Meta-analysis of transcriptomic responses to biotic and abiotic stress in tomato

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A wide range of biotic and abiotic stresses adversely affect plant growth and productivity worldwide. The study of individual genes cannot consider as an effective approach for understanding of tolerance mechanisms since these stresses frequently often in combination and a large number of genes involved in these mechanisms. The availability of high-throughput genomic data has enabled the discovery of the role of transcription factors in regulatory networks. A meta-analysis of biotic and abiotic stress responses was performed by analyzing a total of 391 microarray samples from 23 different experiments and 2336 differentially expressed genes involved in multiple stresses were identified. We identified 1862 genes differentially regulated in response to biotic stress was much greater than that regulated by abiotic stresses, 835 genes, and found 15.4% or 361 differentially expressed genes with conserved expression between abiotic and biotic stresses. The greatest percent of genes related to cellular process (>76% genes), metabolic process (>76% genes) and response to stimulus (>50%). About 4.2% of genes involved in biotic and abiotic stress responses belonged to the transcription factor families. We identified several genes which encode transcription factors that play an important role in abiotic and biotic stress responses. These proteins included JERF1, SlGRAS6, MYB48, SlERF4, EIL2, protein LHY, SlERF1, WRKY 26, bZIP transcription factor, ICE1-like, pti6, EIL3 and WRKY 11. Six of these proteins, JERF1, MYB48, protein LHY, EIL3, EIL2 and WRKY 26 play a central role in these mechanisms. This research promoted a new approach to clarify the expression profiles of various genes under different conditions in plants, detected common genes from differentially regulated in response to these conditions and introduced them as candidate genes for improving plant tolerance through genetic engineering approach.

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ABSTRACT

A wide range of biotic and abiotic stresses adversely affect plant growth and productivity worldwide. The study of individual genes cannot consider as an effective approach for understanding of tolerance mechanisms since these stresses frequently often in combination and a large number of genes involved in these mechanisms. The availability of high-throughput genomic data has enabled the discovery of the role of transcription factors in regulatory networks. A meta-analysis of biotic and abiotic stress responses was performed by analyzing a total of 391 microarray samples from 23 different experiments and 2336 differentially expressed genes involved in multiple stresses were identified. We identified 1862 genes differentially regulated in response to biotic stress was much greater than that regulated by abiotic stresses, 835 genes, and found 15.4% or 361 differentially expressed genes with conserved expression between abiotic and biotic stresses. The greatest percent of genes related to cellular process (>76% genes), metabolic process (>76% genes) and response to stimulus (>50%). About 4.2% of genes involved in biotic and abiotic stress responses belonged to the transcription factor families. We identified several genes which encode transcription factors that play an important role in abiotic and biotic stress responses. These proteins included JERF1, SlGRAS6, MYB48, SlERF4, EIL2, protein LHY, SlERF1, WRKY 26, bZIP transcription factor, ICE1-like, pti6, EIL3 and WRKY 11. Six of these proteins, JERF1, MYB48, protein LHY, EIL3, EIL2 and WRKY 26 play a central role in these mechanisms. This research promoted a new approach to clarify the expression profiles of various genes under different conditions in plants, detected common genes from differentially regulated in response to these conditions and introduced them as candidate genes for improving plant tolerance through genetic engineering approach.

INTRODUCTION

As sessile organisms, plants constantly encounter a wide range of stresses that negatively affect the yield production, survival and growth of plants worldwide (*Sharma et al., 2013*). The stresses are divided into two broad categories, abiotic stresses (AS) including a variety of adverse environmental conditions, (i.e. drought, submergence, salinity, heavy metal contamination, nutrient deficiency, light and extreme temperatures) and biotic stresses (BS) caused by attack of living organisms, (i.e. bacteria, viruses, fungi and nematodes as well as other plants) (*Rastgoo & Alemzadeh, 2011; Liu et al., 2014*). To survive under stress conditions, complex mechanisms have evolved in plants to perceive signals from their environment, transmit through signal transduction pathways and respond to the various stresses that each of which may involve several hundred proteins (*Fujita et al., 2006; Rastgoo, Alemzadeh & Afsharifar, 2011; Atkinson, Lilley & Urwin, 2013; Sharma et al., 2013, Sami & Alemzadeh, 2016*). Functional molecular studies have figured out interactions between biotic stresses and abiotic stresses signaling components with the identification a large number of genes, which are regulated by various types of transcription factors (TFs) (*Kissoudis et al., 2016*). TFs are proteins with critical roles in the control temporal and spatial gene expression during defense response that many of them are induced by more than one stress (*Liu et al., 2014; Shaik & Ramakrishna, 2014; Prasad et al., 2016*).

Better understanding of plant stress response mechanisms under various stresses can give us a higher view of how to improve the worldwide food production (*Sharma et al., 2013*). It is necessary to learn more about molecular mechanisms of transcription factors, MAPKs and ROS signal pathways, and hormone signaling, and study of genes encoding regulatory molecules, especially transcription factors, can be more efficient than single-function genes, because TFs enable to regulate various genes to improve plant tolerance against stresses (*Seki et al., 2002*).

Bioinformatic approach is an emerging and rapidly developing field which allows researchers to analyze biological data (*Khatri & Drăghici, 2005; Chang et al., 2013; Zinati, Alemzadeh & KayvanJoo 2016*). Recent advancements in genome-wide transcriptome analysis methods, such as RNA sequencing and microarrays, enable researchers to simultaneously study the expression of thousands of genes and their coexpression partners under various stresses (*Khatri & Drăghici, 2005; Sharma et al., 2013; Rest et al., 2016*). Meta-analysis can be used to estimate differential gene expression between normal and stress conditions and find out some genes that their products are key molecules in the response to stress (*Tseng, Ghosh & Feingold, 2012; Shaar-Moshe et al., 2015*). Today, with recent advances in high-throughput technology, a large number of biological data has become accessible via online resources, and we are now able to obtain more reliable results with combining information from multiple sources. In this way, statistical methods can be used as useful and powerful tools to identify suitable candidate genes for test under experimental conditions to use in plant breeding programs. The combination of *P*-values is a common method in microarray meta-analysis and recently used in many studies (*Tseng, Ghosh & Feingold, 2012*). A meta-analysis of microarray using 13 experiments querying cytokinin-regulated gene expression changes in *Arabidopsis* combined with empirically defined filtering criteria identified a set of 226 genes differentially regulated by cytokinin (*Bhargava et al., 2013*). In another research project, a meta-analysis of ten studies identified 4015 genes showing significant differential expression in response to water limitation compared to control conditions in *Arabidopsis* (*Rest et al., 2016*).

Moreover, combining gene expression information across species can improve the ability to identify core gene sets with high evolutionary conservation. For example, a new cross-species meta-analysis of progressive drought stress at the reproductive stage was developed using *Arabidopsis*, rice, wheat and barley. Based on this dataset, shared important genes and metabolic pathways involved in whole plant adaptation to progressive drought stress across-species were

identified (*Shaar-Moshe, Hübner & Peleg, 2015*). In this study, 225 differentially expressed genes (DEGs) shared across studies and taxa were identified and gene ontology enrichment and pathway analyses revealed that the shared genes were classified into functional categories involved predominantly in metabolic processes, regulatory function and response to stimulus (*Shaar-Moshe, Hübner & Peleg, 2015*).

