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 affects plant growth and productivity worldwide. The study of individual genes cannot consider as an effective approach for the 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 stresses was much greater than that regulated by abiotic stresses, 835 genes, and found 15.4% or 361 differentially expressed genes with the conserved expression between abiotic and biotic stresses. The greatest percent of genes related to the 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 SlGRAS6 play central roles 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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2   tomato

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24   **ABSTRACT**

25   A wide range of biotic and abiotic stresses adversely affects plant growth and productivity

26   worldwide. The study of individual genes cannot consider as an effective approach for

27   the understanding of tolerance mechanisms since these stresses frequently, often in combination

28   and a large number of genes involved in these mechanisms. The availability of high-throughput

29   genomic data has enabled the discovery of the role of transcription factors in regulatory

30   networks. A meta-analysis of biotic and abiotic stress responses was performed by analyzing a

31   total of 391 microarray samples from 23 different experiments and 2336 differentially expressed

32   genes involved in multiple stresses were identified. We identified 1862 genes differentially

33   regulated in response to biotic stresses was much greater than that regulated by abiotic stresses,

34   835 genes, and found 15.4% or 361 differentially expressed genes with the conserved expression

35   between abiotic and biotic stresses. The greatest percent of genes related to

36   the cellular process (>76% genes), metabolic process (>76% genes) and response to stimulus

37   (>50%). About 4.2% of genes involved in biotic and abiotic stress responses belonged to the

38   transcription factor families. We identified several genes, which encode transcription factors that

39   play an important role in abiotic and biotic stress responses. These proteins included JERF1,

40   SlGRAS6, MYB48, SlERF4, EIL2, protein LHY, SlERF1, WRKY 26, bZIP transcription factor,

41   ICE1-like, pti6, EIL3 and WRKY 11. Six of these proteins, JERF1, MYB48, protein LHY, EIL3,

42   EIL2 and SlGRAS6 play central roles in these mechanisms. This research promoted a new

43   approach to clarify the expression profiles of various genes under different conditions in plants,

44   detected common genes from differentially regulated in response to these conditions and

45   introduced them as candidate genes for improving plant tolerance through genetic engineering

46   approach.

48   **INTRODUCTION**

49   As sessile organisms, plants constantly encounter a wide range of stresses that negatively affect

50   the yield production, survival and growth of plants worldwide (*Sharma et al., 2013*). The stresses

51   are divided into two broad categories, abiotic stresses (AS) including a variety of adverse

52   environmental conditions, (i.e. drought, submergence, salinity, heavy metal contamination,

53   nutrient deficiency, light and extreme temperatures) and biotic stresses (BS) caused by attack of

54   living organisms, (i.e. bacteria, viruses, fungi and nematodes as well as other plants) (*Rastgoo &*

55   *Alemzadeh, 2011; Liu et al., 2014*). To survive under stress conditions, complex mechanisms

56   have evolved in plants to perceive signals from their environment, transmit through signal

57   transduction pathways and respond to the various stresses that each of which may involve several

58   hundred proteins (*Fujita et al., 2006; Rastgoo, Alemzadeh & Afsharifar, 2011; Atkinson, Lilley*

59   *& Urwin, 2013; Sharma et al., 2013, Sami & Alemzadeh, 2016*). Functional molecular studies

60   have figured out interactions between biotic and abiotic stresses signaling components with the

61   identification a large number of genes, which are regulated by various types of transcription

62   factors (TFs) (*Kissoudis et al., 2016*). TFs are proteins with critical roles in the control temporal

63   and spatial gene expression during defense response that many of them are induced by more than

64   one stress (*Liu et al., 2014; Shaik & Ramakrishna, 2014; Prasad et al., 2016*).

65   The better understanding of plant stress response mechanisms under various stresses can give us

66   a higher view of how to improve the worldwide food production (*Sharma et al., 2013*). It is

67   necessary to learn more about molecular mechanisms of transcription factors, MAPKs and ROS

68   signal pathways, and hormone signaling, and study of genes encoding regulatory molecules,

69   especially transcription factors, can be more efficient than single-function genes, because TFs

70   enable to regulate various genes to improve plant tolerance against stresses (*Seki et al., 2002*).

71   The bioinformatic approach is an emerging and rapidly developing field which allows

72   researchers to analyze biological data (*Khatri & Drăghici, 2005; Chang et al., 2013; Zinati,*

73   *Alemzadeh & KayvanJoo 2016*). Recent advances in genome-wide transcriptome analysis

74   methods, such as RNA sequencing and microarrays, enable researchers simultaneously to study

75   the expression of thousands of genes and their coexpression partners under various stresses

76   (*Khatri & Drăghici, 2005; Sharma et al., 2013; Rest et al., 2016*). Meta-analysis can be used to

77   estimate differential gene expression between normal and stress conditions and find out some

78   genes that their products are key molecules in response to the stress (*Tseng, Ghosh & Feingold,*

79   *2012; Shaar-Moshe, Hübner & Peleg, 2015*).

80   Today, with recent advances in high-throughput technology, a large number of biological data

81   has become accessible via online resources, and we are now able to obtain results that more

82   reliable by combining information from multiple sources. In this way, statistical methods can be

83   used as useful and powerful tools to identify suitable candidate genes for test under experimental

84   conditions to use in plant breeding programs. The combination of *P*-values is a common method

85   in microarray meta-analysis and recently used in many studies (*Tseng, Ghosh & Feingold, 2012*).

86   A meta-analysis of microarray using 13 experiments querying cytokinin-regulated gene

87   expression changes in *Arabidopsis* combined with empirically defined filtering criteria identified

88   a set of 226 genes differentially regulated by cytokinin (*Bhargava et al., 2013*). In another

89   research project, a meta-analysis of ten studies identified 4015 genes showing significant

90   differential expression in response to water limitation compared to control conditions in

91   *Arabidopsis* (*Rest et al., 2016*).

92   Moreover, combining gene expression information across species can improve the ability to

93   identify core gene sets with high evolutionary conservation. For example, a new cross-species

94   meta-analysis of progressive drought stress at the reproductive stage was developed using

95   *Arabidopsis*, rice, wheat and barley. Based on this dataset, shared important genes and metabolic

96   pathways involved in whole plant adaptation to progressive drought stress across-species were

97   identified (*Shaar-Moshe, Hübner & Peleg, 2015*). In this study, 225 differentially expressed

98   genes (DEGs) shared across studies and taxa were identified and gene ontology enrichment and

99   pathway analyses revealed that the shared genes were classified into functional categories

100   involved predominantly in metabolic processes, regulatory function and response to stimulus

101   (*Shaar-Moshe, Hübner & Peleg, 2015*).

