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IN SILICO IDENTIFICATION AND CHARACTERIZATION OF CUMULATIVE ABIOTIC STRESS RESPONDING GENES IN TOMATO (*LYCOPERSICUM ESCULENTUM* L.)

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Abstract

Tomato (*Lycopersicum esculentum.* L) is popular worldwide due to its nutritive value and known as protective food. Economically it is very important vegetable of world. Tomatoes are challenged by number of abiotic and biotic factors reducing their ideal growth, development and production. Expression profiling is very significant tool for the study of organism responds due to abiotic changes. The identification of cumulative abiotic stresses genes, responding to three or four abiotic stresses will be a good source of genes for the production of the abiotic stress resistant food crops. In tomato numerous studies have been done to find out the expression of genes under different abiotic stress conditions, using DNA microarray technology. In this research microarray data for four abiotic stresses salt, heat, cold and drought were analyzed using bioinformatics tools to identify cumulative abiotic stress responding genes. Total 248 cumulative abiotic stress responding genes were identified after the study of 64896 ESTs. The identified genes were also characterized on the basis of their function and validated using *Arabidopsis thaliana* as reference organism.

Key words: *L. esculentum*, Microarray, Abiotic stresses, Cumulative genes, GEO.

Introduction

Tomato as a member of family Solanaceae was originated in South America (Ali *et al*., 2013). It is world's second most consumed vegetable after potato (Bhattarai *et al*., 2021) with about seven-thousand five hundred varieties (Zahedi & Ansari, 2012). China is the top tomato producer, with 31% of the total world production, accompanied by India (11%) and the United States (9%) (Heuvelink *et al*., 2020). Tomato as compare to other crops is short period and gives high yield, so very attractive economically (Naika *et al*., 2005). It is an important vegetables both for processing industry or fresh market (Lucier & Glaser, 2010) and highly consumed throughout the world as a fundamental ingredient in a large variety of processed, cooked and raw foods (Babalola *et al*., 2010). Due to its multiple uses tomato demand is continuously growing in the world market (Anon., 2017).

Abiotic stresses are among the main causes of crop loss globally (Martinez *et al*., 2018). Abiotic stresses like salinity, extreme temperatures and drought badly affects plant growth and development (Seki *et al*., 2007). Naturally, multiple abiotic stresses occur at the same time, and plants have distinctive responses to fight against these combination of stresses (Maria *et al*., 2021). In tomato, abiotic stresses negatively affect its quality, productivity and production. Tomato life cycle is highly vulnerable to abiotic stresses like high salinity, drought and extreme temperature (Krishna *et al*., 2019).

Plants have highly efficient and sophisticated strategies to survive with these environmental stresses (Fu & Dong, 2013). For the adjustment of their physiology to cope with abiotic stresses they depends upon the regulation of gene expression (Floris *et al*., 2009). The identification of novel genes expressed as a result of abiotic stresses and their functional genomics during stress adaptation provides basis of effectual strategies in order to ameliorate stress tolerance (Gull *et al*., 2019). Plants have developed mechanisms to prevail over abiotic and biotic stresses through up- or down-regulation of several genes and their products, which plays important role in various defense mechanisms (Hu *et al*., 2006).

DNA microarray technology as a powerful tool was used in numerous organisms for understanding genome-wide gene expression (Auesukaree, 2006). *In silico* identification and characterization of the abiotic stresses responding genes in several organisms has become an important research due to huge data available in the databases (Aceituno *et al*., 2008).

In this study, we identified 248 cumulative abiotic stress responding genes, which showed response in three or more than three abiotic stresses among salinity, heat, drought, and cold through microarray data analysis available at Gene Expression Omnibus, National Centre for Biotechnology Information **(**Barrett *et al*., 2005) using various bioinformatics tools. Out of 248 identified genes, 34% genes are up-regulated and 66% are down-regulated. These identified cumulative stress responding genes will be the good potential candidate genes for engineering and developing the abiotic stress resistant food crops in future.

