Suppression Subtractive Hybridization Technique in Wheat for the Identification of Disease Resistance Differentially Expressed Genes

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Abstract – A resistant variety with high yielding potential is key for increasing crop production to fulfill the food requirement of the ever increasing world populations. Consequently, the aim of plant breeders is to develop high yielding varieties or cultivars that are resistant or tolerant to specific diseases or insects. For developing a resistant variety, it is enormously indispensable to incorporate or introgress the specific resistant genes of that particular disease into the recipient. Suppression subtractive hybridization (SSH) is a powerful technique for the identification of disease specific differentially expressed genes that are expressed in a resistant or susceptible variety. This paper presents a brief review on the SSH technique with examples focusing on the identification of the wheat disease specific differentially expressed genes and their defense mechanisms against fungal pathogens in global wheat cultivars. This review is helpful for wheat researchers for the updated information on the SSH technique for the identification of differentially expressed genes in the global wheat cultivars and varieties. Eventually, the identified genes could be used to develop the disease resistance variety through marker-assisted backcrossing programme or conventional breeding.

Keywords: Differentially expressed genes, expressed sequence tags (ESTs), resistance variety, Suppression subtractive hybridization (SSH), *Triticum aestivum*

Introduction

Wheat is among the top cereal crops and it ranks first in global crop production (Cao et al., 2011). More than half of the total world population depends on wheat to meet their daily food demand. Wheat is affected by several fungal, bacterial and viral diseases. Among these diseases, leaf rust and leaf blight e.g. Bipolaris leaf blight (Bplb) are the major and common fungal diseases distributed worldwide. The aim of plant breeders is to develop high yielding varieties or cultivars that are resistant or tolerant to specific diseases or insects. To develop a resistant variety, it is necessary to incorporate or introgress the resistant gene of that particular disease into the recipient.

The suppression subtractive hybridization (SSH) method is valuable for identifying differentially regulated genes important for cellular growth and differentiation. It is a widely used method for separating DNA molecules that distinguish two closely related DNA samples. Two of the main SSH applications are cDNA subtraction and genomic DNA subtraction. SSH is one of the most powerful and popular methods for generating subtracted cDNA or genomic DNA libraries. It is based primarily on a suppression polymerase chain reaction (PCR) technique and combines normalization and subtraction in a single procedure. The normalization step equalizes the abundance of DNA fragments within the target population, and the subtraction step excludes sequences that are common to the populations being compared. This dramatically increases the probability of obtaining low-abundance differentially expressed cDNAs or genomic DNA fragments, and simplifies the analysis of the subtracted library. The SSH technique is applicable to many comparative and functional genetic

studies for the identification of disease and developmental, tissue-specific or other differentially expressed genes as well as for the recovery of genomic DNA fragments distinguishing the samples under comparison. The SSH technique may result in over 1000-fold enrichment for rare sequences in a single round of subtractive hybridization (Lukyanov et al., 2007).

Differentially expressed genes are those whose expressions are limited to a particular tissue or stage or whose expression patterns changed after a stimulus is received by the organism (Habib, 2011). They are of interest because they often have important functional roles in specific stage or tissue such as attack of wheat plant by leaf blight and leaf rust pathogens at the vegetative stage in which they are expressed (Jones & Harrower, 1998).

The level of expression of all genes of an organism in different types of cells, tissues, developmental stages, or disease processes constitutes essential information for understanding the function of different genes, and to unravel the complex network of biological processes acting in every biological system (Eickhoff et al., 2000). Several challenges pertaining to gene expression studies take account of low abundance, tissue-specific or stage-specific gene expression patterns. By means of a normal gene isolation method such as cDNA library screening, these genes are the most likely overlooked during the isolation process. Therefore, these genes are the most likely to be missing in the database. A number of approaches can be used to increase the opportunity of isolating tissue-specific, stage-specific and low abundance genes by collecting the sample at a particular period of time e.g. leaf blight attack at the vegetative stage and smut disease at the heading stage, manipulating the growth condition e.g. to make the plant more resistant by applying a silicon fertilizer or make the plant more susceptible by using a nitrogen fertilizer to express the particular genes, and pulling specific tissues to increase the mRNA level for the low abundance gene. Studies of such genes are promising as they include key regulatory factors responsible for differentiated phenotypes, developmental progression, or cell growth regulation (Martin & Pardee, 2000).

In SSH two populations of samples, one treated and one controlled, are generally used to compare the expression of genes. The mRNA which is obtained from total RNA, are used as a starting material. First, both mRNA populations are converted into cDNA: the cDNA that contains specific (differentially expressed) transcripts referred as the tester, and the reference cDNA as the driver. The tester and driver cDNAs are hybridized, and the hybrid sequences are then removed. Consequently, the remaining unhybridized cDNAs represent genes that are expressed in the tester yet absent from the driver mRNA. The process is shown in *Figure 1*.

Comparison of SSH with other methods

Several molecular methods exist for isolating low-abundance genes and for identifying differentially expressed genes among biological samples, namely differential display reverse transcription polymerase chain reaction (DDRT-PCR) (Liang & Pardee, 1992), serial analysis of gene expression (SAGE) (Velculescu et al., 1995), microarrays (Shena et al., 1995), and subtraction and normalization method called suppression subtractive hybridization (SSH) (Diatchenko et al., 1996). Each of the methods has specific advantages and each also has inherent disadvantages.

The differential display (DDRT-PCR) method developed by Liang and Pardee (1992) uses a combination of arbitrary PCR primers and oligo (dT)-anchored cDNA primers to amplify cDNA sequences by reverse transcription of mRNA followed by PCR. This method is simpler, quicker and more sensitive and is widely used for the identification of differentially expressed genes when the RNA sample is limited such as in pre-implantation embryos. Although this method requires a relatively small amount of RNA sample, false positive results can generate a large number of false sequences that do not represent differentially expressed genes. The cDNA-AFLP method is sensitive for gene discovery in plants and more reliable than the DDRT-PCR method. The serial analysis of gene expression (SAGE) method allows a rapid, detailed analysis of thousands of transcript (Song, 2003), but much time is required to set up a gene expression profile for a eukaryote (Velculescu et al., 1995). The microarray technique has been widely used for the isolation of differentially expressed genes in model plants such as *Arabidopsis thaliana* and maize (Brosche et al., 2002) but these

techniques are expensive and their use is commonly limited to the most standardized models. Libraries constructed through SSH, followed by colony blot hybridization or cDNA amplified fragment length polymorphism (AFLP), are time consuming because positive results have to be confirmed by an independent technique (Massart & Jijakli, 2006). The combination of SSH and negative subtraction chain (NSC) ensures that target sequences are dramatically and efficiently enriched but may not have enough sensitivity (Li et al., 2005) to direct isolate differentially expressed genes from highly complex eukaryote genomes (Lisitsyn et al., 1993).

