Transcriptome analysis of *Phytophthora infestans* and *Colletotrichum coccodes* in tomato to reveal resistance mechanisms

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Abstract. Tomato is considered as an important commercial crop and protective food and its yield is extremely affected by different pathogens. Such harmful pathogens severely affecting tomato include *Phytophthora infestans* (oomycete) and *Colletotrichum coccodes* (fungi). In this study, gene expression analysis was performed using the microarray datasets available in the public database to investigate differentially expressed genes DEGs under developmental and anatomy conditions and consequently constructing the predicted PPI (Protein-protein interaction) network. Here, a comparison of abundances revealed that 51 genes were differentially expressed due to oomycete and fungi stresses in tomato. Expression analysis revealed that *Soly06g007510.3* was up-regulated whereas *Soly05g008850.3* was down-regulated in all developmental stages and anatomy conditions to manage auxin metabolism under fungi and oomycete infections in tomato. The gene ontology included cellular metabolic and organic substance metabolic processes, cellular biosynthetic, organic substance biosynthetic, and cellular macromolecule metabolic process, chloroplast, intracellular membrane-bounded organelle, heterocyclic compound binding, organic cyclic compound binding, ion binding, binding, and catalytic activity. Analysis of cis-elements illustrated that 12 most general cis-regulatory elements (CATT-box, TATA-box, MYB, MYC, ABRE, G-box, Box 4, AAGAA-motif, MBS, as-1, TGACG-motif, and CGTCA-motif) were identified in promoter region. The highest frequency of cis-elements included CATT-box and TATA-box in response to the pathogens at different growth stages in tomato. Our findings can provide a better insight to the regulatory mechanisms involved in response to fungi and oomycete infection in tomato which can aid to improve the efficiency of developing tomato varieties.

Keywords: Microarray, co-expressed, gene ontology, oomycete, fungi

INTRODUCTION

Tomato, *Solanum lycopersicum*, formerly, *Lycopersicon esculentum* Mill., is one of the most important vegetable crops in the world and a good source of organic nutrients. Tomato is considered as a major constituent in many foods due to its essential nutrients like lycopene, beta-carotene, and flavonoids (Raiola *et al.*, 2014). Its genome has been sequenced and is an excellent model for basic research. Plant diseases cause substantial losses in crop yield throughout the world.

Pathogens can promote infection at different phases such as fruit ripening complete, ripening, and fruit formation, spreading quickly after spores attack the tissues (Prusky *et al.*, 2009). Since plants are constantly exposed to many microbial infections such as fungi and oomycetes, they have developed several defense mechanisms to withstand the microbial attacks such as hardening of cell wall and programmed cell death (Tyler and Rouxel, 2012; Wirthmueller *et al.*, 2013).

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Resistant/tolerant plants possess various immune receptors to detect pathogen-derived molecules containing mediators produced by the pathogens to initiate infection (Stam *et al.*, 2014). There are two procedures for detection of pathogen mediators namely pathogen-associated molecular patterns (PAMPS) and effector-triggered immunity (ETI) which are localized on the plasma membrane and intracellular.

Phytophthora infestans and *Colletotrichum coccodes* can infect both non-ripened and ripened fruits. *Phytophthora infestans* (oomycete) and *Colletotrichum coccodes* (fungi) are the causal agents of late blight and anthracnose on tomato, respectively. From morphology and habitat point of view, fungi and oomycetes have been considered to be similar and traditionally categorized in one class. These pathogens have basic differences from each other such as cell wall composition. For example, cell walls of oomycetes are formed of cellulose rather than chitin as in fungi. Also, fungi have haploid nuclei whereas oomycetes contain diploid nuclei. In addition, oomycetes utilize diaminopimelate (or DAP) pathways for lysine synthesis (Barr, 1983), whereas fungi use α -aminoadipate (or AAA) pathway. Oomycetes and fungi are different in their promoter regions. Oomycetes have an initiator element (19-nt) as its core sequence instead of TATA box in promoter regions in fungi (McLeod *et al.*, 2004). Both pathogens can infect leaves and fruits, cause massive deaths of plants, and result in reduced yield in tomato (Nowicki *et al.*, 2012; Gudero *et al.*, 2018).