Material and Methods

Microarray data mining: In this research, microarray data is comparatively analyzed using different bioinformatics tools. For the current study, it is compulsory to use same microarray platform which should comprise number of series and samples. Hence microarray data mining is the first step to fulfill this criteria.

Tomato is among the plants whose considerable microarray data is available at Gene expression database GEO NCBI (www.ncbi.nlm.nih.gov/geo/). Overall 73 platforms, 314 series and 5175 samples were obtained for tomato microarray data. The available microarray data for

tomato were mined for four abiotic stresses salt, cold, heat and drought and ultimately two platforms were chosen with 8 eight series and 40 samples. These two platforms are GPL1901 and GPL 1902, which were used for downstream studies. The platform GPL1902 is the prolongation of platform GPL1901.The two platforms has total 64896 probes. All the data present in these platforms, series and samples were downloaded and saved.

Experimental design: The creation of experimental design is an essential step for the analysis of microarray data (Joshua *et al*., 2011). It is a very crucial step to generate a concise experimental design, so for the present study brief, logical and comprehensive experimental design was created in order to found best set of abiotic stress responding genes. The available data was assembled into five stress stages according to stress periods. These stress stages were named Initial-1, Initial-2, Middle-1, Middle-2 and Final. In the first stage the Initial-1 the stress periods were in the order of 6 hour, 4 hour, 6 hour and 1 day for salt, cold, heat and drought stresses respectively, while for the remaining stages stress periods were ranged from 12-96 hour and 3-10 days for the four abiotic stresses (Fig. 1).

Tab-delimited data alignment: For tab delimited data alignment, excel sheet was generated for the platform Spot identifiers (IDs). The normalized log2 intensities under all four stresses were entered and aligned. Separate excel sheets were generated for all stress stages. The aligned platform IDs and their log2 gene expression ratios were saved as tab-delimited files (Mochida *et al*., 2009; Barozai & Wahid, 2012).

Data analysis: The tab-delimited data was analyzed through graphic visualization using Multiexperimental viewer tool, available to public at (www.tm4.org/mev) (Quayum *et al*., 2008). MultiExperiment Viewer (MeV) is a freely available software application integrating sophisticated algorithms for visualization, statistical analysis, clustering and classification. It is multipurpose microarray data analysis tool for the analysis of DNA microarray expression data, protein–protein interaction data, array comparative and genomic hybridization experiments (Howe *et al*., 2010), which allows the user to identify the differentially expressed genes. Briefly, MeV was run in Java script and constructed aligned tab-delimited data was loaded in Multiple Array Viewer windows.

