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BREAD WHEAT COMPARATIVE EXPRESSION ANALYSIS IN RESPONSE TO LEAF RUST (*puccinia triticina*) USING MICROARRAY TECHNIQUES

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Bread wheat (*Triticum aestivum* L.) is one of the most cultivated crop worldwide, moreover, it is one of the fundamental food crops that provide more than 50% of the required calories and proteins for billions of people worldwide (Tadesse *et al.* 2017). In addition to its basic caloric value, wheat with its high protein content is one of the most important sources of plant protein in the hu-

man diet (Shiferaw *et al.* 2013). It is the only cereal which produces gluten allowing the production of leavened bread and is the major ingredient in other foods such as biscuits and cakes, pasta, noodles and some breakfast cereal products (Bruins, 2013).

There is a demand for increasing wheat production by 60% in the Central and West Asia and North Africa "CWANA" region brfore 2050 (Tadesse *et al.*, 2017). Egypt is a member of CWANA and faces many challenges; more than 12% of these challenges are due to biotic stresses and crop diseases. The other challenges are related to irrigation problems, fertilizers, lack of purity of seeds and the problem of high labor costs (Othman *et al.*, 2014). Biotic stresses and pathogens including leaf rust threaten wheat cultivation worldwide (Bolton *et al*., 2008). Wheat leaf rusts due to infection by *Puccinia triticina* causes damages and limitation of wheat productivity in many wheat producing areas worldwide, and it is more dangerous than the other three rusts (El Jarroudi *et al*., 2014). The problem of rust is a very old historical problem and there were many archaeological manuscripts dealing with this disease and its impact on wheat productivity (Gill *et al.*, 2019). This was attributed to the reduction of kernels number and weight (Manickavelu *et al*., 2010).

rScientific studies have shown that there is a great variation in the capabilities of different plants to fight and control pathogen infection. Some plants that have the ability to prevent pathogen infection by building chemical and physical barriers that prevent the virus from attacking the plant. On the other hand, some plants fight the pathogen after infection through signal transduction and rapid and effective change in gene expression. The speed of the plant's reaction to attack the pathogen is the indicator of the effectiveness of the resistance, as the plant succeeds in resisting only through a rapid reaction (Manickavelu *et al.*, 2010). They have mentioned in their report that plants with the ability to resist are distinguished by their very fast induction of several signal transduction pathways which can stop pathogenicity, while sensitive plants have a slow response which enable the pathogen from colonization and cause damages which might lead to severe weakness, which in turn leads to death. The resistant process starts upon the plant recognition of pathogen protein elicitors. The interaction of the elicitors with the plant unleashes different mechanisms for primary and secondary signal transduction pathways, which includes but not limited to reactive oxygen species (ROS), ion fluxes, salicylic acid induction and phosphorylation processes, which in turn works to turn on many genes responsible for pathogen resistance (Meng and Zhang, 2013). These signal transduction pathways lead to induction of several genes responsible for the production of proteins and enzymes evolved in the biochemical pathways of pathogen defense mechanisms such as glutathione S-transferase, small GTPase, Nucleolar GTP-binding protein, peroxidases, transcriptome, pathogenesis-related

(PR) proteins, stress-related phytohormones, carbon metabolism, and RNA processing proteins and many others (Liu and Lam, 2019).

Production of a resistant wheat cultivar using breeding programs is an economical acceptable approach. Although, there is high variability in the population of *Puccinia triticina* (Kolmer and Hughes, 2014) availability of tightly linked molecular markers is essential for successful breeding program (Gill *et al.*, 2019).

The ability of a plant to resist the pathogen depends on a complex interaction in the gene expression between the plant and the pathogen genomes (Manickavelu *et al*., 2010). Therefore, the genetic approach was successfully used in reducing the leaf rust losses where more than 80 genes related to leaf rust resistant (*Lr* genes) were characterized and reported (Qureshi *et al*., 2018). Transgenic bread wheat plants with different *Lr* genes have been successfully utilized by breeders in different breeding programs (Loutre *et al*., 2009).

Wheat has been extensively studied for a wide range of agronomic traits widely distributed throughout its genome. Functional genomic approaches were experimented using high-density oligonucleotide and cDNA microarrays. The expression profiles of thousands of genes under this condition can thus be assayed simultaneously. We propose to characterize and catalog genes involved in the response to biotic stresses especially *Puccinia triticinae* in wheat local germplasms on the transcriptome level.

MATERIALS AND METHODS

Plant materials

Ten Egyptian bread wheat cultivars obtained from the National Wheat Program, Crops Res. Institute, Giza were evaluated for resistance to leaf rust disease caused by *Puccinia triticina* at seedling stage under glass-house condition. These cultivars were; Sakha-93, Gemmeiza-7, Gemmeiza-10, Giza168, Shandwel-1, Sids-12, Misr-1, Misr-2, Sakha-94, Giza-160 and the wheat cultivar Morocco was considered control cultivar is susceptible to leaf rust.

