



COMPARATIVE STUDIES ON GENE EXPRESSION OF RICE AND WHEAT IN RESPONSE TO FUNGAL INFECTION

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ABSTRACT

Comparative sequence analysis is a powerful tool to study homologous gene families, define conserved gene functions between orthologs, and identify lineage- and species-specific genes. Most annotations of newly sequenced genomes are based on similarity with sequences for which functional information is available. Apart from conserved sequences, inter-species differences provide important clues about evolutionary history and species-specific adaptations. In our study, two RNA-sequencing data sets of resistant variety of wheat (*Triticum aestivum* L.) after infection with leaf rust fungus, *Puccinia triticina* and resistant variety of rice (*Oryza sativa* L.) after infection with blast fungus, *Magnaporthe oryzae* were compared. 31768 up-regulated genes in wheat and 3902 up-regulated genes in rice were filtered according to fold change more than 3 and removing variants, 250 upregulated genes of wheat and rice were aligned and phylogenetic tree was generated. The result of phylogenetic tree showed close relationship between ten aligned gene pairs of wheat and rice. Two pairs of aligned gene pairs were selected randomly, super family of these pairs were obtained, the result showed that each aligned pair of proteins shared the same protein family and the same annotation and all pairs participate in plant defense pathways. Then, the gene expression of the two pairs were validated by Real-time PCR after infecting wheat with *Puccinia triticina* and rice

with *Magnaporthe grisea*. Each aligned pair of the two pairs shared the same manner of expression with few exceptions in rice.

Keywords: Comparative analysis, RNA-seq, Wheat, Rice, *Puccinia triticina*, *Magnaporthe oryzae*

1. INTRODUCTION

Cereals are the foremost necessary foods for growing human population. Just about fiftieth of consumed calories by the whole human population rely on wheat, rice and maize (**Gnanamanickam, 2009**). Wheat (*Triticum aestivum*) is the first important and strategic cereal crop for the majority of world's populations. It is the most necessary staple food for about two billion people (36% of the globe population). Worldwide, wheat production provides nearly fifty five percent of the carbohydrates and twenty percent of the food calories consumed globally (**Breiman and Graur, 1995**). It exceeds in land area and production every other grain crop (including rice, maize, etc.) and is so, the most important cereal grain crop of the world, which is cultivated over a wide range of climatic conditions. Although rice (*Oryza sativa*) has the second place because of planted area but it serves as the most important food source for Asian countries mainly in South-East parts where it is an economic crop for farmers and workers who grow it on millions of hectares throughout the region (**Gomez, 2001**).

Historically, rice was cultivated ten thousand years ago in the river valleys of South and Southeast Asia and China as it served as the most important food for people.

Wheat and rice production may be severely restricted by biotic and abiotic constraints. Disease is the major biotic stress in several regions. Rusts are among the most dangerous fungal diseases of wheat worldwide due to their wide distribution, capacity to form new races that can attack previously resistant cultivars, under optimal environmental conditions, ability to move long distances, and potential to develop rapidly under optimal environmental conditions. Leaf rust, caused by *Puccinia triticina* Eriks., is the most common rust disease of wheat (*Triticum aestivum* L.) which cause significant yield losses over large geographical areas worldwide (Kolmer, 2005; Marasas et al 2004; Roelfs et al 1992; Saari and Prescott, 1985). Leaf rust happens more regularly and in more world-wide regions than stem rust of wheat (*P. graminis* f. sp. *tritici*) or stripe rust of wheat (*P. striiformis* f. sp. *tritici*) (D'Oliveira and Samborski, 1966).

The production of rice (*Oryza sativa*) worldwide is reduced due to rice blast disease which is caused by the fungal pathogen *Magnaporthe oryzae* (Talbot, 2003 and Skamnioti and Gurr, 2009). The pathogen is able to infect all the aboveground parts of a rice plant at different stages of growth: leaf, collar, node, internode, base, or neck, and other parts of the panicle, and sometimes the leaf sheath.

Plants have evolved a wide range of mechanisms to overcome biotic and abiotic stresses (Jones and Dangl, 2006; Thomma et al 2011; Spoel and Dong, 2012). The molecular response involved in each stress has been revealed comparatively independently, and so understanding of convergence points between biotic and abiotic stress signaling pathways remain rudimentary. However, few studies revealed several molecules, including transcription factors and kinases, as promising candidates for common players involved in crosstalk between stress signaling pathways. Emerging evidence suggests that pathways of hormone signaling regulated by abscisic acid, salicylic acid, jasmonic acid and ethylene, as well as ROS signaling pathways, play key roles in the crosstalk between signals of both biotic and abiotic stress (Fujita et al 2006).