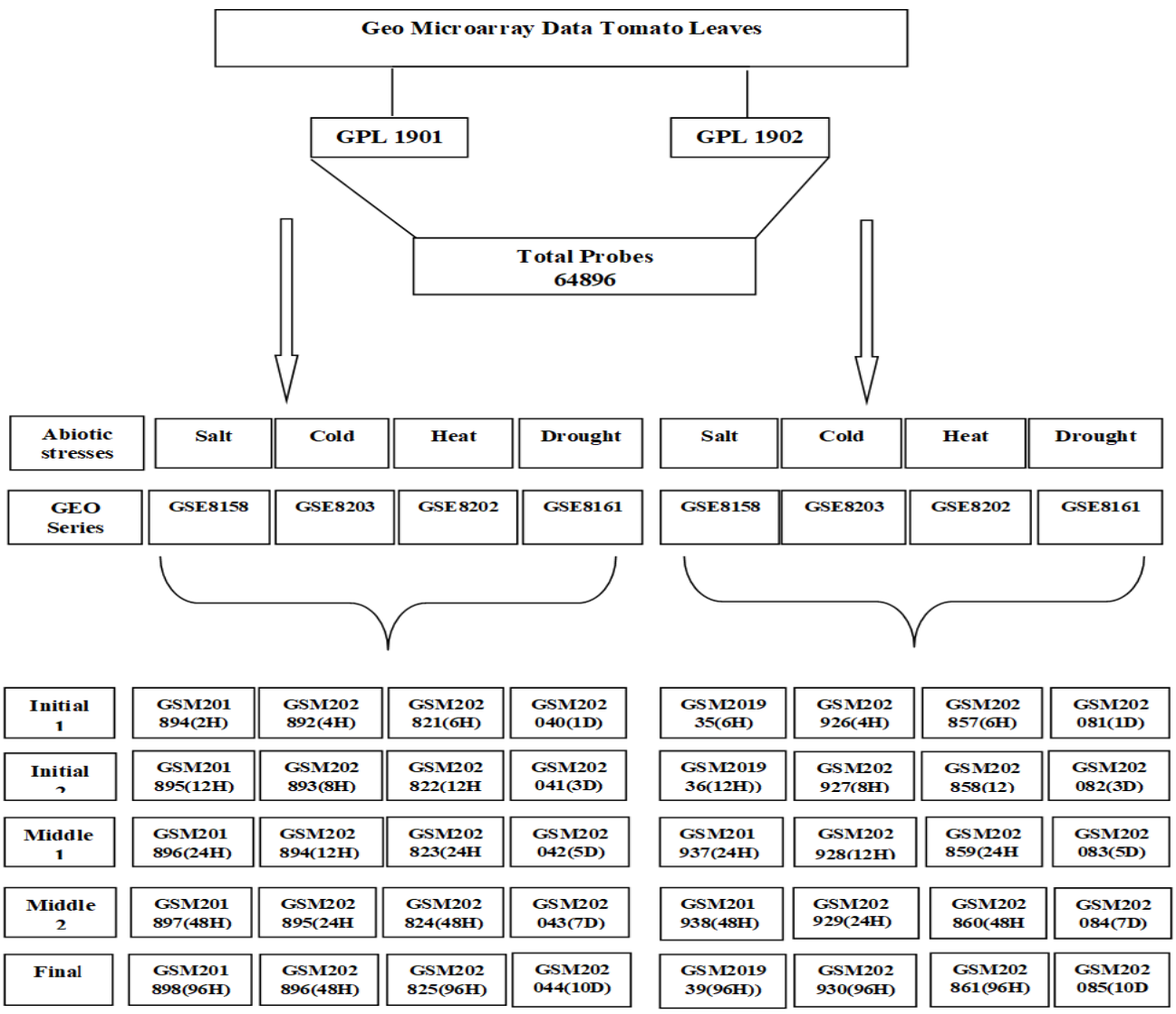

Fig. 1. Experimental Design for the identification of genes in tomato. All the stress stages were based on stressed periods. Analysis was performed to find the correlation of cumulative stress responding genes and stressed periods.

Table 1. Cumulative abiotic stress up-regulated genes in tomato.