Breeding technologies are crucial for improving crop production especially tomato with an exponentially growing population in the face of biotic and abiotic stresses (Tester and Langridge, 2010). The most classic breeding approach to improve tomato has been hybridization followed by selection. An important challenge in classic breeding is linkage drag which the genes of interest may be tightly linked to the unfavorable genes (Fentik, 2017). Classic plant breeding is based on phenotypic selection of elite individuals consequently there are considerable difficulties due to genotype-environment interactions. Besides, testing procedures in classic breeding may be many times more difficult, unreliable, and expensive. There are new approaches for evaluation of plants under stress

conditions such as genomics, marker-assisted selection (MAS), and transcriptomics. MAS is an indirect selection process in genomic regions that are implicated in the expression of traits of interest through molecular markers. However, MAS possesses several limitations such as costly, availability of suitable markers, and staff resources for sample and data handling (Brumlop and Finckh, 2011). It is evident that SSR markers are one of the most important markers utilized in molecular mapping and MAS selection. Microsatellites are multi-allelic and are considered to be one of the best markers for detection of polymorphism. However, development of SSR markers is expensive, laborious, time-consuming (Stepień *et al.*, 2007), and in low frequency in plants (Senan *et al.*, 2014). With the advantages of highly parallel and flexibility, microarray has become increasingly important in crop genetic studies, and it is well suited for applications such as QTL analysis and genotyping (Miller and Tang, 2009). Cutting edge technologies such as microarray is a new powerful tool for studying the molecular basis of interaction on a scale that is impossible using conventional analysis (Lenka *et al.*, 2009). This technique makes it possible to determine the expression of thousands of genes simultaneously.

In the current research, microarray data was used to survey and compare gene expression patterns in tomato pericarp and leaves under two pathogen factors, *Phytophthora infestans* and *Colletotrichum coccodes*. Analysis of differentially expressed genes has created a new perspective on important processes in protecting the pericarp and leaf growth against oomycete and fungi presence in tomato as well as introducing genes to transfer disease resistance into plants. Expression analysis was performed to identify the candidate genes having higher potential value in tomato breeding.

MATERIALS AND METHODS

The microarray data (CEL file) was taken from the GEO/NCBI under the sample numbers GSE33177 and GSE21999. In this study, microarray data was collected from 15 samples and the treatments included tomato leaves from

non-inoculated plants, tomato leaves from inoculated plants with *Phytophthora infestans*, tomato fruit wound inoculation by *Colletotrichum coccodes*, tomato fruit wound control, tomato fruit ammonium treatment, and tomato fruit PBS control in two tomato cultivars from NCBI (<http://www.ncbi.nlm.nih.gov/gds>) (Table 1). Analysis of data was performed by fold change which was based on $\log_2$ ratio, followed by normalizing the data. Then the ratio expression of the genes examined under oomycetes and fungi infections were compared to control and the DEGs were selected by p-value <0.05 as the statistical significance. The filtered data with DEGs >1 was considered as up-regulated genes and DEGs <-1 was considered as down regulated. The DAVID Gene ID Conversion Tool (DICT) is able to convert Affymetrix IDs to gene names and to detect the function of genes affected by fungi and oomycete (<https://david.ncifcrf.gov>). In this study, analysis of data was performed by fold change which was based on $\log_2$ ratio, followed by normalizing the data (the ratio expression of the genes examined under fungi and oomycete infection to control). String (<http://string-db.org/>) was used to identify co-expressed genes and to draw the protein-protein interaction networks (Szklarczyk *et al.*, 2016).

In silico analysis of genes expression

Genevestigator is a web-based system which provides meta-analysis of transcriptomes. Meta-analysis of transcriptomes is based on large-scale and systematic combination of normalized and quality-controlled expression data with experimental context variables using ontologies (e.g. anatomy, development, perturbation). To identify the differential gene expression under pathogen (fungi and oomycete) infections, microarray expression data was explored (<https://genevestigator.com/gv/>; Zimmermann *et al.* 2008) from *Solanum lycopersicum* database using Affymatrix Tomato Genome Array. In addition, 'Perturbations' tool was utilized to find out the differential gene expression under fungi and oomycete infections (<https://genevestigator.com/gv/start/start.jsp>) during developmental stages. The fold change in gene expression was

detected using filter 1 fold as benchmark. Compendium-wide analysis in genevestigator program showing the fold changes in the expression of DEGs under different tissue and stage-specific tissue conditions were used to generate the gene expression heatmap. Red/white color scheme where "Red" color represents up-regulation and "white" color represents down-regulation of respective genes were used to designate the heatmap.