Numerous studies have investigated the environmental stress responses at the transcriptomic level, using RNA sequencing to follow whole-

genome expression in different organisms. RNA-Seq is a recently developed approach that can be used in transcriptome analyses to reveal genome-wide expression profiling and regulation in plant hosts in response to pathogen infection (Han et al 2015). The technique has several advantages over other methods. First, RNA-Sequencing, differs from hybridization-based approaches, it can detect gene transcripts even in the absence of the genome sequence of the target species. Second, RNA-Seq has low background noise (Wang et al 2009). Third, the technology has a higher sensitivity than DNA microarray and can be used to detect a larger dynamic range of expression levels of gene transcripts (Nagalakshmi et al 2008; Mortazavi et al 2008). By following the expression of every gene in the genome, each of these investigations has presented a global view of the physiology of stress defense. Now the opportunity to compare and contrast fungal stress responses have been found. A comparative perspective can reveal the common features of stress defense that have been conserved over millions of years of evolution, reflecting their universal importance in surviving adversities. Conversely, responses that are specific to subsets of species highlight unique defense systems that may have been shaped by the particular habitats of those organisms.

The objectives of the present study were

1. Perform comparative analysis for the RNA-seq data obtained from previous experiments in which the wheat and rice plants will be infected with *Puccinia triticina* and *Magnaporthe grisea*, respectively, using bioinformatics tools.
2. Selection of the redundant – differentially expressed transcripts.
3. Validate the expression profile of the selected transcripts using real-time PCR.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Raw data

Differentially expressed genes data which resulted from RNA-Sequencing of rice (Accession no.: DRA000542) and wheat (Accession no.: PRJEB12497) after infection with *Magnaporthe grisea* and *Puccinia striiformis* f. sp. *tritici*, respectively were downloaded from SRA toolkit on NCBI (<https://www.ncbi.nlm.nih.gov/sra>).

2.1.2. Fungal materials

Puccinia triticina race; TTTBT was kindly obtained from The Dept. of Wheat Diseases Research Dept., Plant Pathology Research Institute, ARC.

Magnaporthe grisea strain was kindly obtained from Plant Pathology Dept., Fac. of Agric., Ain Shams Univ.

2.1.3. Plant materials

Rice cultivar (*Oryza sativa* L.) Giza 177 was used in this study representing a resistant cultivar, while Giza 171 was used as sensitive cultivar to *Magnaporthe grisea*. These cultivars were kindly provided from Rice Research Dept., Field Crops Research Institute, ARC.

Wheat (*Triticum aestivum* L.) varieties; Shandweel 1 representing a resistant variety and Morocco representing a susceptible variety were obtained kindly from Wheat Diseases Research Dept., Pathology Research Institute, ARC.

2.2. Methods

2.2.1. Computational analysis of obtained RNA-Seq data

Differentially expressed genes resulted after infection of rice cultivar *Oryza sativa* L. ssp. *japonica* cv. Nipponbare infected with *Magnaporthe oryzae* isolates; P91-15B and Ina 86-137 as the compatible and incompatible pathogens against Nipponbare, respectively (Accession no.: DRA000542) at 0 h and 24 h after inoculation were downloaded from SRA toolkit on NCBI database website (<https://www.ncbi.nlm.nih.gov/>). Differentially expressed genes resulted after infecting wheat cultivars (Vuka) representing a susceptible variety and a resistant variety (Avocet) with *Puccinia striiformis* f. sp. *tritici* (PST) isolate 87/66 were obtained from SRA toolkit on NCBI database website (Accession no.: PRJEB12497). Up-regulated genes were filtered by removing variants using Microsoft Excel Worksheet (Version: 14.0.4734.1000) to reduce redundancy and removing hypothetical proteins to select proteins which have a known function. Then, extra filtration was applied to remove genes with fold change less than 3 folds. The highest 250 up-regulated genes were selected from previously filtered gene list in rice and wheat. Amino acid sequences of up-regulated genes in rice were downloaded from NCBI Enrez tool (<https://www.ncbi.nlm.nih.gov/>). Amino acid se-

quences of up-regulated genes in wheat were downloaded from Ensembl plants Biomart tool (<http://archive.plants.ensembl.org/index.html>).