1. Biotic stress and sample preparation

According to Salkind (2010), the wheat cultivars were grown in pots in a greenhouse in a randomized complete block design with three replications. The seeds of each cultivar were planted as five seeds per pot in 15 cm diameter pots filled with a mixture of soil, sand and peatmous at the rate of 1: 1: 1. One-week old plantlets were artificially infected with *Puccinia triticinae* (Tarvet and Cassell, 1951). Three biological replications were done for each sample and placed in the greenhouse at 20 to 25°C. The inoculated plants were placed overnight in a dew chamber at 22°C, and then placed on a greenhouse bench with the temperature of 22-25°C. Incubated plants

were irrigated with macronutrient solution (Hoagland and Arnon, 1950). All data for greenhouse screening experiments were statistically analyzed according to Steel and Torrie (1980). The differences between means of plants grown under control versus biotic stress were compared using t-test.

2. Disease assessment

The leaf rust pathotype PTTS was identified according to the method adopted by Long and Kolmer (1989). Infection types were evaluated 12 days after inoculation using the scale of 0 to 4 (Stakman *et al.*, 1962): which 0 = immune, with no visible necrosisor uredinia. ; = hypersensitive fleck with no sporulation, 1 = small uredinia surrounded by necrosis, 2 = small uredinia surrounded by chlorosis, 3 = moderate size uredinia without chlorosis or necrosis, 4 = large uredinia without chlorosis or necrosis. Infection types 0; 1 and 2 are considered as resistant reaction, While, 3 and 4 are susceptible.

3. RNA extraction

RNA was extracted from leaves that were harvested from control as well as three weeks biotic treated plants. Total RNA was extracted by TRI Reagent® RNA Isolation Reagent (Sigma-aldrich corp., Cat. No. T9424).

4. RT-reaction and cDNA cloning

Extracted RNA from the leaf tissues of Gemiza7 (sensitive) and Misr1 (tolerant) was subjected to reverse transcriptase (RT-PCR) reaction to synthesize the first strand cDNA. DNA polymerase I used for cDNA second strand synthesis and the resulted double stranded cDNA fragments were directly ligated into the pJET1.2/blunt cloning vector. *Escherichia coli* strain Jm109 was used in the transformation of the cloning vector. Lethal gene in the multiple cloning site of the cloning vector was used to select the transformed colonies that contain the fragments of interest.

5. Sequencing

Selected cloned cDNA fragments generated from the constructed cDNA library were sequenced using the ABI PRISM Big Dye terminator kit (PE Applied Biosystems, USA) in conjunction with ABI PRISM (310 Genetic Analyzer). The selected inserted fragments were sequenced on both strands.

6. Sequence data analysis

Obtained sequences were analyzed using bioinformatics tools (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to check the redundancy and the quality of the cDNA library. Data was analyzed against GeneBank database using blastn to identify similar DNA sequences also blastx was used to identify similar protein sequences.

7. Microarray experiment

Agilent Wheat Gene Expression Microarray Custom Array 4x44k

(G2519F-022297) was used to measure changes in gene expression. Four chamber slide was used and single dye (Cy3) was detected. Non-infected samples from both varieties were hybridized in two chambers separately and the other two chambers were assigned for the two infected samples. The generated slides were used to identify genes with differential expression under biotic stress treatment.

Total RNA was labelled with One-Color Microarray-Based Gene Expression Analysis (Quick Amp Labeling) with Tecan HS Pro Hybridization kit (G4140-90041) using manufacturer's instruction. RNA was quantified using a NanoDrop-1000 spectrophotometer and quality was monitored with the Agilent 2100 Bioanalyzer.

About 1.5 μg of Cy3-labelled cRNA (specific activity >10.0 pmol Cy3/μg cRNA) was fragmented at 60°C for 30 minutes in a reaction volume of 250 ml containing 1x Agilent fragmentation buffer and 2x Agilent blocking agent following the manufacturer's instructions. Upon completion of the fragmentation reaction, 250 ml of 2x Agilent hybridization buffer was added to the fragmentation mixture and hybridized to Agilent Wheat Gene Expression Microarray (G2519F), for 17 hours at 65°C in a rotating Agilent hybridization oven. After hybridization, microarrays were washed for one minute at room temperature with GE Wash Buffer 1 (Agilent) and 1 minute with 37°C GE Wash buffer 2 (Agilent), then dried immediately by brief centrifugation. Slides were scanned immediately after washing on the Agilent DNA Microarray Scanner using one color scan setting for 4x44k array slides (G2519F). The scanned images were analyzed with Feature Extraction Software 9.5 (Agilent) using default parameters (protocol: GE1v5_95_Feb07).

8. Microarray data analysis

Genespring GX V12.6 was used to microarray data further analysis. The generated raw data file was first imported to Genespring software. Data normalization variance regularization normalization was used. Fold change analysis data were further filtered with fold change threshold more than or equal to 2 fold change. Feature Extraction software (provided by Agilent Technologies) was used to extract features (protocol: GE1-v5_95_Feb07) from the data were done to filter the data to identify genes related to leaf rust treatment.