Development' tool was utilized to detect up-regulated gene expression using microarray dataset in six developmental stages such as fruit ripening complete, ripening, fruit formation, flowering, inflorescence visible, and main shoot growth (<https://genevestigator.com/gv/start/start.jsp>). 'Anatomy' tool was subjected to evaluation of the expression potential of each gene using microarray OS-AFFY-RICE-0 dataset (<http://www.genevestigator.ethz.ch>).

Gene Ontology (GO) Enrichment analysis and KEGG pathway analysis

Classification of DEGs by GO analysis using agriGO (<http://systemsbiology.cau.edu.cn/agriGOv2/>) indicates probable pathways captured by the responsive genes involved in fungi and oomycete infections. The GO database generated an overview of the functional classification of a list of AGI IDs which is used to identify the biological processes, molecular functions, and cellular component. For pathway evaluation of DEGs, String (<http://string-db.org/>) was utilized with p-value cut-off of ≤ 0.05 to visualize fungi and oomycete infections related to changes in the general metabolism.

Analysis of the cis-elements

Promoter regions of 51 genes were analyzed using PlantCare software (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>). For each gene, 1500bp upstream of the transcription initiation region was considered as a promoter sequence. Investigation of cis-regulatory elements including transcription factors was done using the PlantCare database (Lescot *et al.*, 2002).

Table 1. Microarray data of gene expression induced by *P. infestans* and *C. coccodes* infections in tomato.

Accession	Title	Source name	Cultivar	Tissue type
GSE21376	Healthy plants rep1	Tomato leaves from non-inoculated plants	Money Marker	Leaves
GSE21377	Healthy plants rep2	Tomato leaves from non-inoculated plants	Money Marker	Leaves
GSE21378	Healthy plants rep3	Tomato leaves from non-inoculated plants	Money Marker	Leaves
GSE21379	Diseased plants rep1	Tomato leaves from inoculated plants with <i>Phytophthora infestans</i>	Money Marker	Leaves
GSE21380	Diseased plants rep2	Tomato leaves from inoculated plants with <i>Phytophthora infestans</i>	Money Marker	Leaves
GSE21381	Diseased plants rep3	Tomato leaves from inoculated plants with <i>Phytophthora infestans</i>	Money Marker	Leaves
GSE21382	Diseased plants rep4	Tomato leaves from inoculated plants with <i>Phytophthora infestans</i>	Money Marker	Leaves
GSM546597	Tomato fruit wound inoculation by <i>Colletotrichum coccodes</i>	Tomato breaker fruit parenchyma cells	Hazera 1402	Pericarp
GSM546978	Tomato fruit wound inoculation by <i>Colletotrichum coccodes</i> 2	Tomato breaker fruit parenchyma cells	Hazera 1402	Pericarp
GSM546979	Tomato fruit wound control	Tomato breaker fruit parenchyma cells	Hazera 1402	Pericarp
GSM546980	Tomato fruit wound control 2	Tomato breaker fruit parenchyma cells	Hazera 1402	Pericarp
GSM546981	Tomato fruit ammonium treatment	Tomato breaker fruit parenchyma cells	Hazera 1402	Pericarp
GSM546982	Tomato fruit ammonium treatment 2	Tomato breaker fruit parenchyma cells	Hazera 1402	Pericarp
GSM546983	Tomato fruit PBS control	Tomato breaker fruit parenchyma cells	Hazera 1402	Pericarp
GSM546984	Tomato fruit PBS control 2	Tomato breaker fruit parenchyma cells	Hazera 1402	Pericarp

RESULTS AND DISCUSSION

Analysis of co-expressed genes in gene network

The PPI network analysis of 51 genes revealed that the 11 co-expressed genes are involved in auxin metabolism (Tables 2 and 3). Plants are continuously subjected to pathogens in nature and, as a result, different mechanisms have been utilized to defend themselves. Auxin plays multiple roles in plant growth and development including their response to abiotic and biotic stresses (Kunkel and Harper, 2017). Auxin is one