102   Tomato (*Solanum lycopersicum*) is one of the most important food crops belongs to Solanaceae

103   family, one of the largest and most important families of flowering plants. In addition, the tomato

104   is a suitable model system to study environmental signal transduction components involved in

105   biotic and abiotic stresses (*Knapp et al., 2004; Arie et al., 2007*). However, there is no a large-

106   scale transcriptome analysis via meta-analysis of stress response studies in tomato to detect

107   proteins, especially transcription factors, involved in various stresses.

108   In this study, for the first time we performed a large scale comparative transcriptome analysis via

109   meta-analysis of stress response studies in tomato using microarray gene expression data

110   implemented on a single platform (Affymetrix Tomato Genome Array). The goal of this meta-

111   analysis was to detect DEGs involved in biotic and abiotic stresses and detect DEGs shared by

112   both stresses. In addition, we focused on the identification of transcription factors have

113   magnitude effects on the plant cells via up- and down- regulation of various genes under stress

114   conditions. Other analysis methods, such as gene ontology (GO) and Network analysis, were

115   used to explain the complexity of tomato response to abiotic and biotic stresses. We also

116   discussed the functions of the identified transcription factors in stress response.

117

118   METHODS

119   **Selection microarray studies**

120   In this study, a meta-analysis of stress response was performed in tomato using publicly

121   available microarray gene expression data implemented on a single platform (Affymetrix

122   Tomato Genome Array). Expression data of tomato plants exposed to biotic and abiotic stresses

123   were combined. The raw expression data of various experiments were obtained from the Array

124   Express from EBI (https://www.ebi.ac.uk/arrayexpress/).

125   **Identification of differentially expressed genes**

126   Microarray expression data from each source study was pre-processed separately as individual

127   datasets. The raw data were normalized using Robust Multi-array Average (RMA) background

128   correction and quantile normalization. The data were processed in the R program. Meta-analysis

129   of AS and BS was performed separately to identify DEGs involved in stress conditions. Genes

130   that showed significant *P*-value (FDR of 5%), meaning that an estimated 5 percent of the DEGs

131   are false positive, were considered as DEGs (*Campain & Yang, 2010; Chang et al., 2013*). Two

132   methods of the combination of *P*-value have used: Fisher's method and maxP (*Wilkinson, 1951;*

133   *Fisher, 1992*). The Fisher's combined probability test sums the logarithm-transformed *P*-values

134   obtained from individual studies, under the null hypothesis, and follows a chi-squared

135   distribution with 2k degrees of freedom, where k is the number of studies being combined

136   (*Rhodes et al., 2002*). A very small *P*-value in just one study can be adequate to make statistical

137   significance, even if the same genes are not significant in any other study. The maxP method

138   takes maximum *P*-values across studies. It follows a beta distribution with parameters K (number

139   of transcriptomic studies which are combined for a meta-analysis where each study contains G

140   genes) and 1. This method targets on DEGs that have small *p*-values in all studies. (*Chang et al.,*

141   *2013*; *Song & Tseng, 2014*).

142   **Gene Analysis**

143   The genes selected by meta-analysis were further analyzed and characterized. In the first

144   instance, the results were compared with the plant genes in Plant Transcription factor & Protein

145   Kinase Identifier and Classifier, iTAK (http://bioinfo.bti.cornell.edu/cgi-bin/itak/index.cgi) and

146   Plant Transcription Factor, PlantTFDB (http://plntfdb.bio.uni-potsdam.de/v3.0/) databases. The

147   list includes 1845 genes of tomato which encoding the TFs that directly or indirectly involved in

148   signaling   pathway   and   response   to   abiotic   and   biotic   stresses.   Venn's   diagrams

149   (http://bioinfogp.cnb.csic.es/tools/venny/) were used to represent co-occurrence of DEGs. Gene

150   Ontology (GO) enrichment analysis of the DEGs (such as identifying biological processes) was

151   conducted using the AgriGO platform (*Du et al., 2010*).

152   **Network analysis**

153   The   Search   Tool   for   the   Retrieval   of   Interacting   Genes/Proteins,   STRING   10.5

154   (http://www.mybiosoftware.com/string-9-0-search-tool-retrieval-interacting-genesproteins.html)

155   (*Szklarczyk et al., 2014*) database was used to figure out all functional relations between the

156   genes and their occurrence patterns across various genomes. The database makes relations based

157   on several lines of evidences: The empirical evidence from protein–protein interaction assays,

158   co-expression data from NCBI Gene Expression Omnibus (GEO) database, the extraction of

159   information from other databases, coexistence of the genes in the same organisms, conserved

160   gene neighborhood in known genomes, gene fusion events, pathway annotation from other

161   resources such as Kyoto Encyclopedia of Genes and Genomes (KEGG) or Gene Ontology

162   databases, and automated text-mining tools (*Szklarczyk et al., 2014*). STRING calculates a

163 confidence value for those interactions according to the pieces of evidences from above 0.4 to

164 above 0.9, as the medium to highest score, respectively.

165

166 **RESULTS AND DISCUSSION**

167 **Identification of genes involved in biotic and abiotic stresses**

168 After searching in the database, microarray data from 23 different experiments were selected and

169 analyzed. To obtain a global analysis, 391 microarray samples including 213 AS from different

170 categories: drought, heat, salinity, light, hormone, mineral and heavy metals

171 deficiencies/toxicities and 178 BS that caused by viruses, nematode, fungi, bacteria and pests in

172 tomato (wild type and transgenic) were chosen (Table S1). We found a total of 1862 genes

173 differentially regulated in response to biotic stresses, was far greater than that induced by abiotic

174 stresses, 835 genes, with a false discovery rate (FDR) ≤ 0.05 by Fisher's statistical method

175 (Table S1-S3). We also figured out 15.4% or 361 DEGs with the conserved expression between

176 AS and BS by Fisher's statistical method, suggesting that these genes and their associated cell-

177 signaling pathways are regulated in a similar way in a wide range of stresses (Fig. 1A) (Table

178 S5). Using maxP method, a total of 14 genes differentially regulated in response to abiotic

179 stresses and a total of 220 genes differentially regulated in response to biotic stresses were found.

