesting electrophoresis profile. Comparison of cDNA and gDNA profiles revealed that in synthetic *T. aestivum* the *Ae. tauschii*-derived transcript is suppressed, while a *T. turgidum* transcript is more abundant.

Amplification of TC267455, TC267082, TC23840 on gDNA of each genotype suggested that gene deletion events had not occurred in the synthetic hexaploid for any of these genes (Figure 3-4), and gene loss was most likely not responsible for the reduced expression levels measured by microarrays. The *Ae. tauschii*-derived homoeoallele of TC267455 (oligonucleotide feature USDAWHE.02876; 3.4 fold lower; putative Hsp70 binding protein) was not present in the cDNA-SSCP profile, indicating silencing of this homoeoallele in synthetic *T. aestivum*. Although subtle, repression of a *T. turgidum* homoeoallele and/or up-regulation of the *Ae. tauschii* homoeoallele was observed for TC267082 (oligonucleotide feature FGAS.02280; 3.0 fold lower; underdominant; putative serine carboxypeptidase). Results for TC238480 (oligonucleotide feature USDAWHE.05946; 2.7 fold lower; underdominant; putative histidyl-tRNA synthetase) were inconclusive; the cDNA profile had similar band intensities to the gDNA profile when compared across genotypes. In summary, cDNA-SSCP analysis revealed that four out of the six genes characterized show differential expression of homoeologous loci in the

synthetic *T. aestivum*. Although not a purely quantitative assay, cDNA-SSCP also supported non-additive overall transcript levels.

Discussion

Gene expression changes that occur as a result of recent allopolyploidization events are varied in the genera investigated to date. The initial polyploidization process may induce a genomic shock (McCLINTOCK 1984) where rapid changes in genome structure, composition and gene expression are essential for a new species to persevere (WENDEL 2000; CHEN and NI 2006). Some changes are stochastic and may be different in subsequent generations of the same neo-polyploids as reported in allotetraploids of Arabidopsis and wild wheat amphiploids, while other changes seem to be conserved in both natural and synthetic allopolyploids (KASHKUSH *et al*. 2002; HE *et al*. 2003; ADAMS *et al*. 2004; WANG *et al*. 2004, 2006). In allotetraploid cotton, unequal expression of homoeologs is frequent, and often organ-specific (ADAMS *et al*. 2003, 2004), though no substantial genomic changes are observed (LIU *et al*. 2001). Approximately 5% of genes are estimated to be silenced or suppressed in neo-allotetraploid cotton (ADAMS *et al*. 2004), while ~ 25% of genes may display biased homoeolog expression in natural cotton (ADAMS *et al*. 2003). In neo-allotetraploid Arabidopsis, expression changes are estimated to affect from 0.4% up to 11% of genes based on cDNA-AFLP (COMAI *et al*. 2000; WANG *et al*. 2004). Analysis of global expression levels in Arabidopsis synthetic allotetraploids by microarray experiments revealed that ~6% of transcripts (and potentially as high as ~40% depending on statistical methods employed) are expressed in a non-additive manner, relative to mid-parent expression levels (WANG *et al*. 2006). In this study, mid-parent expression levels were quantified by fluorescent labeling of an equal mixture of diploid RNAs in competitive hybridizations against the derived synthetic allotetraploids. Taking a noteworthy step forward on

the central dogma path, ALBERTIN *et al*. (2006) used a proteomics approach to show non-additive accumulation of over 25% of 1600 polypeptides measured in newly-synthesized *Brassica napus* allotetraploids.

In the only investigation documenting wide-spread gene expression changes in neo-allohexaploid wheat to date, HE *et al*. (2003) estimated that ~7% of transcripts are down-regulated and only 0.4% of transcripts are more abundant in synthetic *T. aestivum* using the cDNA-AFLP technique. A limitation to this technique is the primarily qualitative nature of the results, which undoubtedly underestimates the magnitude of quantitative changes that occur upon polyploidization. It is somewhat counterintuitive to conceive of a polyploid advantage in evolutionarily young hexaploid wheat arising predominantly from qualitative gene suppression events. Even so, repression or silencing appears by far to be the most frequent method of non-additive gene regulation in new allopolyploids (reviewed by UDALL and WENDEL 2006). On the other hand, elevated gene expression levels need not be major, as the cumulative effect of subtle expression differences could have a drastic effect on phenotype.

Quantitative determination and comparison of gene expression among ploidy levels is a challenge in the synthetic allohexaploid *T. aestivum* system, due to the unbalanced composition of the progenitor diploid and tetraploid genomes. Identification of an infallible approach to estimate and classify quantitative gene expression changes in a new allohexaploid is daunting, if not impossible, especially given the magnitude of gene expression differences observed between maize inbreds and their F1 hybrid progeny [where underdominant, low-parent dominant, high-parent dominant, and overdominant expression patterns are relatively frequent (SWANSON-WAGNER *et al*. 2006)]. Restating a view presented by BIRCHLER *et al*. (2003) in reference to current efforts to define a molecular basis for heterosis: such difficulties in deciphering how

genes behave and interact in an allohexaploid nucleus may lead some to conclude that such an investigation should not be attempted at present; however, the only way to advance is to "chip away at alternatives".

In the present investigation, synthetic *T. aestivum* gene expression was characterized by hybridization to spotted 70mer oligonucleotide microarrays and cDNA-SSCP. Additive mid-parent expression values were estimated, based on parental *Ae. tauschii* and *T. turgidum* levels, and compared to those observed for the synthetic *T. aestivum*. Similar expression level divergence between synthetic *T. aestivum* and either of its progenitors indicates that neither parental species overwhelmingly biases hexaploid gene expression. This is supported by the better agreement between synthetic *T. aestivum* expression levels and hypothetical $MPV_{1:1}$ expression values, compared to the agreement in expression levels between synthetic *T. aestivum* and either parent. In other words, and not surprisingly, the substantial majority of genes show additive expression in the synthetic allohexaploid nucleus.

Approximately 78% of transcripts were classified as differentially expressed between the *Ae. tauschii* and *T. turgidum* genotypes using per-gene variance analysis. In comparison, ~43% of transcripts were classified as differentially expressed between diploid relatives *Arabidopsis thaliana* and *A. arenosa* using a per-gene variance model (WANG *et al*. 2006). Similarly, ~48% of genes were differentially expressed between diploid relatives *Drosophila melanogaster* and *D. simulans* (RANZ *et al.* 2003). The relatively high estimate of genes differentially expressed between *Ae. tauschii* and *T. turgidum* is not necessarily surprising. After *Triticum*/*Aegilops* divergence ~3 MYA, *T. turgidum* was formed ~0.5-1 MYA as the result of a polyploidization event between *T. urartu* and yet another *Aegilops* species. The genomic shock caused by this polyploidization event, mutation and selection during tetraploid evolution, and subsequent

domestication and plant breeding efforts on *T. turgidum* have undoubtedly accelerated gene expression divergence.

Comparisons to mid-parent expression level estimates suggested that ~16% of genes had non-additive expression in the synthetic *T. aestivum*. Approximately 7% of genes showed reduced expression levels. This estimate is in remarkable agreement with the cDNA-AFLP estimate of ~7.3% in the same synthetic wheat line (HE *et al*. 2003) and with other neo-allopolyploid gene expression investigations (ADAMS *et al*. 2004; WANG *et al*. 2006). More surprisingly, ~9% of genes showed higher expression levels than expected based on additive mid-parent values. Even when compared to high-parent expression levels, a considerable fraction of genes (~1.7%) was expressed greater than two-fold higher (overdominant expression). Considering many significant expression changes likely fall below a two-fold threshold (BIRCHLER *et al*. 2003), and would be ignored in the present study, we speculate that this is still an underestimate of the extent to which relevant changes in gene expression occur.

The contribution of individual homoeoalleles to total homoeologous transcript levels in a synthetic allohexaploid *T. aestivum* was analyzed in order to determine whether differential expression of homoeologous loci is established in early stages of polyploidization. The frequency of differential homoeologous expression we observed (~13%) in seedling leaf tissue by analyzing transcription of 30 homoeoloci by cDNA-SSCP supports the hypothesis that allopolyploidization results in rapid initiation of subfunctionalization (UDALL and WENDEL 2006) and non-additive gene expression in synthetic *T. aestivum*. While it is possible that the frequent preferential expression of homoeoalleles observed to date in natural hexaploid wheat (MOCHIDA *et al*. 2003; BOTTLEY *et al*. 2006) have continued to accumulate due to genetic [e.g. mutation in promoter or enhancer regions after a homoeolog is freed from selective constraints

by genome duplication (KELLOGG 2003; VIETIA 2005)] and epigenetic changes in the brief evolution of hexaploid wheat, our data indicate that allopolyploid formation, *per se*, establishes much of these changes.

By comparing homoeologous transcript abundance in a common nucleus, where *trans*-regulatory variation is controlled, differential expression of homoeoalleles may be attributed to *cis*-acting regulatory variation (COWLES *et al*. 2002). Somewhat surprisingly, cDNA-SSCP analysis of an additional six transcripts (classified as having non-additive expression levels when compared to $MPV_{1:1}$ estimates in microarray experiments) revealed that at least three of the six homoeoloci (50%) display unequal expression of homoeologous transcripts. Although a limited sample, this representation suggests that *cis*-regulatory variation plays an important role in non-additive expression of genes in synthetic *T. aestivum*. *Cis*-regulatory variation may arise from divergent promoter or enhancer regions in the progenitor species, though rapid and widespread epigenetic restructuring in new allopolyploids (LIU and WENDEL 2003; LEVY and FELDMAN 2004; WANG *et al*. 2004) is expected to play a considerable role (see WITTKOPP 2005 for review on sources of cis-/trans-regulatory variation). A majority of differential allelic expression patterns in *Drosophila* interspecific hybrids (WITTKOPP *et al*. 2004) and maize intraspecific hybrids (STUPAR and SPRINGER 2006) is also attributed to *Cis*-acting regulatory variation.

Although this investigation was not specifically conducted to identify particular genes or biological processes affected by polyploidization, the putative annotations of non-additively expressed genes and their similarity to genes differentially expressed in other neo-allopolyploid systems warrants discussion. In their genomwide survey of non-additive gene expression in Arabidopsis allotetraploids, WANG *et al*. (2006) showed that 33 out of 97 heat shock proteins (*HSPs*) in the Arabidopsis genome were differentially expressed relative to midparent values.

More interestingly, 31 out of the 33 were repressed. Nine putative *HSPs* were indentified from the 825 genes in the present study, six of which were repressed greater than two-fold relative to $MPV_{1:1}$ and/or $MPV_{2:1}$ values (Table 3-4). The agreement between the present investigation and the results of WANG *et al*. (2006) suggest that *HSPs* are targeted in early stages of allopolyploidization across diverse species. The role of *HSP90* in modulating expression of genetic variation in both plant and animal species is well established (RUTHERFORD and LINDQUIST 1998; QUEITSCH *et al*. 2002) and may be partially attributed to an epigenetic mechanism (SOLLARS *et al*. 2002). Non-additive expression of glutathione *S*-transferases (GSTs) was documented in allotetraploids of both Arabidopsis and Brassica (WANG *et al*. 2004; ALBERTIN *et al*. 2006), as well as, in natural hexaploid wheat (XU *et al*. 2002). Two of the four putative GSTs included in the 825 genes presently studied were classified as non-additively expressed. Additional examples of non-additively expressed gene families also identified in other allopolyploid systems include putative peptide/protein transporters, cellulose synthase catalytic subunits, kinesin-related proteins, ribosomal proteins, and chlorophyll a/b binding proteins (WANG *et al*. 2004; HEGARTY *et al*. 2005; ALBERTIN *et al*. 2006).

The results presented have several implications, particularly for applied plant scientists. Although many polyploidy-induced regulatory changes may be conserved across several independent polyploidization events and maintained on an evolutionary timeframe (HE *et al*. 2003; ADAMS *et al*. 2004; WANG *et al*. 2004, 2006), a significant fraction of changes are expected to be random or genotype-specific and may provide additional layers of regulatory polymorphism. Because natural hexaploid wheat likely arose from only a few chance hybridization events between *T. turgidum* and *Ae. tauschii* (TALBERT *et al*. 1998), the amount of regulatory variation captured is expected to be significantly less than what exists in the

progenitor species. Systematic efforts to capture additional genetic diversity in wheat breeding programs by utilizing synthetic wheats (WARBURTON *et al*. 2006) have undoubtedly provided a wealth of new regulatory variation. A rapid onset of differential expression of homoeologous loci may also have specific relevance to trait improvement efforts. Several studies utilizing synthetic *T. aestivum* or other amphiploid materials have documented the suppression of disease resistance genes that are highly effective in parental lines (KERBER and GREEN 1980; KERBER 1983; INNES and KERBER 1994; KEMA *et al*. 1995; MA *et al*. 1995; REN *et al*. 1996). We speculate that in some cases this is due to differential regulation of homoeologous loci.

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Figure 3-1. Microarray experimental design.

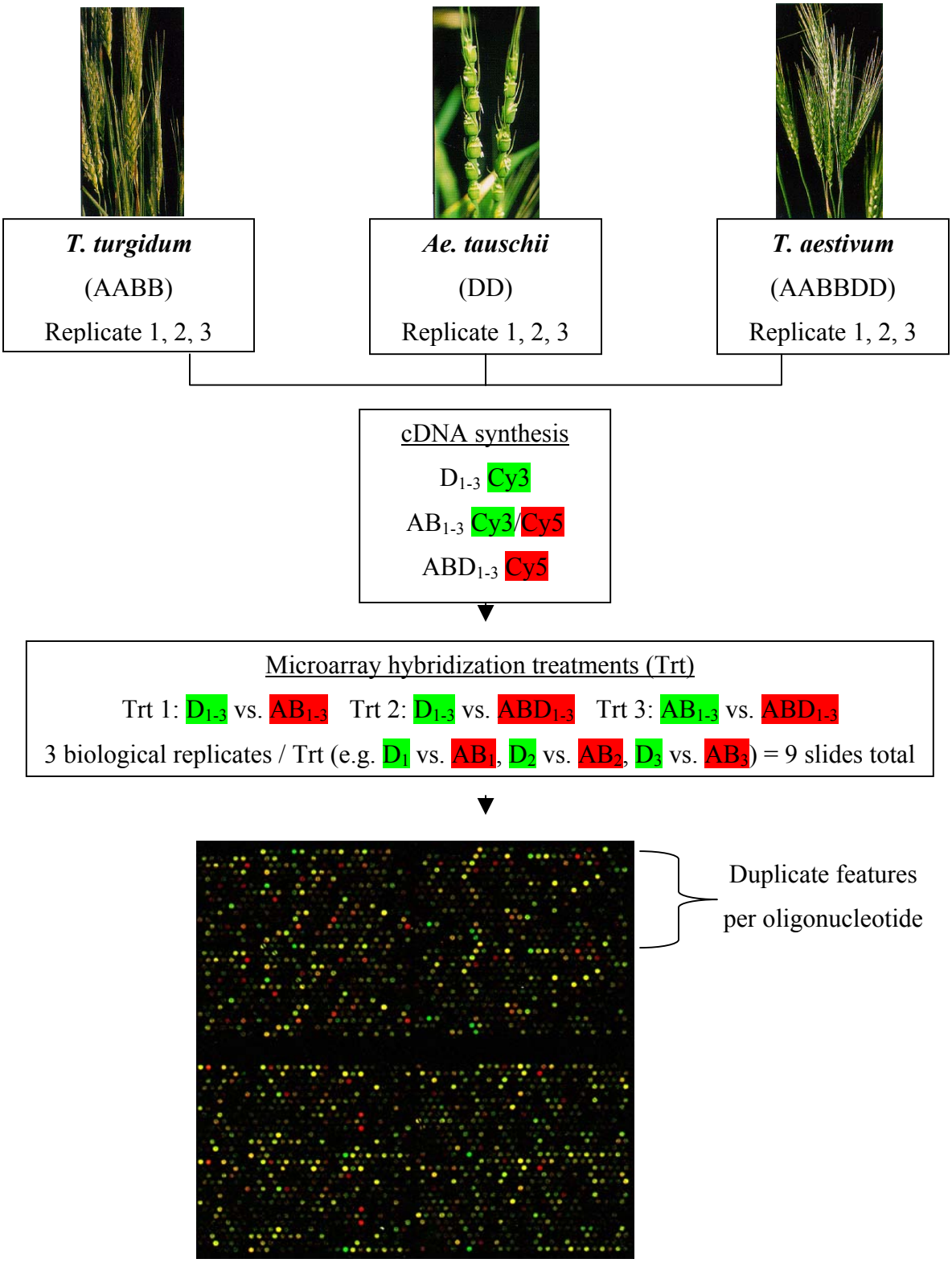

Figure 3-2. cDNA-SSCP analysis of cDNA-AFLP differentially expressed transcript AFLP-23. Although total AFLP-23 transcript levels are similar between diploid *Ae. tauschii*, tetraploid *T. turgidum* and the derived synthetic *T. aestivum*, homoeologous transcripts are differentially expressed in seedling leaves following polyploidization (HE *et al*. 2003). Equal amounts of second-strand cDNAs from *Ae. tauschii* (D), *T. turgidum* (AB) and synthetic *T. aestivum* (ABD) were PCR-amplified with AFLP-23 primers and run on MDE polyacrylamide gels. The cDNA-SSCP technique was suitable to identify suppression of a *T. turgidum* homoeoallele (arrow).