of the up-regulated genes involved in the co-expressed network enhancing auxin level which in turn can antagonize defense responses in some plant-pathogen interactions (Kunkel and Harper, 2017). On the other hand, auxin can also act as a signaling molecule to induce virulence genes required in pathogenesis (Chandler, 2016). It has been reported that auxin is decreased notably upon drought but increased in response to cold and heat stresses in rice and pathogen infection in *Arabidopsis thaliana* (Hout *et al.*, 2014; Ramegowda *et al.*, 2015). Transcriptional regulators like Auxin/Indole-Acetic Acid (Aux/IAAs), Auxin Response Factors (ARFs), and TOPLESS

proteins (TPS) can regulate auxin level in cell-specific transcription (Zouine *et al.*, 2014). It is concluded that auxin might be implicated in pathogen infection response through ARFs (Sun *et al.*, 2015). Similar findings offer that the regulation of auxin signaling could be critical in defense response through the regulation of the

expression of ARF gene in tomato (Kunkel and Harper, 2017). *Sohyc06g007510.3* is one of the responsive genes involved during plant-pathogen interaction. Findings of a research suggested that *Sohyc06g007510.3* is selectively expressed during hormone treatment and pathogen infection (Sharma and Bhatt, 2017).

Table 2. Characterized key genes in response to fungi and oomycete infections.

No.	Gene ID	Description or functional genes	Location
1	<i>Sohyc02g077390.2</i>	Compound inflorescence, S gene	ch2:42336107..42338039
2	<i>Sohyc02g081670.1</i>	DOT	ch2:45542385..45543752
3	<i>Sohyc06g007510.3</i>	Ubiquitin-conjugating enzyme	ch6:48411762..48416262
4	<i>Sohyc07g062840.3</i>	Goblet	ch7:65615837..65618073
5	<i>Sohyc11g072600.2</i>	AP2 transcription factor SIAP2d	ch11:56068300-56071320
6	<i>Sohyc02g064960.3</i>	AP2 transcription factor SIAP2b	ch2:36641329-36645265
7	<i>Sohyc02g083950.3</i>	WUSCHEL-related homeobox-containing protein 4, WUS	ch2:47744436-47748006
8	<i>Sohyc11g013310.2</i>	LAX3 protein	ch11:6272690..6272875
9	<i>Sohyc10g076790.2</i>	LAX4 protein	ch10:59843582-59848957
10	<i>Sohyc03g005355.1</i>	IAA1	ch3:210025-212319
11	<i>Sohyc05g008850.3</i>	Aux/IAA	ch5:3078325-3079636

Ubiquitin-ligase degrades transcriptional repressor Aux/IAA and then releases auxin response factor or ARF to regulate the transcription of auxin-responsive genes (Woodward and Bartel, 2005). LAX3 and LAX4 are transporters of auxin and auxin-inducible genes in which their expressions are down-regulated dramatically under the stress (Péret *et al.*, 2013). In this study, *Sohyc02g077390.2* encoded a transcription factor associated to a gene called *wus* gene which were co-expressed in the gene network. *Wus* gene expression is regulated by abiotic stress and plant hormones. *Wus* gene was another co-expressed gene in the present network which seemed to control auxin signaling output at all levels and places (Figure 1). There is another report that *wus* gene can regulate the expression of a large number of genes implicated in auxin biosynthesis, transport, perception, and response. Also, it has been reported that in SAM, *wus* protein can act as a transcriptional activator or repressor of auxin signaling component on the regulatory environment. In Arabidopsis, *wus* gene seems to act both upstream and downstream of the key ARF transcription factors due to reduced auxin under stress (Ma *et al.*, 2018). Functional studies in NAM/NAC have revealed that many of the NAC TFs regulate immune responses in plants. Knockout of OsNAC6 lead to fungi

penetration of powdery mildew in rice (Jensen *et al.*, 2008). AP2 is involved in several key steps in plant development and response to cold, heat, drought, and pathogen infections through different hormones related to signal transduction pathways such as ethylene, cytokinin and jasmonate (Rashotte *et al.*, 2010; Hu *et al.*, 2013).

Figure 1. Interactions of responsive genes to both fungi and oomycete infections in tomato genotypes where each node and edge represents a protein and interaction, respectively. The web based tool “String” (<http://string-db.org/>) was utilized to predict the interactions.

Table 3. Description of 51 genes in response to oomycete and fungi infections in tomato.