RESULTS AND DISCUSSION

Food security has the highest priority for the Egyptian government to supply the Egyptian food needs. In this context, wheat is the main component of bread, which is the main pillar in the Egyptians food, especially low-income people. Egypt needs to increase the acre productivity from 12.4 ardab to 21.6 ardab/fadan (Othman *et al.,* 2014). Therefore, solving

the problems and challenges facing wheat cultivation in Egypt and increasing its productivity occupy the priorities of research work in Egypt. Biological stresses in general, and fungal infections in particular are of the most important challenges, which represent almost 12% of the challenges facing Egyptian wheat producers (Othman *et al.*, 2014). The current research helps to find fundamental solutions to solve the problem of *Puccinia triticinae* infection which causes the leaf rust in wheat (Qureshi, 2018) through the comparative expression analysis in response to leaf rust using microarray techniques.

Ten bread wheat cultivars were evaluated against leaf rust. Their infection levels were represented in Table (1). Leaf rust inoculation resulted in Giza-168, Sids-12, Misr-1 and Misr-2 were highly resistant (0 infection type, immune). Shandwel-1 had extremely low infection types of; (fleck) to 1. Gemmeiza-10, Sakha94 Sakha-93 and Gemmeiza-7 had moderate infection types (1 and 2). On contrast, both Giza-160 and Morocco cultivars showed susceptible infection type (4). The newest cultivar Misr-1 was selected as highly resistant cultivar, Gemmeiza7 as a moderate resistant and Morocco as highly susceptible to leaf rust at seedling stage.

Biotic stresses have a serious impacts on plants triggering stimulation of plant defense mechanisms of plant immune response (Shamrai, 2014). All plants whether they are being sensitive, moderate tolerance or even highly tolerance have their responses to the biotic stresses. These responses include several molecular events and signaling systems which might lead to adaptation (Kranner *et al.*, 2010). Therefore, studying the differentially expressed genes as the immune response of both varieties (Misr1 and Gemmiza7) has important implications.

Total RNA was successfully isolated from leaves of the Egyptian wheat cultivars Misr1 and Gemmiza7 after treatment to be used in cDNA library construction. Purity and concentrations of isolated RNA were investigated using spectrophotometer and agarose gel, where all RNA samples were within the limits that can be used to start the construction of cDNA library, (Fig. 1).

Purified RNA was used as a template for first strand cDNA synthesis, followed by second strand synthesis. The resulted cDNAs were fractionated regarding to their size and the resulted double stranded cDNA fragments were directly ligated into the pJET1.2 blunt cloning vector for subsequent transformation into *Escherichia coli* strain Jm109 as a bacterial host. Blunt end cloning vector with lethal gene in its multiple cloning site was used to avoid the restriction digestion activities within the inserted fragments. PCR technique was used to identify the different lengths of the inserted fragments into the cloning vector using the specific primers supplied by the pJET 1.2/blunt cloning vector Kit. The fragments with length about 0.5 kb or more were selected for library printing. (Fig. 2) represents the

results of PCR screening process. Selected cloned cDNA fragments were sequenced and analyzed using bioinformaticstools (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Blastn and Blastx are considered very powerful tools in bioinformatics used to compare sequences, derive their functions and their relationship to living things (McGinnis and Madden, 2004). In order to check the quality and the redundancy of the cDNA library, the selected cloned cDNA fragments were sequenced and analyzed using bioinformatics tools (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Blastn analysis has revealed that about 72% of the sequence belongs to wheat transcriptome with e-value ranging from zero to 0.05. However, Blastn only succeeded to identify accession number for a related gene to 17% of the sequences. Repeated sequences ratio was 21% identified as number of well-identified repeated sequences (s) to total number of well identified sequences (n). Blastx didn't show relevant result to blastn. Most of the sequences was related to wheat, barley and rice transcriptomes. Only 48% of the sequences could be identified as related to wheat proteome with e-value ranged from zero to 5.7. Repeated sequences ratio was 9% identified as number of well identified repeated sequences (s) to total number of well identified sequences (n). Blastn was better to identify sequences related to wheat due to the length of the query, while Blastx results was more accurate to identify accession number for a related gene. The results were as expected, most of the sequences were related to wheat, barley and rice transcriptomes (as wheat transcriptome is not fully identified like rice). The rest of the sequences "about 7%", neither blastn nor blastx could identify similar sequence.

Microarray technique is one of the effective techniques used to monitor changes in gene expression, which produces a large amount of analyzable information that contains a massive number of genes, which represents a major challenge to interpreting and linking the results of differences in gene expression (Gupta, 2018). In 2007, there was an attempt to identify the differentially expressed wheat genes upon the infection with *Puccinia triticina* and monitoring the change in their expression profiling at several time intervals using microarray technique (Fofana *et al.*, 2007). In contrast with the study of Fofana and her coworker (2007), a microarray experiment has been designed to explore the differentially expressed genes as a result of infection of two local bread wheat cultivars (Misr1 and Gemmiza7) against leaf rust caused by *Puccinia triticinae*. As mentioned before, the two local cultivars were selected from 10 different Egyptian cultivars (Table 1) as a moderate (Gemmiza7) and a tolerant (Misr1) cultivars for leaf rust.