Platform ID	Gene Bank Acc. No	Putative function
131267	BQ505347	GAST 1 Protein precursor
131531	BQ514118	Fasciclin-like arabinogalactan-protein
137182	BQ121879	Auxin-binding protein
137858	BQ121940	Auxin-binding protein
150491	BQ116319	Alpha-expansin precursor
153340	BQ120474	Brassinosteroid-regulated protein
154541	BQ514933	S-adenosyl-methionine-methyltransferase
211178	BQ505500	Null
210085	BQ120158	Brassinosteroid-regulated protein
131267	BQ505347	GAST1 Protein precursor
135213	BQ111690	Unknown protein
133865	BQ119086	Glucan endo-1 3-beta Glucosidase
136609	BQ119464	RING finger-like protein
150491	BQ116319	Alpha-expansin precursor
161777	BQ120158	Brassino-regulated protein
132450	BQ121938	Acyl carrier protein
137182	BQ121879	Auxin-binding protein
137858	BQ121940	Auxin-binding protein
151174	BQ513588	Putative kinesin light chain gene
153389	BQ115198	50S ribosomal protein, chloroplastic
157769	BQ112998	Unknown protein
161586	BQ512855	Unknown protein
179839	BQ514118	Fasciclin-like arabinogalactan-protein
183297	BQ514547	Unknown protein
185490	BQ121879	Auxin-binding protein
186166	BQ121940	Auxin-binding protein
191710	BQ517120	Intron maturase plasma membrane protein
198799	BQ116319	Alpha-expansin precursor
202360	BQ113763	Delta 9 desaturase-like protein
202104	BQ121741	Plasma membrane protein
204388	BQ113334	Germin like protein precursor
211169	BQ515186	Unknown protein
131354	BQ113375	NAD(P)-binding domain-containing protein
132914	BQ115010	Putative 16kDa membrane protein
132450	BQ121938	Acyl carrier protein
131531	BQ514118	Fasciclin-like arabinogalactan-protein
135409	BQ112396	Ferredoxin-nitrite reductase
134421	BQ111668	mRNA-binding protein precursor
136224	BQ514450	BTB and TAZ domain protein 1
134145	BQ514547	Unknown protein
141883	BQ113260	Peptidyl-prolyl cis-trans isomerase
140781	BQ504679	5-phosphoribosyl-1-pyrophosphate
144149	BQ120711	Ferredoxin-nitrite reductase
143748	BQ508891	Unknown protein
150491	BQ116319	Alpha-expansin precursor
152963	BQ119236	Hypothetical protein
157874	BQ115776	En/Spm-like transposon protein
157987	BQ516853	Unknown protein
131531	BQ514118	Fasciclin-like arabino- protein
134199	BQ519261	Zinc finger family protein
145734	BQ118336	LEXYL1 protein
145534	BQ517278	Epa4p
148106	BQ513478	Serine/threonine protein kinase
150394	BQ512920	Unknown protein
155335	BQ516650	Triose-phosphate/phosphate translocator
161083	BQ519167	Unknown protein
181434	BQ505347	GAST 1 protein precursor.
180342	BQ121929	Genomic DNA chromosome 3
181503	BQ515390	Photosystem I reaction center subunit X
179848	BQ120934	Photosystem I chloroplast precursor
183073	BQ506563	Pectate lyase
186473	BQ112209	Photosystem I reaction center subunit X
187654	BQ516559	Chlorophyll A-B binding protein
190598	BQ120711	Ferredoxin-nitrite reductase
195365	BQ113039	Fructose-1 6-bisphosphatase
200101	BQ120811	Chlorophyll a/b-binding protein
202238	BQ513490	Unknown protein
204777	BQ112286	Histone H3
205820	BQ119107	Ribulose-phosphate 3-epimerase
207032	BQ508194	Protein TIC 62, chloroplastic
207455	BQ111843	Photosystem I chloroplast precursor

Identification of cumulative responding genes: Genes showed expression under three or more than three stresses with log2 signal intensities ≥/≤ 1.0-fold were identified as cumulative abiotic stressed responding genes and saved. The putative functions were assigned to these identified genes by using available information at platform and through BLASTx and BLASTn tools respectively (Altschul, 1990; Stephen *et al*., 1997).

Validation and characterization studies: As a reference organism *Arabidopsis thaliana* was used for validation and characterization studies. Orthologues for identified genes were investigated in *Arabidopsis thaliana* applying blast algorithm. The *Arabidopsis* orthologues were subjected to Genevestigator response viewer for validation, to determine their expression pattern by analyzing the Log2 ratios (Zimmermann *et al*., 2004). For characterization studies, the gene code names (Atg) of *Arabidopsis* orthologues, were entered to Gene Ontology (GO) functional categorization at TAIR web site (http://*Arabidopsis*.org/ tools/bulk/go/ index.jsp), (Berardini *et al*., 2004) based on biological processes, molecular functions and cellular components. The genes annotation list and charts were saved.