No.	Gene ID	Description	No.	Gene ID	Description
1	Solyc07g005330.3	Beta-glucosidase 08	27	Solyc02g064960.3	AP2 transcription factor SIAP2b, AP2b
2	Solyc02g077390.2	S gene, compound inflorescence	28	Solyc06g075510.3	AP2 transcription factor SIAP2e
3	Solyc02g081670.	DOT	29	Solyc11g013810.2	Nitrate reductase
4	Solyc06g007510.3	Ubiquitin-conjugating enzyme	30	Solyc02g083950.3	WUS
5	Solyc06g082600.3	Ubiquitin-conjugating enzyme	31	Solyc06g076410.3	C-4 methyl sterol oxidase
6	Solyc07g062840.3	NAM	32	Solyc01g097440.3	N-glycanase
7	Solyc08g081010.3	GSH1	33	Solyc09g014380.3	LAX1 protein
8	Solyc01g109880.3	bZIP	34	Solyc01g111310.3	LAX2 protein
9	Solyc04g050930.3	Violaxanthin de-epoxidase	35	Solyc11g013310.2	LAX3 protein
10	Solyc08g063080.3	UDP-sulfoquinovose synthase	36	Solyc10g076790.2	LAX4 protein
11	Solyc07g066580.3	Sulfurtransferase	37	Solyc10g055260.2	LAX5 protein
12	Solyc08g005680.3	Dimethylallyltransferase	38	Solyc05g049970.3	Mitogen-activated protein kinase 6 (MAPK6)
13	Solyc04g008380.3	GAGA-binding transcriptional activator	39	Solyc08g081495.1	MAPK7
14	Solyc08g076230.1	GAGA-binding transcriptional activator	40	Solyc07g062080.3	MAPK11
15	Solyc04g081170.3	GAGA-binding transcriptional activator	41	Solyc07g056350.3	MAPK12
16	Solyc01g079620.3	MYB12	42	Solyc01g080240.3	MAPK13
17	Solyc07g066480.3	Molybdenum cofactor sulfurase	43	Solyc04g007710.3	MAPK14
18	Solyc06g073190.3	Fructokinase-2	44	Solyc12g040680.2	MAPK16
19	Solyc07g064680.3	Autophagy-related protein	45	Solyc05g008020.3	MAPK15
20	Solyc08g078820.3	Autophagy-related protein	46	Solyc03g005355.1	IAA1
21	Solyc08g078320.3	Ubiquitin-protein ligases, E2 enzyme	47	Solyc05g008850.3	Aux/IAA
22	Solyc09g092170.2	Beta-galactosidase	48	Solyc02g091840.3	Leucine rich repeat receptor protein kinase CLAVATA1
23	Solyc03g119770.3	TCP9	49	Solyc04g056640.1	RLP
24	Solyc06g069240.2	TCP8	50	Solyc03g059490.1	Receptor like kinase, RLK
25	Solyc02g093150.3	AP2 transcription factor SIAP2c	51	Solyc05g023760.3	Receptor like protein kinase SOL2
26	Solyc11g072600.2	AP2 transcription factor SIAP2d			

***In silico* analysis of DEGs expression**

Meta-analysis of Genevestigator microarray dataset was performed on *S. lycopersicum* DEGs (Figure 2). *Solyc06g007510.3* was the highest expressed gene in almost all stages of developmental stages such as fruit ripening complete, ripening, fruit formation, flowering, inflorescence visible, and main shoot growth whereas, *Solyc05g008850.3* showed the lowest expression during the all stages. *Solyc07g066580.3* and *Solyc08g063080.3* were slightly up-regulated in all developmental stages in tomato (Figure 2). In

course of the inflorescence visible stage most of genes showed almost same level of expression. Expression of all genes were maximal during all the stages of main shoot growth whereas, expression of these genes were minimal during the fruit formation and flowering stages.

Anatomy expression profile showed that the number of down-regulated DEGs in generative cells was higher than the up-regulated DEGs, while the opposite occurred in sympodial inflorescence meristem, shoot apex, and floral meristem under fungi and oomycete stresses.