Ready-made Agilent Wheat Gene Expression Microarray Slide (G2519F) was used. About 43,803 wheat probes are represented, sourced from: RefSeq (Release 31), Sep 2008, UniGene (Build 53), Oct 2008, TIGR Plant Transcript Assemblies (Release 5), Jul 2008 and TIGR

Gene Indices (Release 11), Jul 2008. A TIFF generated image (Fig. 3) shows the microarray slide after hybridization and wash steps. Although, results for Misr1 treatment/control were much better than Gemmiza7 treatment/control results, useful data from the Gemmiza7 slides was collected and interpreted.

Variance regularization normalization was used. Whereas normalization adjusts the median or mean of the log2 (ratio) measurements, stochastic processes will cause the variance of the measured log2 (ratio) values to differ from one region of an array to another or between arrays. A box whisker plot for the imported raw data after normalization is shown in Fig. (4) while a profile plot for the imported raw data after normalization is shown in Fig. (5).

Samples Quality Control QC helps to decide which samples are ambiguous and which are passing the quality criteria. Accordingly, the unreliable samples can be removed from the analysis. Functional annotation analysis for the obtained genes was carried out using Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 on its site (http://david.abcc.ncifcrf.gov/).

Data were further filtered with fold change threshold more than or equal to 2 fold change. Feature Extraction Software (provided by Agilent Technologies) was used to extract features (protocol: GE1-v5_95_Feb07) form the data were done to filter the data to find genes related to leaf rust treatment and have significant variance between treatment and control. About 18203 genes were identified to have changed gene expression according to leaf rust treatment within Misr1 cultivar. While about 11303 genes were identified to change gene expression within Gemmeza7 cultivar.

Venn diagram (Fig. 6) illustrates the intersection of identified genes, the final number of identified genes related to fungal stress tolerance genes are about 6589 genes between both studied cultivars.

Hierarchical clustering (Murtagh and Contreras, 2011) is one of the most widely used clustering techniques for analysis of gene expression data. This method follows an agglomerative approach, where the most similar expression profiles are joined together to form a group. These are further joined in a tree structure, until all data forms a single group. Hierarchical clustering was used for the prediction of the intersected genes. The results of the hierarchical clustering analysis (Fig. 7) shows genes with similar expression grouped within the same clade in the dendrogram. Each clade was saved as separate group or trend. The microarray experiment resulting data were submitted to GeneBank and had got GEO (Gene Expression Omnibus) accession no. GSE55297.

There were 6589 differentially expressed genes in both cultivars that vary in their behavior according to the cultivar. In Misr1 cultivar, there were 3460 up-regulated genes and 3129 down-regulated

genes where in case of Gemmza7 cultivar there were 3249 up-regulated genes 3340 down-regulated genes.

As shown in Fig. (8), within the differentially expressed genes in both varieties, about 1719 genes were up-regulated and about 1948 genes down-regulated in Gimmiza7 cultivar. On the other hand, about 1870 genes were up-regulated and about 1797 genes were down-regulated in Misr1 cultivar. Within the same group of differentially expressed genes in both cultivars there were 1657 genes up-regulated in both cultivars, while 1753 genes were down-regulated in both cultivars. Within the same group, about 213 genes were up-regulated in Gimmiza7 cultivar, while they were down-regulated in Misr1 cultivar and on the contrary, there were about 65 genes down-regulated in Gimmiza7 cultivar, while they were up-regulated in Misr1 cultivar.

In comparison to the microarray experiment reported by Fofana and her coworkers (2007), in the current study about 18203 genes were differentially expressed within Misr1 cultivar and about 11303 genes were differentially expressed within Gemmeza7 cultivar, while their experiment there were about 7728 genes. Many of the genes that were differentially expressed in the current experiment were also differentially expressed in their trial such as; putative hydrolase, nucleotide metabolism, photosynthetic enzymes, energy related genes and protein binding activity.

There was no correlation between the number of differentially expressed genes and the cultivar sensitivity. There were about 6589 genes differentially expressed in both cultivars Figs (6 and 8). These genes include several important candidates that could tolerate leaf rust such as peroxisomal biogenesis factor which has a vital role in the induction of reactive oxygen species that could destroy the invader (Lopez-Huertas *et al.*, 2001); NBS-LRR type resistance protein which has a leucine rich domains and an amino-terminal signaling domain which plays an important role in cell death signaling; *Lr21* resistance protein (Belkhadir *et al.*, 2004); Resistance-related receptor-like kinase which is involved in development and defense mechanisms (Shiu *et al.*, 2004); Clp-like energy-dependent protease precursor which has a role in prokaryotic proteolysis (Gottesman *et al.*, 1990) and several pathogen related proteins. There was a remarkable up-regulation of hydrolase enzymes in both tomato cultivars which have a vital role in cellular methylation and in biotic stress tolerance (Li *et al.*, 2015).