Tomato (*Solanum lycopersicum*) is one of the most important food crops belongs to Solanaceae family, one of the largest and most important families of flowering plants. In addition, tomato is a suitable model system to study environmental signal transduction components involved in biotic and abiotic stresses (*Knapp et al., 2004; Arie et al., 2007*).

In this study, for the first time we performed large scale comparative transcriptome analysis via meta-analysis of stress response studies in tomato using microarray gene expression data implemented on a single platform (Affymetrix Tomato Genome Array). The goal of this meta-analysis was to detect DEGs involved in biotic and abiotic stresses and detect DEGs shared by both stresses. Also, we focused on the identification of transcription factors have magnitude effects on the plant cells via up- and down- regulation of various genes under stress conditions. Other analysis methods, such as gene ontology (GO) and Network analysis, were used to explain the complexity of tomato response to abiotic and biotic stresses. We also discussed functions of the identified transcription factors in stress response.

METHODS

Selection microarray studies

In this study, a meta-analysis of stress response was performed in tomato using publicly available microarray gene expression data implemented on a single platform (Affymetrix Tomato Genome Array). Expression data of tomato plants exposed to biotic and abiotic stresses were combined.

The raw expression data of various experiments were obtained from the Array Express from EBI (https://www.ebi.ac.uk/arrayexpress/).

Identification of differentially expressed genes

Microarray expression data from each source study was pre-processed separately as individual datasets. The raw data were normalized using Robust Multi-array Average (RMA) background correction and quantile normalization. Data were processed in the R program. Meta-analysis of AS and BS was performed separately to identify DEGs involved in stress conditions. Genes that showed significant *P*-value (FDR of 5%), meaning that an estimated 5 percent of the DEGs are false positive, were considered as DEGs (*Campain & Yang, 2010; Chang et al., 2013*). Two methods of combination by *P*-value were used: Fisher's method and maxP (*Wilkinson, 1951; Fisher, 1992*). The Fisher's combined probability test sums the logarithm-transformed *P*-values obtained from individual studies, under null hypothesis, and follows a chi-squared distribution with 2k degrees of freedom, where k is the number of studies being combined (*Rhodes et al., 2002*). A very small *P*-value in just one study can be adequate to make statistical significance, even if the same genes are not significant in any other study. The maxP method takes maximum *P*-values across studies. It follows a beta distribution with parameters K (number of transcriptomic studies which are combined for a meta-analysis where each study contains G genes) and 1. This method targets on DEGs that have small *p*-values in all studies. (*Chang et al., 2013*; *Song & Tseng, 2014*).

Gene Analysis

The genes selected by meta-analysis were further analyzed and characterized. In the first instance, the results were compared with the plant genes in Plant Transcription factor & Protein Kinase Identifier and Classifier, iTAK (http://bioinfo.bti.cornell.edu/cgi-bin/itak/index.cgi) and Plant Transcription Factor, PlantTFDB (http://plntfdb.bio.uni-potsdam.de/v3.0/) databases. The list includes 1845 genes of tomato which encoding the TFs that directly or indirectly involved in

signaling pathway and response to abiotic and biotic stresses. Venn's diagrams (http://bioinfogp.cnb.csic.es/tools/venny/) were used to represent co-occurrence of DEGs. Gene Ontology (GO) enrichment analysis of the DEGs (such as identifying biological processes) was conducted using the AgriGO platform (*Du et al., 2010*).

Network analysis

The Search Tool for the Retrieval of Interacting Genes/Proteins, STRING 10.0 (http://www.mybiosoftware.com/string-9-0-search-tool-retrieval-interacting-genesproteins.html) (*Szklarczyk et al., 2014*) database was used to figure out all functional relations between the genes and their occurrence patterns across various genomes. The database makes relations based on several lines of evidences: Empirical evidence from protein–protein interaction assays, co-expression data from NCBI Gene Expression Omnibus (GEO) database, the extraction of information from other databases, coexistence of the genes in the same organisms, conserved gene neighborhood in known genomes, gene fusion events, pathway annotation from other resources such as Kyoto Encyclopedia of Genes and Genomes (KEGG) or Gene Ontology databases, and automated text-mining tools (*Szklarczyk et al., 2014*). STRING calculates a confidence value for those interactions according to the evidences from above 0.4 to above 0.9, as medium to highest score, respectively.

RESULTS AND DISCUSSION

Identification of genes involved in biotic and abiotic stresses

After searching in the database, microarray data from 23 different experiments were selected and analyzed. To obtain a global analysis, 391 microarray samples including 213 AS from different categories: drought, heat, salinity, light, hormone, mineral and heavy metals deficiencies/toxicities and 178 BS that caused by viruses, nematode, fungi, bacteria and pests in

tomato (wild type and transgenic) were chosen (Table S1). We found a total of 1862 genes differentially regulated in response to biotic stresses, was far greater than that induced by abiotic stresses, 835 genes, with a false discovery rate (FDR) ≤ 0.05 by Fisher's statistical method (Table S1-S3). We also figured out 15.4% or 361 DEGs with conserved expression between AS and BS by Fisher's statistical method, suggesting that these genes and their associated cell-signaling pathways are regulated in a similar way in a wide range of stresses (Fig. 1A) (Table S5). Using maxP method, a total of 14 genes differentially regulated in response to abiotic stresses and a total of 220 genes differentially regulated in response to biotic stresses were found. We figured out 0.9% or 2 DEGs (LES.1399.1 and LES.3969.1) with conserved expression between AS and BS by maxP method (Fig. 1A) (Table S2-S4). Fisher's and maxP methods are two popular microarray meta-analysis methods used in the literature that have different strength for detecting different types of DEGs (*Tseng, Ghosh & Feingold, 2012; Wang et al., 2012*). Our results also showed that the two used methods detected different sets of DEGs, suggesting different assumptions behind the methods. Among these methods, Fisher's method is more popular in the microarray meta-analysis studies (*Wang et al., 2012*) and hence, we were more interested in detecting DEGs across all studies through Fisher's statistical method.

Gene ontology

We focused on the 361 DEGs common to both AS and BS and subjected them to Gene Ontology (GO) and network analysis to explore other possible functions of them. GO enrichment analysis of the DEGs showed that shared DEGs represented genes involved in main biological and cellular processes. Among them, the greatest percentage of genes involved in cellular process (>76% genes), metabolic process (>76% genes) and response to stimulus (>50%). On the other hand, the lowest percentage of genes involved in positive regulation of biological process (5%)

(Fig. 2) (Table S6). The most significant GO terms for cellular process were cellular metabolic process (FDR: 1.8E-37), organic acid metabolic process (FDR: 9.2E-29), cellular ketone metabolic process (FDR: 1.2E-28) in metabolic process were primary metabolic process (FDR: 7.8E-39), cellular metabolic process (FDR: 1.8E-37) and organic acid metabolic process (FDR: 9.2E-29) and in response to stimulus were response to abiotic stimulus (FDR: 3.4E-40), response to biotic stimulus (FDR: 9.4E-26), and response to stress (FDR: 9.4E-26). Some of these gene possess hub roles in DEGs shared by AS and BS networks (Table S7).