2.2.1.1. Multi-sequence alignment (MSA) of protein sequences and phylogenetic analysis

Both retrieved files of amino acids for rice and wheat were combined into one text file then submitted to Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>). The most similar genes according to phylogenetic tree were selected according to similarity score, and then manually curated according to similar function and belonging to the super family.

2.2.1.2. Super family sequence analysis

To find protein family (pfam) of selected genes and active domain, amino acid sequences were submitted to HMMER database website (<http://www.ebi.ac.uk/Tools/hmmer/>).

2.2.1.3. Primers design

Primers for selected genes were designed using primer 3 program (<http://bioinfo.ut.ee/primer3-0.4.0/>).

2.2.2. Preparation of control and infected samples for host plants and pathogens

2.2.2.1. Rice infection with *Magnaporthe oryzae*

Rice variety Giza 177 (*Oryza sativa* L.) was used in this study representing a resistant variety and Giza 171 (*Oryza sativa* L.) as sensitive variety to *Magnaporthe oryzae* infection. The procedure of infection was started by growing *Magnaporthe oryzae* fungus on PDA medium at 26°C for 10-12 days, Plates were transferred to 24°C and incubated for 3 d under a black fluorescence light to induce conidia formation. About 20 ml of sterilized water were pour into the plate and fungal conidia were collect using a painting brush. Rice seeds were soaked in water at 30°C for one day in the dark and then sown in a pot of soil in a greenhouse at 28°C in the day and 23°C at night for 10-14 days. Conidia were suspended in 0.05% Tween 20 and sprayed onto rice seedlings at four-leaf stage with fully expanded top leaves until the uppermost leaf was completely wet. After incubation in a dew chamber at 24°C for 24 h, rice plants were moved back to the greenhouse. Two biological replicates were prepared for control and infected plants. Plant samples were collected after 24 hrs. of infection.

2.2.2.2. Wheat infection with *Puccinia triticina*

This part of study was carried out at the greenhouse of Wheat Diseases Research Dept., Plant Pathology Research Institute, ARC, Egypt during 2017/18 growing season. The two wheat varieties Shandweel 1 representing a resistant variety and Morroco representing a susceptible variety were planted at ten seeds per 10 cm diameter plastic pots. When the first leaf was fully expanded in seven days old seedlings, leaves were rubbed gently between moist fingers with tap water. Then inoculated with the urediniospores of leaf rust pathotype TTTBT which was the most frequent pathotype during 2017/18 growing season (El-Orabey et al 2018). The inoculated seedlings were sprayed gently again with water in order to form a film of free water, which is essential to initiate spore germination and establishment of infection. Finally, the inoculated seedlings were incubated in moist chambers for 24 h at 18 to 20°C and 100% RH, and then moved to greenhouse benches. Greenhouse inoculation was done using the methods and procedures developed by Kolmer et al (2005). Plant samples were taken from leaves at one day post-inoculation (dpi). Two biological replicates were prepared for control and infected plants. Plant samples were collected after 24 hrs of infection.

2.2.3. Total RNA extraction

Total RNA was extracted from harvested samples of each control and infected plant using TRI-sure™ RNA isolation kit (Bioline, Cat. No. BIO-38033).

2.2.4. cDNA synthesis

A unique blend of random hexamer and anchored oligo dT primers provided optimal sensitivity and accuracy of first-strand cDNA synthesis. Anchored oligo dT primers anneal precisely to the junction of the poly-A tail and the gene of interest according to SensiFAST cDNA Synthesis Kit (Bioline, Cat. No. BIO-65054).

2.2.5. Real-time PCR

Differential expression of selected genes was verified by Real-time-PCR using SensiFAST™ SYBR® No-ROX Kit (Bioline, Cat. No. BIO-98020).

2.2.6. Real-time PCR data analysis

The data of Realtime-PCR was analyzed by relative quantification $2^{-\Delta\Delta Ct}$ method. The relative difference in gene expression the $2^{-\Delta\Delta Ct}$ method was used (Livak and Schmittgen, 2001).

3. RESULTS AND DISCUSSION

3.1. Computational analysis of obtained RNA-Seq data

RNA-Seq raw data for differentially expressed genes of rice (**Accession no.: DR000542**) and wheat (**Accession no.: PRJEB12497**) after 24 hrs of infection with *Magnaporthe grisea* and *Puccinia striiformis f. sp. tritici*, respectively, were downloaded from SRA toolkit on NCBI (<https://www.ncbi.nlm.nih.gov/sra>). In wheat 31768 up-regulated genes and 3902 up-regulated genes in rice were filtered according to fold change more than 3 and the variants were removed using Microsoft Excel Worksheet (**version: 14.0.4734.1000**). After filtration, the redundant and hypothetical proteins were removed. The number of genes after filtration was 1087 in wheat and 2428 in rice.