180 We figured out 0.9% or 2 DEGs (LES.1399.1 and LES.3969.1) with the conserved expression

181 between AS and BS by maxP method (Fig. 1A) (Table S2-S4). These methods used in other

182 studies to identify important genes in different physiological processes in other plants. Shaar-

183 Moshe Hübner and Peleg (2015) identified 225 differentially expressed genes across-species by

184 using 17 microarray experiments of drought stress at the reproductive stage of *Arabidopsis*, rice,

185 wheat and barley. Fisher's and maxP methods are two popular microarray meta-analysis methods

186   used in the literature that have different strength for detecting different types of DEGs (*Tseng,*

187   *Ghosh & Feingold, 2012; Wang et al., 2012*). Our results also showed that the two used methods

188   detected different sets of DEGs, suggesting different assumptions behind the methods. Among

189   these methods, Fisher's method is more popular in the microarray meta-analysis studies (*Wang et*

190   *al., 2012*) and hence, we were more interested in detecting DEGs across all studies through

191   Fisher's statistical method.

192

193   **Gene ontology**

194   We focused on the 361 DEGs common to both AS and BS and subjected them to Gene Ontology

195   (GO) and network analysis to explore other possible functions of them. GO enrichment analysis

196   of the DEGs showed that shared DEGs represented genes involved in main biological and

197   cellular processes. Among them, the greatest percentage of genes involved in the cellular process

198   (>76% genes), metabolic process (>76% genes) and response to stimulus (>50%). One of largest

199   functional group of DEGs was associated with metabolic processes (e.g. carbohydrate metabolic

200   process, lipid metabolic process, protein metabolic process, nucleobase, nucleoside, nucleotide

201   and nucleic acid metabolic process, cellular amino acid and derivative metabolic process and

202   regulation of primary metabolic process).

203   In addition, another large functional group of DEGs was associated with cellular processes (e.g.

204   cellular metabolic process, regulation of cellular process and cellular response to stimulus), the

205   show a significant rearrangement in plant metabolism as part of stress adaptation (*Shaar-Moshe,*

206   *Hübner & Peleg, 2015*).

207   On the other hand, the lowest percentage of genes involved in positive regulation of biological

208   process (5%) (Fig. 2) (Table S6). The most significant GO terms for cellular process were

209 cellular metabolic process (FDR: 1.8E-37), organic acid metabolic process (FDR: 9.2E-29),

210 cellular ketone metabolic process (FDR: 1.2E-28) in metabolic process were primary metabolic

211 process (FDR: 7.8E-39), cellular metabolic process (FDR: 1.8E-37) and organic acid metabolic

212 process (FDR: 9.2E-29). Moreover, in response to stimulus were response to abiotic stimulus

213 (FDR: 3.4E-40), response to biotic stimulus (FDR: 9.4E-26), and response to stress (FDR: 9.4E-

214 26). Some of these genes possess important roles in DEGs shared by AS and BS networks (Table

215 S7). A genome-wide transcriptional analysis was performed just for Fe deficiency and was

216 identified 97 DEGs by comparing Fe deficient and Fe-sufficient in roots of tomato. These

217 transcripts are related to the physiological responses to the nutrient stress resulting in an

218 improved iron uptake, including regulatory aspects, translocation, root morphological

219 modification, and adaptation in primary metabolic pathways, such as glycolysis and TCA cycle

220 (*Zamboni et al.,* 2012). In another research was showing that the largest functional groups of

221 DEGs was associated respectively to metabolic processes (e.g. amino acid and carbohydrate

222 metabolism), regulator function (e.g. protein degradation and transcription) and response to the

223 stimulus that identified by the research of Shaar-Moshe Hübner and Peleg (2015).

224 **Network analysis**

225 Network analysis using STRING 10.5 identified the connections among DEGs common to AS

226 and BS (Fig. 3) (Table S7). In addotion, some genes that possess an important role in the three

227 biological pathways; cellular process, metabolic process, response to stimulus were detected. For

228 example, Solyc02g090890.2.1 (*ZE*: plays an important role in resistance to stresses, seed

229 development and dormancy), Solyc04g049350.2.1 (*CS1*: biosynthesis of aromatic amino acids),

230 Solyc07g052480.2.1 (LOC544276: involved in storage lipid mobilization during the growth of

231 higher plant seedling), Solyc07g049530.2.1 (*ACO1*: involved in ethylene biosynthesis via S-

232 adenosyl-L-methionine), Solyc01g099630.2.1 (*XTH1*: an essential component of the primary cell

233 wall, and thereby participates in cell wall construction of growing tissues), Solyc05g055230.1.1

234 (*RPS17*: ribosomal small subunit assembly), Solyc12g098350.1.1 (*infA*: no specific function has

235   so far been attributed to this initiation factor; however, it seems to stimulate more or less all the

236   activities of the other two initiation factors, IF-2 and IF-3), Solyc02g082920.2.1 (*CHI3*: defense

237   against chitin containing fungal pathogens), Solyc06g082600.2.1 (*UBC3*: mediates the selective

238   degradation of abnormal and short-lived proteins), Solyc01g096480.2.1 (LOC543657: probable

239   thiol-disulfide oxidoreductase that may play a role in proper chloroplast development and

240   negatively regulates Cf-9/Avr9-mediated cell death and defense responses), Solyc07g047850.2.1

241   (*CAB4*: it receives and transfers excitation energy to photosystems).

242   The results of network analysis also showed some of the transcription factors have connections

243   to other molecules. Based on pieces of literatures reviewing, detected transcription factors in this

244   study, JERF1, EIL3, EIL2, SlGRAS6, Protein LHY and MYB 48 affected by a wide range of

245   stresses and possibly play essential and significant roles in multiple stress responses which is in

246   agreement with their functional roles in biotic and abiotic stress responses. Expression of *JERF1*

247   was induced by salt, ethylene, MeJA and abscisic acid (ABA) treatments in tomato that may be

248   pointed to a central role for *JERF1* in different signal transduction pathways (*Zhang et al., 2004*).

249   It has been also reported that JERF1 activates ABA biosynthesis related genes such as NtSDR

250   (short-chain dehydrogenase/reductase) and enhanced cold and salt stress tolerance in transgenic

251   tobacco (Wu *et al*., 2007; *Ouyang et al*., 2007). It showed that the expression of *EIL2* and *EIL3*

252   was induced by ethylene and salinity treatments (*Ying et al., 2004*) and SlGRAS6 is one of the

253   most important members of the tomato GRAS family in disease resistance and mechanical stress

254   (*Mayrose et al., 2006*). Different studies have shown the large family of MYB has essential roles

255   in plant growth, development, primary and secondary metabolism and response to BS and AS

256   (*Oh, Park & Han, 2003; Van et al., 2008; Dou et al., 2016*). It also reported that LHY controls

257   the expression of a large number of ABA-responsive genes by binding directly to their promoters

258   (*Huang et al.,* 2007). These results support that the detected transcription factors in our study

259   might play an important role in biotic and abiotic stress responses.