Network analysis

Network analysis using STRING 10.0 identified the connections among DEGs common to AS and BS (Fig. 3) (Table S7). For example, Solyc02g090890.2.1 (*ZE*: plays an important role in resistance to stresses, seed development and dormancy), Solyc04g049350.2.1 (*CS1*: biosynthesis of aromatic amino acids), Solyc07g052480.2.1 (LOC544276: involved in storage lipid mobilization during the growth of higher plant seedling), Solyc07g049530.2.1 (*ACO1*: involved in ethylene biosynthesis via S-adenosyl-L-methionine), Solyc01g099630.2.1 (*XTH1*: an essential component of the primary cell wall, and thereby participates in cell wall construction of growing tissues), Solyc05g055230.1.1 (*RPS17*: ribosomal small subunit assembly), Solyc12g098350.1.1 (*infA*: no specific function has so far been attributed to this initiation factor; however, it seems to stimulate more or less all the activities of the other two initiation factors, IF-2 and IF-3), Solyc02g082920.2.1 (*CHI3*: defense against chitin containing fungal pathogens), Solyc06g082600.2.1 (*UBC3*: mediates the selective degradation of abnormal and short-lived proteins), Solyc01g096480.2.1 (LOC543657: probable thiol-disulfide oxidoreductase that may play a role in proper chloroplast development and negatively regulates Cf-9/Avr9-mediated cell death and defense responses), Solyc07g047850.2.1 (*CAB4*: it receives and transfers excitation energy to photosystems).

Identification of transcription factors involved in biotic and abiotic stresses

In this research, because of the importance of TFs as a powerful tool for the manipulation of complex metabolic pathways, we tried to identify genes encoding them. According to the PlantTFDB, 1845 genes from tomato encoding TFs were identified and classified into 58 families that directly or indirectly involved in signaling and response to AS and BS (Table S8). Based on our results, from the 1862 genes response to biotic stresses selected by Fisher's statistical method, 93 genes (5%) encode TFs (Fig.1C) (Table S9). ERF family has the greatest number of genes (18 genes), and C3H, FAR1, GATA, LBD, MIKC-MADS, PHD, SLP protein, TALE, TIFE and YABBY families have the lowest number of genes (each family has only one gene) (Table 1). From the 835 genes response to abiotic stresses selected by Fisher's statistical method, 31 of those (3.7%) encode TFs (Fig. 1B) (Table S10). ERF and NAC families have the greatest number of genes (5 genes), and ARF, BBR-BPC, GRAS, NF-NY and VOZ families have the lowest number of genes (each family has only one gene) (Table 1).

Since TFs are the key regulators of plant growth, development and metabolisms via up- and down- regulation of various genes, identification of the genes encoding these proteins and transferring them into plants to improve their stress tolerance would be interest. These TFs (JERF1, Pti6, SlERF1, SlERF4, SlGRAS6, MYB48, protein LHY, EIL2, EIL3, WRKY 11, WRKY 26, ICE1-like and bZIP transcription factor) can be used as candidate genes in molecular breeding programs. Because, these TFs are common to abiotic and biotic stresses and our results indicate they are affected by a wide range of stresses and possibly play essential and significant roles in multiple stress responses which is in agreement with their functional roles in biotic and abiotic stress responses (*Gu et al., 2002; Ying et al., 2004; Zhang et al., 2004; Borrás-Hidalgo et al., 2005; Mayrose et al., 2006; Wu et al., 2007; Yáñez et al., 2009; Zhang et al., 2010; Sharma et al., 2010; Chen et al., 2012; Kim, Stork & Mudgett, 2013; Feng et al., 2013; Li, Luan & Liu,*

2015). The results of network analysis also showed some of these proteins have central roles in biotic and abiotic stress responses (Fig. 3). The two-way Venn diagram indicated that the number of common TFs between biotic and abiotic stresses was 13 while 18 and 80 TFs were uniquely identified in abiotic and biotic stresses, respectively (Fig. 4).

ERF family

ERF (Ethylene-responsive transcription factors) has been shown to play a key role during plant development and stress tolerance mechanisms. Overexpression of ERF family genes in different plant species such as *Arabidopsis*, tobacco, rice and tomato has been shown to increase tolerance to a wide range of biotic and abiotic stresses (*Sharma et al., 2010*). Tomato has 112 AP2/ERF superfamily genes (*Sharma et al., 2010; Sharma et al., 2013*). Our results showed that 4 of 13 transcription factors shared between AS and BS, were belong to ERF family including JERF1, SlERF4, SlERF1 and pti6 (Table 2).

JERF1, Jasmonate Ethylene Response Factor 1, has critical roles in plant abiotic stress responses, such as salinity, low temperature, dehydration and various biotic stresses in different plant species (*Zhang et al., 2004; Wu et al., 2007; Zhang et al., 2010*). It has been shown that the expression of *JERF1* in tomato was induced by salt, ethylene, MeJA and abscisic acid (ABA) treatments that may be pointed to a central role for *JERF1* in different signal transduction pathways (*Zhang et al., 2004*). Overexpression of *JERF1* enhanced the tolerance of transgenic tobacco and rice plants to high salinity, low temperature, osmotic stress and pathogen attack by regulating the expression of downstream stress-responsive genes and a number of pathogenesis-related (PR) genes due to binding to DRE/CRT and GCC-box *cis*-elements (*Wu et al., 2007; Zhang et al., 2010*). Expressing *JERF1* in tobacco induced expression of GCC box-containing genes such as *CHN50*, *Prb-1b*, *GLA*, *NtSDR* and *osmotin* that subsequently resulted in enhanced tolerance to salt stress and low temperature (*Zhang et al., 2004; Wu et al., 2007*). *JERF1* up-regulated the expression of

genes related to osmolyte synthesis such as *OsP5CS* and *OsSPDS2* that increased the production of osmolytes, also induced the expression of *OsABA2* and *Os03g0810800* genes (containing DRE element in their promoter regions), which encode two key enzymes involved in ABA biosynthesis and increased the synthesis of ABA in transgenic rice that resulted in elevated tolerance to drought (*Zhang et al., 2010*). JERF1 may also be located downstream of EIN3 in the ethylene signaling pathway (*Zhang et al., 2004*). It has been previously reported that SlERF4, an ERF in tomato, is a XopD, a type III secretion effector from *Xanthomonas campestris*, destabilize SlERF4 and this task has been proposed to promote bacterial growth (*Kim, Stork & Mudgett, 2013*). SlERF4 reduces ethylene production and, in turn, limits disease symptom development (*Kim,* Stork & Mudgett, *2013*) (Table 2). Pti6 is belong to the ERF family in the plant that bind specifically to the GCC-box *cis* element present in the promoter regions of many PR genes and induced the expression of a wide range of these genes that have key roles in the plant defense. It also showed that the expression of jasmonic acid- and ethylene-regulated genes, such as *PR3*, *PR4*, *PDF1.2*, and *Thi2.1*, was differently affected by *Pti4* or other similar genes, *Pti5*, or *Pti6* (*Gu et al., 2002; Chakravarthy et al., 2003*) (Table 2). The gene encoding *SlERF1* was another detected gene that probably has an important role in response to various stresses. It has been shown that the overexpression of Sl*ERF1* in *Arabidopsis* can confer resistance to necrotrophic fungi like *Plectosphaerella cucumerina* and *Botrytis cinerea* (*Mayrose et al., 2006*) (Table 2).