In the SSH method subtraction and normalization are performed simultaneously (Mahalingam et al., 2003) thus equalizing the abundance of target cDNAs in the subtracted population (Diatchenko et al., 1996). The subtraction method overcomes the problems of differences in mRNA abundance by incorporating a hybridization step that normalizes (equalizes) sequence abundance during the course of subtraction by standard hybridization kinetics. The SSH method eliminates any intermediate step(s) for the physical separation of single and double stranded cDNAs in one round of subtractive hybridization.
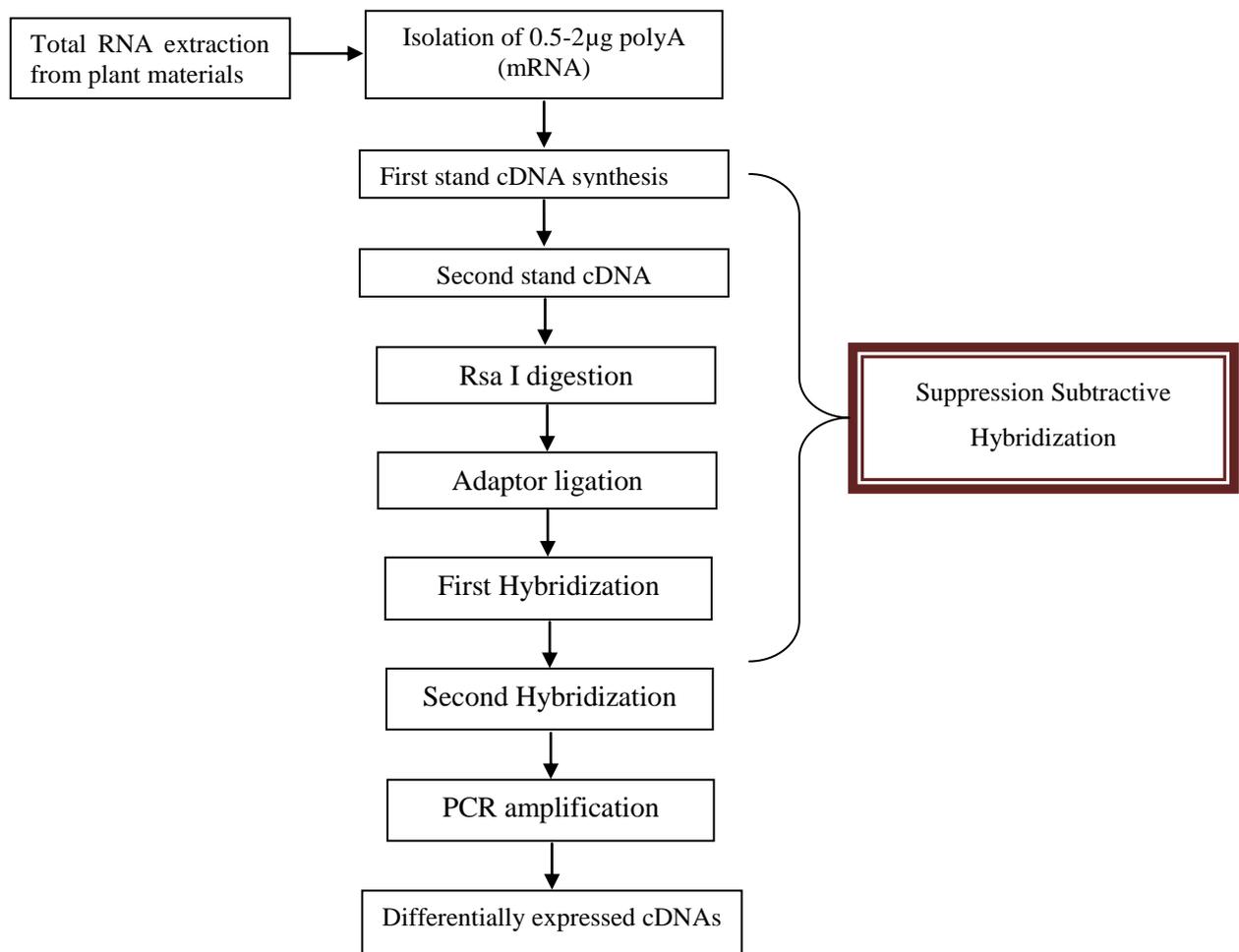

Figure 1: Brief overview of the suppression subtractive hybridization (SSH)

Powdery Mildew Resistance Genes Identification by SSH
Powdery mildew of wheat is caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *Tritici* Em. Marchal (*Bgt*) =*Erysiphe graminis*DC. *Ex Merat*f. sp. *Tritici* Em.Marchal. It is the most devastating fungal disease of common wheat worldwide (Alam et al., 2011). Due to this disease, global yield loss is accounted for 13 to 34% (Griffey et al., 1993; Leath & Bowen, 1989). A marker-assisted selection is a common molecular procedure for the identification of mildew resistance gene of wheat. The use

of resistant cultivars is the more effective and environmentally safe approach to avoid the yield loss due to powdery mildew. The resistance to powdery mildew in wheat is mainly defined as two types: monogenic (vertical) or race specific resistance, and adult plant resistance (APR) (Alam et al., 2011). The adult plant resistance is also known as "slow mildewing" (Shaner, 1973) and "partial resistance" (Hautea et al., 1987). Approximately more than 60 genes/alleles for resistance to powdery mildew have been identified and located on 18 different chromosomes in bread wheat. The 29 resistance genes/alleles have been tagged with common molecular markers such as RFLPs, RAPDs, AFLPs, STS and SSRs (Alam et al., 2011). However, identification of powdery mildew resistance genes using SSH is very limited. In a comparison of SSH technique with conventional cDNA library construction using a powdery mildew resistance wheat cultivar (BaiNong 321/Mardler BC5F4), Luo et al. (2002a) concluded that the subtracted cDNA library is a more advantageous technique in gene expression profiling of disease resistance than the simple library construction procedure in rich and specific DRR (disease-resistance-related) genes. They found 760 ESTs from the subtracted cDNA library and the redundancy of disease resistance genes such as those related to photosynthesis and ribosome are less than those in conventional cDNA library. About 54.1% function-known ESTs in the SSH cDNA library were identified to be DRR genes by screening. There were 247 clones of the SSH cDNA library that had positive signal in the repeated hybridizations with the pathogen uninfected probe. The identified DRR genes are distributed in the whole procedure of powdery mildew resistance, but they are mainly focused on the SAR (systemic of acquired resistance). Luo et al. (2002b) also conducted another study using the Bainong 3217 × Mardler BC_5F_4 wheat line for the construction of a cDNA library with an inoculation of powdery mildew pathogen (*Erysiphegraminis* DC) at the initial stage of plant by the SSH technique. Totally 199 differentially expressed ESTs with related diseases were obtained out of 760 ESTs based on BLASTn and BLASTx comparison sequencing analysis. The obtained powdery mildew resistance genes in wheat were predominant in terms of kinds (anti-oxidation substances and cell protection mechanism) and quantity. The average size was 83 to 276 bp and 91 ESTs were known functional homology in nature (34.0% of total ESTs), and their functions were mainly involved in energy metabolisms and protein synthesis (*Table 1*).