260

261   **Identification of transcription factors involved in biotic and abiotic stresses**

262   In this research, because of the importance of TFs as a powerful tool for the manipulation of

263   complex metabolic pathways, we have tried to identify genes encoding them. According to the

264   PlantTFDB, 1845 genes from tomato encoding TFs were identified and classified into 58

265   families that directly or indirectly involved in signaling and response to AS and BS (Table S8).

266   Based on our results, from the 1862 genes response to biotic stresses selected by Fisher's

267   statistical method, 93 genes (5%) encode TFs (Fig.1C) (Table S9). ERF family has the greatest

268   number of genes (18 genes), and C3H, FAR1, GATA, LBD, MIKC-MADS, PHD, SLP protein,

269   TALE, TIFE and YABBY families have the lowest number of genes (each family has only one

270   gene) (Table 1). From the 835 genes response to abiotic stresses selected by Fisher's statistical

271   method, 31 of those (3.7%) encode TFs (Fig. 1B) (Table S10). ERF and NAC families have the

272   greatest number of genes (5 genes), and ARF, BBR-BPC, GRAS, NF-NY and VOZ families

273   have the lowest number of genes (each family has only one gene) (Table 1).

274   Since TFs are the key regulators of plant growth, development and metabolisms via up- and

275   down- regulation of various genes, identification of the genes encoding these proteins and

276   transferring them into plants to improve their stress tolerance would be interesting. These TFs

277   (JERF1, Pti6, SlERF1, SlERF4, SlGRAS6, MYB48, protein LHY, EIL2, EIL3, WRKY 11,

278   WRKY 26, ICE1-like and bZIP transcription factor) can be used as candidate genes in molecular

279   breeding programs. Because, these TFs are common to abiotic and biotic stresses and also, our

280   results indicate they are being affected by a wide range of stresses. In addition, they possibly

281   play essential and significant roles in multiple stress responses which is in agreement with their

282   functional roles in biotic and abiotic stress responses (*Gu et al., 2002; Ying et al., 2004; Zhang et*

283   *al., 2004; Borrás-Hidalgo et al., 2005; Mayrose et al., 2006; Wu et al., 2007; Yáñez et al., 2009;*

284   *Zhang et al., 2010; Sharma et al., 2010; Chen et al., 2012; Kim, Stork & Mudgett, 2013; Feng et*

285   *al., 2013; Li, Luan & Liu, 2015*). The results of network analysis also showed some of these

286   proteins have central roles in biotic and abiotic stress responses (Fig. 3). The two-way Venn

287   diagram indicated that the number of common TFs between biotic and abiotic stresses was 13

288   while 18 and 80 TFs were uniquely identified in abiotic and biotic stresses, respectively (Fig. 4).

289   **ERF family**

290   The ERF (Ethylene-responsive transcription factors) has been shown to play a key role during

291   plant development and stress tolerance mechanisms. Overexpression of ERF family genes in

292   different plant species such as *Arabidopsis*, tobacco, rice and tomato has been shown to increase

293   tolerance to a wide range of biotic and abiotic stresses (*Sharma et al., 2010*). The tomato has 112

294   AP2/ERF superfamily genes (*Sharma et al., 2010; Sharma et al., 2013*). Our results showed that

295   4 of 13 transcription factors shared between AS and BS, were belong to ERF family, including

296   JERF1, SlERF4, SlERF1 and pti6 (Table 2).

297   JERF1, Jasmonate Ethylene Response Factor 1, has critical roles in plant abiotic stress responses,

298   such as salinity, low temperature, dehydration and various biotic stresses in different plant

299   species (*Zhang et al., 2004; Wu et al., 2007; Zhang et al., 2010*). It has been shown that the

300   expression of *JERF1* in tomato was induced by salt, ethylene, MeJA and abscisic acid (ABA)

301   treatments that may be pointed to a central role for *JERF1* in different signal transduction

302   pathways (*Zhang et al., 2004*). Overexpression of *JERF1* enhanced the tolerance of transgenic

303   tobacco and rice plants to high salinity, low temperature, osmotic stress and pathogen attack by

304   regulating the expression of downstream stress-responsive genes and a number of pathogenesis-

305   related (PR) genes due to binding to DRE/CRT and GCC-box *cis*-elements (*Wu et al., 2007;*

306   *Zhang et al., 2010*). Expressing *JERF1* in tobacco induced expression of GCC box-containing

307   genes such as *CHN50*, *Prb-1b*, *GLA*, *NtSDR* and *osmotin* that subsequently resulted in enhanced

308   tolerance to salt stress and low temperature (*Zhang et al., 2004; Wu et al., 2007*). *JERF1* up-

309   regulated the expression of genes related to osmolyte synthesis such as *OsP5CS* and *OsSPDS2*,

310 which, has increased the production of osmolytes. Also it induced the expression of *OsABA2* and

311 *Os03g0810800* genes (containing DRE element in their promoter regions), which encode two

312 key enzymes involved in ABA biosynthesis and increased the synthesis of ABA in transgenic

313 rice that resulted in elevated tolerance to drought (*Zhang et al., 2010*). JERF1 may also be

314 located downstream of EIN3 in the ethylene signaling pathway (*Zhang et al., 2004*). It has been

315 previously reported that SlERF4, an ERF in tomato, is a XopD, a type III secretion effector from

316 *Xanthomonas campestris*, destabilize SlERF4 and this task has been proposed to promote

317 bacterial growth (*Kim, Stork & Mudgett, 2013*). SlERF4 reduces ethylene production and, in

318 turn, limits disease symptom development (*Kim,* Stork & Mudgett, *2013*) (Table 2). Pti6

319 belonged to the ERF family in the plant that binds specifically to the GCC-box *cis* element

320 present in the promoter regions of many PR genes and induced the expression of a wide range of

321 these genes that have key roles in the plant defense. It also showed that the expression of

322 jasmonic acid- and ethylene-regulated genes, such as *PR3*, *PR4*, *PDF1.2*, and *Thi2.1*, was

323 differently being affected by *Pti4* or other similar genes, *Pti5*, or *Pti6* (*Gu et al., 2002;*

324 *Chakravarthy et al., 2003*) (Table 2). The gene encoding *SlERF1* was another detected gene that

325 probably has an important role in response to various stresses. It has been shown that the

326 overexpression of Sl*ERF1* in *Arabidopsis* can confer resistance to necrotrophic fungi like

327 *Plectosphaerella cucumerina* and *Botrytis cinerea* (*Mayrose et al., 2006*) (Table 2).