GRAS family

SlGRAS6 was another identified transcription factor that is one of the most important members of tomato GRAS family in disease resistance and mechanical stress (*Mayrose et al., 2006*). SlGRAS6 is required for tomato disease resistance to the bacterial pathogen *Pseudomonas syringae* pv. tomato. It has been shown that the suppression of *SlGRAS6* gene expression through virus-induced gene silencing reduced resistance of tomato to *P. syringae* pv. tomato (*Mayrose et al., 2006*) (Table 2).

MYB family and MYB-related family

MYB is a large TF family in plants that have essential roles in plant growth, development, primary and secondary metabolism and response to BS and AS (*Oh, Park & Han, 2003; Van et al., 2008; Dou et al., 2016*). Most of MYB genes involved in response to various abiotic stresses, belong to the R2R3-type group. With the comparison of *OsMYB48-1* expression with other MYB proteins, a special role for this gene in response to abiotic stresses in rice can be suggested. It has been cleared that its expression was strongly induced by ABA, H_2O_2, PEG, and dehydration stresses, while slightly induced by salt and cold stresses (*Xiong et al., 2014*). It was reported that the overexpression of *OsMYB48-1* regulated the expression level of some stress responsive-genes, such as *RAB21*, *RAB16D, RAB16C* and *LEA3* under drought stress conditions (*Xiong et al., 2014*) (Table 2). The LHY (LATE ELONGATED HYPOCOTYL) transcription factor plays a critical role in the regulation of low temperature stress response via controlling expression of *CBF 1, 2* and *3*, *COR27* and *COL1* genes and contribute to plant defense through the circadian regulation of stomatal aperture (*Grundy, Stoker & Carré, 2015; Seo & Mas, 2015*). It was also cleared that LHY controls the expression of a large number of ABA-responsive genes by binding directly to their promoters (*Huang et al., 2007*) expression of the *CBF 1* (Table 2).

EIL family

It has been shown that the expression of *EIL2* and *EIL3*, which encode transcription factors in ethylene signal transduction, was induced by ethylene and salinity treatments (*Ying et al., 2004*). It also reported that its ortholog in tobacco is required for resistance against *Peronospora hyoscyami* (*Borrás-Hidalgo et al., 2006*) (Table 2).

WRKY family

Different studies have shown that the members of WRKY family contribute multiple biological processes such as plant growth and development and also have important roles in a wide range of biotic and abiotic stresses such as drought, salt, invasion of pathogens, implying that the members of this family may be putative regulators in response to various BS and AS (*Huang et al., 2012; Li, Luan & Jin, 2012*). Two of 13 transcription factors, *WRKY11* and *WRKY26*, which involved in both AS and BS belong to WRKY family (Table 2). In other plant species, it also showed that the members of this family have a key role in abiotic and biotic stress responses (*Li et al., 2011; Chen et al., 2012*). It was reported that *OsWRKY11* involved in drought and heat tolerance in rice (*Wu et al., 2009*). It was shown that, in *Arabidopsis thaliana*, *WRKY26* positively regulates the cooperation between heat shock proteins and ethylene-activated signaling pathways that mediate responses to heat stress (*Li et al., 2011*). It has also been shown that the promoters of some heat tolerance-related genes including *Hsp* and *Hsf* genes such as *HsfA2, HsfB1, Hsp101, and MBF1c* contain W-box sequences that are recognized by WRKY proteins (*Li et al., 2011*).

ICE1-like bHLH family

ICE1-like (inducer of CBF expression 1) encodes a MYC-type basic helix-loop-helix (bHLH) transcription factor (*Chinnusamy et al., 2003*). ICE1 is an important inducer of *CBF3/DREB1A* which regulates cold stress tolerance (*Chinnusamy et al., 2003; Huang et al., 2015; Feng et al., 2013*) (Table 2). In *Arabidopsis*, *ice1* mutants showed decreased cold and chilling tolerance, whereas *ICE1* overexpression in *Arabidopsis* increased the cold tolerance of transgenic plants (*Chinnusamy et al., 2003*). Furthermore, osmotic and salt tolerance were also much markedly increased in transgenic tobacco carrying *SlICE1* from tomato by increasing the expression level of *DREB1/CBF* and their target genes (*Feng et al., 2013*) (Table 2).

bZIP family

Transcription factors of the basic leucine zipper (bZIP) family regulate various biological processes in plant growth and development, including morphogenesis and seed formation, as well as in abiotic and biotic stress responses by binding to promoters of specific target genes to up- or down- regulate their expression (*Li et al., 2015*). WRKY transcription factors, basic leucine zipper domain (bZIP) and MYB transcription factors involved in plant defense against pathogens (Alves *et al*., 2013) (Table 2). Transcription factor bZIP11 is a member of S1 class of the bZIP family, and it was shown that *bZIP11* mRNA translation is repressed in response to sucrose (*Hummel et al., 2009*). It has been shown that sucrose repression of *bZIP11* mRNA translation depends on the presence of the 5'-leader sequence upstream of *bZIP11* gene (*Hummel et al., 2009*). It has also been shown that sucrose signaling has an important role in the translational control of *bZIP11* in response to stress conditions (*Hummel et al., 2009*).

These reports implied that these transcription factors are important regulators of biological processes and may be useful in the breeding of plants to improve their tolerance against adverse stresses. The presence or absence of the transcription factors across various plant species and other organisms including bacteria, archaea and other eukaryotes is shown in figure 5. A high homology was observed for all transcription factors except protein LHY between *Solanum lycopersicum* and *Solanum tuberosum* and also the highest percentage of similarity was found between *Vitis vinifera*, *Populus trichocarpa* and *Glycine max*. The results showed that GRAS6 is the only TF present in bacteria with a low similarity to that of tomato GRAS6. Just three of the identified TFs, ICE1-like, protein LHY and MYB48, are present in opisthokonta that the homology between MYB48 and those of opisthokonata was the highest. MYB48 is the only protein which is present in all organisms except bacteria and archaea (Fig. 5). It has been cleared that MYB is one of the largest and most diverse transcription factor families, which are key

factors in regulatory networks controlling metabolism, development and responses to BS and AS (Roy, 2016). The members of MYB family found in all eukaryotes and molecular studies in *Arabidopsis* has been shown the role of this family in the epigenetic regulation of stress responses in plants (Roy, 2016). The results showed MYB48 as one of the members of MYB family possesses a hub role in the network of abiotic and biotic responses (Fig. 3). In addition to MYB48, three other TFs, WRKY11, WRKY26 and protein LHY, were observed in *Amoebozoa* with low similarity to those of tomato (Fig. 5). As well as *Amoebozoa*, the WRKYs were observed in *Giardia lamblia*, also known as *Giardia intestinalis* (Fig. 5). It was previously thought that WRKYs are specific to plants and algae (*Eulgem & Somssich, 2007*) but recent studies show their presence in human protozoan parasite *G. lamblia* and *D. discoideum* (slime mold) (*Wu et al., 2005*). It has been reported that WRKYs have also important roles in other physiological processes. It was shown that the expression of genes encoding proteins of cell wall increased, when a *WRKY-like* gene overexpressed in *G. lamblia* (*Pan et al., 2009*).

The results showed that protein LHY present in all eukaryotes except *G. lamblia*, *Trichomonas vaginalis* and *Trypanosomatidea* (Fig. 5) and also suggested a central role for this protein in the network of abiotic and biotic responses (Fig. 3). The results of other studies also support an important role for this protein and suggested that this protein involved in circadian clock control (*Lu et al., 2009*).