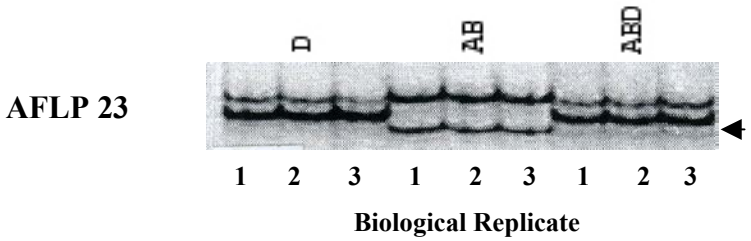

Figure 3-3. Differentially expressed homoeologous transcripts identified by cDNA-SSCP analysis of 30 arbitrary homoeologs. Equal amounts of second-strand cDNAs from *Ae. tauschii* (D), *T. turgidum* (AB) and synthetic *T. aestivum* (ABD), as well as an *in vitro* synthetic control (AB+D cDNAs equally mixed; not shown for TC270558) were PCR-amplified with conserved primers for each locus and run on MDE polyacrylamide gels. Arrows indicate homoeoalleles with differential expression.

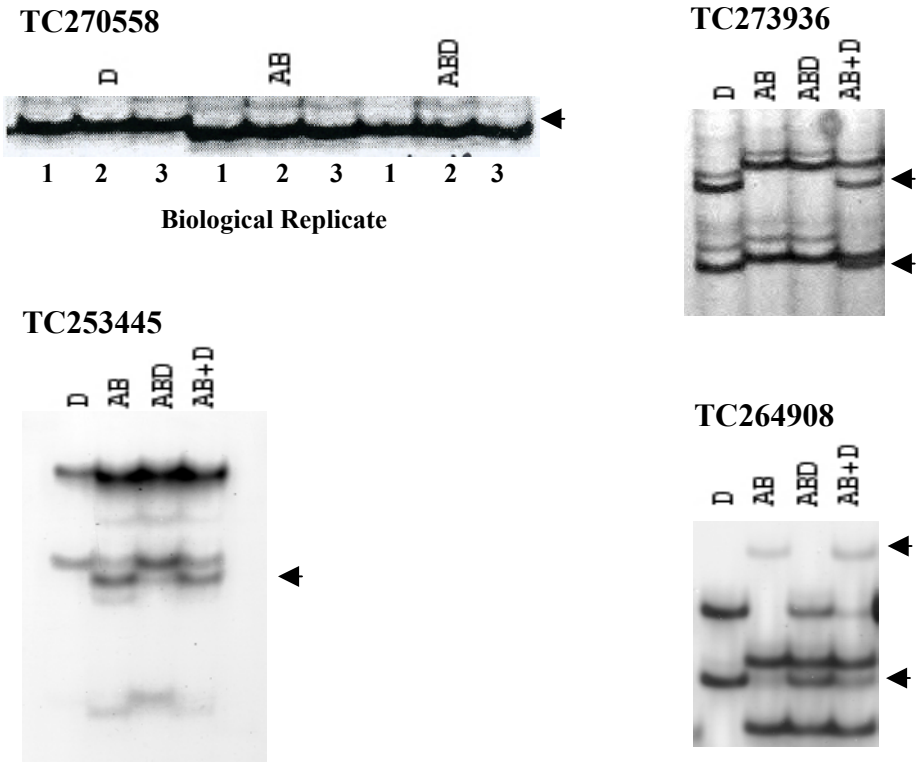

Figure 3-4. cDNA-SSCP analysis of six genes with non-additive expression levels based on microarray experiments. Assays were performed on three transcripts that were classified as up-regulated (panel A) and three transcripts that were classified as down-regulated (panel B) in synthetic *T. aestivum* compared to $MPV_{1:1}$ values. A) TC252860 corresponds to oligonucleotide feature FGAS.02071 that was expressed 2.3 fold higher in synthetic *T. aestivum*. TC267682 corresponds to oligonucleotide feature FGAS.03312 that was expressed 3.7 fold higher. TC262784 corresponds to oligonucleotide feature FGAS.00736 that was expressed 2.6 fold higher. B) TC267455 corresponds to oligonucleotide feature USDAWHE.02876 that was expressed 3.4 fold lower in synthetic *T. aestivum*. TC267082 corresponds to oligonucleotide feature FGAS.02280 that was expressed 3.0 fold lower in synthetic *T. aestivum*. TC238480 corresponds to oligonucleotide feature USDAWHE.05946 that was expressed 2.7 fold lower. Arrows indicate differential expression of homoeologs. Two independent replicates are pictured for the first two homoeoloci in each group to demonstrate reproducibility of results [*in vitro* synthetic controls (AB+D) were not included in second replicate]. Images of both single-stranded and heteroduplex conformations are shown for products of cDNA amplification with TC267682 and TC267082.

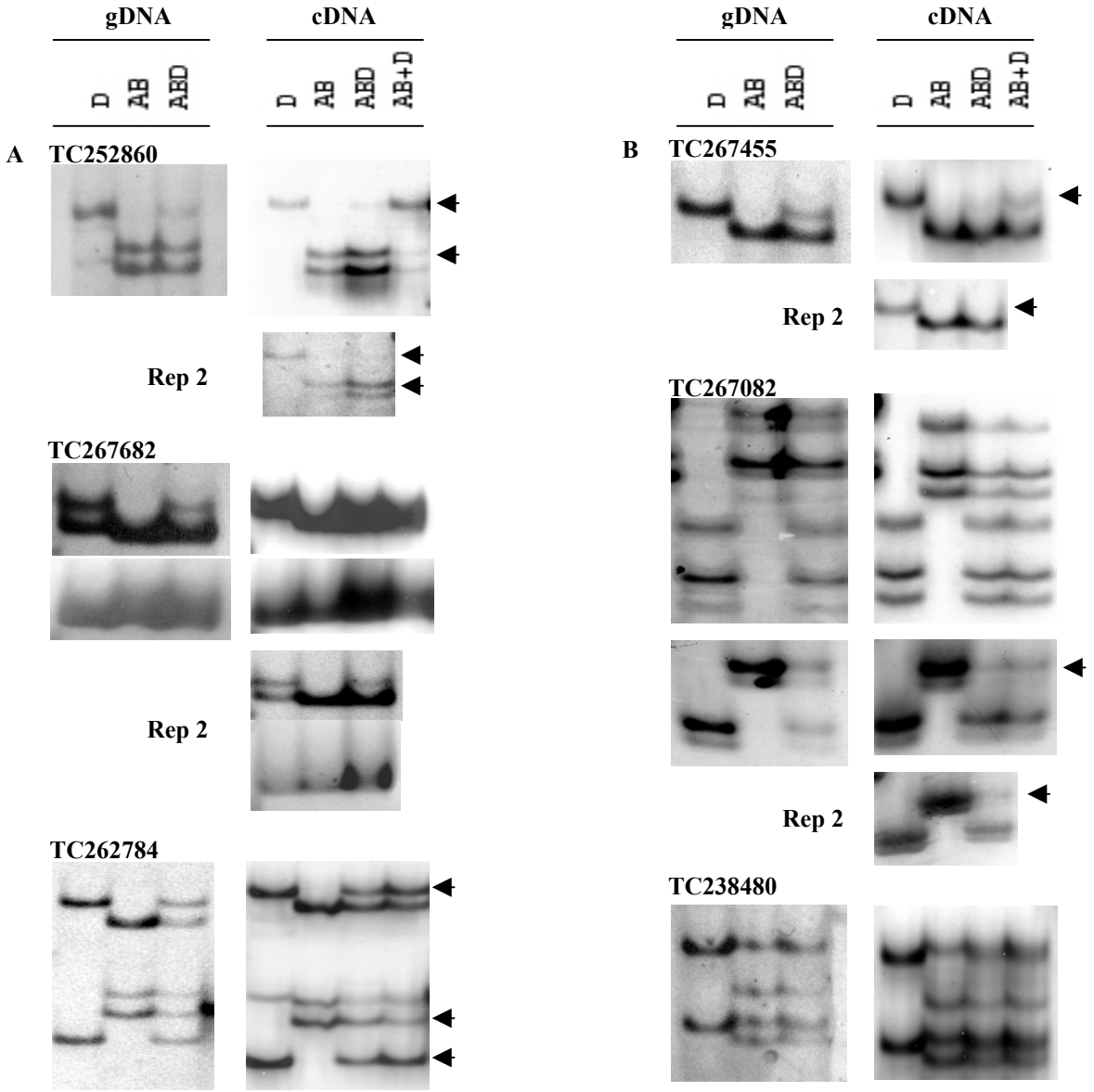

Table 3-1. Primer sequences used for cDNA-SSCP analysis of arbitrarily-selected homoeologous transcripts.

Locus ID	Forward Primer	Reverse Primer
BE605005	AGCCACCTTCTTACCCGATT	CCACCACATCTGCCTAAGGT
BF145701	ATCAGCTTCCTCCTCAGCAA	CAGGTCGATCACCGTCTTCT
BF482744	TGAGCTAATCCACCGGAATC	CAGCAGTAACACGTCGCAGT
BF484422	TCTTCAAGGGGAGGTTCCTT	ACTGCTTGCGTAGGCTGAAT
BG274398	GACAAGGAGAAGCAGGAGGA	TTGATCACCTCACCGTTGTC
TC232798	AGCCCTGAGGGTGAAAAGAT	CTCAGCAATCGTCATCTCCA
TC234825	GAAGTTCCCAGCCCAGCTAT	AGCGGCATATCCTTCATCAC
TC235913	ATGGGCCAGGACATATCAAA	CCCACCAAAAGAAGTCCAGA
TC237579	TGAGCGACTACGAGGAGGAT	CAGCTTCAACTTCGCTGTCA
TC237653	CAGCTCCTGCACATTTTCAA	TCTTCGCAGAGATTCGCTTT
TC237750	AGAAGCTTTGCCATTGGTGT	TCCCCGACCTTGTGGTAATA
TC241345	AACGACCTCGACATCCACAT	TCCTGCAGTTTGTTGTCTCG
TC241859	AGCATCCTTCTGGACGAGAG	GTCAGCAACTCCAGCATGAC
TC246766	AGACCCTCGCCTTGATCC	GCCTCGAAGTCCTCCTCTTC
TC249042	CACAGAGCGTGATGAGAGGA	GGTCTTCGGTAGCCATCAGA
TC251243	GGCTAAGGCAAGTGAACCAG	AGGCAGTGGTGGTAGCATCT
TC251989	CAGCTAGACGTCCGATGTGA	GCTTTGGGACAGTGGCTCTA
TC252254	GTAGAGGCTGCAAGGGCTTA	CCCTTTTGGGACTTTGTGAA
TC252388	GCTATTGCAGAGGCCAAGAC	ACATGGAACCGAAATCGAAC
TC253445	ACAGGAGGTTGGATGAGTGG	CGCAAACTAGCAGCATCAAG
TC257394	CTGTCTCGCGTTTGTTACGA	GGGTGAGAAAGCATAGAACGA
TC263227	TGCGTCTACTTGACCATCCA	TTATGTCCCTGTGGCAAACA
TC263837	GCGTGGACCAACTATGGACT	GCCCATTACTCTGGTGGAAA
TC264761	TATGCCGCTCTCTTGGTTCT	AAGCCACTTAGATCGCCTTG
TC264908	GCAAAGGCCAAGAATCAAAG	AGGCTAACCCTGGAAGGAAA
TC265165	GGCAGAGCAAGTGTTTCACA	CTTTCTTGCCATTGCTCCAT
TC265180	CGCCTTCATAAATCGTCACC	AGCCGTCTGATTGGCAGTAG
TC270558	TCCCTTTCCATTTCAAGCAC	CAGTGCCAAGTGGTTTCCTT
TC273472	CCGCTTCACTGGAAATCCTA	AGGATGATGCCAAATCAAGG
TC273936	GCGCCAATAGCTTTGTCTTT	TTCCACAAACACGTGCAAAC

Table 3-2. Correlation coefficients, *r*, among gene expression levels observed for *Ae. tauschii* (D), *T. turgidum* (AB), and synthetic *T. aestivum* (ABD) across hybridization treatments one (Trt 1), two (Trt 2) and three (Trt 3).

	D-Trt 2	AB-Trt 1	AB-Trt 3	ABD-Trt 2	ABD-Trt 3
D-Trt 1	0.94***	0.30***	0.26***	0.65***	0.67***
D-Trt 2	.	0.37***	0.34***	0.70***	0.70***
AB-Trt 1	.	.	0.97***[a]	0.75***	0.75***
AB-Trt 3	.	.	.	0.69***	0.71***
ABD-Trt 2	.	.	.	.	0.97***

***Correlation significant at $P < .0001$.

[a] *T. turgidum* was labeled with Cy5 in treatment one, with Cy3 in treatment three.

Table 3-3. Proportion of variation ($R^2 \times 100$) in gene expression levels explained between genotypes [*Ae. tauschii* (D), *T. turgidum* (AB), and synthetic *T. aestivum* (ABD)] and by mid-parent expression models $MPV_{1:1}$ and $MPV_{2:1}$.

	AB	ABD	$MPV_{1:1}$	$MPV_{2:1}$
		%		
D	11	49	.	.
AB	.	54	.	.
ABD	.	.	77	74

Table 3-4. Differentially expressed (≥two-fold) oligonucleotide features in microarray experiments, their translated blast annotation and expression fold change in synthetic *T. aestivum* relative to additive mid-parent expression model estimates $MPV_{1:1}$ and $MPV_{2:1}$. Down and up arrows indicate lower and higher expression, respectively, in synthetic *T. aestivum* than expected based on mid-parent estimates.