328 **GRAS family**

329 SlGRAS6 was another identified transcription factor that is one of the most important members

330 of tomato GRAS family in disease resistance and mechanical stress (*Mayrose et al., 2006*).

331 SlGRAS6 is required for tomato disease resistance to the bacterial pathogen *Pseudomonas*

332 *syringae* pv. tomato. It has been shown that the suppression of *SlGRAS6* gene expression through

333   virus-induced gene silencing reduced resistance of tomato to *P. syringae* pv. tomato (*Mayrose et*

334   *al., 2006*) (Table 2).

MYB family and MYB-related family

336   The MYB is a large TF family in plants that have essential roles in plant growth, development,

337   primary and secondary metabolism and response to BS and AS (*Oh, Park & Han, 2003; Van et*

338   *al., 2008; Dou et al., 2016*). Most of MYB genes involved in response to various abiotic stresses,

339   belong to the R2R3-type group. With the comparison of *OsMYB48-1* expression with other MYB

340   proteins, a special role for this gene in response to the abiotic stresses in rice can be suggested. It

341   has been shown that its expression was strongly induced by ABA, H_2O_2, PEG, and dehydration

342   stresses, while slightly induced by salt and cold stresses (*Xiong et al., 2014*). It was reported that

343   the overexpression of *OsMYB48-1* regulated the expression level of some stress responsive-

344   genes, such as *RAB21*, *RAB16D*, *RAB16C* and *LEA3* under drought stress conditions (*Xiong et*

345   *al., 2014*) (Table 2). The LHY (LATE ELONGATED HYPOCOTYL) transcription factor plays

346   a critical role in the regulation of low temperature stress response via controlling expression of

347   *CBF 1, 2* and *3*, *COR27* and *COL1* genes and contribute to plant defense through the circadian

348   regulation of stomatal aperture (*Grundy, Stoker & Carré, 2015; Seo & Mas, 2015*). It was also

349   shown that LHY controls the expression of a large number of ABA-responsive genes by binding

350   directly to their promoters (*Huang et al., 2007*).

EIL family

352   It has been shown that the expression of *EIL2* and *EIL3*, which encode transcription factors in

353   ethylene signal transduction, was induced by ethylene and salinity treatments (*Ying et al., 2004*).

354   It also reported that its ortholog in tobacco is required for resistance against *Peronospora*

355   *hyoscyami* (*Borrás-Hidalgo et al., 2006*) (Table 2).

356   **WRKY family**

357   Different studies have shown that the members of WRKY family contribute multiple biological

358   processes such as plant growth and development. They also have important roles in a wide range

359   of biotic and abiotic stresses such as drought, salt, invasion of pathogens, implying that the

360   members of this family may be putative regulators in response to various BS and AS (*Huang et*

361   *al., 2012; Li, Luan & Jin, 2012*). Two of 13 transcription factors, *WRKY11* and *WRKY26*, which

362   involved in both AS and BS belong to the WRKY family (Table 2). In other plant species, it also

363   showed that the members of this family have a key role in abiotic and biotic stress responses (*Li*

364   *et al., 2011; Chen et al., 2012*). It was reported that *OsWRKY11* involved in drought and heat

365   tolerance in rice (*Wu et al., 2009*). It was shown that, in *Arabidopsis thaliana*, *WRKY26*

366   positively regulates the cooperation between heat shock proteins and ethylene-activated signaling

367   pathways that mediate responses to heat stress (*Li et al., 2011*). It has also been shown that the

368   promoters of some heat tolerance-related genes including *Hsp* and *Hsf* genes such as *HsfA2,*

369   *HsfB1, Hsp101, and MBF1c* contain W-box sequences that are recognized by WRKY proteins

370   (*Li et al., 2011*).

371   **ICE1-like bHLH family**

372   *ICE1*-like (inducer of CBF expression 1) encodes a MYC-type basic helix-loop-helix (bHLH)

373   transcription factor (*Chinnusamy et al., 2003*). ICE1 is an important inducer of *CBF3/DREB1A*

374   which regulates cold stress tolerance (*Chinnusamy et al., 2003; Huang et al., 2015; Feng et al.,*

375   *2013*) (Table 2). In *Arabidopsis*, *ice1* mutants showed decreased cold and chilling tolerance,

376   whereas *ICE1* overexpression in *Arabidopsis* increased the cold tolerance of transgenic plants

377   (*Chinnusamy et al., 2003*). Furthermore, osmotic and salt tolerance were also much markedly

378   increased in transgenic tobacco carrying *SlICE1* from tomato by increasing the expression level

379   of *DREB1/CBF* and their target genes (*Feng et al., 2013*) (Table 2).

bZIP family

381   Transcription factors of the basic leucine zipper (bZIP) family regulate various biological

382   processes in plant growth and development, including morphogenesis and seed formation, as

383   well as in abiotic and biotic stress responses by binding to promoters of specific target genes to

384   up- or down- regulate their expression (*Li et al., 2015*). WRKY transcription factors, basic

385   leucine zipper domain (bZIP) and MYB transcription factors involved in plant defense against

386   pathogens (Alves *et al.*, 2013) (Table 2). Transcription factor bZIP11 is a member of the S1 class

387   of the bZIP family, and it was shown that *bZIP11* mRNA translation is repressed in response to

388   sucrose (*Hummel et al., 2009*). It has been shown that sucrose repression of *bZIP11* mRNA

389   translation depends on the presence of the 5'-leader sequence upstream of *bZIP11* gene (*Hummel*

390   *et al., 2009*). It has also been shown that sucrose signaling has an important role in the

391   translational control of *bZIP11* in response to stress conditions (*Hummel et al., 2009*).