CONCLUSION

Transcriptome-wide studies by meta-analysis allowed us to narrow down DEGs to small number. DEGs represent the genes in different networks and signaling pathways that enable us to figure out which genes play the major role in various stresses and determine the overlap between biotic and abiotic stress response pathways.

This study was designed to identify genes have important roles in abiotic and biotic tolerance mechanisms in tomato and focused on those shared by both stresses. We identified 1862 and 835 genes differentially regulated in response to biotic and abiotic stresses, respectively, and 361 genes identified in this work are to be involved in cross talk between biotic and abiotic stress responses. Since transcription factors regulate the expression of a wide range of genes under stress conditions, we focused on genes which encode transcription factors and it was revealed that close to 5% of identified genes encoding TFs. Among them, the expression of genes encoding JERF1, GRAS6, MYB48, SlERF4, EIL2, protein LHY, SlERF1, WRKY 26, bZIP transcription factor, ICE1-like, pti6, EIL3 and WRKY 11 are affected by a diverse set of biotic and abiotic stresses. This study highlighted the importance of detected TFs as regulators of various stress responses, reinforcing the role of these TFs in regulating plant tolerance to a board spectrum of stresses (*Borrás-Hidalgo et al., 2005; Yáñez et al., 2009; Sharma et al., 2010; Feng et al., 2013; Li, Luan & Liu, 2015*). The results suggest that detected transcription factors in this work play important roles in plant tolerance response to various stresses and data generated will be helpful in conducting functional genomics studies to figure out their precise role during plant stress response.

The salient features of this study include 1) a valuable approach to identify the expression profiles of various genes under different conditions in plants; 2) meta-analysis can be used to characterize candidate genes for both biotic and abiotic stress tolerance; 3) identified genes are the potential candidates for genetic manipulation of plants to improve their tolerance to biotic and abiotic stresses.

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Table 1(on next page)

Transcription factors and their binding sites

Transcription factor and binding site for each, in biotic and abiotic stresses. TF: Transcription factor, TFBS: Transcription factor binding site.

TF family	NO. of TF in abiotic	NO. of TF in biotic	TFBS
ARF	1	3	TGTCTC auxin response elements (AuxRE)
BBR-BPC	1	-	(GA/TC)8 and GAGA-binding
bHLH	3	9	E-box (5'-CANNTG-3')
C2H2	-	9	a DNA element that contains an AGCT core
C3H	-	1	Unknown
ERF	5	18	GCC box is an 11 bp sequence (TAAGAGCCGCC)
EIL	3	2	EIL2 BS in ERF1 (TTCAAGGGGGCATGTATCTTGAA)
FAR1	-	1	FBS for FHY3-FAR1 binding site
GATA	-	1	WGATAR (W = T or A; R = G or A) motifs
GRAS	1	7	cis-element AATTT
HSF	-	3	that consists in a tandem of inverted repeats of the sequence GAA, generating a perfect
LBD	-	1	HSE, TTCnnGAAnnTTC core sequence CGGC
MIKC_MADS	-	1	keratin-like coiled-coil domain
MYB and MYB _related	2	8	(CNGTTR), (GKTWGTTR), TAACPy sequence (only one AAC sequenc) and (GKTWGGTR; R, A or G; K, G or T; W, A or T) and EE, AGATATTT
NAC	5	4	NAC recognition site (NACRS), NAC binding element (SNBE) with a longer and variable sequence[T/A]NN[C/T][T/C/G]TNNNNNNNA[A/C]GN[A/C/T][A/T])
NF-YB	-	3	CCAAT binding
NF-YC	1	-	core nucleotide sequence CCAAT
PHD	-	1	Unknown
SPL proteins	-	1	SBP-box, TNCGTACAA
TALE	2	1	Pbx:Meinox binding site (CTGTCA**A**TCA)
TCP	-	2	GGNCCCAC sequences and s G(T/C)GGNCCC
TIFY	-	1	Unknown

VOZ	1	-	GCGTNx7ACGC
WRKY	3	9	W box (TTGACY; Y, C or T)
YABBY	-	1	WATNATW (W = T or A; R = G or A)
ZIP and HD-ZIP	3	6	motif AATNATT

Table 2 (on next page)

Detected transcription factors

Detected transcription factors are affected by a diverse set of biotic and abiotic stresses.

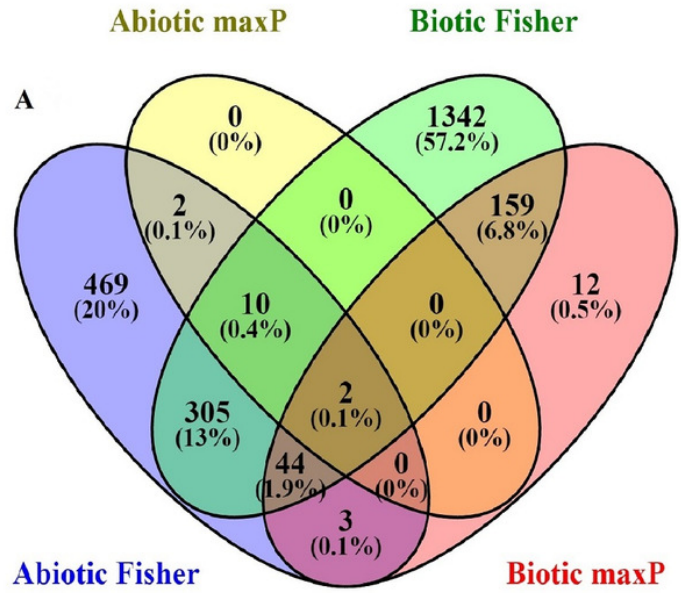
Transcription factor family	Transcription factor	Function	References
ERF	JERF1	salinity, low temperature, drought and various biotic stresses	Zhang *et al*., 2004; Wu *et al*., 2007; Sharma *et al*., 2010; Zhang *et* al., 2010
	SlERF4	abiotic stress and pathogen (Xanthomonas campestris)	Sharma *et al*., 2010; Kim, Stork & Mudgett, 2013
	Pti6	pathogen	Gu *et al*., 2002; Chakravarthy *et al*., 2003; Sharma *et al*., 2010
	SlERF1	abiotic stress and pathogen (Plectosphaerella cucumerina and Botrytis cinerea)	Gu *et al*., 2002; Mayrose *et al*., 2006
GRAS	SlGRAS6	abiotic stress, mechanical stress and pathogen (Pseudomonas syringae pv. tomato)	Mayrose *et al*., 2006
MYB family and MYB-related family	MYB48	salt , drought , cold and pathogen	Xiong *et al*., 2014
	LHY	low temperature and pathogen	Huang *et al*., 2007; Alves *et al*., 2013; Grundy, Stoker & Carré, 2015; Seo & Mas, 2015
EIL	EIL2	salinity and ortholog in tobacco resistance against pathogene (Peronospora hyoscyami)	Ying *et al*., 2004; Borrás-Hidalgo *et al*., 2006
	EIL3	salinity and ortholog in tobacco resistance against pathogene (Peronospora hyoscyami)	Ying *et al*., 2004; Borrás-Hidalgo *et al*., 2006
WRKY	WRKY11	drought , heat tolerance and pathogen	Wu *et al*., 2009; Li *et al*., 2011; Chen *et al*., 2012; Alves *et al*., 2013
	WRKY26	heat stress and pathogen	Li *et al*., 2011; Chen *et al*., 2012; Alves *et al*., 2013
ICE1-like bHLH	ICE1-like	cold , chilling, osmotic and salt	Chinnusamy *et al*., 2003; Feng *et al*., 2013; Huang et al., 2015
bZIP	bZIP11	abiotic stress and pathogen	Hummel *et al*., 2009; Alves *et al*., 2013