Oligonucleotide	Translated blast annotation	*E* value	$MPV_{1:1}$ fold	$MPV_{2:1}$ fold
FGAS.00200	putative arabinogalactan-like protein [*Oryza sativa*]	4×10^{-41}	5.0↓	5.9↓
FGAS.00233	beta-1,3-glucanase precursor [*Triticum aestivum*]	1×10^{-143}	1.8	2.5↓
FGAS.00291	putative class III chitinase [*Oryza sativa*]	1×10^{-123}	4.0↑	5.2↑
FGAS.00308	unnamed protein product [*Triticum aestivum*]	5×10^{-64}	1.5	2.0↓
FGAS.00311	OSJNBa0088H09.5 [*Oryza sativa*]	4×10^{-68}	1.9	2.2↑
FGAS.00329	putative ubiquitin-specific protease UBP12 [*Arabidopsis thaliana*]	1×10^{-121}	2.2↓	2.0↓
FGAS.00351	unnamed protein product [*Debaryomyces hansenii* CBS767]	7×10^{-01}	2.4↑	1.8
FGAS.00359	unknown protein [*Oryza sativa*]	9×10^{-16}	1.5	2.3↑
FGAS.00413	OSJNBb0002J11.20 [*Oryza sativa*]	1×10^{-173}	2.2↓	2.9↓
FGAS.00439	10.2 kDa photosystem I polypeptide [*Hordeum vulgare*]	2×10^{-48}	2.3↑	1.8
FGAS.00455	glutamine synthetase isoform GSr2 [*Triticum aestivum*]	1×10^{-104}	2.1↓	1.4
FGAS.00458	P0410E03.24 [*Oryza sativa*]	1×10^{-133}	2.7↑	3.6↑
FGAS.00484	OSJNBa0036B21.19 [*Oryza sativa*]	9×10^{-90}	3.1↓	3.0↓
FGAS.00500	putative nucleic acid-binding protein [*Oryza sativa*]	2×10^{-78}	2.1↑	1.5
FGAS.00509	OSJNBa0013K16.16 [*Oryza sativa*]	5×10^{-33}	1.7	2.3↑
FGAS.00581	unknown [*Arabidopsis thaliana*]	6×10^{-90}	1.6	2.0↑
FGAS.00589	delta-24-sterol methyltransferase [*Triticum aestivum*]	1×10^{-179}	1.8	2.0↓
FGAS.00704	putative multiple stress-responsive zinc-finger protein [*Oryza sativa*]	1×10^{-56}	2.2↑	1.6
FGAS.00733	unnamed protein product [*Hordeum vulgare* subsp. vulgare]	1×10^{-149}	1.6	2.3↑
FGAS.00736	putative transketolase 1 [*Oryza sativa*]	0	2.6↑	2.6↑
FGAS.00748	protein H2B153 [*Triticum aestivum*]	2×10^{-47}	3.5↓	2.3↓
FGAS.00794	HvPIP1 5 [*Hordeum vulgare*]	1×10^{-151}	2.9↓	2.1↓
FGAS.00983	histone H4 [*Triticum aestivum*]	2×10^{-38}	2.4↓	3.0↓
FGAS.01044	putative molybdopterin oxidoreductase [*S. thermophilum*]	3	2.8↓	1.9
FGAS.01155	peptidylprolyl isomerase [*Triticum aestivum*]	1×10^{-137}	2.1↓	2.4↓
FGAS.01188	putative transposase [*Oryza sativa*]	4×10^{-08}	2.3↓	1.5
FGAS.01201	mitogaligin [*Homo sapiens*]	2×10^{-01}	2.1↑	1.6
FGAS.01238	farnesylated protein 1 [*Hordeum vulgare*]	7×10^{-84}	3.7↑	2.8↑
FGAS.01252	predicted protein [*Arabidopsis thaliana*]	5	4.4↑	5.4↑
FGAS.01461	OSJNBa0018M05.18 [*Oryza sativa*]	0	2.3↓	1.5
FGAS.01595	hypothetical protein [*Oryza sativa*]	9×10^{-75}	2.0↑	2.2↑
FGAS.01640	putative 1-deoxy-D-xylulose 5-phosphate synthase [*Oryza sativa*]	5×10^{-69}	1.9	2.0↓
FGAS.01719	putative 3-oxoacyl synthase I chloroplast precursor [*Oryza sativa*]	1×10^{-156}	2.0↓	2.3↓
FGAS.01749	nucleoid DNA-binding protein cnd41-like protein [*Oryza sativa*]	4×10^{-35}	2.3↓	1.7
FGAS.01797	OSJNBb0116K07.16 [*Oryza sativa*]	1	2.5↑	2.2↑

Oligonucleotide	Translated blast annotation	*E* value	$MPV_{1:1}$ fold	$MPV_{2:1}$ fold
FGAS.01811	SGT1 [*Hordeum vulgare*]	0	1.9	2.1↓
FGAS.01834	Hypothetical protein MGC75959 [*Xenopus tropicalis*]	2	3.0↑	2.2↑
FGAS.01843	putative glucan endo-1 3-beta-D-glucosidase [*Oryza sativa*]	1×10^{-124}	2.0↑	2.2↑
FGAS.01868	high light protein [*Hordeum vulgare*]	1×10^{-56}	2.0↑	3.0↑
FGAS.01956	putative glyoxalase [*Oryza sativa*]	1×10^{-145}	2.0↑	1.7
FGAS.01961	HSP70 [*Triticum aestivum*]	1×10^{-123}	3.2↓	3.9↓
FGAS.02071	respiratory burst oxidase homolog [*Solanum tuberosum*]	1×10^{-102}	2.3↑	2.4↑
FGAS.02082	putative GDSL-like lipase/acylhydrolase [*Oryza sativa*]	8×10^{-59}	2.6↑	2.7↑
FGAS.02083	EREBP-4 like protein [*Oryza sativa*]	9×10^{-92}	2.2↑	2.4↑
FGAS.02094	hypothetical protein [*Oryza sativa*]	2×10^{-18}	2.1↓	1.4
FGAS.02280	serine carboxypeptidase I CP-MI [*Hordeum vulgare*]	0	3.0↓	3.1↓
FGAS.02301	Chaperonin CPN60-1 mitochondrial precursor (HSP60-1)	0	1.7	2.2↓
FGAS.02352	HSP70 [*Triticum aestivum*]	3×10^{-71}	2.3↓	2.9↓
FGAS.02472	putative metal-binding protein [*Oryza sativa*]	2×10^{-26}	2.2↓	1.5
FGAS.02524	putative late embryogenesis abundant protein [*Oryza sativa*]	2×10^{-63}	1.4	2.0↑
FGAS.02529	putative oxalyl-CoA decarboxylase [*Oryza sativa*]	5×10^{-72}	2.3↑	3.0↑
FGAS.02689	putative NADPH-dependent reductase [*Oryza sativa*]	0	3.3↑	3.4↑
FGAS.02807	putative thioredoxin-like protein CDSP32 [*Oryza sativa*]	1×10^{-118}	3.1↑	2.4↑
FGAS.02902	glutathione-S-transferase 19E50 [*Triticum aestivum*]	1×10^{-115}	3.2↓	2.1↓
FGAS.02907	AT3g20810/MOE17_10 [*Arabidopsis thaliana*]	4×10^{-77}	4.4↑	4.9↑
FGAS.03035	ENSANGP00000005326 [*Anopheles gambiae*]	4×10^{-01}	2.8↑	2.2↑
FGAS.03249	CAA30376.1 protein [*Oryza sativa*]	6×10^{-37}	2.0↓	1.4
FGAS.03312	sucrose synthase [Zea mays]	1×10^{-179}	3.7↑	2.9↑
FGAS.03376	putative oxidoreductase [*Oryza sativa*]	1×10^{-157}	2.3↓	3.1↓
FGAS.03401	putative protein kinase SPK-3 [*Oryza sativa*]	1×10^{-69}	2.0↑	2.5↑
FGAS.03426	B1139B11.9 [*Oryza sativa*]	1×10^{-47}	8.6↓	6.8↓
FGAS.03461	OSJNBa0070C17.13 [*Oryza sativa*]	1×10^{-66}	2.2↑	1.7
FGAS.03477	No match		2.1↑	1.6
FGAS.03494	expressed protein [*Oryza sativa*]	1×10^{-28}	2.1↑	2.3↑
FGAS.03623	PREDICTED: similar to KIAA1237 protein [*Pan troglodytes*]	1	3.3↑	2.5↑
FGAS.03836	peptidylprolyl isomerase [*Triticum aestivum*]	0	1.9	2.5↓
FGAS.04018	OSJNBb0093G06.10 [*Oryza sativa*]	1×10^{-131}	2.6↓	3.4↓
FGAS.04019	PB1 domain-containing protein [Oikopleura dioica]	4×10^{-01}	2.2↑	1.6
FGAS.04044	diadenosine 5' 5'''-P1 P4-tetraphosphate hydrolase [*Hordeum vulgare*]	1×10^{-109}	3.4↓	2.3↓
FGAS.04167	pyruvate dehydrogenase kinase 1 [*Oryza sativa*]	1×10^{-144}	1.9	2.2↓
FGAS.04177	csAtPR5 [Aegilops tauschii]	2×10^{-11}	2.1↑	2.4↑
FGAS.04295	dentin sialophosphoprotein-related [*Arabidopsis thaliana*]	2×10^{-06}	5.6↓	5.8↓
FGAS.04337	putative PP 2A regulatory subunit isoform 1 [*Oryza sativa*]	2×10^{-69}	2.1↑	2.5↑
FGAS.04429	histone H4 - [*Triticum aestivum*]	3×10^{-41}	2.7↓	3.3↓

Oligonucleotide	Translated blast annotation	*E* value	$MPV_{1:1}$ fold	$MPV_{2:1}$ fold
FGAS.04591	codanin I [*Homo sapiens*]	2×10^{-01}	1.9	2.2↑
FGAS.04600	aldehyde dehydrogenase [*Oryza sativa*]	6×10^{-82}	2.4↓	1.6
FGAS.04645	OSJNBa0053K19.8 [*Oryza sativa*]	1×10^{-141}	2.5↑	3.6↑
FGAS.04703	hypothetical protein [*Arabidopsis thaliana*]	1×10^{-29}	2.0↑	2.0↑
FGAS.04901	putative transposable element [*Oryza sativa*]	9×10^{-10}	1.8	2.0↑
FGAS.04940	peudo-response regulator-like [*Oryza sativa*]	1×10^{-136}	2.4↑	2.6↑
FGAS.05144	putative endoxyloglucan transferase [*Oryza sativa*]	2×10^{-69}	2.3↑	2.5↑
FGAS.05165	expressed protein (with alternative splicing) [*Oryza sativa*]	2×10^{-14}	1.9	2.2↑
FGAS.05261	unknown protein [*Oryza sativa*]	7×10^{-25}	2.5↑	1.9
FGAS.05296	OSJNBa0016O02.14 [*Oryza sativa*]	3×10^{-30}	2.2↑	2.9↑
FGAS.05334	serine/threonine protein kinase [*Oryza sativa*]	4×10^{-15}	2.4↓	1.6
FGAS.05377	unknown protein [*Oryza sativa*]	9×10^{-03}	2.5↓	3.0↓
FGAS.05402	Structure Of Glutathione S-Transferase Iii In Apo Form	1×10^{-52}	2.0↑	1.5
FGAS.05522	70 kDa heat shock protein [*Arabidopsis thaliana*]	1×10^{-104}	2.1↓	2.6↓
FGAS.05593	putative Cell elongation protein DIMINUTO [*Oryza sativa*]	1×10^{-48}	2.7↓	3.6↓
FGAS.05814	putative auxin-repressed protein [*Prunus armeniaca*]	1×10^{-09}	2.4↑	1.8
FGAS.05953	CG17104-PA [*Drosophila melanogaster*]	8×10^{-01}	2.0↓	1.4
FGAS.05981	putative serine/threonine protein kinase [*Oryza sativa*]	2×10^{-22}	1.9	2.8↑
FGAS.06027	heat shock protein 80 [*Triticum aestivum*]	1×10^{-104}	2.1↓	2.3↓
FGAS.06175	unknown protein [*Oryza sativa*]	1×10^{-149}	2.2↑	2.4↑
FGAS.06261	putative ankyrin [*Oryza sativa*]	2×10^{-30}	1.7	2.0↓
FGAS.06388	putative cellulose synthase catalytic subunit [*Hordeum vulgare*]	0	1.8	2.0↑
FGAS.06861	putative auxin-repressed protein [*Prunus armeniaca*]	2×10^{-06}	1.4	2.0↑
FGAS.06871	gibberellin-induced receptor-like kinase TMK [*Oryza sativa*]	0	2.1↓	2.4↓
FGAS.06913	pseudo-response regulator-like [*Oryza sativa*]	9×10^{-16}	3.0↑	3.6↑
FGAS.06924	No match	0	28.8↑	23.1↑
FGAS.07086	putative ribosomal protein [*Oryza sativa*]	4×10^{-78}	2.8↑	2.1↑
FGAS.07114	kinesin heavy chain [Zea mays]	1×10^{-114}	2.5↓	2.8↓
FGAS.07270	unknown protein [*Oryza sativa*]	4	2.0↑	1.5
FGAS.07275	LOC445881 protein [*Xenopus laevis*]	2×10^{-02}	2.7↑	2.1↑
FGAS.07437	proteasome alpha subunit [*Oryza sativa*]	1×10^{-114}	2.5↓	1.7
FGAS.07603	Isoflavone reductase homolog IRL	4×10^{-55}	1.4	2.0↑
FGAS.07831	putative thylakoid lumenal chloroplast precursor [*Oryza sativa*]	3×10^{-24}	1.9	2.3↑
FGAS.07893	cell division inhibitor-like [*Oryza sativa*]	8×10^{-80}	1.8	2.1↑
FGAS.07907	hypothetical protein CNBC0740 [*Cryptococcus neoformans*]	5	1.9	2.1↑
FGAS.07936	putative receptor-like protein kinase [*Oryza sativa*]	2×10^{-44}	2.1↑	1.8
FGAS.07985	unknown protein [*Oryza sativa*]	4×10^{-32}	2.7↑	2.6↑
FGAS.08233	urease [*Oryza sativa*]	1×10^{-43}	2.4↓	1.7
FGAS.08506	similar to vesicle transport protein [*Oryza sativa*]	1×10^{-92}	3.7↓	4.1↓

Oligonucleotide	Translated blast annotation	*E* value	$MPV_{1:1}$ fold	$MPV_{2:1}$ fold
FGAS.09294	unknown protein [*Oryza sativa*]	1×10^{-06}	3.1↑	3.5↑
FGAS.09542	P0439E07.14 [*Oryza sativa*]	6×10^{-48}	1.9	2.1↑
FGAS.09592	cysteine endopeptidase precursor [*Ricinus communis*]	5×10^{-10}	2.1↓	1.4
FGAS.10103	putative cellulose synthase catalytic subunit [*Hordeum vulgare*]	1×10^{-170}	1.6	2.0↑
USDAWHE.00119	fatty aldehyde dehydrogenase 1 [*Zea mays*]	6×10^{-23}	2.0↑	1.5
USDAWHE.00122	peptide transport protein [*Hordeum vulgare*]	1×10^{-14}	2.3↓	1.5
USDAWHE.00165	putative cytochrome P450 [*Oryza sativa*]	5×10^{-43}	1.6	2.0↓
USDAWHE.00204	OSJNBa0060N03.9 [*Oryza sativa*]	1×10^{-29}	5.7↓	7.6↓
USDAWHE.00222	unnamed protein product [*Oryza sativa*]	2×10^{-43}	2.5↑	1.9
USDAWHE.00239	serine/threonine protein kinase SAPK9 [*Oryza sativa*]	4×10^{-35}	1.9	2.4↑
USDAWHE.00320	DNA topoisomerase II [*Arabidopsis thaliana*]	3×10^{-55}	4.6↓	5.1↓
USDAWHE.00433	SMC4 protein [*Oryza sativa*]	9×10^{-08}	2.6↓	3.0↓
USDAWHE.00768	putative Nuclear pore complex protein Nup155 [*Oryza sativa*]	5×10^{-71}	2.0↓	1.9
USDAWHE.00837	putative farnesyl-pyrophosphate synthetase [*Oryza sativa*]	2×10^{-48}	2.5↓	1.7
USDAWHE.01121	putative naphthoate synthase [*Oryza sativa*]	4×10^{-65}	2.0↓	1.4
USDAWHE.01509	plastid sigma factor SIG5 [*Oryza sativa*]	1×10^{-61}	5.3↓	6.3↓
USDAWHE.01598	hypothetical protein [*Oryza sativa*]	6×10^{-15}	2.1↑	2.3↑
USDAWHE.01861	OJ1656_A11.3 [*Oryza sativa*]	1×10^{-09}	1.6	2.4↑
USDAWHE.01866	OSJNBa0006A01.3 [*Oryza sativa*]	2×10^{-43}	2.2↑	1.7
USDAWHE.02072	putative chalcone synthase [*Oryza sativa*]	2×10^{-43}	3.1↓	4.1↓
USDAWHE.02208	B1015E06.23 [*Oryza sativa*]	3×10^{-28}	2.2↑	1.6
USDAWHE.02554	protein kinase homolog [*Oryza sativa*]	5×10^{-41}	1.7	2.3↑
USDAWHE.02876	putative Hsp70 binding protein [*Oryza sativa*]	6×10^{-44}	3.4↓	3.9↓
USDAWHE.02888	unnamed protein product [*Oryza sativa*]	3×10^{-22}	11.1↑	8.7↑
USDAWHE.03020	OSJNBb0034I13.13 [*Oryza sativa*]	5×10^{-68}	2.0↑	2.3↑
USDAWHE.03113	putative drought-induced protein [*Oryza sativa*]	3×10^{-14}	2.5↓	1.7
USDAWHE.03193	P0439E11.13 [*Oryza sativa*]	8×10^{-56}	2.1↑	2.0↑
USDAWHE.03214	unknown protein [*Oryza sativa*]	5×10^{-39}	1.8	2.1↑
USDAWHE.03577	putative high-affinity potassium transporter [*Hordeum vulgare*]	3×10^{-12}	3.9↓	4.1↓
USDAWHE.03603	putative pyrophosphate-dependent PPFK subunit [*Oryza sativa*]	1×10^{-104}	1.8	2.4↑
USDAWHE.03631	PREDICTED OJ1150_A11.17 gene product [*Oryza sativa*]	5×10^{-36}	2.2↑	2.5↑
USDAWHE.03687	Rar1 [*Hordeum vulgare*]	1×10^{-126}	2.2↓	1.5
USDAWHE.03913	OSJNBa0070M12.6 [*Oryza sativa*]	2×10^{-44}	2.2↓	1.5
USDAWHE.04015	MADS6 [*Lolium perenne*]	1×10^{-106}	5.6↓	3.8↓
USDAWHE.04083	putative endo-1 3 1 4-beta-D-glucanase' [*Oryza sativa*]	2×10^{-64}	2.1↑	1.6
USDAWHE.04104	probable beta-1 3-glucanase [*Triticum aestivum*]	9×10^{-58}	2.1↑	2.2↑
USDAWHE.04152	expressed protein [*Oryza sativa*]	1×10^{-19}	1.6	2.2↓
USDAWHE.04169	unnamed protein product [*Oryza sativa*]	4×10^{-32}	4.1↑	3.1↑
USDAWHE.04398	phosphate transporter 6 [*Hordeum vulgare*]	2×10^{-64}	2.0↓	1.3