Dramatic change in the expression profile of the regulatory genes has been observed in both cultivars. These changes include gene responsible for DNA metabolic process, protein modification en-

zymes, ribonucleotide binding proteins, ATP binding proteins, transcription regulators and proteins involved in the regulation of macromolecules. These categories of genes are involved in the initiations of signaling system and regulation of plant response to the stress conditions (Verma *et al.*, 2013).

The effectiveness of resistance is not only due to integration of gene action (Kranner *et al.*, 2010) and signal transduction (Liu and Lam, 2019) but also to the time taken by the cultivar to induce all involved genes as the plant succeeds in resistance only through a rapid reaction (Manickavelu *et al.*, 2010). This was clear as well from the results reported by Fofana *et al.* (2007) because they took the time factor in their consideration.

SUMMARY

Wheat is one of the major food crops in the world and a foundation of human nutrition worldwide. In addition to its basic caloric value, wheat with its high protein content is one of the most important sources of plant protein in the human diet. Egypt faces many challenges in wheat production and pest management. Besides, there is increasing concern over environmental issues. These issues will continue to be important into the future. Two local bread wheat Cultivars (Misr1 and Gemmiza7) against leaf rust (*Puccinia triticinae*) were examined. About 18203 genes were differentially expressed in response to leaf rust treatment in the cultivar Misr1 and 11303 genes in Gemmeiza7 cultivar. Within the differentially expressed genes, 6589 genes were differentially expressed in both cultivars which were vary in their behavior according to the cultivar. Misr1 showed 3460 up-regulated genes and 3129 down-regulated genes, while Gemmiza7 had 3249 up-regulated genes and 3340 down-regulated genes. We also discovered 486 genes with opposite expression between the two studied cultivars.

The current study resulted in a huge pool of genes associated with resistance to biological stresses and fungal resistant, which needs more studies to draw an integrated picture of genetic interference that leads to resistance to biological stresses. The current study also suggested the need to study the time component as an influencing factor in the resistance of biological stresses.

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REFERENCES

Belkhadir, Y., R. Subramaniam and J. Dangl (2004). Plant disease resistance protein signaling: NBS–LRR proteins and their partners. Current Opinion in Plant Biology, 7: 391-399.

Bolton, M.D., J. A. Kolmer and D. F. Garvin (2008). Wheat leaf rust caused by *Puccinia Triticina*. Mol. Plant Pathol, 9: 563-575.

Bruins, M. J. (2013). The clinical response to gluten challenge: a review of the literature. Nutrients, 5: 4614-4641.

El Jarroudi, M., L. Kouadio, P. Delfosse and B. Tychon (2014). Brown rust disease control in winter wheat: I. Exploring an approach for disease progression based on night weather conditions. Environ. Sci. Pollut. Res., 21: 4797-4808.

Fofana, B., T. W. Banks, B. McCallum, S.E. Strelkov and S. Cloutier (2007). Temporal gene expression profiling of the wheat leaf rust pathosystem using cDNA microarray reveals differences in compatible and incompatible defence pathways. Int J Plant Genomics, 1-13.

Gill, H. S., C. Li, J. S. Sidhu, W. Liu, D. Wilson, G. Bai, B. S. Gill and S.K. Sehgal (2019). Fine mapping of the wheat leaf rust resistance gene Lr42. Int. J. Mol. Sci., 20: 2445-2457.

Gottesman, S., C. Squires, E. Pichersky, M. Carrington, M. Hobbs, J. Mattick, B. Dalrymple, H. Kuramitsu, T. Shiroza and Foster T. (1990). Conservation of the regulatory unit for the ClpA ATP-dependent proteases in prokaryotes and eukaryotes. Proceedings of the National Academy of Sciences of the United States of America, 87: 3513-3517.

Gupta, V. (2018). Comparative analysis of cancer gene using microarray gene expression data. International Journal of Advanced Research and Development, 3: 155-165.

Hoagland, D. R. and D. I. Arnon (1950). The water-culture method for growing plants without soil. California Agricultural Experiment Station, Circular-347.

Kolmer, J. A. and M. E. Hughes (2014). Physiologic specialization of *Puccinia Triticina* on wheat in the United States in 2012. Plant Dis., 98: 1145-1150.

Kranner, I., F. V. Minibayeva, R. P. Beckett and C.E. Seal (2010). Tansley review: What is stress? Concepts, definitions and applications in seed science. New Phytologist, 188: 655-673.

Li, X., L. Huang, Y. Hong, Y. Zhang, S. Liu, D. Li, H. Zhang, and F. Song (2015). Co-silencing of tomato S-adenosylhomocysteine hydrolase genes confers increased immunity against *Pseudomonas syringae* pv. Tomato DC3000 and enhanced tolerance to drought stress. Front Plant Sci., 6: (717) 1-13.