A study was conducted by Ma et al. (2015) using two wheat lines, L693 and L1095 (Liu et al., 2014), which were resistant and susceptible to powdery mildew to understand physiological changes during the host–pathogen interaction as well as gene expression, photosynthetic and chlorophyll fluorescence parameters, chlorophyll content, and antioxidant activity, specific to the resistance response to powdery mildew. Wheat (*Triticum aestivum* L.) photosynthesis- and energy-related pathways are directly affected by powdery mildew. From their investigation, 58 and 26 ESTs were identified from forward and reverse SSH cDNA libraries constructed from *Blumeriagraminis* (DC.) Speerf. sp. *Tritici* Em. Marchal infected L693 and L1095 leaves respectively, and their average length with detailed information are given in Table 1. A total of 9% of the ESTs in the forward library was high homology, further classified into carbon metabolism, photosynthesis pathway, nitrogen metabolism, protein modification pathway, and transport of metabolic products. In contrast, 50% in the reverse cDNA library showed high homology and further classified into photosynthesis, cell wall catabolism, nitrogen metabolism, and carbon metabolism related functions.

A wheat line N0308 with resistance to powdery mildew was used for the construction of a cDNA library by the SSH technique (Alam et al., 2014). The leaves were inoculated by *Bgt*race E09 at the two-leaf stage. In response to *Bgt*infection in wheat line N0308, the differentially expressed genes were identified, and a total of 175 positive clones from the library were sequenced, and 90 expressed sequence tags (ESTs) were subjected to clustering, BLAST alignment, functional annotation, and classification into different categories. Among these, 7 ESTs were differentially expressed and associated with the resistance reaction of powdery mildew by using semi-quantitative reverse transcription-polymerase chain reaction based on BLAST search analysis, whereas 5 genes (sulfatase, pathogenesis-related protein 17, betacarbonic anhydrase 2, thioredoxin h-like protein, and coronatine-insensitive) transcripts were induced at the early stage at 72 h after *Bgt* infection. Two genes violaxanthin de-epoxidase and gag-pol-polyprotein were at the highest level at 12 and 18 hours post-

infection respectively, which suggested that these genes are induced at an early stage of infection and are transcriptionally activated for the host defense response (*Table 1*).

Another related study was conducted by Michel et al. (2006) using susceptible wheat (*Triticumaestivum* L.) cultivar Fidel and inoculated with a Swiss field isolate of the powdery mildew fungus *Blumeriagraminisf.sp. tritici* for the construction of a cDNA library by the SSH technique. After the differential sequencing analysis, 158 uni-gene sequences were obtained and among these 71 (45%) exhibited > 90% sequence identity to ESTs present on the Affymetrix wheat gene, and their average size were 80 bp (Table 1).

Table 1: Differentially expressed genes in wheat cultivars against powdery mildew disease

Cultivar/ Variety	Name of the disease with pathogen	Identified genes	Forward library of SSH L693 as the tester and L1095 as the driver		Reverse library of SSH L693 as the driver and L1095 as the tester		Post inoculation time	References
			No.	Average length (bp)	No.	Average length (bp)		
L693 L1095	Powdery mildew (*Blumeriagra minis* f.sp. *trit ici*)	ESTs Singletons Contigs UniESTs	58 20 9 29	215 188 299 238	26 6 5 11	322 190 338 255	24, 48 and 72 hours	Ma et al., 2015
Semi-quantitative reverse transcription-PCR								
N0308	Powdery mildew, (*Blumeriagra minis*f. sp*tritici*(*Bgt*) race E09)	ESTs	7	200-100	-		24, 48, and 72hours	Alam et al., 2014
Forward Library of SSH								
"Bainong 3217 × Mardler" BC$_5$F$_4$ pop ulations	Powdery mildew (*Erysiphegra minis* DC)	ESTs	91	83-276	Functional homologus in nature		24, 48 and 72 hours	Luoet al., 2002b
Fidel	Powdery mildew (*Blumeriagra minis*f.sp. *tritici*)	ESTs	71	80	Affymetrix wheat gene in nature		-	Michel et al., 2006

Fusarium graminearum resistance genes identification by SSH

Fusarium head blight (FHB) is a serious disease of wheat caused by *Fusariumgraminearum.* Resistance to FHB in wheat is mainly defined by Type I (resistance to initial infection) and Type II (resistance to spread of the disease within a spike) (Mesterhazy, 1995) identified in several wheat genotypes. Multiple quantitative trait loci (QTL) mapping has been used in the identification of regions of the wheat genomes referred to as *Fhb*1in resistant cultivar Sumai 3, and its derivatives (Bai et al., 1999; Waldron et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2002; Zhou et al., 2002; Liu et al., 2006). A study was conducted by Bernardo et al. (2007) for the identification of the expression patterns of transcriptomes from wheat spikes of FHB resistant cultivar Ning 780, and susceptible cultivar Clark were used after 72 hours of inoculation with *Fusarium graminearum*. From this investigation, 44 significantly and differentially expressed genes between cv. Ning7840 and cv. Clark, and maximum differentially expressed genes were found in susceptible libraries compared to resistance libraries. The resistant cultivar Ning7840 related to the susceptive cultivar Clark during the early fungal stressed on the basis of up-regulation defence related genes. Three up-regulated unknown function genes in cv. Ning7840 were investigated which played a significant role for enhancing the FHB resistance. Muhovski et al. (2012) used *Fusariumgraminearum* infected spikes of a resistant

Belgian winter wheat (*Triticumaestivum* L.) variety Centenaire and susceptible genotype Robigus for the construction of a SSH cDNA library, exhibiting Type II resistance to FHB. From this investigation, 43 differentially expressed transcripts were identified and these were classified in different categories according to their predicted functions, including proteins involved in defence response, signaling, transport of molecules, metabolism and proteins with unknown functions. An ontime-course gene expression analysis on 12 selected genes was carried out between the FHB resistant genotype Centenaire and the susceptible genotype Robigus in order to validate the SSH screening. The selected transcripts were differentially expressed between the resistant and the susceptible genotypes at three-time points (24, 48 and 72 h) after the inoculation with the pathogen showing real-time quantitative polymerase chain reaction. The transcripts' accumulation rates were higher in the FHB resistant as compared to the susceptible one.

Another study was conducted by Kong et al. (2005) involving *F. graminearum* and Chinese wheat cultivar Ning7840 (resistance to FHB) using the SSH for identifying the differentially expressed genes. From this study, 24 differentially expressed genes were identified between this fungus and wheat interaction, out of which 8 showed homology with genes from the pathogen, and 16 with wheat genes, including those encoding cytochrome P450, actin depolymerizing factors, chitinase, histone H4, pyruvate decarboxylase, and S-adenosylmethionine decarboxylase.