Oligonucleotide	Translated blast annotation	*E* value	$MPV_{1:1}$ fold	$MPV_{2:1}$ fold
USDAWHE.04572	hypothetical protein [*Oryza sativa*]	1×10^{-58}	3.6↓	2.4↓
USDAWHE.04807	putative ribulokinase [*Oryza sativa*]	1×10^{-117}	1.8	2.1↑
USDAWHE.04995	putative protein of gibberellin-stimulated transcript [*Oryza sativa*]	3×10^{-35}	2.1↓	2.8↓
USDAWHE.05201	Hypothetical protein [*Oryza sativa*]	3×10^{-25}	2.8↓	3.7↓
USDAWHE.05320	putative basic blue copper protein [*Oryza sativa*]	1×10^{-37}	1.8	2.4↓
USDAWHE.05462	hypothetical protein [*Oryza sativa*]	3×10^{-99}	2.3↑	1.8
USDAWHE.05477	serine carboxypeptidase III [*Hordeum vulgare*]	0	2.0↓	1.7
USDAWHE.05594	putative chlorophyll a/b-binding protein type II [*Oryza sativa*]	1×10^{-117}	2.7↑	3.6↑
USDAWHE.05735	PREDICTED P0724B10.24 gene product [*Oryza sativa*]	5×10^{-44}	1.8	2.2↑
USDAWHE.05935	OSJNBa0004N05.2 [*Oryza sativa*]	3×10^{-27}	2.2↓	2.8↓
USDAWHE.05946	histidyl-tRNA synthetase [*Triticum aestivum*]	1×10^{-73}	2.7↓	2.7↓
USDAWHE.05947	putative NF-E2 inducible protein [*Oryza sativa*]	2×10^{-36}	3.5↓	4.1↓
USDAWHE.06455	putative aldehyde dehydrogenase [*Arabidopsis thaliana*]	1×10^{-61}	2.1↑	1.8
USDAWHE.06535	hypothetical protein [*Phaseolus vulgaris*]	1×10^{-14}	2.3↑	2.6↑
USDAWHE.06694	seven transmembrane protein Mlo4 [*Zea mays*]	8×10^{-55}	2.8↑	2.1↑
USDAWHE.06826	putative Band 7 protein [*Oryza sativa*]	3×10^{-26}	1.7	2.2↓
USDAWHE.06880	ASR-like protein 1 [*Hevea brasiliensis*]	2×10^{-17}	2.5↑	3.3↑
USDAWHE.06899	putative glyoxysomal FA beta-oxidation protein [*Oryza sativa*]	9×10^{-56}	2.0↓	1.3
USDAWHE.07029	OsCDPK protein [*Oryza sativa*]	6×10^{-80}	2.6↑	2.1↑
USDAWHE.07114	putative GPI-anchored protein [*Oryza sativa*]	2×10^{-44}	3.6↑	2.8↑

Table 3-5. Oligonucleotide features displaying over/under-dominant expression (≥two-fold) in microarray experiments, their putative annotation (function) and expression fold change in synthetic *T. aestivum* relative to high/low-parent levels. Down and up arrows indicate lower and higher expression, respectively, in synthetic *T. aestivum* than expected based on high/low-parent values.

Oligonucleotide	Blast annotation/putative function	Expect	Over/under-dominant fold
FGAS.00200	putative arabinogalactan-like protein [*Oryza sativa*] (signaling, development, cell-wall anchored)	4×10^{-41}	2.3↓
FGAS.00291	putative class III chitinase [*Oryza sativa*] (PR protein, defense, stress response, development)	1×10^{-123}	2.4↑
FGAS.00484	OSJNBa0036B21.19 [*Oryza sativa*] (hypothetical non-cell-autonomous protein pathway2; cell to cell communication)	9×10^{-90}	2.9↓
FGAS.00736	putative transketolase 1 [*Oryza sativa*] (calvin cycle)	0	2.4↑
FGAS.01252	predicted protein [*Arabidopsis thaliana*] (adh1-adh2 region, novel transposable element, contains TIR)	5	2.9↑
FGAS.02082	putative GDSL-like lipase/acylhydrolase [*Oryza sativa*] (lipid metabolism)	8×10^{-59}	2.4↑
FGAS.02280	serine carboxypeptidase I CP-MI [*Hordeum vulgare*] (protein turnover)	0	2.6↓
FGAS.02689	putative NADPH-dependent reductase [*Oryza sativa*]	0	2.9↑
FGAS.02907	AT3g20810/MOE17_10 [*Arabidopsis thaliana*] (hypothetical; possible transcription factor; transferase activity)	4×10^{-77}	3.4↑
FGAS.03312	sucrose synthase [*Zea mays*]	1×10^{-179}	2.1↑
FGAS.03426	B1139B11.9 [*Oryza sativa*] (hypothetical; putative regulator of gene silencing;EF-hand, calcium binding motif)	1×10^{-47}	3.1↓
FGAS.04295	dentin sialophosphoprotein-related [*Arabidopsis thaliana*] (expressed protein)	2×10^{-06}	5.1↓
FGAS.05144	putative endoxyloglucan transferase [*Oryza sativa*] (cell wall structure/remodeling)	2×10^{-69}	2.0↑
FGAS.06913	pseudo-response regulator-like [*Oryza sativa*] (hypothetical signal transduction; transcriptional regulation)	9×10^{-16}	2.1↑
FGAS.06924	None (Repeat database: Jura 2 DNA transposon, MITE, tourist)	8×10^{-50}	16.5↑
FGAS.07985	unknown protein [*Oryza sativa*] (hypothetical)	4×10^{-32}	2.5↑

Oligonucleotide	Blast annotation/putative function	Expect	Over/under-dominant fold
FGAS.08506	similar to vesicle transport protein [*Oryza sativa*] (protein trafficking)	1×10^{-92}	2.6↓
FGAS.09294	unknown protein [*Oryza sativa*] (hypothetical)	1×10^{-06}	2.3↑
USDAWHE.00320	DNA topoisomerase II [*Arabidopsis thaliana*]	3×10^{-55}	3.2↓
USDAWHE.01509	plastid sigma factor SIG5 [*Oryza sativa*] (plastid RNA polymerase subunit)	1×10^{-61}	2.5↓
USDAWHE.02888	unnamed protein product [*Oryza sativa*] (hypothetical helicase-related)	3×10^{-22}	6.1↑
USDAWHE.03577	putative high-affinity potassium transporter [*Hordeum vulgare*]	3×10^{-12}	3.2↓
USDAWHE.04169	unnamed protein product [*Oryza sativa*] (hypothetical invertase/pectin methylesterase inhibitor)	4×10^{-32}	2.1↑
USDAWHE.05946	histidyl-tRNA synthetase [*Triticum aestivum*] (protein synthesis)	1×10^{-73}	2.6↓

Appendix A - Mean Fluorescence Intensities in Microarray Hybridization Experiments

Table A-1. Oligonucleotide features, their translated blast annotations, and hybridization intensities (expression levels) measured for synthetic *Triticum aestivum* and parental *T. turgidum* and *Aegilops tauschii* lines. Additive mid-parent $MPV_{1:1}$ expression values were calculated by averaging *T. turgidum* and *Ae. tauschii* intensities. $MPV_{2:1}$ values were calculated by a weighted 2:1 average of *T. turgidum* and *Ae. tauschii* intensities.

		Mean of intensity levels*			Additive values	
Oligonucleotide	**Translated blast annotation**	***T. aestivum***	***T. turgidum***	***Ae. tauschii***	**$MPV_{1:1}$**	**$MPV_{2:1}$**
FGAS.00200	putative arabinogalactan-like protein [Oryza sativa]	89	688	204	446	527
FGAS.00201	OSJNBa0093F16.12 [Oryza sativa]	3273	3857	1056	2456	2923
FGAS.00208	putative cellulose synthase [Oryza sativa]	1615	712	1765	1238	1063
FGAS.00214	xyloglucan endo-1;4-beta-D-glucanase (EC 3.2.1.-) - wheat	265	578	161	370	439
FGAS.00233	beta-1;3-glucanase precursor [Triticum aestivum]	311	1140	10	575	763
FGAS.00250	OSJNBa0038J17.24 [Oryza sativa]	1634	3826	10	1918	2554
FGAS.00258	xyloglucan endotransglycosylase (XET) [Hordeum vulgare subsp. vulgare]	713	900	10	455	604
FGAS.00263	putative peptide transport protein [Oryza sativa]	351	886	107	497	627
FGAS.00266	putative cellulose synthase catalytic subunit [Hordeum vulgare]	7679	4088	12022	8055	6732
FGAS.00287	PREDICTED P0506F02.119 gene product [Oryza sativa]	562	911	10	461	611
FGAS.00291	putative class III chitinase [Oryza sativa]	1120	93	463	278	216
FGAS.00300	histone deacetylase 2 isoform b [Zea mays]	1126	2181	15	1098	1459
FGAS.00307	histone H3.2 protein [Oryza sativa]	5188	47	12910	6478	4335
FGAS.00308	unnamed protein product [Triticum aestivum]	1132	3377	10	1694	2255
FGAS.00310	double C2-like domains; beta [Homo sapiens]	398	682	12	347	458
FGAS.00311	OSJNBa0088H09.5 [Oryza sativa]	1867	583	1373	978	846
FGAS.00323	putative metal-binding protein [Oryza sativa]	1413	1949	362	1156	1420
FGAS.00326	hypothetical protein [Oryza sativa]	390	23	1132	578	393
FGAS.00329	putative ubiquitin-specific protease UBP12 [Arabidopsis thaliana]	194	340	506	423	395
FGAS.00334	putative glycine-rich RNA-binding protein 2 [Oryza sativa]	1347	597	1042	820	745

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.00335	putative cysteine conjugate beta-lyase [Oryza sativa]	1885	2036	611	1323	1561
FGAS.00338	putative acyl-CoA oxidase [Oryza sativa]	368	747	12	379	502
FGAS.00341	monodehydroascorbate reductase [Oryza sativa]	902	1776	369	1072	1307
FGAS.00350	putative Aconitate hydratase [Oryza sativa]	5712	6045	3240	4643	5110
FGAS.00351	unnamed protein product [Debaryomyces hansenii CBS767]	681	555	10	283	374
FGAS.00359	unknown protein [Oryza sativa]	1819	8	2380	1194	799
FGAS.00365	putative class III chitinase [Oryza sativa]	325	434	88	261	319
FGAS.00373	OSJNBb0048E02.12 [Oryza sativa]	2579	14	6179	3096	2069
FGAS.00375	phosphoglucomutase [Triticum aestivum]	2372	3573	10	1792	2386
FGAS.00378	unknown protein [Oryza sativa]	629	1023	194	609	747
FGAS.00394	unknown protein [Oryza sativa]	327	729	153	441	537
FGAS.00401	putative acyl-CoA oxidase [Oryza sativa]	1375	91	4105	2098	1429
FGAS.00402	At5g51110/MWD22_5 [Arabidopsis thaliana]	1543	2103	638	1371	1615
FGAS.00413	OSJNBb0002J11.20 [Oryza sativa]	305	1301	19	660	873
FGAS.00415	unknown protein [Oryza sativa]	2553	2681	1460	2071	2274
FGAS.00425	10A19I.15 [Oryza sativa]	1315	1907	10	958	1275
FGAS.00432	putative peroxidase [Oryza sativa]	1276	1840	335	1087	1338
FGAS.00439	10.2 kDa photosystem I polypeptide [Hordeum vulgare]	7324	6032	287	3159	4117
FGAS.00441	unknown [Hordeum vulgare]	272	19	671	345	236
FGAS.00451	OsRad6 [Oryza sativa]	2453	1334	2994	2164	1888
FGAS.00453	putative amino acid transport protein [Oryza sativa]	656	322	571	446	405
FGAS.00455	glutamine synthetase isoform GSr2 [Triticum aestivum]	2081	17	8602	4310	2879
FGAS.00458	P0410E03.24 [Oryza sativa]	877	72	587	330	244
FGAS.00480	OSJNBa0093F12.14 [Oryza sativa]	2286	139	5449	2794	1909
FGAS.00484	OSJNBa0036B21.19 [Oryza sativa]	148	431	494	462	452

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.00500	putative nucleic acid-binding protein [Oryza sativa]	2028	1968	10	989	1315
FGAS.00509	OSJNBa0013K16.16 [Oryza sativa]	1142	172	1143	658	496
FGAS.00578	putative pfam00106; adh_short; short chain dehydrogenase [Oryza sativa]	465	243	253	248	246
FGAS.00581	unknown [Arabidopsis thaliana]	4030	1302	3584	2443	2063
FGAS.00589	delta-24-sterol methyltransferase [Triticum aestivum]	1172	2979	1199	2089	2385
FGAS.00595	putative protein phosphatase 2C [Oryza sativa]	1319	1271	757	1014	1100
FGAS.00610	putative cytochrome c oxidase subunit [Triticum aestivum]	393	528	26	277	361
FGAS.00623	wheatwin1 [Triticum aestivum]	851	1673	10	842	1119
FGAS.00627	Carbonic anhydrase; chloroplast precursor (Carbonate dehydratase)	694	802	287	544	630
FGAS.00635	unknown protein [Oryza sativa]	727	97	1527	812	574
FGAS.00642	hypersensitive-induced reaction protein 1 [Hordeum vulgare subsp. vulgare]	345	141	690	416	324
FGAS.00644	putative ADP-ribosylation factor [Oryza sativa]	336	28	1019	523	358
FGAS.00704	putative multiple stress-responsive zinc-finger protein [Oryza sativa]	904	817	14	416	549
FGAS.00722	putative photosystem II subunit (22KDa) precursor [Oryza sativa]	1009	1024	17	520	688
FGAS.00723	phosphoinositide-specific phospholipase C [Oryza sativa]	785	294	1159	726	582
FGAS.00733	unnamed protein product [Hordeum vulgare subsp. vulgare]	545	23	655	339	233
FGAS.00736	putative transketolase 1 [Oryza sativa]	540	221	186	204	210
FGAS.00741	WSI76 protein induced by water stress [Oryza sativa]	524	158	988	573	435
FGAS.00748	protein H2B153 [Triticum aestivum]	559	7	3919	1963	1311
FGAS.00761	chloroplast RelA homologue 1 [Oryza sativa]	322	417	100	259	312
FGAS.00794	HvPIP1;5 [Hordeum vulgare subsp. vulgare]	176	106	900	503	371
FGAS.00813	caffeic acid O-methyltransferase [Triticum aestivum]	1159	110	2973	1541	1064
FGAS.00883	hypothetical protein XP_489464 [Mus musculus]	434	142	1053	597	445
FGAS.00955	10A19I.3 [Oryza sativa]	186	387	313	350	362
FGAS.00969	putative phophatidylethanolamine binding protein [Oryza sativa]	927	327	1800	1064	818