392   These reports implied that these transcription factors are important regulators of biological

393   processes and may be useful in the breeding of plants to improve their tolerance against adverse

394   stresses. The presence or absence of the transcription factors across various plant species and

395   other organisms including bacteria, archaea and other eukaryotes is shown in figure 5. A high

396   homology was observed for all transcription factors except protein LHY between *Solanum*

397   *lycopersicum* and *Solanum tuberosum* and the highest percentage of similarity was found

398   between *Vitis vinifera*, *Populus trichocarpa* and *Glycine max*. The results showed that GRAS6 is

399   the only TF present in bacteria with a low similarity to that of tomato GRAS6. Just three of the

400   identified TFs, ICE1-like, protein LHY and MYB48, are present in opisthokonta that the

401   homology between MYB48 and those of opisthokonata was the highest. MYB48 is the only

402   protein, which is present in all organisms except bacteria and archaea (Fig. 5). It has been shown

403   that MYB is one of the largest and most diverse transcription factor families, which are the key

404   factors in regulatory networks controlling metabolism, development and responses to BS and AS

405   (Roy, 2016). The members of MYB family found in all eukaryotes and molecular studies in

406   *Arabidopsis* has been shown the role of this family in the epigenetic regulation of stress

407   responses in plants (Roy, 2016). The results showed MYB48 as one of the members of MYB

408   family possesses an important role in the network of abiotic and biotic responses (Fig. 3). In

409   addition to MYB48, three other TFs, WRKY11, WRKY26 and protein LHY, were observed in

410   *Amoebozoa* with low similarity to those of tomato (Fig. 5). As well as *Amoebozoa*, the WRKYs

411   were observed in *Giardia lamblia*, also known as *Giardia intestinalis* (Fig. 5). It was previously

412   thought that WRKYs are specific to plants and algae (*Eulgem & Somssich, 2007*) but recent

413   studies show their presence in human protozoan parasite *G. lamblia* and *D. discoideum* (slime

414   mold) (*Wu et al., 2005*). It has been reported that WRKYs have also important roles in other

415   physiological processes. It was shown that the expression of genes encoding proteins of cell wall

416   increased, when a *WRKY-like* gene overexpressed in *G. lamblia* (*Pan et al., 2009*).

417   The results showed that protein LHY present in all eukaryotes except *G. lamblia*, *Trichomonas*

418   *vaginalis* and *Trypanosomatidea* (Fig. 5) and also suggested a central role for this protein in the

419   network of abiotic and biotic responses (Fig. 3). The results of other studies also support an

420   important role for this protein and suggested that this protein involved in circadian clock control

421   (*Lu et al., 2009*).

422

CONCLUSION

Transcriptome-wide studies by meta-analysis allowed us to narrow down DEGs to a 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, which, have important roles in abiotic and biotic tolerance mechanisms in tomato and focused on those shared by both stresses. The novelty of the work presented here is that a large meta-analysis was used to identify important genes in tomato as a model of Solanaceae crops. Meta-analysis has been previously used to figure out important genes that play critical roles in response to biotic or abiotic stresses in other plants such as *Arabidopsis* (*Shaar-Moshe, Hübner & Peleg, 2015; Rest et al., 2016*). However this is for the first time that the tomato was selected as the target plant to detect genes involved in a wide range of biotic and abiotic stress responses. In previous studies, usually one or a few stresses were considered, but here, a wide range of stresses was studied simultaneously. 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 being affected by a diverse set of biotic and abiotic stresses. This study highlighted the importance of detected TFs as regulators of

445 various stress responses, reinforcing the role of these TFs in regulating plant tolerance to a board

446 spectrum of stresses (*Borrás-Hidalgo et al., 2005; Yáñez et al., 2009; Sharma et al., 2010; Feng*

447 *et al., 2013; Li, Luan & Liu, 2015*). The results suggest that detected transcription factors in this

448 work play important roles in plant tolerance response to various stresses and data generated will

449 be helpful in conducting functional genomics studies to figure out their precise role during plant

450 stress response.

451 The salient features of this study include 1) a valuable approach to identify the expression

452 profiles of various genes under different conditions in plants; 2) meta-analysis can be used to

453 characterize candidate genes for both biotic and abiotic stress tolerance; 3) Identification of

454 important genes involved in various biotic and abiotic stresses; 4) we have identified genes

455 which are the potential candidates for genetic manipulation of plants to improve their tolerance

456 to biotic and abiotic stresses.

457

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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 (CTGTCAATCA)
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

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

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.
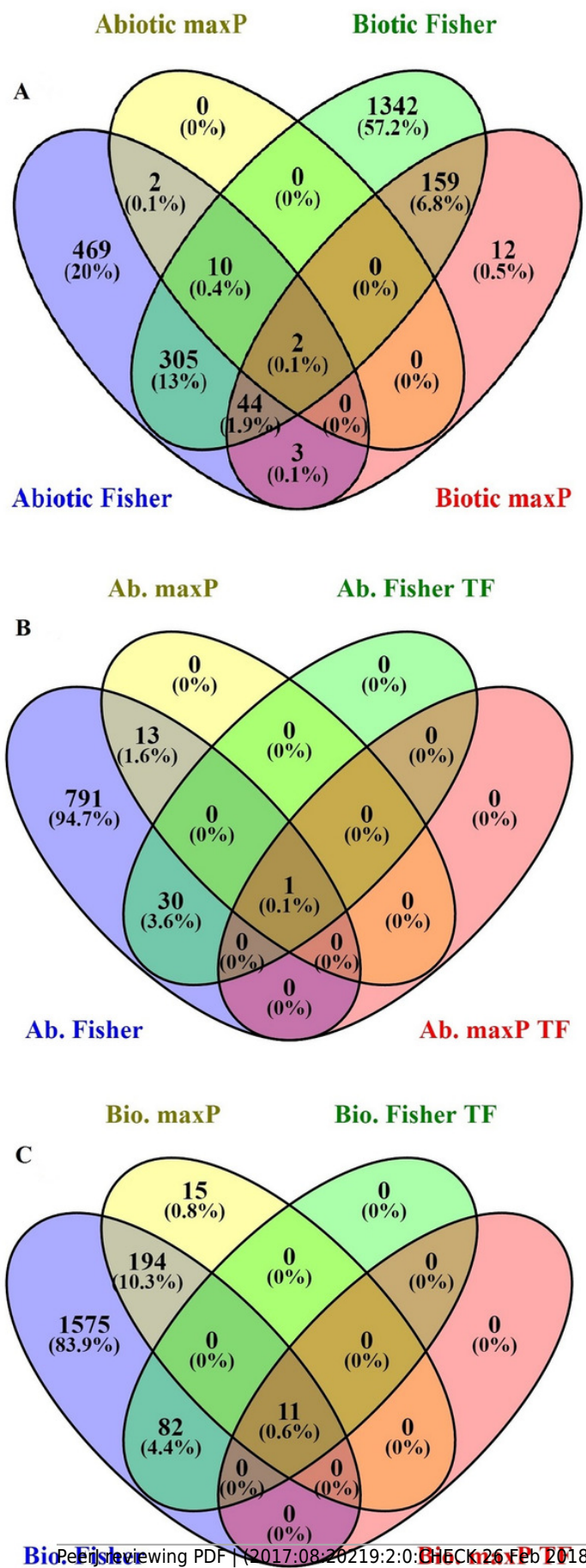
Figure 2