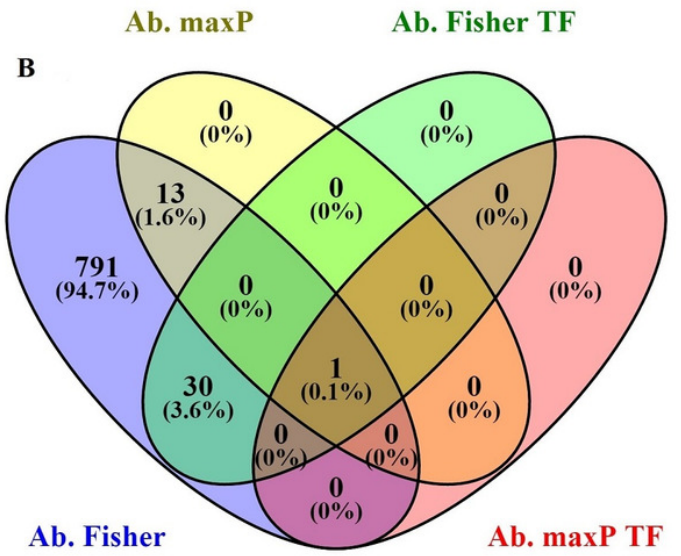
1

Figure 1

Identification of genes involved in biotic and abiotic stresses

Comparison of differentially expressed genes (DEGs) under abiotic and biotic stress responses, (A) Four-way Venn diagrams showing co-occurrence of DEGs in response to various abiotic and biotic stresses by two meta-analytical approaches: Fisher and maxP methods. (B) Four-way Venn diagrams showing number of transcription factors DEGs in all identified abiotic stresses by two different meta-analytical statistical methods: Fisher and maxP. (C) Four-way Venn diagrams showing number of transcription factors DEGs in all identified biotic stresses by two different meta-analytical statistical methods: Fisher and maxP


A
Abiotic maxP
Biotic Fisher
Abiotic Fisher
Biotic maxP
0 (0%)
1342 (57.2%)
2 (0.1%)
0 (0%)
159 (6.8%)
469 (20%)
10 (0.4%)
0 (0%)
12 (0.5%)
305 (13%)
2 (0.1%)
0 (0%)
44 (1.9%)
0 (0%)
3 (0.1%)


B
Ab. maxP
Ab. Fisher TF
Ab. Fisher
Ab. maxP TF
0 (0%)
0 (0%)
13 (1.6%)
0 (0%)
0 (0%)
791 (94.7%)
0 (0%)
0 (0%)
0 (0%)
30 (3.6%)
1 (0.1%)
0 (0%)
0 (0%)
0 (0%)
0 (0%)

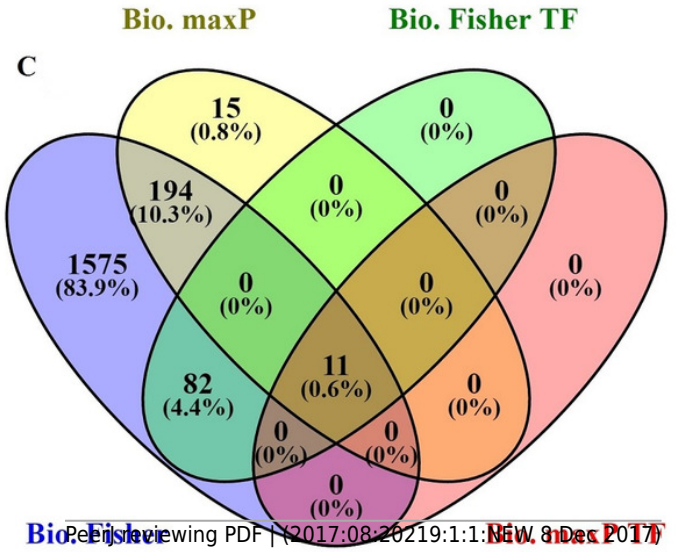

C
Bio. maxP
Bio. Fisher TF
Bio. Fisher
Bio. maxP TF
15 (0.8%)
0 (0%)
194 (10.3%)
0 (0%)
0 (0%)
1575 (83.9%)
0 (0%)
0 (0%)
0 (0%)
82 (4.4%)
11 (0.6%)
0 (0%)
0 (0%)
0 (0%)
0 (0%)

Figure 2

Gene ontology

Gene Ontology analysis. Frequency of most representative biological process terms. Com: Differentially expressed genes obtained by common genes in biotic and abiotic stresses, AS: Differentially expressed genes obtained by abiotic stresses, BS: Differentially expressed genes obtained by biotic stresses. BG: The frequency of these terms in the reference genes, Tomato Locus set. Gene Ontology analysis made in the AgriGO platform (FDR = 5%). More details in Table S10 and Table S11