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.00983	histone H4 - wheat	243	1026	124	575	725
FGAS.00991	Similar to ATP-citrate-lyase [Arabidopsis thaliana]	397	176	533	354	295
FGAS.01002	putative extensin [Arabidopsis thaliana]	1758	3059	1387	2223	2502
FGAS.01023	unknown protein [Oryza sativa]	1018	207	1257	732	557
FGAS.01044	putative molybdopterin oxidoreductase [Symbiobacterium thermophilum]	680	9	3793	1901	1270
FGAS.01059	putative uricase [Oryza sativa]	883	772	1772	1272	1105
FGAS.01082	P0466H10.24 [Oryza sativa]	501	232	855	543	440
FGAS.01084	putative peptide transporter protein [Oryza sativa]	423	520	12	266	351
FGAS.01110	unknown protein [Oryza sativa]	609	868	10	439	582
FGAS.01111	aldehyde dehydrogenase [Oryza sativa]	598	693	352	523	580
FGAS.01128	superal1 [Zea mays]	438	19	992	506	344
FGAS.01130	none	1976	96	4910	2503	1701
FGAS.01132	putative MLH1 protein [Arabidopsis thaliana]	223	61	621	341	247
FGAS.01155	peptidylprolyl isomerase [Triticum aestivum]	530	1610	600	1105	1273
FGAS.01168	OSJNBa0032F06.16 [Oryza sativa]	283	214	611	412	346
FGAS.01188	putative transposase [Oryza sativa]	297	7	1354	681	456
FGAS.01201	mitogaligin [Homo sapiens]	1066	1017	10	514	682
FGAS.01205	unknown protein [Oryza sativa]	706	816	48	432	560
FGAS.01224	hypothetical protein [Oryza sativa]	342	486	16	251	329
FGAS.01229	putative sphingosine kinase [Oryza sativa]	347	19	867	443	302
FGAS.01235	methionine adenosyltransferase (EC 2.5.1.6) - barley	962	864	2153	1508	1293
FGAS.01238	farnesylated protein 1 [Hordeum vulgare subsp. vulgare]	2033	1092	10	551	732
FGAS.01239	putative adenylyl cyclase associated protein [Oryza sativa]	1026	2087	268	1178	1481
FGAS.01252	predicted protein [Arabidopsis thaliana]	1531	156	537	347	283
FGAS.01296	OSJNBb0072M01.18 [Oryza sativa]	1771	3058	1327	2193	2481

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.01316	unknown protein [Oryza sativa]	3395	440	5169	2805	2016
FGAS.01318	hypothetical protein [Oryza sativa]	2357	24	4265	2144	1437
FGAS.01361	auxin-induced protein [Arabidopsis thaliana]	390	130	370	250	210
FGAS.01386	putative GTP-binding protein [Oryza sativa]	4591	6872	3021	4947	5589
FGAS.01411	putative F-box protein (SKP1 interacting partner 3-related) [Oryza sativa]	417	676	233	454	528
FGAS.01420	putative transcriptional corepressor LEUNIG [Oryza sativa]	661	9	2468	1238	828
FGAS.01421	zinc finger protein family-like [Oryza sativa]	3555	1711	3231	2471	2218
FGAS.01427	unknown protein [Oryza sativa]	419	810	69	440	563
FGAS.01461	OSJNBa0018M05.18 [Oryza sativa]	223	17	987	502	340
FGAS.01540	DNA binding protein [Triticum aestivum]	710	998	166	582	721
FGAS.01578	zinc metalloproteinase-like [Oryza sativa]	783	1250	605	928	1035
FGAS.01595	hypothetical protein [Oryza sativa]	3123	1165	1980	1573	1437
FGAS.01615	polyubiquitin [Oryza sativa]	1673	2463	989	1726	1972
FGAS.01640	putative 1-deoxy-D-xylulose 5-phosphate synthase [Oryza sativa]	351	768	586	677	708
FGAS.01718	unknown protein [Oryza sativa]	347	35	966	500	345
FGAS.01719	putative 3-oxoacyl-[acyl-carrier-protein] synthase I [Oryza sativa]	198	545	261	403	451
FGAS.01730	PREDICTED: phospholipase C; gamma 2 [Pan troglodytes]	374	411	25	218	283
FGAS.01733	farnesylated protein 3 [Hordeum vulgare subsp. vulgare]	629	197	1243	720	546
FGAS.01738	hypothetical protein [Plasmodium falciparum 3D7]	1291	305	3389	1847	1333
FGAS.01742	Eukaryotic translation initiation factor 5A-3 (eIF-5A 3)	840	1560	29	795	1050
FGAS.01743	OSJNBa0032F06.22 [Oryza sativa]	698	1191	387	789	923
FGAS.01749	nucleoid DNA-binding protein cnd41-like protein [Oryza sativa]	507	162	2186	1174	837
FGAS.01766	B1147A04.10 [Oryza sativa]	3141	530	3983	2257	1681
FGAS.01774	BRITTLE CULM1 [Oryza sativa (indica cultivar-group)]	10590	4680	14658	9669	8006
FGAS.01775	none	1783	2288	10	1149	1529

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.01797	OSJNBb0116K07.16 [Oryza sativa]	1091	622	255	439	500
FGAS.01811	SGT1 [Hordeum vulgare]	2329	5707	3159	4433	4858
FGAS.01819	unnamed protein product [Oryza sativa]	693	1013	19	516	682
FGAS.01821	unknown protein [Oryza sativa]	505	415	1117	766	649
FGAS.01833	transcription factor Myb2 [Triticum aestivum]	842	465	1081	773	671
FGAS.01834	Hypothetical protein MGC75959 [Xenopus tropicalis]	2094	1400	10	705	937
FGAS.01843	putative glucan endo-1;3-beta-D-glucosidase [Oryza sativa]	4134	1504	2554	2029	1854
FGAS.01868	high light protein [Hordeum vulgare]	724	27	683	355	245
FGAS.01894	hypothetical protein [Oryza sativa]	312	529	215	372	424
FGAS.01941	putative SMA-9 class B [Oryza sativa]	611	735	159	447	543
FGAS.01956	putative glyoxalase [Oryza sativa]	619	477	131	304	362
FGAS.01961	HSP70 [Triticum aestivum]	143	761	160	461	561
FGAS.01962	putative 60S ribosomal protein L18a [Oryza sativa]	512	161	1567	864	630
FGAS.02029	unknown protein [Oryza sativa]	360	593	205	399	463
FGAS.02030	none	2689	4535	864	2700	3312
FGAS.02037	40S subunit ribosomal protein [Oryza sativa]	3704	9243	58	4651	6182
FGAS.02041	expressed protein [Arabidopsis thaliana]	273	29	942	485	333
FGAS.02044	purine nucleotide binding protein [Guillardia theta]	450	487	42	264	339
FGAS.02048	unknown protein [Oryza sativa]	986	525	942	734	664
FGAS.02050	ent-kaurene synthase-like protein 1 [Hordeum vulgare subsp. vulgare]	383	23	818	421	288
FGAS.02061	expressed protein [Oryza sativa]	316	403	206	305	338
FGAS.02065	protein phosphatase 2 (f... [Pan troglodytes]	295	103	600	352	269
FGAS.02071	respiratory burst oxidase homolog [Solanum tuberosum]	512	176	278	227	210
FGAS.02073	probable ferrochelatase (EC 4.99.1.1) - barley	535	1181	13	597	791
FGAS.02082	putative GDSL-like lipase/acylhydrolase [Oryza sativa]	1315	460	544	502	488

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.02083	EREBP-4 like protein [Oryza sativa]	429	136	255	196	176
FGAS.02086	unknown protein [Oryza sativa]	340	498	13	255	336
FGAS.02092	none	428	6	1645	826	552
FGAS.02094	hypothetical protein [Oryza sativa]	213	32	849	440	304
FGAS.02098	ascorbate peroxidase [Hordeum vulgare subsp. vulgare]	555	646	175	410	489
FGAS.02211	serine carboxylase II-2 [Hordeum vulgare subsp. vulgare]	376	19	1373	696	471
FGAS.02244	putative Proline synthetase associated protein [Arabidopsis thaliana]	790	840	10	425	564
FGAS.02263	putative ribosomal protein L35A [Oryza sativa]	413	737	132	434	535
FGAS.02279	major nitrogen regulation protein - Penicillium chrysogenum (strain Q176)	377	612	64	338	429
FGAS.02280	serine carboxypeptidase I; CP-MI [Hordeum vulgare subsp. vulgare]	581	1917	1517	1717	1784
FGAS.02296	putative PGPD14 protein (pollen germination related protein) [Oryza sativa]	943	1075	67	571	739
FGAS.02301	Chaperonin CPN60-1; mitochondrial precursor (HSP60-1)	1084	3371	316	1844	2353
FGAS.02335	B1156H12.22 [Oryza sativa]	3148	1068	9079	5074	3739
FGAS.02352	HSP70 [Triticum aestivum]	265	1041	198	619	760
FGAS.02380	unknown protein [Oryza sativa]	853	1117	558	837	931
FGAS.02382	similar to Arabidopsis thaliana T6D22.10~unknown protien [Oryza sativa]	539	667	207	437	513
FGAS.02417	B12Dg1 [Hordeum vulgare subsp. vulgare]	550	670	38	354	460
FGAS.02440	methionine synthase [Hordeum vulgare subsp. vulgare]	2036	1305	3504	2405	2038
FGAS.02469	proline rich protein [Lycopersicon esculentum]	1124	657	803	730	705
FGAS.02472	putative metal-binding protein [Oryza sativa]	252	51	1059	555	387
FGAS.02506	putative ribosomal protein L6 [Oryza sativa]	3104	2788	1264	2026	2280
FGAS.02511	ATP-dependent Clp protease ATP-binding subunit precursor [Oryza sativa]	691	1060	437	748	852
FGAS.02517	peroxidase [Oryza sativa]	645	1062	15	539	713
FGAS.02524	putative late embryogenesis abundant protein [Oryza sativa]	1016	62	1425	743	516
FGAS.02529	putative oxalyl-CoA decarboxylase [Oryza sativa]	2437	310	1842	1076	821

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.02532	putative P18 [Oryza sativa]	4984	3072	5454	4263	3866
FGAS.02544	ozone-responsive stress-related protein-like [Oryza sativa]	384	634	10	322	426
FGAS.02549	putative endo-1;4-beta-xylanase X-1 [Oryza sativa]	1546	1355	744	1050	1151
FGAS.02558	none	871	1096	693	894	961
FGAS.02612	unnamed protein product [Triticum aestivum]	1567	734	1298	1016	922
FGAS.02689	putative NADPH-dependent reductase [Oryza sativa]	1054	281	366	323	309
FGAS.02709	methylenetetrahydrofolate reductase; 3-partial [Oryza sativa]	1039	478	2081	1279	1012
FGAS.02771	Putative Squalene monooxygenase [Oryza sativa]	536	1309	208	758	942
FGAS.02800	leucine-rich repeat resistance protein-like protein [Gossypium hirsutum]	379	620	129	375	456
FGAS.02807	putative thioredoxin-like protein CDSP32 [Oryza sativa]	3369	2119	37	1078	1425
FGAS.02815	hypothetical protein [Oryza sativa]	377	54	1148	601	418
FGAS.02826	40S subunit ribosomal protein [Oryza sativa]	4289	2581	11251	6916	5471
FGAS.02836	OSJNBa0084K01.11 [Oryza sativa]	443	718	11	364	482
FGAS.02850	putative chloroplast ribosomal protein L1 [Oryza sativa]	603	245	522	384	338
FGAS.02889	unknown [Arabidopsis thaliana]	797	814	10	412	546
FGAS.02902	glutathione-S-transferase 19E50 [Triticum aestivum]	861	30	5397	2713	1819
FGAS.02907	AT3g20810/MOE17_10 [Arabidopsis thaliana]	637	102	187	145	131
FGAS.02912	putative copper chaperone [Oryza sativa]	442	569	44	306	394
FGAS.02923	ATP synthase beta subunit [Triticum aestivum]	1118	7	2257	1132	757
FGAS.02925	none	626	693	298	495	561
FGAS.02930	OSJNBb0006L01.5 [Oryza sativa]	838	313	869	591	498
FGAS.02936	gamma-type tonoplast intrinsic protein [Triticum aestivum]	3261	4361	1185	2773	3302
FGAS.02945	mitochondrial aldehyde dehydrogenase ALDH2 [Hordeum vulgare]	514	527	138	333	397
FGAS.02975	unknown protein [Arabidopsis thaliana]	404	541	164	352	415
FGAS.03008	hypothetical protein [Oryza sativa]	1419	1413	514	964	1113

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.03018	putative serine peptidase [Oryza sativa]	889	1441	10	726	964
FGAS.03028	putative BAG domain containing protein [Oryza sativa]	553	257	708	483	408
FGAS.03031	unknown protein [Oryza sativa]	688	77	922	499	358
FGAS.03033	EREBP transcription factor [Triticum aestivum]	391	101	958	529	387
FGAS.03035	ENSANGP00000005326 [Anopheles gambiae]	397	263	21	142	182
FGAS.03048	PREDICTED B1370C05.32 gene product [Oryza sativa]	1217	880	2548	1714	1436
FGAS.03100	unknown protein [Oryza sativa]	392	24	1300	662	450
FGAS.03220	PREDICTED: hypothetical protein XP_529452 [Pan troglodytes]	1037	1884	15	949	1261
FGAS.03221	non-ribosomal peptide synthetase [Pseudomonas syringae]	8167	17130	12	8571	11424
FGAS.03249	CAA30376.1 protein [Oryza sativa]	518	9	2093	1051	704
FGAS.03260	ornithine aminotransferase [Vitis vinifera]	363	631	84	357	449
FGAS.03261	ADP-ribosylation factor 1 [Arabidopsis thaliana]	1402	2334	22	1178	1564
FGAS.03271	putative glutathione S-transferase [Oryza sativa]	1799	707	2103	1405	1172
FGAS.03285	putative LRR-containing F-box protein [Oryza sativa]	736	632	291	462	519
FGAS.03294	KH domain-containing protein NOVA-like [Oryza sativa]	819	1235	535	885	1001
FGAS.03297	glycoprotein B [Psittacid herpesvirus 1]	903	1576	10	793	1054
FGAS.03310	putative cytochrome 450 [Oryza sativa]	1426	1799	10	905	1203
FGAS.03312	sucrose synthase [Zea mays]	763	370	42	206	260
FGAS.03339	methionine synthase [Hordeum vulgare subsp. vulgare]	382	166	1109	638	480
FGAS.03354	splicing factor 4-like protein [Oryza sativa]	730	800	535	668	712
FGAS.03355	6-phosphofructo-2-kinase [Oryza sativa]	597	259	581	420	367
FGAS.03376	putative oxidoreductase [Oryza sativa]	1079	5007	13	2510	3342
FGAS.03397	permease; putative [Bacillus cereus ATCC 10987]	581	1398	10	704	935
FGAS.03401	putative protein kinase SPK-3 [Oryza sativa]	590	124	463	293	237
FGAS.03426	B1139B11.9 [Oryza sativa]	113	347	1593	970	762

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.03461	OSJNBa0070C17.13 [Oryza sativa]	498	412	42	227	289
FGAS.03467	hypothetical protein [Cicer arietinum]	1430	1396	670	1033	1154
FGAS.03477	none	613	549	27	288	375
FGAS.03480	none	439	1261	10	636	844
FGAS.03486	vacuolar proton-inorganic pyrophosphatase [Hordeum brevisubulatum]	807	232	1455	843	639
FGAS.03494	expressed protein [Oryza sativa]	10096	3846	5583	4714	4425
FGAS.03505	Histidinol-phosphate/aminotransferase [Dechloromonas aromatica RCB]	468	554	13	283	374
FGAS.03508	putative WD repeat protein [Oryza sativa]	438	754	10	382	506
FGAS.03518	putative 60S ribosomal protein L36 [Oryza sativa]	424	1078	10	544	722
FGAS.03549	putative alanine aminotransferase [Oryza sativa]	277	73	772	422	306
FGAS.03554	nucleic acid binding protein [Oryza sativa]	660	1246	210	728	901
FGAS.03606	similar to Dictyostelium discoideum (Slime mold). Spore coat protein SP96	722	421	754	588	532
FGAS.03623	PREDICTED: similar to KIAA1237 protein [Pan troglodytes]	1530	912	10	461	612
FGAS.03650	zinc finger-like [Oryza sativa]	238	409	102	255	307
FGAS.03670	salt-inducible putative protein serine/threonine/tyrosine kinase [Zea mays]	327	545	276	410	455
FGAS.03694	hemagglutinin	421	437	290	364	388
FGAS.03703	At5g51110/MWD22_5 [Arabidopsis thaliana]	1278	2842	12	1427	1899
FGAS.03765	putative gamma hydroxybutyrate dehydrogenase [Oryza sativa]	747	355	1026	690	579
FGAS.03836	peptidylprolyl isomerase [Triticum aestivum]	236	856	28	442	580
FGAS.03854	unnamed protein product [Oryza sativa]	927	539	1649	1094	909
FGAS.03957	unknown protein [Arabidopsis thaliana]	695	816	370	593	667
FGAS.03969	hypothetical protein 2 (rRNA external transcribed spacer) - mouse	912	500	1607	1053	869
FGAS.03978	membrane lipoprotein lipid attachment site-like [Oryza sativa]	711	162	1028	595	450
FGAS.04015	ubiquitin-activating enzyme E1-like [Oryza sativa]	548	778	487	633	681
FGAS.04018	OSJNBb0093G06.10 [Oryza sativa]	395	1983	42	1012	1336