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.
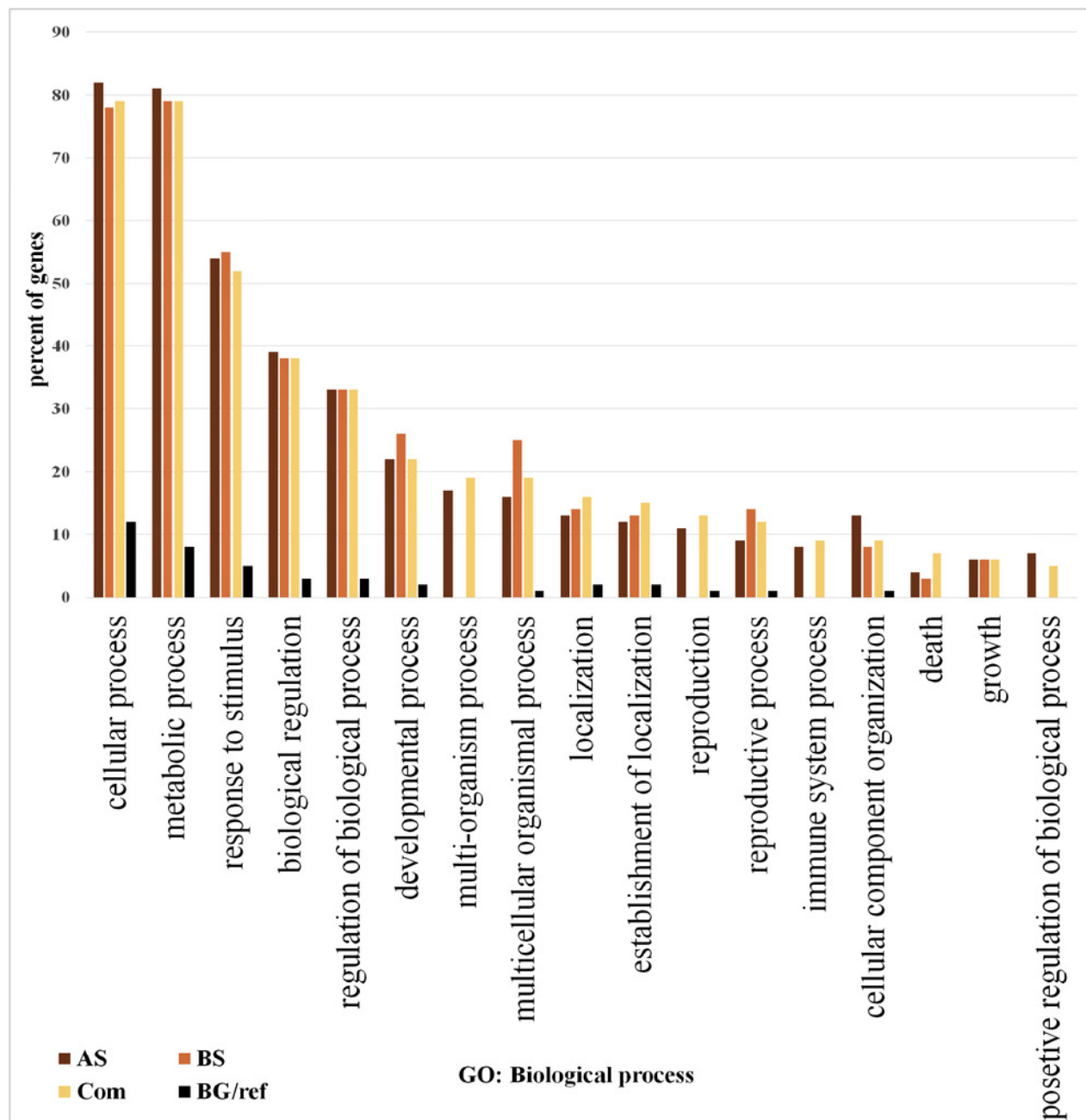
Figure 3