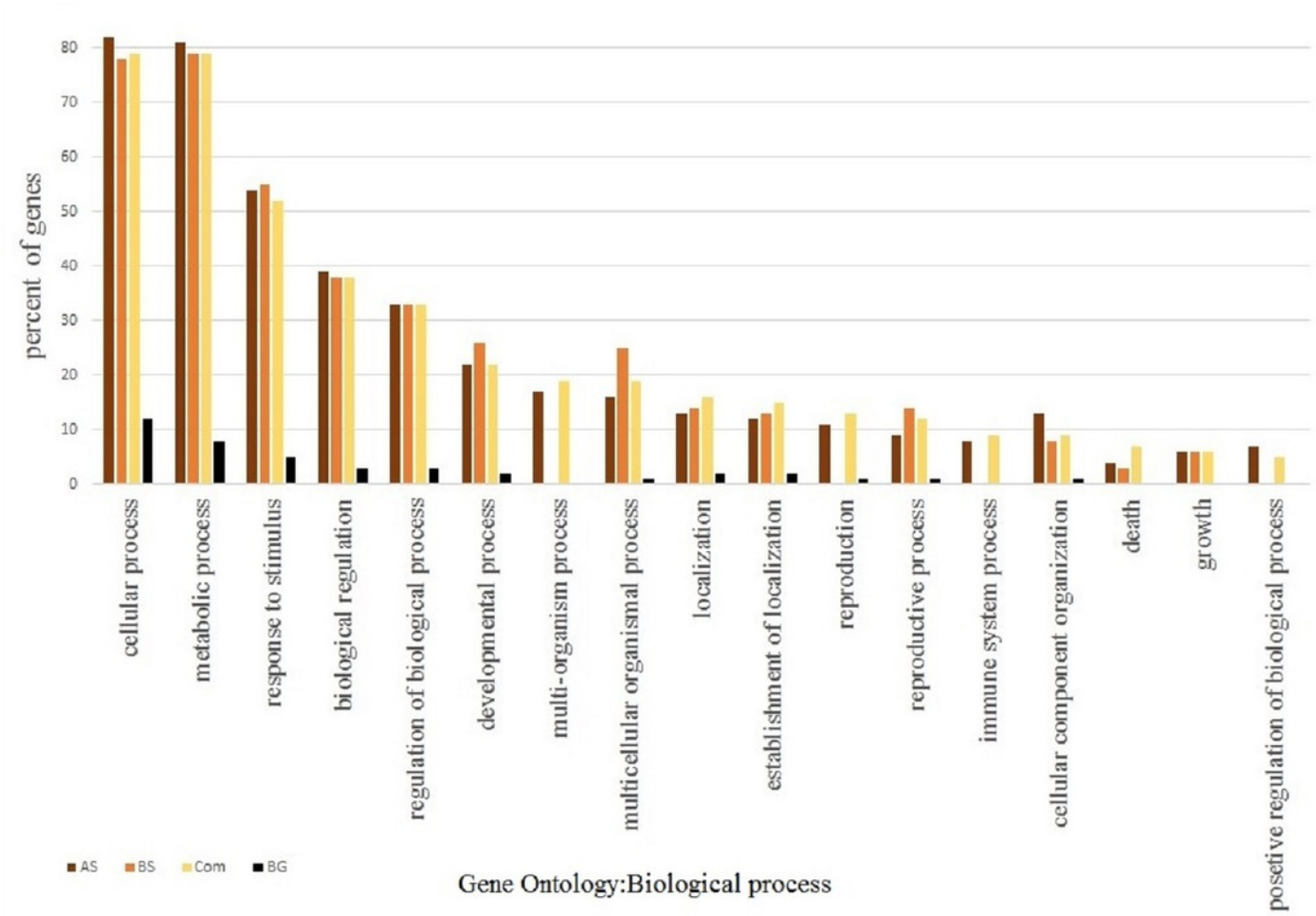


Figure 3

Network analysis to clear all connections for differentially expressed genes shared by abiotic and biotic stresses and determine genes possessing hub roles

Network analysis of the 361 common DEGs identified in biotic and abiotic stresses. Network is made by STRING10.0 database and represents all connections of those genes with a confidence score > 0.4. The connection colors show the types of evidence for concluding association: recurring neighborhood in various genomes (green line), co-occurrence of those genes in the same organisms (dark blue), co-expression (black), experimental protein–protein interaction data (pink), pathway characterized by other databases (light blue) and literature text-mining (yellow). A number above hub represent TF. 1) JERF1, 2) MYB48, 3) protein LHY, 4) EIL3, 5) EIL2, 6) WRKY26

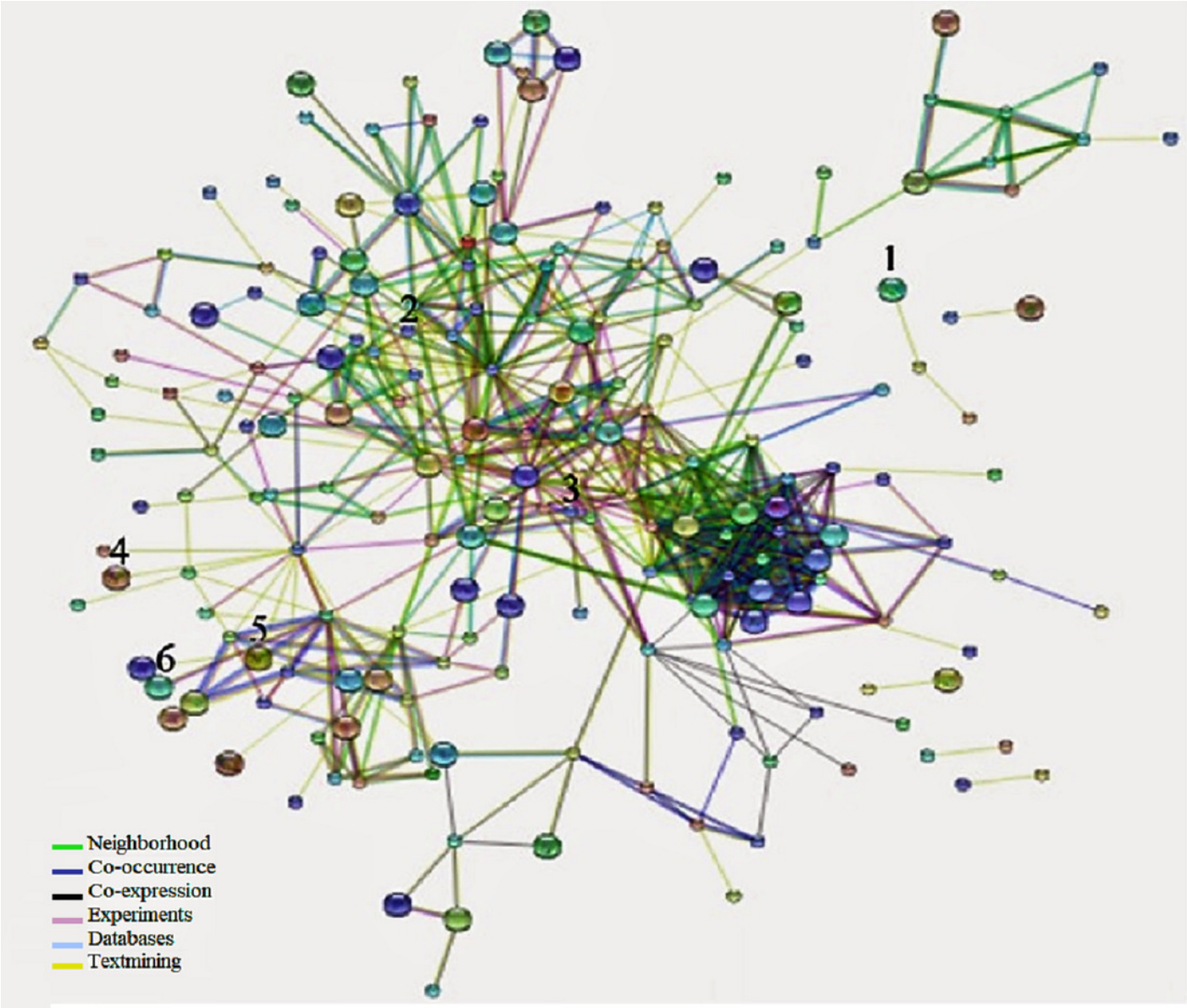

1
2
3
4
5
6
Neighborhood
Co-occurrence
Co-expression
Experiments
Databases
Textmining

Figure 4

Common transcription factors in biotic and abiotic stresses

Two-way Venn diagram showing the common transcription factors between abiotic and biotic stresses