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.04019	PB1 domain-containing protein [Oikopleura dioica]	1019	925	19	472	623
FGAS.04041	probable ubiquitin activating enzyme 2 [imported] - Picea mariana (fragment)	730	1332	10	671	892
FGAS.04044	diadenosine 5';5'''-P1;P4-tetraphosphate hydrolase [Hordeum vulgare]	772	10	5197	2603	1739
FGAS.04077	annexin p35 [Zea mays]	3358	3988	1162	2575	3046
FGAS.04094	putative potassium channel beta subunit [Oryza sativa]	1171	56	3128	1592	1080
FGAS.04101	putative protein phosphatase type-2C [Oryza sativa]	720	1064	398	731	842
FGAS.04125	cytosolic glutathione reductase [Triticum monococcum]	238	64	799	431	309
FGAS.04135	conserved hypothetical protein [Ralstonia eutropha]	1094	1384	479	932	1082
FGAS.04167	pyruvate dehydrogenase kinase 1 [Oryza sativa]	269	776	249	513	601
FGAS.04168	T10O24.10 [Arabidopsis thaliana]	326	599	15	307	405
FGAS.04177	csAtPR5 [Aegilops tauschii]	511	135	361	248	210
FGAS.04179	soluble acid invertase [Hordeum vulgare]	2302	833	2114	1473	1260
FGAS.04183	putative calcium-dependent protein kinase [Oryza sativa]	202	90	584	337	254
FGAS.04190	unknown [Oryza sativa]	479	733	14	374	494
FGAS.04194	sterol carrier protein 2-like [Oryza sativa]	1165	11	4549	2280	1524
FGAS.04207	OSJNBb0051N19.2 [Oryza sativa]	906	14	2959	1487	996
FGAS.04226	PREDICTED OJ1634_H04.108 gene product [Oryza sativa]	508	570	99	335	413
FGAS.04232	unknown protein [Oryza sativa]	467	16	1241	628	424
FGAS.04249	OSJNBa0067K08.18 [Oryza sativa]	828	1526	711	1118	1254
FGAS.04252	immunophilin [Zea mays]	1187	72	4015	2044	1386
FGAS.04281	chorismate synthase 2 [Lycopersicon esculentum]	284	192	465	329	283
FGAS.04295	dentin sialophosphoprotein-related [Arabidopsis thaliana]	183	1133	922	1028	1063
FGAS.04312	putative elongation factor 1 beta [Hordeum vulgare subsp. vulgare]	2022	4139	672	2405	2983
FGAS.04318	Malate dehydrogenase; glyoxysomal precursor	635	402	1300	851	701
FGAS.04337	putative protein phosphatase 2A 48 kDa regulatory subunit [Oryza sativa]	899	258	583	421	366

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.04375	high mobility group protein [Triticum aestivum]	360	42	630	336	238
FGAS.04393	putative Pib [Oryza sativa]	631	1131	13	572	758
FGAS.04400	pyrrolidone carboxyl peptidase-like protein [Oryza sativa]	358	573	225	399	457
FGAS.04416	hypothetical protein Krad07001071 [Kineococcus radiotolerans SRS30216]	592	1068	10	539	715
FGAS.04422	unknown [Arabidopsis thaliana]	400	545	282	413	457
FGAS.04423	putative disease resistance protein [Oryza sativa]	3548	1878	2230	2054	1995
FGAS.04429	histone H4 - wheat	317	1379	350	865	1036
FGAS.04446	unknown [Arabidopsis thaliana]	1307	1685	10	847	1126
FGAS.04517	serine protease-like protein [Oryza sativa]	516	74	1167	621	439
FGAS.04580	GLP_291_53790_56330 [Giardia lamblia ATCC 50803]	581	30	1354	692	471
FGAS.04591	codanin I [Homo sapiens]	2040	599	1554	1077	918
FGAS.04600	aldehyde dehydrogenase [Oryza sativa (indica cultivar-group)]	415	8	2018	1013	678
FGAS.04645	OSJNBa0053K19.8 [Oryza sativa]	787	20	620	320	220
FGAS.04660	chalcone isomerase [Hordeum vulgare subsp. vulgare]	2438	1048	2783	1915	1626
FGAS.04685	122aa long hypothetical protein [Pyrococcus horikoshii OT3]	714	949	10	479	636
FGAS.04701	At5g49900 [Arabidopsis thaliana]	229	442	256	349	380
FGAS.04703	hypothetical protein [Arabidopsis thaliana]	2993	1415	1562	1489	1464
FGAS.04722	unknown protein [Oryza sativa]	446	572	10	291	385
FGAS.04744	S-adenosylmethionine decarboxylase 2 [Oryza sativa]	352	520	11	265	350
FGAS.04749	cell wall protein Awa1p [Saccharomyces cerevisiae]	364	469	130	299	356
FGAS.04753	putative vacuolar protein sorting; Vps29p [Oryza sativa]	1269	1347	624	986	1106
FGAS.04802	ribosomal protein L19 [Triticum aestivum]	2288	1183	2338	1761	1568
FGAS.04811	hypothetical protein [Oryza sativa]	924	1003	313	658	773
FGAS.04901	putative transposable element [Oryza sativa]	1178	463	860	662	595
FGAS.04917	unknown protein [Arabidopsis thaliana]	576	13	1135	574	387

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.04940	peudo-response regulator-like [Oryza sativa]	514	150	285	218	195
FGAS.04963	OSJNBb0022F23.7 [Oryza sativa]	418	226	434	330	295
FGAS.05000	putative clathrin coat assembly protein AP17 [Oryza sativa]	868	49	2002	1025	700
FGAS.05016	expressed protein [Oryza sativa]	624	862	334	598	686
FGAS.05047	lipid-binding serum glycoprotein family protein [Arabidopsis thaliana]	504	249	383	316	294
FGAS.05062	OSJNBb0042I07.2 [Oryza sativa]	747	20	1411	716	484
FGAS.05144	putative endoxyloglucan transferase [Oryza sativa]	2300	805	1170	988	927
FGAS.05165	expressed protein (with alternative splicing) [Oryza sativa]	1109	401	744	572	515
FGAS.05227	hypothetical protein [Oryza sativa]	517	412	980	696	602
FGAS.05251	nonribosomal peptide synthetase [Streptomyces vinaceus]	487	13	857	435	294
FGAS.05258	OSJNBa0035M09.17 [Oryza sativa]	708	757	10	383	508
FGAS.05261	unknown protein [Oryza sativa]	2771	2238	10	1124	1496
FGAS.05273	unknown protein [Oryza sativa]	588	1001	10	506	671
FGAS.05296	OSJNBa0016O02.14 [Oryza sativa]	5503	756	4249	2502	1920
FGAS.05333	pyrimidine 5'-nucleotidase -related [Oryza sativa]	278	103	636	370	281
FGAS.05334	serine/threonine protein kinase [Oryza sativa]	308	19	1477	748	505
FGAS.05377	unknown protein [Oryza sativa]	855	3519	698	2109	2579
FGAS.05402	Structure Of Glutathione S-Transferase Iii In Apo Form	386	361	33	197	252
FGAS.05415	unnamed protein product [Oryza sativa]	865	1251	331	791	944
FGAS.05506	hypothetical protein [Oryza sativa]	728	15	2519	1267	850
FGAS.05518	unnamed protein product [Candida glabrata CBS138]	756	796	10	403	534
FGAS.05522	70 kDa heat shock protein [Arabidopsis thaliana]	186	667	116	392	484
FGAS.05533	putative H+/Ca2+ exchanger [Oryza sativa]	306	455	93	274	334
FGAS.05582	hypothetical protein [Oryza sativa]	384	770	10	390	516
FGAS.05585	lysophospholipase - like [Oryza sativa]	1567	686	3355	2020	1576

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.05593	putative Cell elongation DIMINUTO (Cell elongation Dwarf1) [Oryza sativa]	331	1805	10	908	1207
FGAS.05608	hypothetical protein [Oryza sativa]	642	1308	419	864	1012
FGAS.05613	clathrin assembly protein AP17-like protein [Oryza sativa]	597	677	15	346	456
FGAS.05657	phytoene dehydrogenase-like protein [Oryza sativa]	420	10	865	437	295
FGAS.05664	putative SHOOT1 protein [Oryza sativa]	1670	2101	85	1093	1429
FGAS.05700	putative purple acid phosphatase [Oryza sativa]	315	63	574	318	233
FGAS.05728	unknown protein [Oryza sativa]	507	421	155	288	332
FGAS.05736	gibberellin responsive protein	4970	8286	2214	5250	6262
FGAS.05765	putative peptidyl-prolycis-trans isomerase protein [Oryza sativa]	748	388	839	613	538
FGAS.05768	ferredoxin-NADP+ reductase [Oryza sativa]	719	1072	540	806	895
FGAS.05776	putative ubiquitin [Oryza sativa]	2127	2637	757	1697	2010
FGAS.05794	glycosyltransferase [Triticum aestivum]	578	798	86	442	560
FGAS.05814	putative auxin-repressed protein [Prunus armeniaca]	561	451	11	231	304
FGAS.05843	probable fructose-bisphosphate aldolase precursor; chloroplast - rice	11060	19642	12	9827	13099
FGAS.05941	TPA: class III peroxidase 135 precursor [Oryza sativa]	1021	638	1616	1127	964
FGAS.05953	CG17104-PA [Drosophila melanogaster]	1308	48	5242	2645	1779
FGAS.05968	putative SKP1 protein [Triticum aestivum]	1919	3058	727	1892	2281
FGAS.05981	putative serine/threonine protein kinase [Oryza sativa]	628	14	650	332	226
FGAS.06027	heat shock protein 80 [Triticum aestivum]	5454	14910	7511	11211	12444
FGAS.06079	protein H2B-6 [Triticum aestivum]	277	682	13	348	459
FGAS.06119	At1g12390 [Arabidopsis thaliana]	429	183	993	588	453
FGAS.06130	apoptosis-associated nuclear protein [Homo sapiens]	463	626	48	337	434
FGAS.06136	putative transmembrane protein [Arabidopsis thaliana]	436	156	979	567	430
FGAS.06175	unknown protein [Oryza sativa]	3110	1102	1704	1403	1303
FGAS.06187	putative uricase [Oryza sativa]	666	945	11	478	634

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.06204	unknown protein [Oryza sativa]	340	532	115	324	393
FGAS.06216	ovule development protein aintegumenta (ANT)-like [Oryza sativa]	432	648	190	419	495
FGAS.06226	putative NADPH-dependent retinol dehydrogenase/reductase [Oryza sativa]	622	1207	13	610	809
FGAS.06231	putative protein kinase [Oryza sativa]	409	560	85	323	402
FGAS.06261	putative ankyrin [Oryza sativa]	167	464	92	278	340
FGAS.06307	unknown protein [Oryza sativa]	351	168	449	308	261
FGAS.06368	SCAR2 -like [Oryza sativa]	667	284	932	608	500
FGAS.06381	unknown protein [Oryza sativa]	516	990	13	501	664
FGAS.06388	putative cellulose synthase catalytic subunit [Hordeum vulgare]	1553	638	1096	867	791
FGAS.06408	peroxisomal membrane protein OsPex14p [Oryza sativa]	483	646	12	329	435
FGAS.06435	unknown protein [Oryza sativa]	538	513	704	609	577
FGAS.06470	nuclear protein-like [Arabidopsis thaliana]	909	1393	806	1099	1197
FGAS.06525	none	716	1035	113	574	728
FGAS.06536	putative single-strand binding protein [Oryza sativa]	777	974	39	506	662
FGAS.06546	OSJNBa0044K18.10 [Oryza sativa]	576	860	10	435	577
FGAS.06561	P0460C04.14 [Oryza sativa]	533	622	12	317	419
FGAS.06595	hypothetical protein [Oryza sativa]	329	18	1028	523	355
FGAS.06641	hypothetical protein (repetitive element TCb1 No.10) - Caenorhabditis briggsae	2438	3661	10	1835	2444
FGAS.06781	Hypothetical protein CBG09801 [Caenorhabditis briggsae]	856	489	1091	790	690
FGAS.06785	26S proteasome regulatory particle triple-A ATPase subunit2b [Oryza sativa]	853	61	2081	1071	734
FGAS.06861	putative auxin-repressed protein [Prunus armeniaca]	619	58	798	428	305
FGAS.06871	gibberellin-induced receptor-like kinase TMK [Oryza sativa]	551	1614	686	1150	1305
FGAS.06913	pseudo-response regulator-like [Oryza sativa]	2388	430	1139	785	666
FGAS.06924	none	2592	157	23	90	112
FGAS.06976	putative actin related protein 2 [Oryza sativa]	356	655	13	334	441

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.06992	Auxin-binding protein 4 precursor (ABP)	410	639	13	326	430
FGAS.07033	late embryogenesis abundant protein [Picea glauca]	471	719	215	467	551
FGAS.07053	unknown protein [Oryza sativa]	501	830	12	421	557
FGAS.07074	hypothetical protein [Oryza sativa]	961	1046	505	775	865
FGAS.07086	putative ribosomal protein [Oryza sativa]	1086	765	12	389	514
FGAS.07087	heterokaryon incompatibility protein HET-C [Neurospora crassa]	381	384	117	251	295
FGAS.07092	putative 15.9 kDa subunit of RNA polymerase II [Oryza sativa]	429	793	12	403	533
FGAS.07102	guanylate kinase [Nicotiana tabacum]	964	1239	656	947	1044
FGAS.07114	kinesin heavy chain [Zea mays]	173	586	264	425	479
FGAS.07126	P0505D12.24 [Oryza sativa]	598	809	11	410	543
FGAS.07136	P0519D04.18 [Oryza sativa]	425	168	651	410	329
FGAS.07182	putative phytochrome P450 [Oryza sativa]	466	456	48	252	320
FGAS.07230	unnamed protein product [Tetraodon nigroviridis]	559	23	1292	658	446
FGAS.07252	aldehyde dehydrogenase [Oryza sativa]	301	737	16	376	496
FGAS.07270	unknown protein [Oryza sativa]	646	634	19	326	429
FGAS.07275	LOC445881 protein [Xenopus laevis]	554	393	15	204	267
FGAS.07297	putative calcium-dependent protein kinase [Oryza sativa]	413	419	926	672	588
FGAS.07351	B1078G07.35 [Oryza sativa]	259	134	388	261	219
FGAS.07404	putative actin-depolymerizing factor [Oryza sativa]	9640	12548	6463	9505	10519
FGAS.07415	putative vesicle transport v-SNARE protein [Oryza sativa]	1829	1167	2860	2014	1731
FGAS.07437	proteasome alpha subunit [Oryza sativa]	351	14	1726	870	584
FGAS.07480	db83 protein [Danio rerio]	616	22	1972	997	672
FGAS.07564	ripening-related protein-like [Oryza sativa]	464	192	629	411	338
FGAS.07589	similar to serine protease (AF097709) [Oryza sativa]	822	1320	10	665	883
FGAS.07603	Isoflavone reductase homolog IRL	390	19	542	281	193