Solyc05g008850.3 was down regulated during all the stage-specific tissues such as hypodermis, outer epidermis, parenchyma, seed, locular tissue, endocarp, leaf, flower, columella, generative cell, radical pericarp, ovary wall, fruit, placenta, pericarp, leaflet, inner pericarp, ovule, callus, pistil, placenta, vascular tissue, roots, floral meristem, sympodial inflorescence meristem, and shoot apex. ARF plays a critical role in the transcriptional activation or repression of auxin-responsive genes or IAA through direct binding to their promoters (Bouzroud *et al.*, 2018). Auxin is implicated in plant developmental processes and response to environmental stresses (Liu *et al.*, 2014). It has been reported that the auxin level was reduced notably upon drought condition but was enhanced in response to heat in rice and during pathogen infections in *Arabidopsis* (Schmeiz *et al.*, 2004; Du *et al.*, 2013). Based on another study, auxin is as an active player in plant defense against pathogens (Navarro *et al.*, 2006). Our findings agreed with Bouzroud *et al.* (2018) and Saidi and Hajibarat (2019) that suggested auxin could be implicated in pathogen infections through ARFs.

Solyc06g007510.3 had the highest expression in all of tissue specific (Figure 3). The ubiquitin-dependent protein degradation pathway is implicated in hormone regulation, senescence, morphogenesis, and pathogen defense (Devoto *et al.*, 2003). Previous studies showed that ubiquitination can also play a critical role in plant tolerance against different biotic and abiotic stresses (Zhou *et al.*, 2010; Jeon *et al.*, 2012). It has been reported that the expression of *Solyc06g007510.3* genes was observed in different tissues of tomato genotypes, a finding which is in agreement to our results (Sharma and Bhatt, 2017). A recent study revealed that the *Solyc06g007510.3* was positively regulating immunity due to pathogen attack and hormone treatment in tomato (Hamera *et al.*, 2014). E2 ubiquitin-conjugating enzyme was expressed in response to ABA and auxin hormone (Sharma and Bhatt, 2017).

Gene Ontology enrichment analysis

Gene ontology analysis revealed that pathogen-responsive genes were preferentially involved in

the biological processes including metabolic and cellular processes, cellular metabolic process, organic substance metabolic process, cellular biosynthetic process, organic substance biosynthetic process, primary metabolic process, and cellular macromolecule metabolic process which acted as indices of significant biological pathways underlying the fungi and oomycetes responses of plants (Figure 4). Similar results were recently observed for tomato- *X. perforans* interaction (Du *et al.*, 2015). The cellular component is involved in intracellular membrane-bounded organelle and chloroplast. The molecular function is related to binding, ion binding, organic cyclic compound binding, heterocyclic compound binding, and catalytic activity (Figure 4). Our results revealed a similar activation of the basic response related genes in fungi and oomycete infections (Tan *et al.*, 2015). These pathways can overlap with tomato responses to pathogens which agrees with the genes involved in bacterial and fungal tolerance in tomato (McCormick *et al.*, 1986; Tan *et al.*, 2013).

KEGG analysis of DEGs

To characterize and evaluate the active biological pathways of the identified DEGs, gene classification was performed on the basis of KEGG analysis (Kanehisa *et al.*, 1999). Only significant pathway categories were selected and listed in Figure 5. The pathways involving the highest number of DEGs were plant hormone signal transduction, amino sugar and nucleotide sugar metabolism, autophagy, and cysteine and methionine metabolism. Another survey has reported that the genes responding to *V. dahliae* in tomato include hormone signal transduction, glutathione metabolism, and phenylalanine metabolism which are similar to those major pathways implicated in plant and pathogen interactions (Sun *et al.*, 2014). Therefore, the DEGs implicated in these pathways can be considered as gene candidates for genetic engineering and breeding programs for tomato genetic improvements for fungi and oomycete infections.

Figure 2. Heat map results of differentially expressed genes in the microarray experiment. Each row represented log₂-fold change of the relative gene expression. Up and down-regulated expression genes are represented by red and white colors, respectively.

Figure 3. Anatomy expression profiles of responsive genes at different developmental stages under fungi and oomycete infections in *S. lycopersicum*.

Figure 4. Gene Ontology (GO) categories of DEGs based on molecular function, biological processes, and cellular component in response to both fungi and oomycete infections in Money Marker and Hazera 1402 tomato genotypes.

Figure 5. Diagram of significantly enriched KEGG pathways of DEGs by fungi and oomycete infections.