Network analysis of the DEGs identified in biotic and abiotic stresses

Network analysis of the 361 common DEGs identified in biotic and abiotic stresses. Network was generated by STRING (version 10.5) 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: co-occurrence of those genes in the same organisms (dark blue), co-expression (black), experimental protein–protein interaction data (pink) and literature text-mining (yellow). A number above hub represent TF. 1) JERF1, 2) MYB48, 3) protein LHY, 4) EIL3, 5) EIL2, 6) WRKY26.
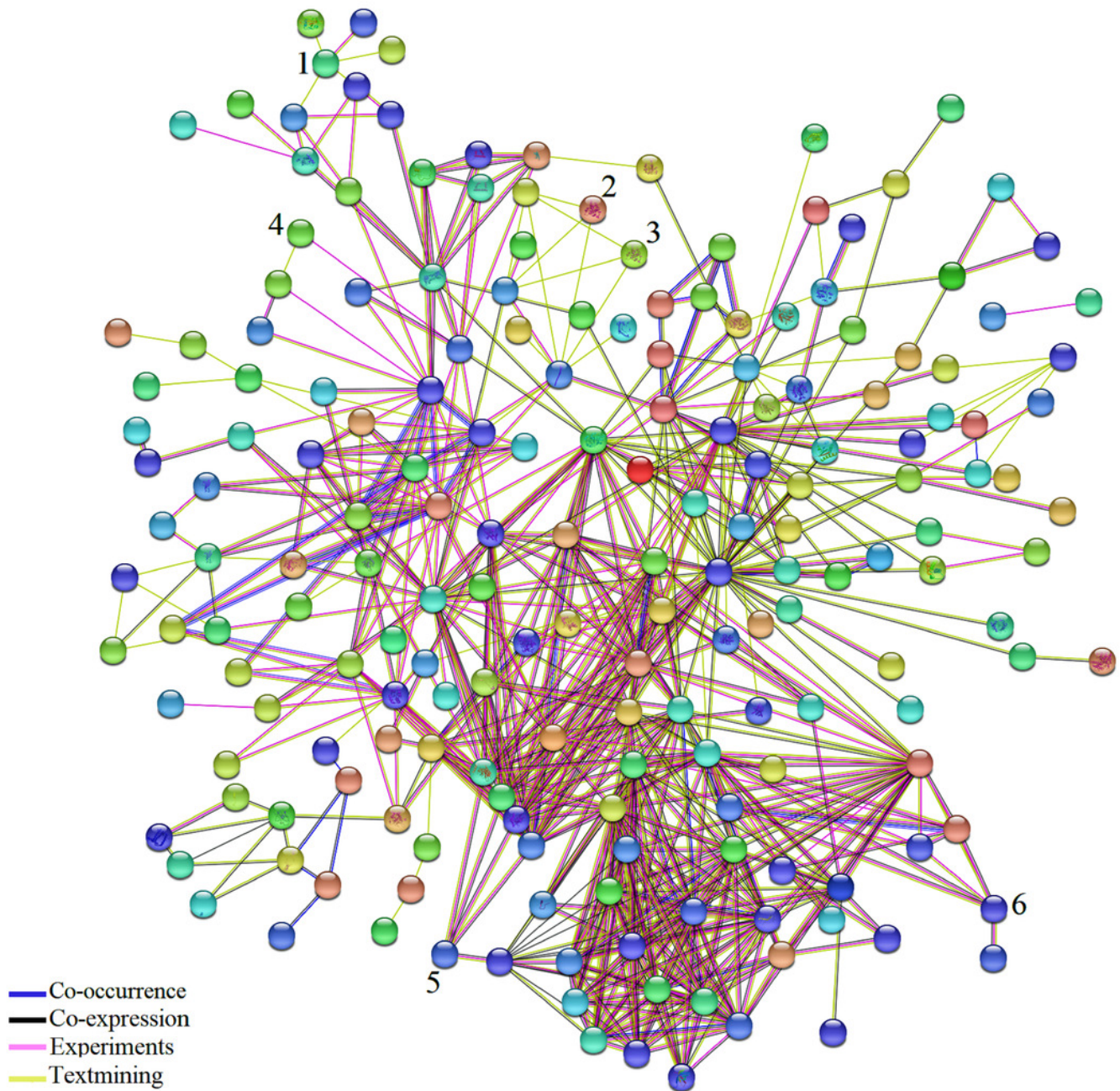
Figure 4

Common transcription factors in biotic and abiotic stresses

Two-way Venn diagram showing the common transcription factors between abiotic and biotic stresses.
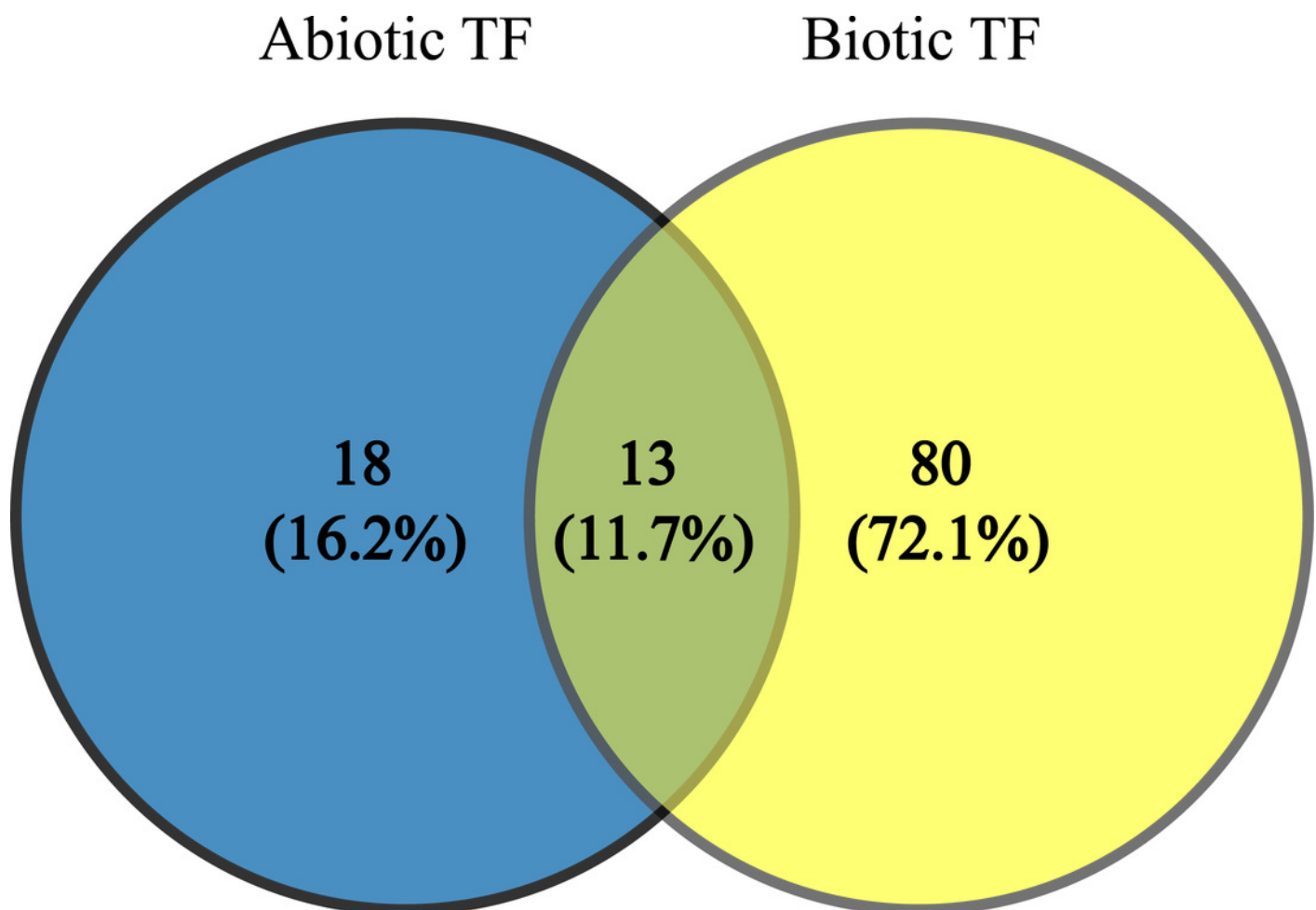
Figure 5

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).