Liu, J. and H. Lam (2019). Signal transduction pathways in plants for resistance against pathogens. International Journal of Molecular Sciences, 20: 2335-2341.

Long, D. L. and J. A. Kolmer (1989). A North American system of nomenclature for *Puccinia recondita* f. sp. tritici. Phytopathology, 79: 525-529.

Lopez-Huertas, E., W. Charlton, B. Johnson, I. Graham and A. Baker (2001). Stress induces peroxisome biogenesis genes. The EMBO journal, 19: 6770-6777.

Loutre, C., T. Wicker, S. Travella, P. Galli, S. Scofield, T. Fahima, C. Feuillet and B. Keller (2009). Two different CC-NBS-LRR genes are required for *Lr10* mediated leaf rust resistance in tetraploid and hexaploid wheat. Plant J., 60: 1043-1054.

Manickavelu, A., K. Kawaura, K. Oishi, T. Shin-I, Y. Kohara, N. Yahiaoui, B. Keller, A. Suzuki, K. Yano, and Y. Ogihara (2010). Comparative gene Expression analysis of susceptible and resistant near-isogenic lines in common wheat infected by *Puccinia triticina*. DNA Research, 17: 211-222.

McGinnis, S. and T. L. Madden (2004). BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Research, 32: 20-25.

Meng, X. and S. Zhang (2013). MAPK cascades in plant disease resistance signaling. Annu. Rev. Phytopathol., 51: 245-266.

Murtagh, F. and P. Contreras (2011). Methods of hierarchical clustering. Computing Research Repository, 1-21.

Othman, A. Z., N. Y. Solieman and A. A. Hassa (2014). Study of the most important factors affecting the production of wheat crop in the new lands in Egypt (Nubaria Area). Middle East Journal of Agriculture Research, 3: 20-26, ISSN 2077-4605.

Qureshi, N., H. Bariana, V. V. Kumran, S. Muruga, K. L. Forrest, M. J. Hayden and U. Bansal (2018). A New leaf rust resistance gene *Lr79* mapped in chromosome 3BL from the durum wheat landrace Aus26582. Theor. Appl. Genet., 131: 1091-1098.

Salkind, N. J. (2010). Encyclopedia of research design Thousand Oaks, CA: SAGE Publications, Inc. doi: 10.4135/9781412961288

Shamrai, S. (2014). Plant immune system: Basal immunity. Cytology and Genetics, 48: 258-271.

Shiferaw, B., M. Smale, H. J. Braun, E. Duveiller, M. Reynolds and G. Muricho. (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Sec., 5: 291-317.

Shiu, S., W. M. Karlowski, R. Pan, Y. Tzeng, K.F. X. Mayer and W. Li (2004). Comparative analysis of the receptor-Like kinase family in arabidopsis and rice. The Plant Cell, 16: 1220-1234.

Stakman, E. C., D. M. Stwart and W. Q. Loegering (1962). Identification of physiologgic races of *Puccinia graminis* var. tritici. U.S. Dept. Agric. Serv. E, 617-53.

Steel, R. G. D. and J. H. Torrie (1980). Principles and procedures of statistics. A biometrical approach. 2nd edition. McGraw-Hill, New York, USA: 20-90.

Tadesse, W., H. Halila, M. Jamal, S. El-Hanafi, S. Assefa, T. Oweis and M. Baum (2017). Role of sustainable wheat production to ensure food security in the CWANA region. Journal of Experimental Biology and Agricultural Sciences, 5: 15-32.

Tarvet, I. and R. C. Cassell (1951). The use of cyclone in race identification of microscopic particles. Phyto-pathology, 41: 282-285.

Verma, S., S. Nizam and P. Verma (2013). Biotic and abiotic stress signaling in Plants. Chapter 2 in book, M. Sarwat *et al.* (eds.), Stress Signaling in Plants: Genomics and Proteomics Perspective, (1), 10.1007/978-1-4614-6372-6-2.

Table (1): Infection type of Leaf rust reactions of plant materials.

Cultivars	Infection types
Sakha-93	2
Gemmeiza-7	2
Gemmeiza-10	1
Giza168	0
Shandwel-1	1
Sids-12	0
Misr-1	0
Misr-2	0
Sakha-94	1
Giza-160	4
Morocco	4

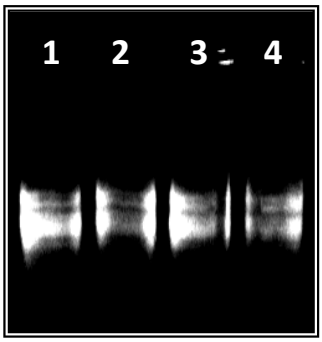

Fig. (1): Agarose gel electrophoresis of total RNAs isolated from the most tolerant and moderate cultivars with and without control artificial infection. 1: Misr1, 2: Gemmiza7 3: Misr1 Control, 4: Gemmiza7 Control.