3.1.1. Multi-sequence alignment (MSA) of protein sequences and phylogenetic analysis

In the present study, protein MSA was performed for the highest 250 up-regulated genes selected from previously filtered gene list in rice and wheat. Alignments for selected proteins were obtained by a gap-opening penalty of 10 and a gap extension penalty of one. Data resulted from MSA (**Fig. 1**) was used to obtain phylogenetic tree for all 500 genes and the results of phylogenetic tree showed close relationships between ten pairs of proteins (**Fig. 2**).

3.1.2. Super family sequence analysis

To allocate the conserved protein domains, amino acid sequences of the two selected pairs of proteins were submitted to HMMER database website (<https://www.ebi.ac.uk/Tools/hmmer/>). The results showed the presence of one domain as shown in (**Table 2 and Fig. 3, 4**) shared between each two aligned proteins and all domains represented two protein families; Protein kinase and GDSL-like lipase/Acylhydrolase.

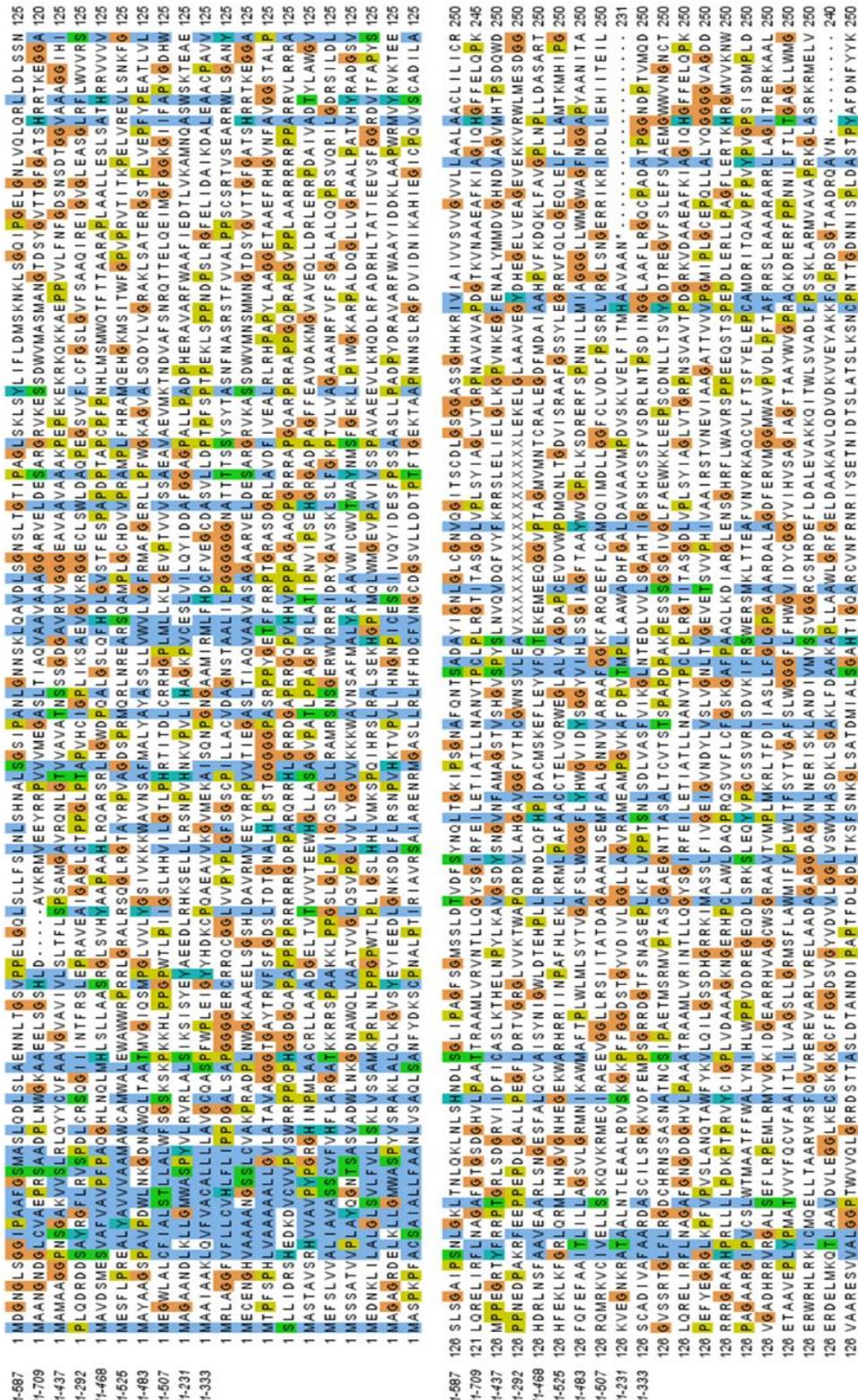


Fig. 1. Multi-sequence alignment which represent the conserved regions in the two selected pairs of genes in wheat and rice