A related study was carried by Golkari et al. (2009) for the investigation of QTL-specific differential gene expression between the resistant Chinese cultivar Sumai-3 and two susceptible near isogenic lines (NILS) inoculated with *Fusariumgraminearum* by the SSH technique. From this investigation, 25 wheat uni-genes were found to express differentially, responsible of *F. graminearum* infection. Three pathogenesis-related (PR) encoding gene proteins, viz., β-1, 3-glucanase (PR-2), wheatwins (PR-4) and thaumatin-like proteins (PR-5) were identified, which showed a significant .,=regulation in genotype Sumai-3 3BS region. Simultaneously, susceptible genotypes NILS have less of these three genes, and the gene activity indicateds the importance of both the 2AL and 3BS regions in the activation of effective defense responses for the infection of *F. graminearum*. Significant up-regulation of phenylalanine ammonia-lyase was also detected only in the resistant Sumai-3, and all expressed differentially genes were quantitative in nature in both the resistant cultivar (Sumai-3) and susceptive lines (NILS).

The resistant Chinese cultivar Sumai-3 was also used for the construction of another subtracted library by Al Taweel et al. (2014) to clone and identify the differentially expressed genes between FHB-inoculated and water-inoculated wheat spikes. After the EST sequencing based on the BLAST homology search programme (BLASTn and BLASTx), 31 ESTs were obtained in differentially expressed genes and among these, 7 genes were selected based on their putative involvement in pathogen-defence response and/or high signal expression in differential screening (Table 2). Another gene expression was studied by Goswami et al. (2006) for preparing a subtraction library using the susceptible wheat cultivar Norm, inoculated with a highly aggressive *F. graminearum* strain (NRRL 31084) or mock-inoculated(less aggressive strain NRRL 28303) with water for identification and functional analysis of novel genes potentially involved in the pathogenicity, and also for the identification of fungal genes differentially expressed in *planta* during interactions between wheat and a highly or less aggressive strain. They found four differentially expressed genes (*Abc*2, *Lyp*1, *Rrr*1 and *Zbc*1) potentially involved in the pathogenesis and development of this fungus in wheat cultivar Norm (*Table 2*). Based on BLASTx comparisons, *Abc*2 and *Zbc*1 appeared to encode protein and *Lyp*1, *Rrr*1 appeared putative regulatory in nature. Different differentially expressed gene details are given in Table 2 with respective wheat cultivars.

Table 2: Differentially expressed genes in wheat cultivars against Fusarium head blight (FHB) disease

Cultivars/ Varieties	Name of the disease with pathogen	Identified genes			Post inoculation Time	References
		Using method for library construction	No.	Nature		
Clark and Ning 780		(Forward Library of SSH) Clark as a tester andNing 780 as a driver	44	Homology	72 hours	Bernardo et al., 2007
Robigus and Centenaire		Robigus as a tester and Centenaire as a driver	43	Homology	24, 48, and 72 hours	Muhovski et al., 2012
Ning7840		Forward and Reverse subtracted cDNAs	24	8 genes homology	2, 6, 12, 24, 36, 72, and 96 hours	Kong et al., 2005
Sumai-3 and its isogenic lines (NILS)	Fusarium head blight (*Fusariumgra minearum*)	Isogenic line of Sumai as a tester and Sumai as a driver	25	Quantative in nature with up-regulation		Golkari et al., 2009
Sumai-3		Quantitative real time reverse transcriptage PCR	31	Up-regulated putative defense genes	6, 12, 24, 36, 48, 72, and 144 hours	Al‑Taweel et al., 2014
Norm		(Reverse Library of SSH) NRRL 28303-inoculated wheat heads as a tester and NRRL 31084-inoculated wheat as a driver.	4	Putative and protein regulator in nature	48 hours	Goswami et al., 2006

Leaf rust of wheat and SSH

Pucciniatriticina is the most common and widely distributed pathogen among the three wheat rusts. Globally, leaf rust damages are greater than stem and stripe rust (Goswami et al., 2005; Huerta-Espino et al., 2011). More than 70 leaf rust resistance genes are identified which can be pyramiding to develop a resistant variety of wheat through marker-assisted selection programmes (MAS) (Kolmer, 1996). The use of SSH method for the identification of differentially expressed genes in the case of leaf rust disease is very few. A study was conducted by Venkatappa et al. (2003) to understand the molecular basis of wheat-rust interactions by using SSH to construct a cDNA library. From this study, 26 differentially expressed genes were identified, and non-ribosomal protein genes were induced in rust-infected leaves. Among these genes, 21 of the induced genes were from the rust fungus, indicating that the majority of the induced genes were rust *PIGs* that were likely to play a role in parasitism. Some of the induced genes were homologous to known *PIGs* or virulence genes in other fungi, suggesting similarities in parasitism among different fungi. Eight clones correspond to novel PIGs that have not been reported in any organism. Another finding was observed by Li et al. (2008) using leaf rust resistance wheat cultivar TcLr41 as a driver and a susceptible host Thatcher as a tester to identify the differentially expressed genes by the SSH method. From their investigation, 240 differential expression sequence tags were obtained and 137 qualified ESTs showed high homology (Table 3) with the function known genes or ESTs. After the analyses, these genes with their functions were further classified into glutathione-S-transferase, mitogen-activated protein kinase, W1R1 protein, serine/threonine protein kinase, lipid transfer protein, thaumatin-like protein, GTP-binding protein, chitinase, and calreticulin for the involvement in the process of the incompatible interaction between wheat TcLr41 and the *Pucciniatriticina* race.

Stripe rust of wheat and SSH

Stripe rust is one of the most economically destructive diseases in common wheat (*Triticumaestivum*L.) worldwide caused by *Pucciniastriiformis*f. sp. *tritici*(*Pst*). The identification of stripe-rust resistance genes is comparatively more difficult than other rust pathogens due to the frequent emergence of new virulent races (Dadrezaei et al., 2013). Although more than 40 stripe-rust resistance genes have been identified using different molecular marker breedings, approaches derived from wheat and wheat related species such as *Triticum spelta album* (*Yr*5), *Secalis cereale* (*Yr*9), *Triticum dicoccoides* (*Yr*15, *Yr*35) (Cao et al., 2008; Peng et al., 2000) *Triticum tauschii* (*Yr*28) (Marais et al., 2005) and A*egilops* species (*Yr*37, *Yr*38 and *Yr*40) (Peng et al., 2000; Singh et al., 2000; Marais et al., 2006) identification of differentially expressed genes for stripe rust in wheat using SSH technique is very rare. A study was conducted by Zeng et al. (2010) using wheat germplasm NR1121 infected by Chinese *Pst*race CYR32 as a tester and susceptible Mingxian169 and as a driver (control) for the identification of differentially expressed genes in response to *Pst* infection in wheat by the SSH technique (Table 3). A total of 550 positive clones from the library was identified and subjected to sequencing. The 162 ESTs were grouped into different cluster patterns based on BLAST alignment and functional annotation and classified into different categories. Among these, eight genes were detected by semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and real-time polymerase chain reaction. The expression of six gene (acylcoenzyme A synthetase, glutathione S transferase, Lipid transfer protein, ubiquitin protein ligase 2, cytochrome P450, and serine/threonine protein kinase SNT7) transcripts were induced and up-regulated to their highest levels at 24 hours after *Pst* infection, while those of two genes (serine Hydroxy-methyl transferase and S-adenosylmethionine decarboxylase) were significantly expressed at 48 hours (hpi). These genes were transcriptionally activated for the host defence response and highly induced at an early stage of infection.