Analysis of cis-regulatory elements in the promoter regions

In order to obtain more information regarding the regulatory elements involved in response to signaling molecules, their promoter regions were analyzed for frequency and types of cis-elements. The characterized cis-regulatory elements (CREs) included the seven most common CREs

found in the promoter sequences of the -1 <DEGs<1 fold change (CAAT-box, TATA-box, TGACG motif, MYB, MYC, ABRE, and G-Box). CREs are binding sites for transcription factors involved in regulation of transcription. Surveys on association of CREs provide clearer understanding of the promoter function and help to provide better insight about regulation of gene

expression (Hernandez-Garcia and Finer, 2014; Saidi and Hajibarat, 2019). Description of the first 12 most frequently occurring cis-elements of the total detected elements is provided in Figure 6. Our analysis showed that among CREs, CATT-box and TATA-box were the highest number of CRE whereas, the least number of CRE was observed in CGTCA-motif genes under fungi and oomycete infections, respectively.

MYB proteins include the largest family of TFs in plants and are involved in gene expression related to development, disease resistance, and abiotic stress tolerance (Li *et al.*, 2009). In Arabidopsis, AtMYB96 mediated ABA signals as a tolerance mechanism to pathogen infections using inducing salicylic acid biosynthesis (Nagaraju *et al.*, 2019). A report has shown that expression of some MYB family members are regulated by auxin (Shin *et al.*, 2007). MYC transcription factors are major transcriptional regulators in the expression of jasmonate (JA)-responsive genes, positive regulator in wound resistance genes and negative regulators during the expression of pathogen defense genes (Van *et al.*, 2009). MYB and WRKY can activate the expression of MYC transcription factor under stress and as a result MYC activates the transcription of JAZ protein. JAZ protein quickly aids to promote the release and activation of MYC. ABRE can induce ABA expression which in turn acts as a binding site for bZIP family transcription factors (Lenka *et al.*, 2009). ABRE motifs, bZIP, AuxRE, MYC and MYB recognition sites were found to be significantly enriched in the auxin-responsive promoters from Arabidopsis and rice (Berendzen *et al.*, 2012; Abe *et al.*, 2003).

Recently, it has been pointed out that MYB Related Elements (MREs) MREs, bZIP Response Elements (ZREs), and ARF have to function together as potential quantitative elements in auxin-mediated transcription in Arabidopsis and rice (Berendzen *et al.*, 2012; Abe *et al.*, 2003). Based on another study, auxin level and expression act as the primary regulator of pathogen stress response in fungal pathogen infected plants (Yin *et al.*, 2014). Our results can suggest that MYB, MYC, G-box, and ABRE have key roles in auxin signaling for pathogen-inducible genes. This analysis confirmed that

specific and composite motif containing AuxREs, ZREs, G-box related MYC element, are importantly enriched in the promoters of auxin inducible genes and in some auxin-regulated gene families (Berendzen *et al.*, 2012). Both DNA motifs (G-box and TGACG motif) are found in several plant promoters bZIP proteins (Saidi and Hajibarat, 2019). G-box, also known as ABRE (ABA-responsive element), is a binding site for bZIP proteins which has been identified as binding proteins (Hatton *et al.*, 1995). BZIP, bHLH, and G-box enhance the possibility of being co-regulated by other transcription factors to regulate gene expression under pathogen infections (Saidi and Hajibarat, 2018; Liu *et al.*, 2017). The TGACG motif, known as 'as1 element', is also involved in MeJA response which is present in the promoter of some plant genes activated during defense under pathogen infections (Zhang *et al.*, 1999).

CONCLUSION

In this study, *in silico* analysis of DEGs tomato was performed during fungi and oomycete infections, and the presence of different types of CREs were identified. Consequently, analysis of gene network of co-expressed genes, expression patterns in stage specific-tissue, and expression in stages of development were performed using bioinformatics tools. Our findings showed that 12 important CREs were implicated in auxin management to minimize damage in tomato. Most co-expressed genes were involved in synthesis, metabolism, and degradation of auxin which in turn controls auxin concentration under fungi and oomycete infections. Auxin was down and up regulated by ARFs and E2-enzymes during all staged of specific tissues, respectively. Thus, these up or down regulations of auxin can make it possible to prevent pathogen growth through regulation of auxin concentration. Our findings suggested that genes involved in auxin signaling pathway can provide a better overview of the transcriptome changes of tomato cultivars under pathogen infections, which can provide insights as to how tomato evolved to adopt with pathogen infections.

Figure 6. Histogram showing the frequencies of responsive genes of CREs under fungi and oomycete infections in *S. lycopersicum*.

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