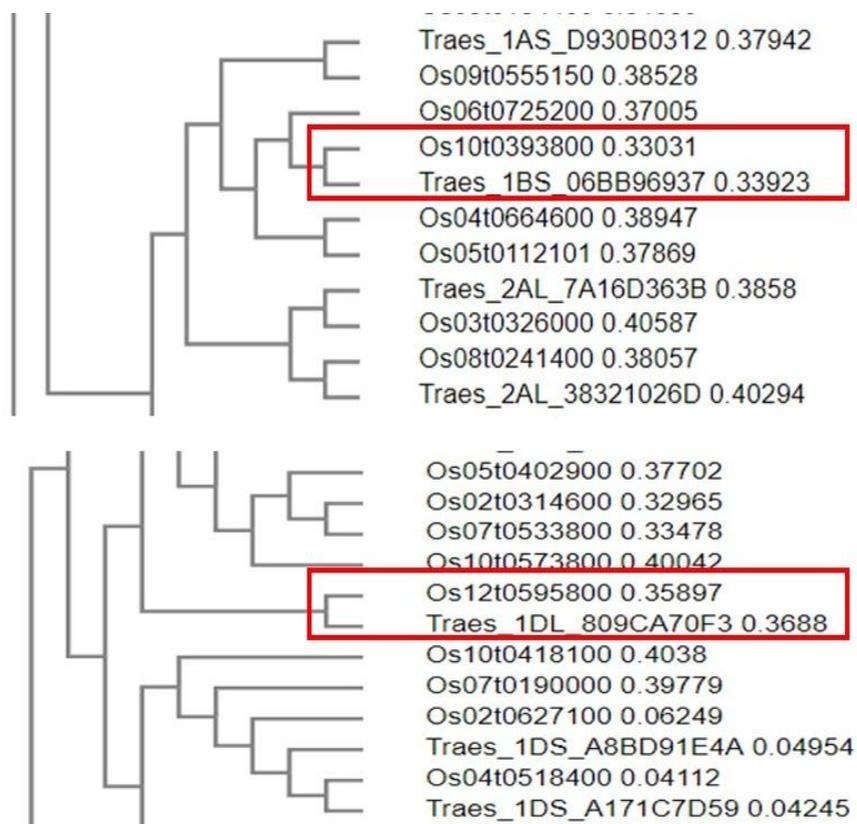


Fig. 2. Phylogenetic tree of the selected gene pairs in wheat and rice

Table 1. Primer sequences used in the quantitative real-time PCR analysis to determine expression patterns of genes involved in the wheat-*Puccinia triticina* and rice-*Magnaporthe oryzae* interaction

Gene	Primer pair	Fragment length
Actin	F: 5'TGACGTGGATATCAGGAAGG3' R: 5'GCTGAGTGAGGCTAGGATGG3'	196 bp
Os10t0393800	F: 5'GAGGAGGAAGATCATGGCGA3' R: 5'GAGAGGTATCATCCCCGGC3'	205 bp
Os12t0595800	F: 5'ATACCGCCAGCAACTTCAAC3' R: 5'CATCCTGGACATGGTTTCGG3'	218 bp
Traes_1BS_06BB96937	F: 5'CTTACAACCTCATGCCGGGG3' R: 5'AGCTTTACTCTGGGCTTGA3'	157 bp
Traes_1DL_809CA70F3	F: 5'GATTCCATGTCCGCGAAACA3' R: 5'GTCCCAGTCCAGCTTCTTCT3'	221 bp

Table 2. Conserved protein domains and accession numbers of the selected genes from wheat and rice plants

Query	Identifier	Description	Accession no.	Start	End
Os12t0595800	Pkinase	Protein kinase domain	PF00069.24	380	658
Traes_1DL_809CA70F3	Pkinase	Protein kinase domain	PF00069.24	290	564
Os10t0393800	Lipase_GDSL	GDSL-like lipase/Acylhydrolase	PF00657.21	34	369
Traes_1BS_06BB96937	Lipase_GDSL	GDSL-like lipase/Acylhydrolase	PF00657.21	104	409