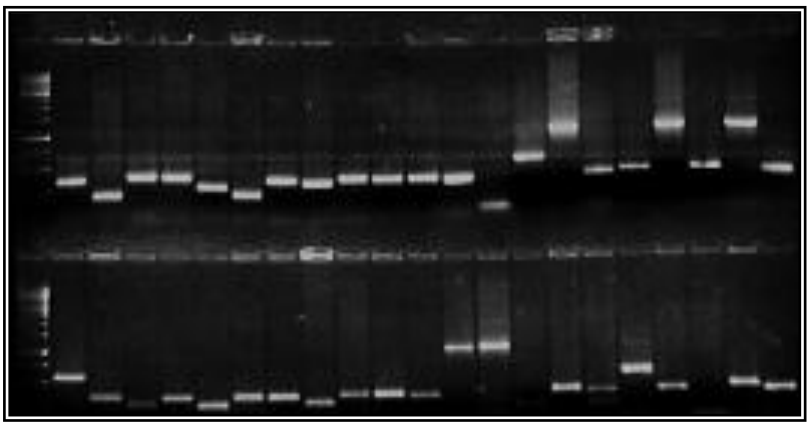

Fig. (2): Agarose gel electrophoresis showing examples of PCR screening for different cloned cDNA fragments.

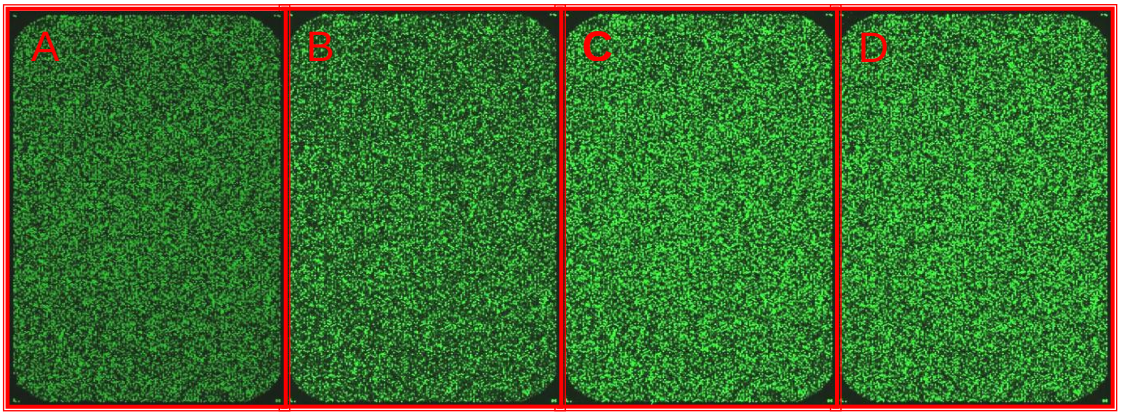

Fig. (3): A TIFF generated image shows the microarray slide after hybridization and wash steps. A) Misr1 Treated, B) Misr1 Control, C) Gemmiza7 Treated and D) Gemmiza7 Control.

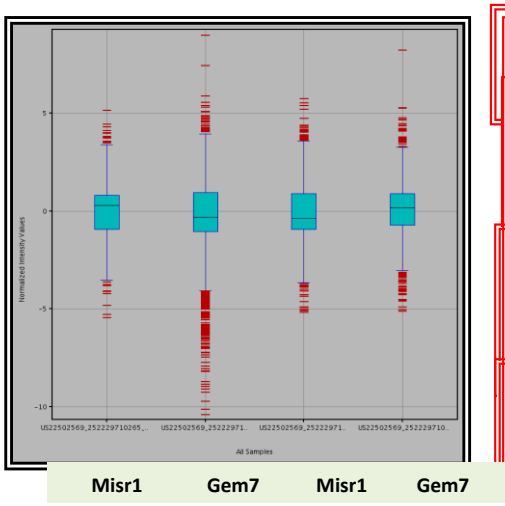

Fig. (4): Box whisker plot for the imported raw data after

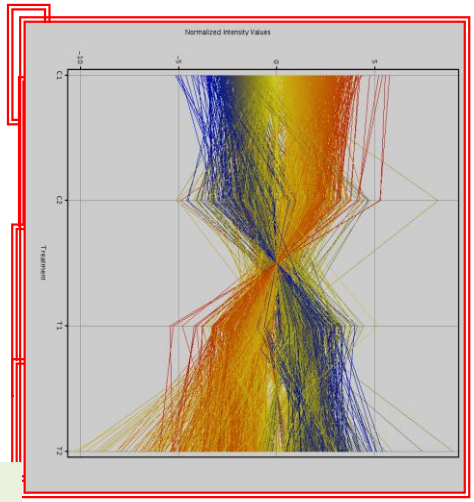

Fig. (5): Shows a profile plot for the imported raw data after normalization.