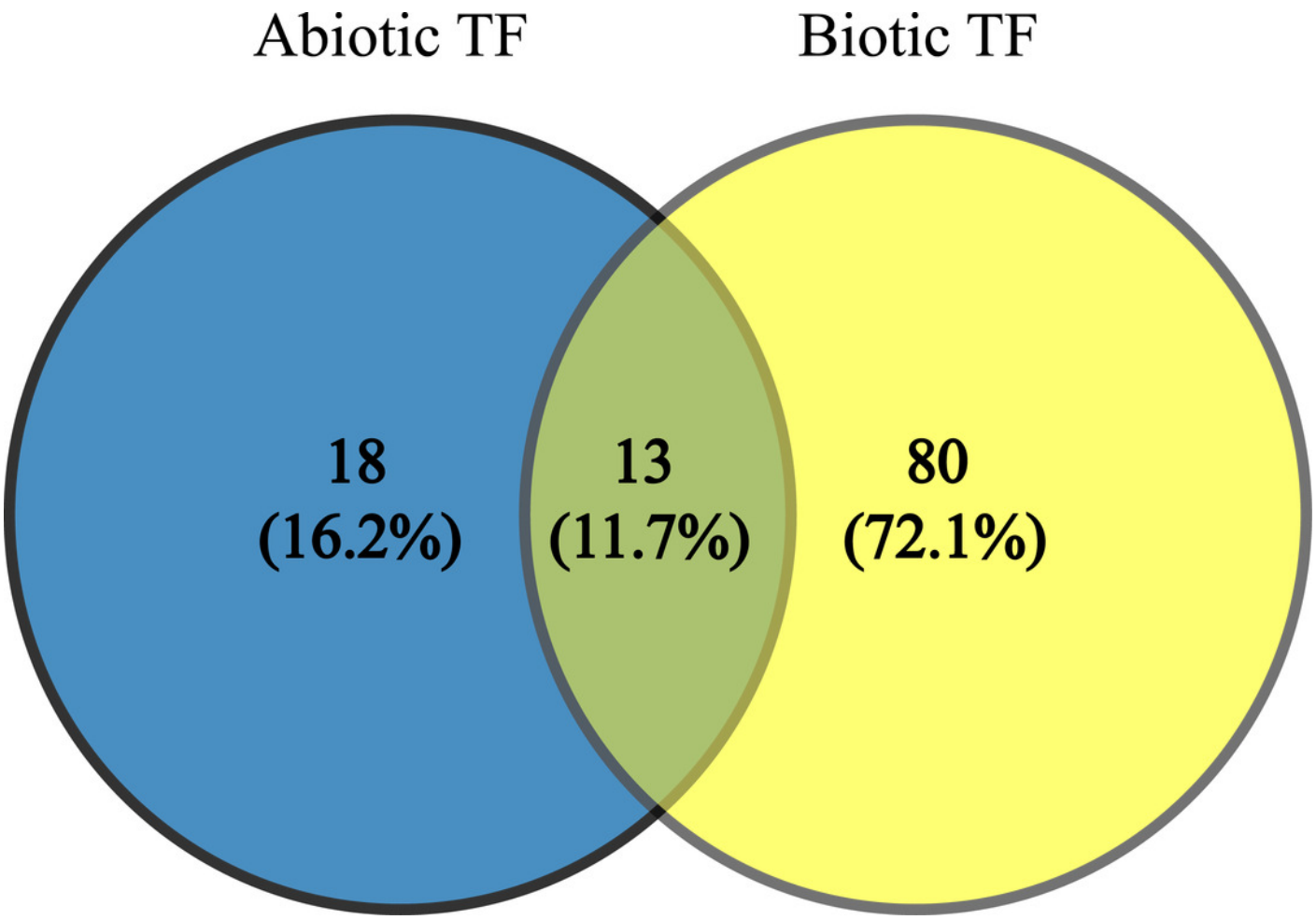
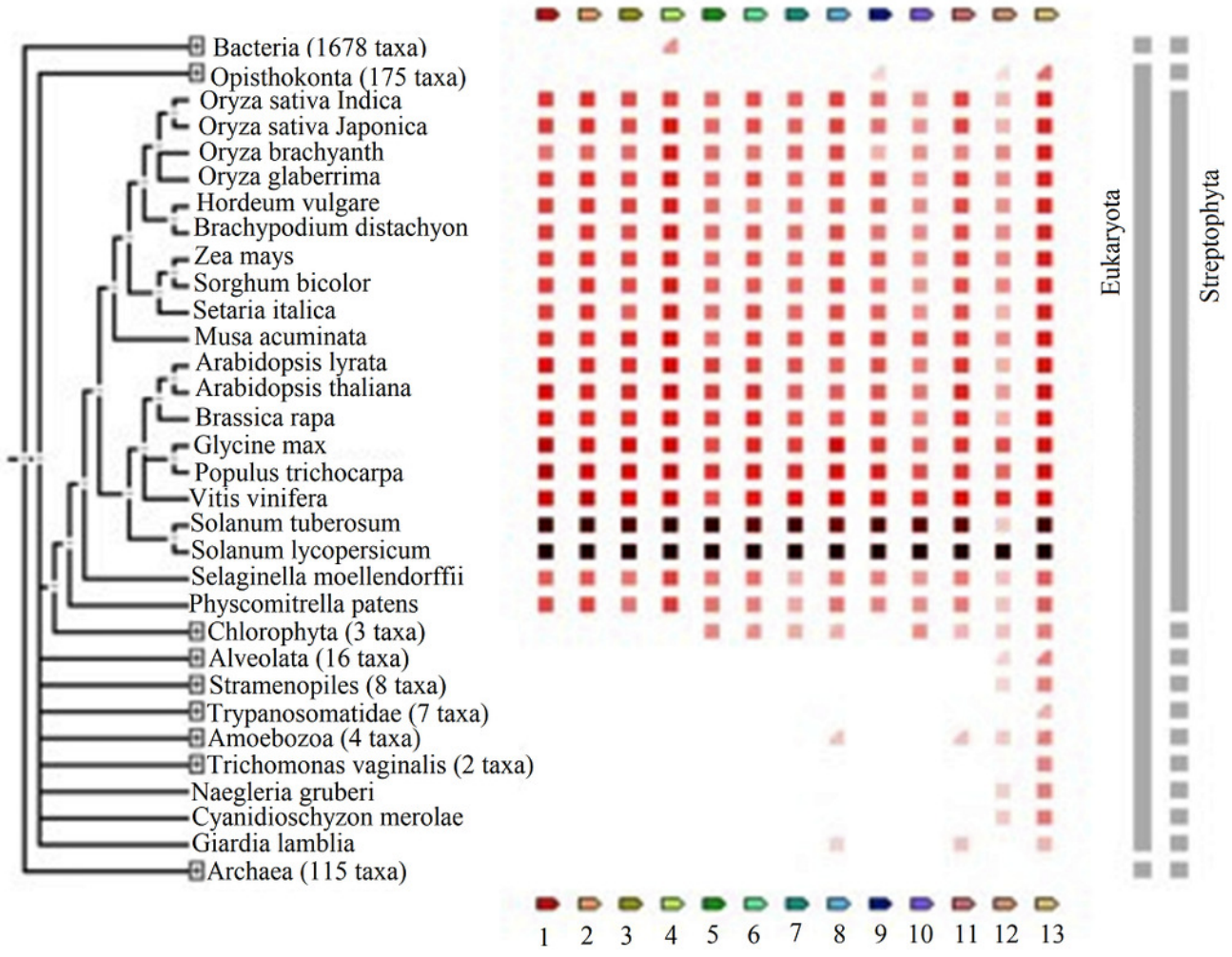


Figure 5

The presence or absence of the transcription factors across various organisms

Transcription factor occurrence patterns across various genomes. 1) EIL2, 2) EIL3, 3) ZIP, 4) GRAS6, 5) SlERF4, 6) SlERF1, 7) JERF1, 8) WRKY26, 9) ICE1-like, 10) pti6, 11) WRKY11, 12) protein LHY, 13) MYB48. The intensity of the color of the red square reflects the amount of conservation of the homologous protein in the species. White color (No similarity detectable) and black red color **(**100 similarity detectable)