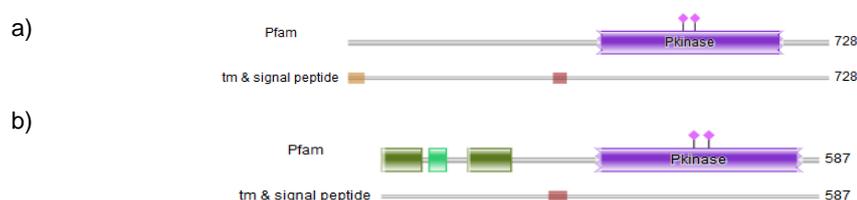


Fig. 3. Protein kinase domain of (a) Os12t0595800 and (b) Traes_1DL_809CA70F3

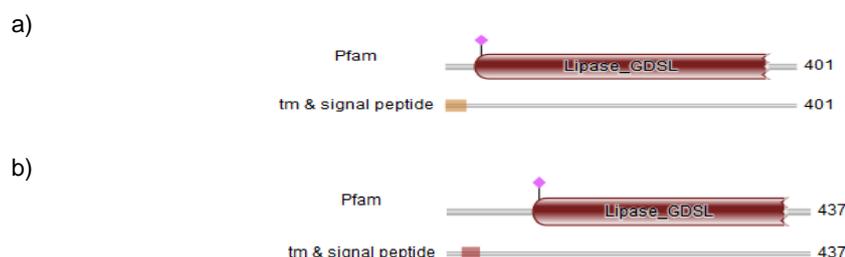


Fig. 4. GDSL-like lipase domain of (a) Os10t0393800 and (b) Traes_1BS_06BB96937

3.1.3. Validation of gene expression

Two differentially expressed genes (**Table 3 and Fig. 5**) of wheat and rice after infection with *Puccinia triticina* and *Magnaporthe oryzae*, respectively, were validated by real-time PCR. The fold changes of each transcript accumulation were determined by comparing infected resistant genotypes to its non-infected control using the comparative cycle threshold method ($2^{-\Delta\Delta C_t}$). The results showed that receptor like kinase gene was up regulated in rice and wheat and GDSL-like Lipase was up regulated in wheat and down regulated in rice.

Plants are attacked by several biotic stresses such as microbial pathogens and other herbivores. To defend against such attackers, Plants have developed a complex defense system against di-

verse pests and pathogens and possess an array of pattern recognition receptors (PRRs) that sense the danger and consequently initiate a defense program that prevents further damage and spreading of the pest. Characteristic pathogenic structures, so-called microbe-associated molecular patterns (MAMPs), serve as signals that allow the plant to sense invaders (**Andersen et al 2018; Albert, 2013**). In the present study, the two differentially expressed genes represented two annotated protein families including Protein kinase and GDSL-like lipase/Acylhydrolase. These proteins play important roles in fungal resistance in wheat and rice. Receptor-like kinases are a large superfamily of proteins are involved in the response to pathogens, growth, and development. Also, plant receptor-like kinases have been identified to act in both broad-spectrum, elicitor-initiated defense re-

sponses and as dominant resistance (R) genes in race-specific pathogen defense. Most defense-related receptor-like kinases are of the leucine-rich repeat (LRR) subclass although new data are highlighting other classes of RLKs as important players in defense responses (Goff and Ramonell, 2007; Delteil et al 2016; Rajaraman et al 2016). MAPK is one of the receptors which activate signaling mechanisms that are common to many cellular processes. The general model of MAPK signaling, membrane-bound Ras proteins facilitates the conversion of GTP to GDP, phosphorylating MAPKKK (Raf) proteins. This in turn phosphorylates MAPKK (MEK) proteins, leading to the phosphorylation of MAPK (ERK) proteins which control the synthesis and/or signaling of defense hormones, the activation of defense genes, the synthesis of antimicrobial metabolites, the stomatal closure, and hypersensitive response (HR)-like cell death, among other defense responses (Meng and Zhang, 2013).

In our study, receptor like kinase gene was up regulated in rice and wheat, this result is consistent with Harkenrider et al (2016), where some of wall-associated kinase genes were upregulated and enhanced the resistance to *Xanthomonas oryzae* and *Magnaporthe oryzae*. In contrast, *OsWAK112d*; one of the wall-associated kinase genes were negative regulator for *Magnaporthe oryzae* resistance in rice.