Another SSHcDNA library was constructed from *Pst* infected seedling leaves of resistant wheat cultivar Shaanmai 139 (Zhang et al., 2011). They obtained a total of 84 expressed sequence tags with high quality and alignment of the sequences identified in 56 Unigenes. After the analysis by BLASTx search, 46 Unigenes showed homologous and encoding proteins of known functions such as signal transmission, energy and metabolism, transcription regulation, phenylpropanoid pathway, and defence response (Table 3). The wheat cultivar Suwon11 was inoculated with stripe rust (*Puccinia striifromis*f. sp. *tritici*) strains CYR23 and CYR31 for the construction of SSH cDNA library to analyze the expression patterns of wheat HSP70 (Heat Shock Proteins) gene in wheat plants exposed under abiotic stress (Duan et al., 2011). From this study, the differentially expressed gene designated as *TaHSC70* was identified during an incompatible and compatible interaction with wheat-stripe rust and was up-regulated by the MeJA treatment. The identified gene *TaHSC70* played a role of stress-related responses, predicted to encode a protein of 690 amino acids, and also demonstrated in a homolog of chloroplast HSP70s by the phylogenetic analysis (*Table 3*).

The Chinese winter wheat cultivar Xingzi 9104 (XZ) (XY is susceptible to CYR32 at the seedling stage, but is resistant at the adult-plant stage) inoculated with high aggressive *Pst* race CYR32, was used for the construction of SSH cDNA library to identify differentially expressed genes involved in wheat-*Pst* interactions on adult plants (Huang et al., 2013). From this study, a large number of genes were found involved in defence and signal transduction based on putative functions. Twelve genes specifically expressed in inoculated adult plants of XZ were isolated and identified, and these genes are valuable candidates for functional analyses of stripe rust APR in wheat cultivar XZ.

Wheat common bunt and SSH

A common bunt is caused by *Tilletia caries* (DC) Tul.,*T. foetida* (Wallr) Liro., and *T. controversa*, respectively, and can still cause yield and quality losses of wheat (Zeng et al., 2010). Very few molecular markers have been used for the identification of common bunt resistance genes in wheat. More than 15 specific common bunt resistance genes named Bt genes have been identified using different molecular markers which can be used to breed resistant cultivars (Ciuca, 2011). The use of SSH technique for the identification of differentially expressed genes is very rare. A related study was conducted by Zhen-Xiang et al. (2005), using susceptible wheat line Neepaw and resistant line

BW553 for the identification of differentially expressed genes against wheat common bunt disease (pathogen: *Tilletiatritici)*. They identified 193 differentially genes (168 up-graded and 25 downgraded genes) (Table 3) and sequenced them. Among these, more than 70% sequences had significant homology to genes of known functions, and these homology sequences have been categorized into three based on their cellular functions, viz., 59% cellular metabolism and development, 24% in abiotic/biotic stress responses, as well as 3% involved in transcription and signal transduction responses. Furthermore, two putative resistance genes and a transcription factor were identified among the up-regulated sequences.

Bipolaris sorokiniana of wheat and SSH

Bipolaris sorokiniana is a dominant pathogen causing leaf blight or blotch on wheat and yield losses up to 15% in Bangladesh (Alam et al., 1998), and average yield losses range from 15.5 to 19.6% in the world wheat production (Dubin & Van Ginkel 1991; Chowdhury et al., 2013). To date only eight resistant genes have been identified in wheat against *Bipolaris sorokiniana* pathogen by using SSR and microsatellites marker-assisted breeding. However, only one research was conducted using the SSH technique for the identification of differentially expressed genes in bread wheat. A resistant wheat (*Triticum aestivum*L.) genotype, BH1146 and one of the F$_2$ lines (line 47) of the cross between BH 1146 and *Bipolaris sorokiniana* susceptible genotype Sonalika were selected for the preparation of cDNA library using the SSH technique (Chowdhury et al., 2013). From this study, 1000 clones were obtained and among these, 480 clones after the colony PCR was subjected to sequencing. The sequences were analysed by the BLAST comparison analysis for homology search against the gene bank non-redundant database. Out of 480 clones, 36% of the clones (172 clones) were high quality clones having > 200 base pair size. They further used 172 clones for sequence analysis and found that all the 172 clones matched with ESTs of different plant species in the NCBI database. High redundancy for putative puroindoline b-protein, putative NADH dehydrogenase and hypothetical protein OSJ 021-069 were observed in the BLAST search. Finally 48 differentially expressed genes were identified (Table 3) and these were further classified into 7 categories based on their putative functions, viz., plant defense related genes, genes which have role in signal transduction, genes associated with transcription and translation, energy related genes, genes involved in metabolism and unknown functional. Furthermore, out of 48 differentially expressed genes, 20 gene sequences matched with *Arabdiopsis* spp, 5 matched with *Brassica* spp. and 3 matched with *Triticum aestivum.* In addition to defense-related genes analysis, out of 48 differentially expressed clones, 12 clones (25%) were classified as plant defense responsive genes based on their putative functions observed in NCBI blast search, while 21% genes were having metabolism related functions, 17% genes were related to transcription/translation and 10% genes had energy related functions and cellular transport each. Only 4% genes were related to signal transduction while 13% were unknown genes. For the clones of SSH, 135 matched with putative puroindoline b proteins from *Triticum aestivum* which are encoded by puroindoline gene (*pin B* gene). This gene contains tryptophan rich hydrophobic domain, which is responsible for grain hardness and also has antimicrobial properties having lipid binding sites.