		Mean of intensity levels*			Additive values	
Oligonucleotide	**Translated blast annotation**	***T. aestivum***	***T. turgidum***	***Ae. tauschii***	**$MPV_{1:1}$**	**$MPV_{2:1}$**
FGAS.07687	unknown protein [Oryza sativa]	400	986	16	501	663
FGAS.07705	hypothetical protein Krad07001202 [Kineococcus radiotolerans SRS30216]	937	6	3023	1514	1012
FGAS.07734	unknown protein [Oryza sativa]	313	526	162	344	405
FGAS.07746	P0701D05.19 [Oryza sativa]	427	416	261	338	364
FGAS.07824	OSJNBb0015G09.12 [Oryza sativa]	547	773	61	417	536
FGAS.07831	putative thylakoid lumenal 16.5 kDa protein [Oryza sativa]	647	163	511	337	279
FGAS.07843	putative Exopolygalacturonase precursor [Oryza sativa]	2994	2467	1044	1755	1993
FGAS.07893	cell division inhibitor-like [Oryza sativa]	501	133	438	285	235
FGAS.07907	hypothetical protein CNBC0740 [Cryptococcus neoformans B-3501A]	20362	7157	14634	10895	9649
FGAS.07926	unknown protein [Oryza sativa]	542	954	10	482	640
FGAS.07936	putative receptor-like protein kinase [Oryza sativa]	401	308	66	187	227
FGAS.07937	P0681B11.17 [Oryza sativa]	361	19	1113	566	384
FGAS.07985	unknown protein [Oryza sativa]	431	173	150	161	165
FGAS.08087	hypothetical protein PC102570.00.0 [Plasmodium chabaudi]	329	81	457	269	207
FGAS.08170	putative thioredoxin-like U5 small ribonucleoprotein [Oryza sativa]	2047	973	2409	1691	1451
FGAS.08206	AT5g13430/T22N19_80 [Arabidopsis thaliana]	507	271	645	458	395
FGAS.08233	urease [Oryza sativa (indica cultivar-group)]	213	79	955	517	371
FGAS.08248	none	551	211	1089	650	503
FGAS.08284	OSJNBa0070O11.6 [Oryza sativa]	337	168	666	417	334
FGAS.08335	somatic embryogenesis related protein [Dactylis glomerata]	456	465	329	397	420
FGAS.08363	transcription factor Hap5a-like protein [Arabidopsis thaliana]	1291	46	2055	1050	715
FGAS.08382	unknown protein [Oryza sativa]	902	1132	10	571	758
FGAS.08408	hypothetical protein [Oryza sativa]	960	1060	10	535	710
FGAS.08442	P0460H02.3 [Oryza sativa]	407	625	10	317	420
FGAS.08500	unknown protein [Oryza sativa]	288	73	561	317	236

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.08506	similar to vesicle transport protein [Oryza sativa]	252	1218	652	935	1030
FGAS.08550	OSJNBa0088H09.15 [Oryza sativa]	278	305	643	474	418
FGAS.08605	histone deacetylase HDA101 [Zea mays]	1000	1434	792	1113	1220
FGAS.08623	putative actin-binding protein [Oryza sativa]	478	849	12	431	570
FGAS.08639	P0451D05.23 [Oryza sativa]	679	420	901	660	580
FGAS.08641	putative mitochondrial carrier protein [Oryza sativa]	455	335	658	497	443
FGAS.08692	PREDICTED: similar to hypothetical protein [Homo sapiens]	600	795	10	402	533
FGAS.08714	COG4227: Antirestriction protein [Novosphingobium aromaticivorans]	484	753	36	395	514
FGAS.08726	caffeoyl-CoA O-methyltransferase [Oryza sativa]	260	567	10	289	382
FGAS.08733	OSJNBb0020J19.7 [Oryza sativa]	323	531	93	312	385
FGAS.08784	putative pyruvate dehydrogenase E1 alpha subunit [Oryza sativa]	1106	498	1908	1203	968
FGAS.08785	putative subtilisin-like proteinase [Oryza sativa]	1345	2718	1214	1966	2217
FGAS.08799	hypothetical protein [Oryza sativa]	354	93	395	244	193
FGAS.08826	putative conserved oligomeric Golgi complex component [Oryza sativa]	301	403	184	293	330
FGAS.08874	hypothetical protein SYNW1017 [Synechococcus sp. WH 8102]	402	10	805	408	275
FGAS.08889	unknown protein [Oryza sativa]	2054	1928	615	1272	1491
FGAS.08918	putative serine/threonine-specific protein kinase [Zea mays]	876	1230	458	844	973
FGAS.08953	PREDICTED OJ1081_B12.117 gene product [Oryza sativa]	351	388	223	305	333
FGAS.08991	profilaggrin - rat (fragment)	521	527	10	268	354
FGAS.09017	unknown protein [Oryza sativa]	931	1027	10	518	688
FGAS.09036	OSJNBa0043L09.30 [Oryza sativa]	449	663	490	577	606
FGAS.09090	putative inositol phosphate kinase [Oryza sativa]	519	873	10	441	585
FGAS.09096	ubiquitin-specific protease 8; putative (UBP8) [Arabidopsis thaliana]	443	700	429	565	610
FGAS.09156	putative cytochrome b561 [Oryza sativa]	710	754	15	384	507
FGAS.09169	OSJNBb0091E11.23 [Oryza sativa]	528	1058	524	791	880

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.09256	putative enhancer of rudimentary [Oryza sativa]	296	659	95	377	471
FGAS.09294	unknown protein [Oryza sativa]	899	191	397	294	260
FGAS.09345	hypothetical protein K02F6.9 - Caenorhabditis elegans	1357	2268	10	1139	1515
FGAS.09360	Wasp-binding protein [Mus musculus]	486	577	14	296	389
FGAS.09368	glutamate-ammonia ligase (EC 6.3.1.2); cytosolic - barley (fragment)	1333	2602	633	1617	1945
FGAS.09390	Ribosomal protein S7 [Hordeum vulgare subsp. vulgare]	284	742	10	376	498
FGAS.09394	silencing group B protein [Zea mays]	460	903	598	750	801
FGAS.09405	histone H3.2 protein [Oryza sativa]	4211	10437	15	5226	6963
FGAS.09478	OSJNBa0042I15.14 [Oryza sativa]	3379	4710	2418	3564	3946
FGAS.09487	hypothetical protein [Pisum sativum]	735	1098	10	554	736
FGAS.09542	P0439E07.14 [Oryza sativa]	1347	476	946	711	632
FGAS.09592	cysteine endopeptidase precursor [Ricinus communis]	415	19	1691	855	576
FGAS.09650	potyviral capsid protein interacting protein 2a [Nicotiana tabacum]	787	329	1181	755	613
FGAS.09669	troponin T [Mizuhopecten yessoensis]	1197	1913	10	961	1279
FGAS.09695	hypothetical protein glr2037 [Gloeobacter violaceus PCC 7421]	266	13	965	489	330
FGAS.09741	unknown protein [Oryza sativa]	1122	689	1931	1310	1103
FGAS.09771	putative growth regulator [Oryza sativa]	275	25	745	385	265
FGAS.09780	DNA binding protein [Triticum aestivum]	645	1185	13	599	794
FGAS.09793	PREDICTED P0048D08.116 gene product [Oryza sativa]	389	603	19	311	408
FGAS.09877	P0679C12.24 [Oryza sativa]	540	177	996	586	450
FGAS.09929	TPA: cellulose synthase-like C1 [Oryza sativa]	329	602	186	394	463
FGAS.09943	SCAR2 -like [Oryza sativa]	252	116	558	337	263
FGAS.09979	TPA: PDR4 ABC transporter [Arabidopsis thaliana]	889	1340	13	677	898
FGAS.09988	putative SP3D [Oryza sativa]	683	891	11	451	598
FGAS.10103	putative cellulose synthase catalytic subunit [Hordeum vulgare]	543	155	525	340	278

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
FGAS.10146	unknown protein [Oryza sativa]	281	546	100	323	397
FGAS.10150	isoamylase precursor; glycogen 6-glucanohydrolase [Triticum aestivum]	698	1327	363	845	1006
FGAS.10187	small heat shock protein Hsp23.6 [Triticum aestivum]	264	411	296	353	372
lcl|TC147236	Actin; Wheat - Positive Control	537	643	301	472	529
lcl|TC147663	Tubulin beta-2; Wheat - Positive Control	566	929	10	470	623
lcl|TC170782	70 kDa heat shock-related protein; Wheat - Positive Control	381	192	451	322	279
lcl|TC174039	RuBisCo subunit binding protein beta subunit; Wheat - Positive Control	514	621	130	376	458
USDAWHE.00015	P0503C12.12 [Oryza sativa]	737	393	1159	776	648
USDAWHE.00116	ATP synthase C chain (Lipid-binding protein) (Subunit III)	2477	1865	6640	4252	3457
USDAWHE.00117	none	3412	2700	8711	5706	4704
USDAWHE.00119	fatty aldehyde dehydrogenase 1 [Zea mays]	662	636	35	335	436
USDAWHE.00122	peptide transport protein - barley	505	8	2316	1162	777
USDAWHE.00165	putative cytochrome P450 [Oryza sativa]	271	783	87	435	551
USDAWHE.00168	putative NIC2 [Oryza sativa]	234	108	695	402	304
USDAWHE.00171	Putative DEAD/DEAH box RNA helicase protein [Oryza sativa]	374	883	10	447	592
USDAWHE.00180	Putative ABC transporter [Oryza sativa]	632	402	1032	717	612
USDAWHE.00187	P0665A11.11 [Oryza sativa]	628	866	18	442	583
USDAWHE.00204	OSJNBa0060N03.9 [Oryza sativa]	137	1558	10	784	1042
USDAWHE.00222	unnamed protein product [Oryza sativa]	554	423	18	221	288
USDAWHE.00239	serine/threonine protein kinase SAPK9 [Oryza sativa]	787	163	676	420	334
USDAWHE.00320	DNA topoisomerase II [Arabidopsis thaliana]	128	766	416	591	650
USDAWHE.00433	SMC4 protein [Oryza sativa]	152	557	241	399	452
USDAWHE.00590	putative glycoprotein 3-alpha-L-fucosyltransferase [Triticum aestivum]	294	113	672	392	299
USDAWHE.00591	bifunctional nuclease [Zinnia elegans]	434	78	1360	719	505
USDAWHE.00599	OSJNBa0006A01.2 [Oryza sativa]	299	555	25	290	378

		Mean of intensity levels*			Additive values	
Oligonucleotide	**Translated blast annotation**	***T. aestivum***	***T. turgidum***	***Ae. tauschii***	**$MPV_{1:1}$**	**$MPV_{2:1}$**
USDAWHE.00633	unknown protein [Oryza sativa]	2380	3530	1596	2563	2885
USDAWHE.00677	Putative serine/threonine protein kinase [Oryza sativa]	214	52	724	388	276
USDAWHE.00743	putative Hydroquinone glucosyltransferase [Oryza sativa]	694	188	1770	979	715
USDAWHE.00758	putative nuclease I [Oryza sativa]	375	735	44	390	505
USDAWHE.00768	putative Nuclear pore complex protein Nup155 [Oryza sativa]	280	437	704	571	526
USDAWHE.00772	unknown protein [Oryza sativa]	592	201	639	420	347
USDAWHE.00799	hypothetical protein [Oryza sativa]	410	10	1126	568	382
USDAWHE.00837	putative farnesyl-pyrophosphate synthetase [Oryza sativa]	208	25	1012	519	354
USDAWHE.00855	putative SNF5 homolog BSH (bsh) [Oryza sativa]	349	497	65	281	353
USDAWHE.00922	putative mitochondrial carrier [Oryza sativa]	672	397	858	628	551
USDAWHE.01022	unknown protein [Oryza sativa]	447	364	764	564	497
USDAWHE.01063	OSJNBa0088I22.12 [Oryza sativa]	380	329	917	623	525
USDAWHE.01121	putative naphthoate synthase [Oryza sativa]	244	41	914	477	332
USDAWHE.01229	csAtPR5 [Aegilops tauschii]	320	535	20	277	363
USDAWHE.01279	putative leucine zipper protein [Oryza sativa]	352	544	290	417	459
USDAWHE.01282	major facilitator superfamily antiporter [Oryza sativa]	755	209	1177	693	531
USDAWHE.01292	putative mitogen-activated protein kinase 4 [Oryza sativa]	322	106	407	256	206
USDAWHE.01308	putative polygalacturonase [Oryza sativa]	473	130	496	313	252
USDAWHE.01324	putative cellulose synthase catalytic subunit [Hordeum vulgare]	1149	777	1713	1245	1089
USDAWHE.01432	unknown protein [Arabidopsis thaliana]	330	487	95	291	357
USDAWHE.01446	alanyl-tRNA synthetase [Arabidopsis thaliana]	636	360	631	495	450
USDAWHE.01447	putative CER3 [Oryza sativa]	1264	1125	670	898	974
USDAWHE.01456	putative glutathione S-transferase [Oryza sativa]	437	566	15	290	382
USDAWHE.01474	putative protein kinase [Oryza sativa]	1463	531	1484	1007	849
USDAWHE.01509	plastid sigma factor SIG5 [Oryza sativa]	105	856	259	558	657

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
USDAWHE.01598	hypothetical protein [Oryza sativa]	4487	1686	2507	2096	1959
USDAWHE.01674	pollen allergen Tri a 4 [Triticum aestivum]	2494	1270	3000	2135	1846
USDAWHE.01742	putative zinc binding protein [Oryza sativa]	580	468	244	356	393
USDAWHE.01748	putative dihydroneopterin aldolase [Oryza sativa]	397	503	42	273	349
USDAWHE.01749	TAF12 [Arabidopsis thaliana]	1267	1383	658	1020	1141
USDAWHE.01778	hypothetical protein [Oryza sativa]	505	645	10	327	433
USDAWHE.01783	putative dehydration-responsive protein RD22 [Oryza sativa]	647	616	301	458	511
USDAWHE.01833	putative disease resistance protein/ dirigent protein-related [Oryza sativa]	595	1022	206	614	750
USDAWHE.01855	putative WD40 repeat protein [Oryza sativa]	337	386	170	278	314
USDAWHE.01861	OJ1656_A11.3 [Oryza sativa]	352	14	421	217	150
USDAWHE.01866	OSJNBa0006A01.3 [Oryza sativa]	667	585	21	303	397
USDAWHE.02018	Hypothetical protein [Oryza sativa]	560	137	781	459	352
USDAWHE.02072	putative chalcone synthase [Oryza sativa]	162	978	24	501	660
USDAWHE.02122	putative cytochrome P450 [Oryza sativa]	999	512	1617	1065	880
USDAWHE.02161	putative ARE1 protein [Oryza sativa]	390	62	1064	563	396
USDAWHE.02162	putative immunophilin / peptidyl-prolyl cis-trans isomerase [Oryza sativa]	228	60	656	358	259
USDAWHE.02163	putative ABA-responsive protein [Oryza sativa]	658	456	1172	814	695
USDAWHE.02208	B1015E06.23 [Oryza sativa]	1165	1069	14	541	717
USDAWHE.02215	cell wall invertase 1 [Oryza sativa (indica cultivar-group)]	163	385	73	229	281
USDAWHE.02229	unknown [Oryza sativa]	557	924	17	470	621
USDAWHE.02231	hypothetical protein [Oryza sativa]	316	36	755	396	276
USDAWHE.02281	hypothetical protein [Oryza sativa]	388	702	10	356	472
USDAWHE.02285	unknown protein [Oryza sativa]	581	608	242	425	486
USDAWHE.02363	hypothetical protein [Oryza sativa]	1251	1753	62	907	1189
USDAWHE.02367	Unknown protein [Oryza sativa]	370	691	15	353	466

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
USDAWHE.02370	putative GA2-oxidase [Oryza sativa]	6141	2745	6874	4809	4121
USDAWHE.02386	glutaredoxin-like [Oryza sativa]	626	780	18	399	526
USDAWHE.02406	18S subunit ribosomal protein [Triticum aestivum]	443	279	698	488	418
USDAWHE.02446	unnamed protein product [Arabidopsis thaliana]	899	1018	234	626	757
USDAWHE.02447	selenium-binding protein-like [Oryza sativa]	2984	1501	3048	2275	2017
USDAWHE.02466	unknown protein [Oryza sativa]	1293	3164	60	1612	2130
USDAWHE.02554	protein kinase homolog - rice	844	155	815	485	375
USDAWHE.02564	PREDICTED OJ1699_E05.22-1 gene product [Oryza sativa]	649	702	287	495	564
USDAWHE.02574	putative protein kinase [Oryza sativa]	390	1044	20	532	703
USDAWHE.02787	flavonoid 3';5'-hydroxylase [Eustoma grandiflorum]	218	46	764	405	285
USDAWHE.02876	putative Hsp70 binding protein [Oryza sativa]	189	914	358	636	729
USDAWHE.02888	unnamed protein product [Oryza sativa]	1096	180	18	99	126
USDAWHE.02919	unknown [Arabidopsis thaliana]	316	101	580	340	261
USDAWHE.02927	calmodulin-like [Oryza sativa]	409	633	55	344	441
USDAWHE.02998	fructose-1; 6-bisphosphatase [Saccharum hybrid cultivar H65-7052]	475	12	1568	790	530
USDAWHE.03020	OSJNBb0034I13.13 [Oryza sativa]	1756	542	1233	887	772
USDAWHE.03098	unknown protein [Oryza sativa]	860	11	2096	1054	706
USDAWHE.03113	putative drought-induced protein [Oryza sativa]	257	51	1214	633	439
USDAWHE.03120	OSJNBa0038O10.23 [Oryza sativa]	976	268	1800	1034	779
USDAWHE.03170	putative pectinacetylesterase [Oryza sativa]	733	577	1392	984	848
USDAWHE.03176	putative auxin response factor 7a [Oryza sativa]	1400	41	4022	2031	1368
USDAWHE.03193	P0439E11.13 [Oryza sativa]	1335	708	580	644	665
USDAWHE.03214	unknown protein [Oryza sativa]	350	115	277	196	169
USDAWHE.03224	putative disease resistance protein [Oryza sativa]	914	450	1623	1036	841
USDAWHE.03234	chloride channel [Oryza sativa]	448	107	831	469	349