Lipids are major structural components of prokaryotic and eukaryotic membranes that also function as energy stores. Additionally, membrane lipids are precursors for signaling molecules that regulate development, growth, and stress response (Laxalt and Munnik, 2002; Wang, 2004;

Ryu 2004; Shah 2005; Gillaspay, 2011). Lipases play a role in resistance response to pathogens in plants in the synthesis and perception of salicylic acid that is crucial for wound and pathogen-induced defense response (Shah, 2005).

In the present study, GDSL-like Lipase was the second gene confirmed by qRT-PCR and the results showed that the expression of that gene was upregulated in wheat as expected but down regulated in rice unexpectedly. These results are compatible with Gao et al (2017) who identified GDSL lipases, *OsGLIP1* and *OsGLIP2* in rice immune responses and reported that expression of *OsGLIP1* and *OsGLIP2* was suppressed by pathogen infection and salicylic acid (SA) treatment, simultaneous down-regulation of *OsGLIP1* and *OsGLIP2* increased plant resistance to both bacterial and fungal pathogens. Whereas, disease resistance in *OsGLIP1* and *OsGLIP2* overexpression plants was significantly compromised, proposing that both genes act as negative regulators of disease resistance. In Arabidopsis, GDSL lipase 1 (*GLIP1*) was shown to modulate systemic immunity through the regulation of ethylene signaling components and plays a critical role in necrotroph-induced systemic resistance, and once activated, can elicit a broad-spectrum resistance to multiple pathogens (Kwon et al 2009; Kim et al 2013; Kim et al 2014). Arabidopsis GDSL lipase (*GLIP1*) was reported to contribute in defense against the necrotrophic fungus *Alternaria brassicicola* (Oh et al 2005). Another GDSL lipase, *GLIP2*, has been suggested to play a role in resistance to *Erwinia carotovora* via negative regulation of auxin signaling (Lee, 2009).

Table 3. The measured gene expression of *Triticum aestivum* and *Oryza sativa* after 24 hours of infection with *Puccinia triticina* and *Magnaporthe oryzae*, respectively

Trans. ID	Control			Treatment			$\Delta\Delta CT$	RQ
	CT	CTH	ΔCT	CT gene	CTH	ΔCT		
Traes_1BS_06BB96937	20.750	25.435	-4.685	21.260	29.875	-8.615	-3.930	15.2422
Os10t0393800	11.440	21.23	-9.79	9.655	19.19	-9.535	0.255	0.8379
Traes_1DL_809CA70F3	28.980	25.435	3.545	30.155	29.875	0.280	-3.265	9.6130
Os12t0595800	22.905	21.23	1.675	20.565	19.19	1.375	-0.3	1.2311

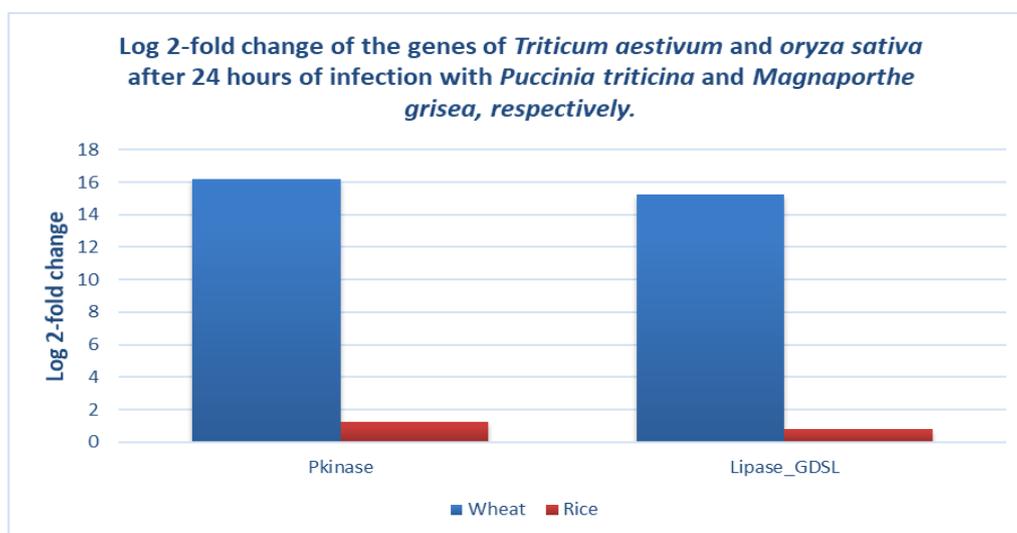


Fig. 5. Log 2-fold change of gene expression of the selected genes in *Triticum aestivum* and *Oryza sativa* after 24 hours of infection with *Puccinia triticina* and *Magnaporthe oryzae*, respectively.