Table 3: Differentially expressed genes in wheat cultivars against rust, common bunt and Bipolaris leaf blight of wheat

Cultivars/ Varieties	Name of the disease with pathogen	SSH library	Identified genes			Post inoculation time	References
			Type	No.	Nature		
Thatcher and TcLr41	Leaf rust of wheat (*Pucciniatriticina*)	Thatcher as a tester and TcLr41 as a driver	ESTs	137	High homology in nature	-	Li et al., 2008
		Semi quantitative RT-PCR and real-time PCR					
NR1121 and Mingxian 169	Strip rust of wheat (*Pucciniastriiformis*f. sp. *tritici race* CYR 32)	NR1121 as a tester and Mingxian 169 as a driver	ESTs	162	80-750 bpi in average length	24 hours	Zeng et al., 2010

Shaanmai 139	Stripe rust of wheat (*Pucciniastriiformis* f. sp.*tritici* race CY 32)	Semi-quantitative reverse transcription-PCR	Unigene	56	Defense related gene expression	-	Zhang et al., 2011
Suwon11	Stripe rust of wheat (*Pst*- strains CYR23 andCYR31)	Forward and Reverse library of SSH	The gene *TaHSC70*	1	Homology and stress related response	-	Duan et al., 2011
Xingzi 9104 (XZ)	Stripe rust of wheat (*Pst*-strains CYR32)	Quantitative RT-PCR	ESTs	12	Defense and signal transduction related response	-	Huang et al., 2013
Forward library of SSH							
Neepaw and BW553	Wheat common bunt (*Pucciniatritici*)	Neepaw as a tester and BW553 as a driver	-	168 25	Up-graded Down graded	-	Zhen-Xiang et al., 2005
F₂ line 47 of BH1146 and Sonalika	Bipolaris Leaf blight of wheat (*Bipolarissorok aniana*)	Sonalika as a tester and F₂ line of BH1146 as a driver	ESTs	48	Average length 200 bp	-	Chowdhury et al., 2013

Advantages of SSH

The molecular mechanisms of host pathogen interaction through SSH is useful. The SSH is used to enrich the rare cDNA transcripts by more than 1000 fold (Honnareddy et al., 2014). The construction of cDNA library by the SSH technique includes more genes related to disease resistance and defense reaction comparing to an ordinary cDNA library by any other molecular approaches (Luo et al., 2002c). The SSH is the powerful method for studying the function of genes involved in a particular disease a well as the development of plants and/or pathogens with the differential expression of tissue-specific proteins etc (Diatchenko et al., 1999). It is also used for identification of differentially expressed genes between hybrids and their parents which are responsible for the heterosis (Muhovski et al., 2012).

Limitation of SSH

The SSH is laborious multi-step technique (Alice et al., 2013). For getting stable hybridization results, experiments must be repeated several times and a large amount of mRNA and radioactive isotope are needed (Yao et al., 2005). It is totally dependent on different enzymes and reagents, so the purity of enzymes and the use of reagent are highly significant for getting the actual findings. The construction of cDNA libraries is one of the key factors of this technique for obtaining the differentially expressed sequences and is an essential need for the identification of genes involved in contrasting situations (Yao et al., 2005). The identification of expressed genes requires two or more than two sample at a time, and hence the handling of these samples are more complicated compared to other methods.

Direction for further research

This review presents a good number of differentially expressed genes identified by wheat researchers using the SSH technique against different fungal diseases of wheat. The updated information will be helpful to further design the experiments to develop durable disease resistant varieties against fungal pathogen through marker-assisted backcrossing (MABC) or gene transformation through genetic engineering. These could be planned against specific pathogen, or combination of pathogens using gene pyramiding.

Conclusion

The use of a resistant cultivar of any crop is the sustainable and environmentally friendly approach to enhance the production of any crop. The SSH is an easy and powerful approach to identify resistant genes of any resistance cultivar. Ultimately, these resistant genes may be used to develop a new resistant variety or cultivar by gene pyramiding using different molecular approaches. This technique

is also helpful to identify pathogen-host interaction mechanism. Through this paper, researchers will be enormously benefited to find out the different resistant wheat cultivars in the world, and in brief they can obtain clear information about the differential expressed genes and their functional activities.

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References

Alam, K. B., Banu, S. P., & Shaheed, M. A. (1998). The occurrence and significance of spot blotch disease in Bangladesh. In: *Helminthosporium* blights of wheat: spot blotch and tan spo*t* (Duveiller E, Dubin HJ, Reeves J, McNab A, eds.) (pp. 63-66). CIMMYT, Mexico D.F.

Alam, M. A., Hongpo, W., Hong, Z., & Ji1 W. Q. (2014). Differential expression of resistance to powdery mildew at the early stage of development in wheat line N0308. *Genetics and Molecular Research*, *13(2)*, 4289-4301.Retrieved from http://dx.doi.org/10.4238/2014.June.9.15.

Alam, M. A., Xue, F., Wang, C., & Ji, W. (2011). Powdery mildew resistance genes in wheat: Identification and Genetic Analysis. *Journal of Molecular Biology Research*, *1*, 20-39.

Alice, C., Brammer, S. P., Martinelli, J. A., Grando, M. F., & D'AvilaDenardin,N. (2013). Gene expression: A review on methods for the study of defense-related gene differential expression in plants. *American Journal of Plant Science*, *4*, 64-73.

Al Taweel, K., Dilantha, F. W. G., & Brule Babel, A. L. (2014). Transcriptome profiling of wheat differentially expressed genes exposed to different chemotypes of Fusariumgraminearum. *Theoretical and Applied Genetics*, *127*, 1703–1718. doi: 10.1007/s00122-014-2333-8

Anderson, J. A., Stack, R. W., Liu, S., Waldron, B. L., Fjeld,A. D., Coyne, C., Moreno-Sevilla, B., Fetch, J. M., Song,Q. J., Cregan, P. B., & Frohberg, R. C., (2001). DNA markers for Fusarium head blight resistance QTLs in two wheat populations. *Theoretical Applied and Genetics*, *102*, 164-1168.

Bai, G., Kolb, F. L., Shaner, G., & Domier, L. L. (1999). Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. *Phytopathology*, *89*, 343-348.

Bernardo, A., Bai, G.,Guo, P., Xiao, K., Guenzi, A. C., & Ayoubi, P. (2007). Fusarium graminearum induced changes in gene expression between Fusarium head blight-resistant and susceptible wheat cultivars. *Functional Integrative Genomics*, *7*, 69–77.

Brosche, M., Schuler, M. A., Kalbina, I., Connor, L., & Strid, A. (2002). Gene regulation by low level UV-B radiation: identification by DNA array analysis. *Photochemical and Photobiological Sciences, 1*, 656-664.

Buerstmayr, H., Lemmens, M., Hartl, L., Doldi, L., Steiner, B., Stierschneider, M., &Ruckenbauer, P. (2002). Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). *Theoretical and Applied Genetics*, *104*, 84-91.

Cao, A. L., Xing, X., Wang, X., Yang, W., & Wang, Y. (2011). Serine/threonine kinasgeneStpk-V, a key member of powdery mildew resistance gene Pm21, confers powdery mildew resistance in wheat. *Proceedings of National Academic of Science*, *108(19)*, 7727-7732.