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
USDAWHE.03335	similarity to ABC transporter gb|U92650 [Arabidopsis thaliana]	408	941	501	721	794
USDAWHE.03337	At2g20920/F5H14.11 [Arabidopsis thaliana]	866	803	204	504	604
USDAWHE.03471	putative cyclophilin (70.8 kD) (cyp-15) [Oryza sativa]	418	278	670	474	409
USDAWHE.03492	ubiquitin-conjugating enzyme [Oryza sativa]	334	792	27	410	537
USDAWHE.03504	putative ubiquitin carboxyl-terminal hydrolase [Arabidopsis thaliana]	349	406	197	301	336
USDAWHE.03515	OSJNBa0063C18.20 [Oryza sativa]	254	35	855	445	308
USDAWHE.03527	unnamed protein product [Oryza sativa]	492	1137	13	575	762
USDAWHE.03535	unknown protein [Oryza sativa]	308	517	174	345	403
USDAWHE.03577	putative high-affinity potassium transporter [Hordeum vulgare]	138	628	443	536	566
USDAWHE.03603	putative phosphofructokinase alpha subunit [Oryza sativa]	427	67	409	238	181
USDAWHE.03631	PREDICTED OJ1150_A11.17 gene product [Oryza sativa]	442	115	292	203	174
USDAWHE.03687	Rar1 [Hordeum vulgare]	223	49	938	493	345
USDAWHE.03747	salt-inducible protein kinase [Zea mays]	612	231	1154	692	538
USDAWHE.03755	unknown protein [Oryza sativa]	2566	3010	1765	2387	2595
USDAWHE.03770	B1051E10.38 [Oryza sativa]	1028	1044	478	761	855
USDAWHE.03826	hypothetical protein [Oryza sativa]	514	1014	10	512	679
USDAWHE.03854	putative Potential phospholipid-transporting ATPase 8 [Oryza sativa]	326	114	545	330	258
USDAWHE.03889	P0020E09.16 [Oryza sativa]	290	451	31	241	311
USDAWHE.03913	OSJNBa0070M12.6 [Oryza sativa]	247	21	1071	546	371
USDAWHE.03927	unknown protein [Oryza sativa]	821	888	218	553	665
USDAWHE.03929	OSJNBa0006M15.13 [Oryza sativa]	551	1080	10	545	723
USDAWHE.03933	sarcoplasmic reticulum protein (with alternative splicing) [Oryza sativa]	881	1953	13	983	1307
USDAWHE.03971	putative RNA helicase [Oryza sativa]	1100	415	1097	756	642
USDAWHE.04015	MADS6 [Lolium perenne]	247	55	2690	1372	933
USDAWHE.04040	apospory-associated protein C-like [Oryza sativa]	589	28	1381	704	479

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
USDAWHE.04057	P0421H07.9 [Oryza sativa]	329	411	13	212	279
USDAWHE.04082	putative LytB protein [Oryza sativa]	1745	2668	520	1594	1952
USDAWHE.04083	putative endo-1;3;1;4-beta-D-glucanase' [Oryza sativa]	636	544	71	307	386
USDAWHE.04104	probable beta-1;3-glucanase (EC 3.2.1.-) - wheat	34025	14197	17578	15887	15324
USDAWHE.04152	expressed protein [Oryza sativa]	511	1651	10	831	1104
USDAWHE.04169	unnamed protein product [Oryza sativa]	1335	641	10	326	431
USDAWHE.04175	putative protein kinase [Oryza sativa]	1455	1324	758	1041	1135
USDAWHE.04215	putative MATE efflux family protein [Oryza sativa]	335	671	11	341	451
USDAWHE.04271	OSJNBa0010D21.13 [Oryza sativa]	277	117	630	373	288
USDAWHE.04343	alpha-N-acetylglucosaminidase [Nicotiana tabacum]	275	137	410	273	228
USDAWHE.04347	putative DNA-binding protein [Oryza sativa]	647	717	214	466	550
USDAWHE.04374	unknown protein [Oryza sativa]	601	763	346	554	624
USDAWHE.04386	hypothetical protein [Oryza sativa]	870	33	1904	968	657
USDAWHE.04398	phosphate transporter 6 [Hordeum vulgare subsp. vulgare]	755	76	2894	1485	1015
USDAWHE.04487	isoflavone reductase-like protein	1979	609	4206	2407	1808
USDAWHE.04492	putative plastid ribosomal protein S6 precursor [Oryza sativa]	791	1102	10	556	738
USDAWHE.04499	unnamed protein product [Oryza sativa]	314	220	795	507	411
USDAWHE.04525	putative heat shock protein [Oryza sativa]	253	587	195	391	456
USDAWHE.04539	OSJNBa0043L09.24 [Oryza sativa]	495	69	1159	614	432
USDAWHE.04549	putative ankyrin-like protein [Oryza sativa]	446	756	15	386	509
USDAWHE.04569	putative vacuolar sorting receptor protein [Oryza sativa]	526	80	1121	601	427
USDAWHE.04572	hypothetical protein [Oryza sativa]	231	7	1673	840	562
USDAWHE.04600	similar to AP2 domain containing protein RAP2.10 (AF003103) [Oryza sativa]	373	629	21	325	426
USDAWHE.04629	stem rust resistance protein [Hordeum vulgare subsp. vulgare]	369	21	895	458	312
USDAWHE.04658	unknown protein [Oryza sativa]	883	110	2276	1193	832

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
USDAWHE.04680	unknown protein [Oryza sativa]	2030	2670	1148	1909	2163
USDAWHE.04748	putative calcium sensor protein [Oryza sativa]	399	652	13	333	439
USDAWHE.04760	unknown [Arabidopsis thaliana]	330	570	44	307	395
USDAWHE.04787	putative protein [Arabidopsis thaliana]	357	586	22	304	398
USDAWHE.04807	putative ribulokinase [Oryza sativa]	562	191	438	315	273
USDAWHE.04811	OSJNBa0089N06.22 [Oryza sativa]	577	1035	655	845	908
USDAWHE.04825	B1358B12.20 [Oryza sativa]	531	902	70	486	625
USDAWHE.04848	putative urease accessory protein G [Oryza sativa]	296	11	1113	562	378
USDAWHE.04865	PREDICTED P0048D08.116 gene product [Oryza sativa]	1081	3	2967	1485	991
USDAWHE.04983	putative peroxidase [Oryza sativa]	441	791	292	542	625
USDAWHE.04994	putative mature anther-specific protein LAT61 [Oryza sativa]	280	484	37	261	335
USDAWHE.04995	putative protein of gibberellin-stimulated transcript [Oryza sativa]	1238	5036	282	2659	3451
USDAWHE.05001	putative ubiquitin carrier protein E2 [Oryza sativa]	374	481	72	277	345
USDAWHE.05030	unknown protein [Oryza sativa]	1226	1554	12	783	1040
USDAWHE.05045	Putative leucine-rich repeat transmembrane protein kinase [Oryza sativa]	974	2187	10	1099	1462
USDAWHE.05079	unknown protein [Oryza sativa]	653	604	267	436	492
USDAWHE.05084	P0446G04.14 [Oryza sativa]	1207	1485	520	1002	1163
USDAWHE.05135	Putative GATA-1 zinc finger protein [Oryza sativa]	638	546	1466	1006	853
USDAWHE.05152	auxin-binding protein - oat	446	669	10	339	449
USDAWHE.05201	Hypothetical protein [Oryza sativa]	267	1453	65	759	990
USDAWHE.05218	putative acetyl-CoA C-acyltransferase [Oryza sativa]	778	56	2293	1175	802
USDAWHE.05245	putative ubiquitin-conjugating enzyme E2 [Oryza sativa]	420	631	10	321	424
USDAWHE.05260	putative peroxisomal membrane protein 22 kDa [Oryza sativa]	664	929	157	543	672
USDAWHE.05272	kinesin heavy chain [Zea mays]	562	126	916	521	389
USDAWHE.05273	unknown protein [Oryza sativa]	351	451	18	234	307

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
USDAWHE.05291	2-on-2 hemoglobin [Hordeum vulgare]	421	851	10	430	571
USDAWHE.05320	putative basic blue copper protein [Oryza sativa]	287	1022	10	516	684
USDAWHE.05344	laccase LAC5-6 [Lolium perenne]	566	120	802	461	347
USDAWHE.05356	GDSL-motif lipase/hydrolase-like [Oryza sativa]	337	228	638	433	365
USDAWHE.05369	S-adenosylmethionine synthetase [Oryza sativa]	1036	789	2252	1520	1277
USDAWHE.05427	OSJNBa0084K01.16 [Oryza sativa]	269	124	908	516	386
USDAWHE.05441	Putative fiddlehead-like protein [Oryza sativa]	477	662	45	354	457
USDAWHE.05451	alpha-tubulin [Nicotiana tabacum]	301	108	486	297	234
USDAWHE.05462	hypothetical protein [Oryza sativa]	600	480	41	261	334
USDAWHE.05475	unknown protein [Oryza sativa]	408	604	19	312	409
USDAWHE.05477	serine carboxypeptidase III; CP-MIII [Hordeum vulgare subsp. vulgare]	4603	4960	13450	9205	7790
USDAWHE.05491	receptor protein kinase PERK1-like protein [Oryza sativa]	246	497	216	356	403
USDAWHE.05499	OSJNBa0020P07.8 [Oryza sativa]	1086	1201	623	912	1008
USDAWHE.05508	putative serine palmitoyltransferase [Oryza sativa]	21381	23666	10154	16910	19162
USDAWHE.05517	unknown protein [Arabidopsis thaliana]	331	17	960	489	331
USDAWHE.05528	putative receptor-like kinase Xa21-binding protein 3 [Oryza sativa]	849	8	2851	1430	956
USDAWHE.05529	unknown protein [Oryza sativa]	428	671	10	341	451
USDAWHE.05546	thioredoxin-like [Oryza sativa]	584	17	2129	1073	721
USDAWHE.05556	ubiquitin activating enzyme-like protein [Arabidopsis thaliana]	1109	1909	456	1183	1425
USDAWHE.05574	putative 4-alpha-glucanotransferase [Oryza sativa]	996	1739	140	940	1206
USDAWHE.05594	putative chlorophyll a/b-binding protein type II [Oryza sativa]	1656	178	1030	604	462
USDAWHE.05602	putative beta-alanine synthase [Oryza sativa]	1110	1861	10	936	1244
USDAWHE.05720	OSJNBa0063C18.19 [Oryza sativa]	1118	1895	945	1420	1579
USDAWHE.05735	PREDICTED P0724B10.24 gene product [Oryza sativa]	832	213	706	460	378
USDAWHE.05797	putative NADPH:adrenodoxin oxidoreductase precursor [Oryza sativa]	282	25	918	471	323

Oligonucleotide	Translated blast annotation	Mean of intensity levels*			Additive values	
		T. aestivum	*T. turgidum*	*Ae. tauschii*	$MPV_{1:1}$	$MPV_{2:1}$
USDAWHE.05802	nuclear protein-like [Arabidopsis thaliana]	425	907	11	459	609
USDAWHE.05831	putative Myb-related transcription factor [Oryza sativa]	7048	3729	5069	4399	4176
USDAWHE.05908	hypothetical protein [Oryza sativa]	566	690	12	351	464
USDAWHE.05909	putative Hsp70 binding protein [Oryza sativa]	3132	7111	1774	4443	5332
USDAWHE.05935	OSJNBa0004N05.2 [Oryza sativa]	250	1005	106	555	705
USDAWHE.05946	histidyl-tRNA synthetase [Triticum aestivum]	205	575	529	552	560
USDAWHE.05947	putative NF-E2 inducible protein [Oryza sativa]	140	750	238	494	579
USDAWHE.05950	OSJNBa0052O12.6 [Oryza sativa]	655	301	419	360	340
USDAWHE.06010	putative SP3D [Oryza sativa]	1381	15	5238	2626	1756
USDAWHE.06066	Similar to GDP-mannose pyrophosphorylase A [Arabidopsis thaliana]	315	656	41	349	451
USDAWHE.06106	unknown protein [Arabidopsis thaliana]	666	669	125	397	488
USDAWHE.06153	MAP kinase-like protein [Oryza sativa]	693	737	32	384	502
USDAWHE.06196	LEDI-5c protein [Lithospermum erythrorhizon]	833	967	10	488	648
USDAWHE.06304	type 1 rice metallothionein-like gene [Oryza sativa]	1278	1724	144	934	1198
USDAWHE.06413	putative dolichol-phosphate (beta-D) mannosyltransferase 2 [Oryza sativa]	497	10	1523	766	514
USDAWHE.06438	predicted protein [Magnaporthe grisea 70-15]	295	14	854	434	294
USDAWHE.06455	putative aldehyde dehydrogenase [Arabidopsis thaliana]	610	467	108	287	347
USDAWHE.06478	hypothetical protein FG06967.1 [Gibberella zeae PH-1]	883	985	28	506	666
USDAWHE.06526	It is a member of GTP1/OBG family PF|01018. [Arabidopsis thaliana]	484	916	29	472	620
USDAWHE.06535	hypothetical protein [Phaseolus vulgaris]	873	267	476	372	337
USDAWHE.06607	P0414E03.8 [Oryza sativa]	368	670	15	342	452
USDAWHE.06650	putative dehydrogenase precursor [Oryza sativa]	214	437	193	315	356
USDAWHE.06694	seven transmembrane protein Mlo4 [Zea mays]	857	596	14	305	402
USDAWHE.06706	putative SEC23 [Oryza sativa]	326	58	715	387	277
USDAWHE.06826	putative Band 7 protein [Oryza sativa]	739	2442	10	1226	1631

		Mean of intensity levels*			Additive values	
Oligonucleotide	**Translated blast annotation**	***T. aestivum***	***T. turgidum***	***Ae. tauschii***	**$MPV_{1:1}$**	**$MPV_{2:1}$**
USDAWHE.06880	ASR-like protein 1 [Hevea brasiliensis]	2641	342	1752	1047	812
USDAWHE.06887	putative permease 1 [Oryza sativa]	1021	707	3270	1989	1561
USDAWHE.06899	putative glyoxysomal fatty acid beta-oxidation protein [Oryza sativa]	537	13	2097	1055	707
USDAWHE.06928	putative dehydrogenase precursor [Oryza sativa]	250	492	348	420	444
USDAWHE.06929	putative chloroplastic RNA-binding protein [Oryza sativa]	888	1287	10	648	861
USDAWHE.06966	P0468B07.18 [Oryza sativa]	10511	4393	8905	6649	5897
USDAWHE.07013	unknown protein [Oryza sativa]	456	340	780	560	487
USDAWHE.07029	OsCDPK protein [Oryza sativa]	715	495	53	274	348
USDAWHE.07051	calcium-dependent protein kinase ZmCPK11 [Zea mays]	347	498	167	332	387
USDAWHE.07096	ethylene-responsive factor EF-Ts precursor [Lycopersicon esculentum]	665	1046	208	627	766
USDAWHE.07099	aldehyde oxidase (EC 1.2.3.1) 2 - maize	660	58	1308	683	474
USDAWHE.07103	OSJNBa0004N05.7 [Oryza sativa]	380	622	46	334	430
USDAWHE.07105	putative ABA-induced protein [Cynodon dactylon]	314	124	587	356	279
USDAWHE.07109	unknown protein [Oryza sativa]	529	814	391	603	673
USDAWHE.07114	putative GPI-anchored protein [Oryza sativa]	1284	670	35	353	459
USDAWHE.07149	diadenosine 5';5'''-P1;P4-tetraphosphate hydrolase [Hordeum vulgare]	1522	2182	11	1096	1458
USDAWHE.07152	hypothetical protein UM02227.1 [Ustilago maydis 521]	588	805	395	600	669
USDAWHE.07164	similar to ethylene-forming-enzyme-like dioxygenase. [Oryza sativa]	894	496	958	727	650
USDAWHE.07175	putative CDPK-related protein kinase [Oryza sativa]	811	1297	815	1056	1136

* Mean intensity values calculated from 12 hybridization events (two features/slide x three biological replicates/treatment x two treatments/genotype)