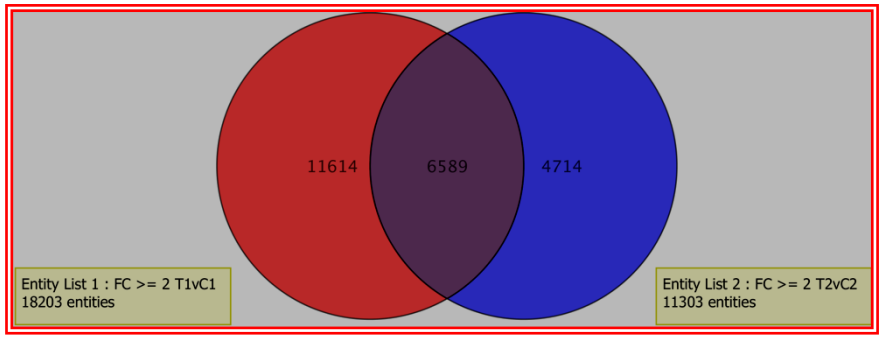

Fig. (6): The intersection of identified 6589 genes that their expression changed according to leaf rust treatment between both studied varieties.

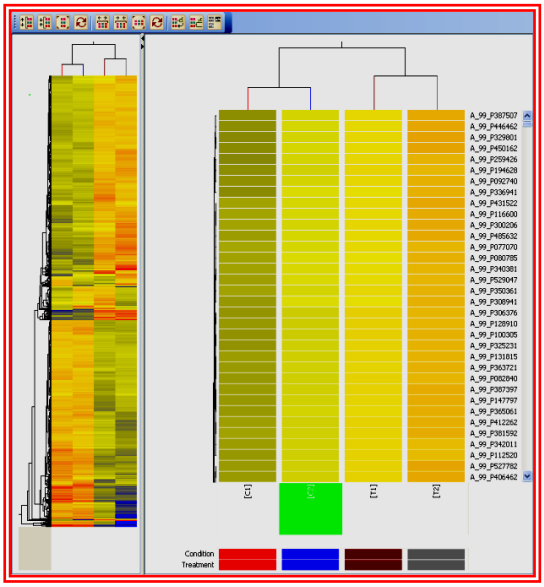

Fig. (7): Dendrogram shows the results of Hierarchical clustering analysis.

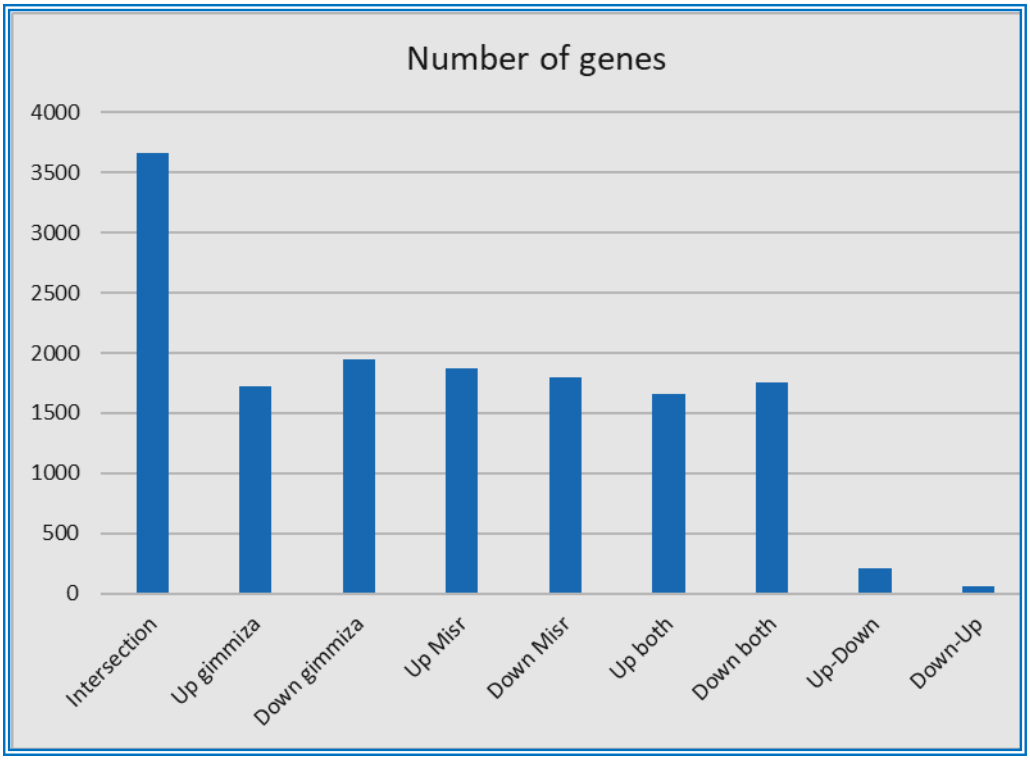

Fig. (8): Number of genes from the intersected genes that up and down-regulated in each cultivar as well as up and down on both of them.