Cao, Z., Deng, Z., Wang, M., Wang, X., Jing, J., Zhang, X., Shang, H., & Zhenqi, L. (2008). Inheritance and molecular mapping of an alien stripe-rust resistance gene from a wheat-Psathyrostac hyshuashanica translocation lines. *Plant Science*, *174*, 544–549.

Chowdhury, A. K., Singh, G., Tyagi1, B. S., Ojhal, A., Dhar,T., & Bhattacharya, P. M. (2013). Spot blotch disease of wheat: a new thrust area for sustaining productivity. *Journal of Wheat Research*, *5(2)*, 1-11.

Ciuca, M. (2011). A preliminary report on the identification of SSR markers for Bunt (Tilletia sp.) resistance in wheat. *Czech Journal of Genetics and Plant Breeding*, *47(Special Issue)*, S142–S145.

Dadrezaei, S.T., Nazari, K., Afshari, F., & Goltapeh, E. M. (2013). Phenotypic and molecular characterization of wheat leaf rust resistance gene Lr34 in Iranian wheat cultivars and advanced lines. *American Journal of Plant Science*, *4*, 1821-1833.

Diatchenko, L., Lau Y. F., Campbell, A. P., Chenchik, A., Moqadam, F., Huang, B., Lukyanov, S, Lukyanov, K., Gurskaya, N., Sverdlov, E. D., & Siebert, P. D. (1996). Suppression subtractive hybridization: a method for generating differentially regulated or tissue specific cDNA probes and libraries. *Proceedings of National Academic Science*, *93*, 6025-6030.

Diatchenko, L., Lukyanov, S., Lau, Y. F., & Siebert, P. D. (1999). Suppressive subtraction hybridization: A versatile method for identifying differentially expressed genes. *Methods in Enzymology, 303*, 349-380.

Duan, Y.-H., Guo, J., Ding. K., Wang, S.-J., Zhang, H., Dai, X.-W., Chen, Y.-Y. Huang, L.-L., & Kang, Z.-S. (2011). Characterization of a wheat HSP70 gene and its expression in response to stripe rust infection and abiotic stresses. *Molecular Biology Reports*, *38*, 301–307. doi: 10.1007/s11033-010-0108-0

Dubin, H. J., & Ginkel, M. V. (1991). The status of wheat diseases and disease research in warmer areas. In: Saunders DA (ed.) wheat for the non-traditional warmer areas (pp. 125-145). CIMMYT, Mexico, D.F.

Eickhoff, H., Schuchhardt, J., Ivanov, I., Meier-Ewert, S., O'Brien, J., MAlik, A., Tandon, N., Wolski, E-W., Rohlfs, E., Nyarsik, L., Reinhardt, R., Nietfeld, W., & Lehrach, H. (2000). Tissue gene expression analysis using arrayed normalized libraries.*Genome Research*, *10*, 1230-1240.

Golkari, S., Gilbert, J., Ban, T., & Procunier, D. (2009). QTL-specific microarray gene expression analysis of wheat resistance to Fusarium head blight in Sumai-3 and two susceptible NILs. *Genome*, *52(5)*, 409-418.

Goswami, R. S., Xu, J. R., Trail, F., Hilburn, K., & Kistler, H. C. (2006). Genomic analysis of host-pathogen interaction between Fusariumgraminearumand wheat during early stages of disease development. *Microbiology*, *152(6)*, 1877-1890. doi: 10.1099/mic.0.28750-0

Griffey, C. A., Das, M. K., & Stromberg, E. L. (1993). Effectiveness of adult-plant resistance in reducing grainyield loss to powdery mildew in winter wheat. *Plant Disease*, *77*, 618-622. Retrieved from http://dx.doi.org/10.1094/PD-77-0618

Habib, S. H. (2011). *Morphological and molecular analysis of "Truncated leaf syndrome" somaclone in oil palm (Elaeis guieensis)* (Unpublished doctoral dissertation). Universiti Putra Malaysia.

Hautea, R. A., Coffman, W. R., Sorrells, M. E., & Bergstrom, G. C. (1987). Inheritance of partial resistance to powdery mildew in spring wheat. *Theoretical and Applied Genetics*, *73*, 609-615. Retrieved from http://dx.doi.org/10.1007/BF00289202

Honnareddy, N., Aggarwal, R. Sharma, S., Gupta, S., Singh, G., Bashyal, B. M., & Ranebennur, H. (2014). Isolation and identification of defense responsive genes in wheat during incompatible interaction with *Bipolaris sorokiniana* (*Cochliobolus sativus*) using SSH Technique. *Vegetos, 27(3)*, 11-20.

Huang, X.-L., Maa, J.-B., Chen, X. Wang, X.-J., Ding, K., Han, D.-J., Qu, Z.-P., Huang, L.-L., & Kang, Z.-S. (2013). Genes involved in adult plant resistance to stripe rust in wheat cultivar Xingzi 9104. *Physiological and Molecular Plant Pathology*, *81*, 26-32.

Huerta-Espino, Singh, R. P., German, S. S., McCallum, B. D., Park, R. F., Chen, W. Q., Bhardwaj,S. C.,& Goyeau, H. (2011). Global status of wheat leaf rust caused by *Puccinia triticina. Euphytica*, *179(1)*, 143-160.

Jones, J. T., & Harrower, B.E. (1998). A comparison of the efficiency of differential display and cDNAs-AFLPs as tools for the isolation of differentially expressed parasite genes. *Fundamental and Applied Nematology*, *21*, 81-88.

Kolmer, J. A. (1996). Genetics of resistance to leaf rust. *Annual Review of Phytopathology*, *34*, 435-455.

Kong, L., Anderson, J. M., & Ohm, H. W. (2005). Induction of Wheat Defense and Stress-Related Genes in Response to *Fusarium graminearum. Genome, 48(1)*, 29-40. Retrieved from http://dx.doi.org/10.1139/g04-097.

Leath, S., & Bowen, K. L. (1989). Effects of powdery mildew, triadimenol seed treatment, and triadimefon foliar sprays on yield of winter wheat in North Carolina. *Phytopathology, 79(2)*, 152-155. Retrieved from http://dx.doi.org/10.1094/Phyto-79-152

Li, L., Techel, D., Gretz, N., & Hilderbrandt, A. (2005). A novel transcriptome subtraction method for the detection of differentially expressed genes in highly complex eukaryotes. *Nucleic Acids Research*, *33*, 1-5.

Li, X., Liu, D. Q., & Yang, W. X. (2008). *Differential expression of wheat leaf rust resistance gene Lr41*. (Doctoral dissertation). Agricultural University of Hebei. China.

Liang, P., & Pardee, A. B. (1992). Differential display of eukaryote messenger RNA by means of the polymerase chain reaction. *Science*, 257, 967-971.

Lisitsyn, N., Lisitsyn, N., &Wigler, M. (1993). Cloning the differences between two complex genomes. *Science*, *259*, 946-951.