4. CONCLUSION

Our present study provides an informative comparative study between the two plants under study; rice after infection with blast fungus and wheat after infection with leaf rust fungus. Thousands of genes were the start point of our study, these genes filtered and aligned to obtain the most common genes between wheat and rice in response to fungal infection which means; despite of different plants, different pathogenic fungi and different plant pathogen interaction responses, there were some pathways work in the same manner in the plants under study. After computational analysis, two genes of up-regulated genes which share the same annotation were selected from each plant to be validated, four weeks germinated plants were infected with the pathogenic fungi, the samples were collected after 24 hr. of infection. Then, the gene expression of the selected genes was validated by real time PCR method after reverse transcription. The two genes of wheat were up-regulated, while one gene was up regulated and the other gene was down-regulated in rice. The present study validated that some plants can respond to stresses in the same attitude, even they are different and such variation of results may be because using different plant varieties and different fungal strains than varieties and strains used in the researches of raw data.

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دراسات مقارنة علي التعبير الجيني في الأرز والقمح بعد الإستجابة للعدوي الفطرية

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الموجز

حدثت زيادة التعبير الجيني للجينات في القمح، وفي نبات الأرز حدثت زيادة للتعبير الجيني لاحدي الجينات (Pkinase) وانخفاض في التعبير الجيني للجين الآخر (Lipase_GDSL) ويمكن تلخيص أهم النتائج المتحصل عليها كالتالي: تم التحقق من التعبير الجيني لاثنتين من الجينات المشتركة بين الارز والقمح بعد العدوي بفطر اللفحة وفطر صدا الأوراق علي التوالي بالرغم من اختلاف النباتات واختلاف الفطريات في التطفل ودورة الحياة وبالرغم من التباعد الزمني بين الأسلاف للنباتين. حدثت زيادة في التعبير الجيني للجينين في القمح بعد الإصابة بفطر صدا الأوراق *Puccinia triticina*. حدثت زيادة في التعبير الجيني لواحد من الجينين في الارز بعد الإصابة بفطر اللفحة *Magnaporthe oryzae* وانخفاض التعبير الجيني للجين الآخر. التنوع في النتائج واختلافها عن البيانات الأولية قد يكون بسبب استخدام اصناف مختلفة من النباتات واستخدام سلالات مختلفة من الفطريات. بعض النباتات قد تواجه الضغوط البيئية كالممرضات بطريقة متشابهة الي حد كبير رغم اختلاف تلك النباتات.

الكلمات الدالة: التحليل المقارن، تسلسل الرنا، القمح، الأرز، صدا الأوراق، اللفحة

يهدف هذا البحث الي مقارنة وتحديد ووصف وعزل الجينات ذات الصلة الممرضة من القمح المصاب بفطر صدا الأوراق *Puccinia triticina* والأرز المصاب بفطر اللفحة *Magnaporthe grisea*. وقد تم الحصول على البيانات الأولية من الدراسات السابقة لتجارب RNA-Sequencing التي أظهرت تعبيراً تفاضلياً في جينات القمح والأرز بعد الإصابة بـ *Puccinia triticina* و *Magnaporthe oryzae* على التوالي. كانت آلاف الجينات نقطة البداية في دراستنا، وتمت تصفية هذه الجينات ومحاذاتها للحصول على الجينات الأكثر شيوعاً بين القمح والأرز بعد الإستجابة للعدوي الفطرية، على الرغم من اختلاف النباتات واختلاف الفطريات الممرضة واختلاف استجابات النباتين للفطرين، كانت هناك بعض المسارات تعمل بنفس الطريقة في النباتات قيد الدراسة. بعد التحليل بإستخدام برامج المعلوماتية الحيوية، تم إختيار إثنين من الجينات التي زاد تعبيرها الجيني بعد العدوي الفطرية والتي تشترك في نفس الوظيفة في النباتين محل الدراسة ليتم التأكد من التعبير الجيني لها. تمت عدوي الأرز بعمر أربعة أسابيع بفطر اللفحة *Magnaporthe oryzae* والقمح بفطر صدا الأوراق *Puccinia triticina* وتم جمع العينات النباتية بعد 24 ساعة.

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