Liu, S., Zhang, X. Pumphrey, M. O., Stack, R. W., Gill, B. S., & Anderson J. A. (2006). Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. *Functional Integrative Genomics*, *6*, 83-89.

Liu, Z., Xu, M., Xiang, Z., Li, X., Chen, W., & Luo, P. (2014). Registration of the novel wheat linesL658, L693, L696, and L699, with resistance to fusarium head blight, stripe rust, and powdery mildew. *Journal of Plant Registrations, 9(1)*, 121-4. Retrieved from http://dx.doi.org/10.3198/jpr2014.01.0003crg.

Lukyanov, S.A., Rebrikov, D., & Buzdin, A. A. (2007). Suppression subtractive hybridization. *Nucleic Acids Hybridization, 3*, 53–84.

Luo M., Kong, X. Y., Liu, Y., Zhou, R. H., & Jia, J. Z. (2002a). cDNA libraries construction and screening in gene expression profiling of disease resistance in wheat. *Yi ChuanXueBao, 29(9)*, 814-9.

Luo, M., Kong, X., Huo, N., Zhou, R., & Jia, J. (2002b). Gene expression profiling related to powdery mildew resistance in wheat with the method of suppression subtractive hybridization. *Chinese Science Bulletin, 47(23)*, 1990-1994.

Luo, M., Kong, X.Y., Jiang T., Jia C., Zhou R.H., & Jia, J.Z. (2002c). Analysis of resistance to powdery mildew in wheat based on expressed sequence tags (ESTs) technique (in Chinese). *Acta BotanicaSinica, 44*, 567-572

Ma, L. X., Zhong, S. F., Liu, N., Chen, W. Q., Liu, T. G., Li, X., Zhang, M., Ren, Z. L., Yang, J. Z., & Luo, P. G. (2015). Gene expression profile and physiological and biochemical characterization of hexaploid wheat inoculated with *Blumeria graminis* f. sp. *tritici. Physiological and Molecular Plant Biology, 90*, 39-48.

Mahalingam, R., Gomez-Buitrago, A., Eckardt, N., Shah, N., Guevara-Garcia, A., Philip Day, R. R., & Fedoroff, N. V. (2003). Characterization of the stress/defense transcriptome of Arabidopsis. *Genome Biology*. *4*, 1-14.

Marais, G. F., Pretorius, Z.A., Wellings, C.R., McCallum, B., & Marais, A. S. (2005). Leaf rust and stripe rust resistance genes transferred to common wheat from *Triticum dicoccoides*. *Euphytica*, *143*, 115–123.

Martin, K. J., & Pardee, A. B. (2000). Identifying expressed genes. Proceedings of the National Academy of Science, *97*, 3789-3791.

Massart, S., & Jijakli, M. H. (2006). Identification of differentially expressed genes by cDNA Amplified Fragment Length Polymorphism in the biocontrol agent *Pichia anomala* (Strain Kh5). *Phytopathology*, *96*, 80-86.

Mesterhazy, A. (1995). Types and components of resistance to Fusarium head blight of wheat. *Plant Breeding*, *114*, 377-386.

Michel, K., Abderhalden, O., Bruggmann, R., & Dudler, R. (2006). Transcriptional changes in powdery mildew infected wheat and Arabidopsis leaves undergoing syringolin-triggered hypersensitive cell death at infection sites. *Plant Molecular Biology*, *62*, 561–578. doi 10.1007/s11103-006-9045-7.

Muhovski Y., Batoko, H., & Jacquemin, J. M. (2012). Identification, characterization and mapping of differentially expressed genes in a winter wheat cultivar (Centenaire) resistant to *Fusarium graminearum* infection. *Molecular Biology Reports, 39(10)*, 9583-9600. doi: 10.1007/s11033-012-1823-5. Epub 2012 Jun 21.

Peng, J. H., Fahima Roder T., Huang, Q.Y., Dahan, A., Li, Y. C., Grama, A., & Nevo,E. (2000). High-density molecular map of chromosome region harboring stripe-rust resistance genes YrH52 and Yr15 derived from wild emmer wheat *Triticum dicoccoides.Genetica*, *109*, 199–210.

Schena, M., Shalon, D., Davis, R. W., & Brown, P. O. (1995). Quantative monitoring of gene expression patterns with a complementary DNA microarrays. *Science*, *270*, 467-470.

Shaner, G. (1973). *Evaluation of slow-mildewing resistance of Knox wheat in the field*. *Phytopathology*, *63*, 867-872. Retrieved from http://dx.doi.org/10.1094/Phyto-63-867

Singh, R. P., Nelson, J.C., & Sorrells, M. E. (2000). Mapping of *Yr28* and other genes for resistance to stripe rust in wheat. *Crop Science*, *40*, 1148-1155.

Song, J. (2003). What a wise SAGE once said about gene expression. *Bio-Tech Journals.1*, 99-104.

Velculescu, V. E., Zhang, L., Vogelstein, B., & Kinzler, K.W. (1995). Serial analysis of gene expression. *Science, 270*, 487.

Venkatappa, K., Thara, P., John, Fellers, & Zhou J.-M. (2003). In planta induced genes of *Puccinia triticina. Molecular Plant Pathology, 4(1),* 51-56.

Waldron, B. L., Moreno-Sevilla, B., Anderson, J. A., Stack, R. W., & Frohberg, R. C. (1999). RFLP mapping of QTL for Fusarium head blight resistance in wheat. *Crop Science*, *39*, 805-811.

Yao, Y., Ni, Z., Zhang, Y., Chen, Y., Ding, Y., Han, Z., Liu, Z., & Sun, Q. (2005). Identification of differentially expressed genes in leaf and root between wheat hybrid and its parental inbreds using PCR-based cDNA subtraction. *Plant Molecular Biology*, *58*, 367–384.

Zeng, X., Changyou, W., Ali, M., Hong, Z., Xinlun, L., Weiyani, L., & Wanquan, J. (2010). Profiling gene expression patterns of stripe rust *(Puccinia striformis f. sp. tritici)* resistance gene in new wheat germplasm. *Pakistan Journal of Botany, 42(6)*, 4253-4266.

Zhang, H., Hu, Y. Wang, C., & Ji, W. (2011). Gene Expression in wheat induced by inoculation with *Puccinia striiformis West. Plant Molecular Biology Reporter*, *29*, 458–465. doi: 10.1007/s11105-010-0245-6.

Zhen-Xiang L., Danis, A., Gaudet, M., Frick, B., Fuchalski, B. G., & Larochie, A. (2005). Identification and characterization of genes differentially expressed in the resistance reaction in wheat infected with Tilletiatritici, the common bunt pathogen. *Journal of Biochemistry and Molecular Biology*, *38(4)*, 420-431.

Zhou, W., Kolb, F. L., Bai, G., Shaner, G., & Domier, L. L. (2002). Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. *Genome*, *45*, 719-727.