Results and Discussion

Cumulative abiotic stress responding genes: *In-silico* study by using bioinformatics tool is an appropriate approach for the identification of cumulative abiotic stress responding genes (Barozai & Husnain 2011; Barozai & Wahid, 2012). By using similar approach, the tomato microarray data mining, filtering and analysis have resulted 248 cumulative abiotic stress responding genes out of 64896 probes (Fig. 1 & Fig. 2a, 2b). Out of these 248 genes 29% genes are up-regulated (Table 1) and 71% are down-regulated (Table 2). These results suggest that tomato genes have changed their expression under abiotic stresses. Same results were reported for potato (Barozai & Wahid, 2012) and Arabidopsis (Seki *et al*., 2007).

The 29% up-regulated genes are significant set of genes with strong potential of resistance for abiotic stresses. Some of the main genes are Serine/threonine protein kinase, Brassinosteroid-regulated protein, photosynthetic proteins, ribosomal proteins and transcription factors. Most of these genes had showed up-regulation under drought, cold and salinity observing the expression profiles of *Arabidopsis* genes (Seki *et al*., 2002). Brassinosteroid tolerance was conferred by Kagale *et al*., (2007) in *Brassica napus* and *Arabidopsis thaliana* under abiotic stresses particularly cold and drought. Barozai *et al*., (2014) reported the up-regulation of ribosomal proteins in *Saccharomyces cerevisiae* as a result chemical stresses. According to many researchers (Fujita *et al*, 2009; Barozai & Husnain, 2011) the transcription factors as an important family of proteins are associated with abiotic stress tolerance. The current study also resulted into the up-regulation of the same family members such as zinc finger protein and ring finger-like protein against abiotic stresses. Barozai & Wahid, 2012 reported the up-regulation of these family members in potato under various abiotic stresses. The role of chlorophyll a/b-binding proteins is very significant in photosynthesis.

Dittami *et al*., (2010) suggested that chlorophyll a/b-binding proteins have significant role in plant stress tolerance. In this research many members of chlorophyll a/b binding protein as up-regulated genes are identified.

Among 71% down-regulated genes the significant genes include metabolic-related proteins, binding proteins, protein inhibitors, transcription factors, cell kinases, hypothetical proteins, chloroplast proteins and DNA repair proteins genes. Barozai & Wahid, (2012) also reported most of the above protein classes as down-regulated genes in potato under similar stress conditions, while Seki *et al*., (2002), reported similar genes as down regulated genes in *Arabidopsis* under three stresses (cold, salt and drought).

Comparative study of responding genes: The comparative study of identified up and down-regulated genes under different stress stages have shown defensive to offensive strategic approach as shown in (Fig. 3). As compared to the up-regulated genes, more down-regulated genes are observed in Initial-1, Initial-2 and Middle-1 stress stages. It suggests the tomato plants adopted defensive approach to switch off their gene expression from Initial to Middle stress stages. As the stresses protracted to Final stage, the tomato plants turned to offensive response. These results are similar as the earlier studies of Urano *et al*., 2010; Barozai & Wahid, 2012.

The distribution of up-regulated genes under five stressed stages showed that most of the genes (52%) have responded to heat- cold-drought; followed by heat-salt-drought (30%), salt-heat-cold (8%), salt-cold-drought (6%) and heat-cold-salt-drought (4%), while the down- regulated genes dispersion under five stressed stages is observed with nearly equal genes (21-24%) responding to salt-cold-drought, heat-cold-drought, heat-cold-salt respectively; followed by heat-cold-salt-drought (17%) and heat-salt-drought (15%). Same results were given by many researchers (William *et al*., 2006; Barozai & Wahid, 2012).

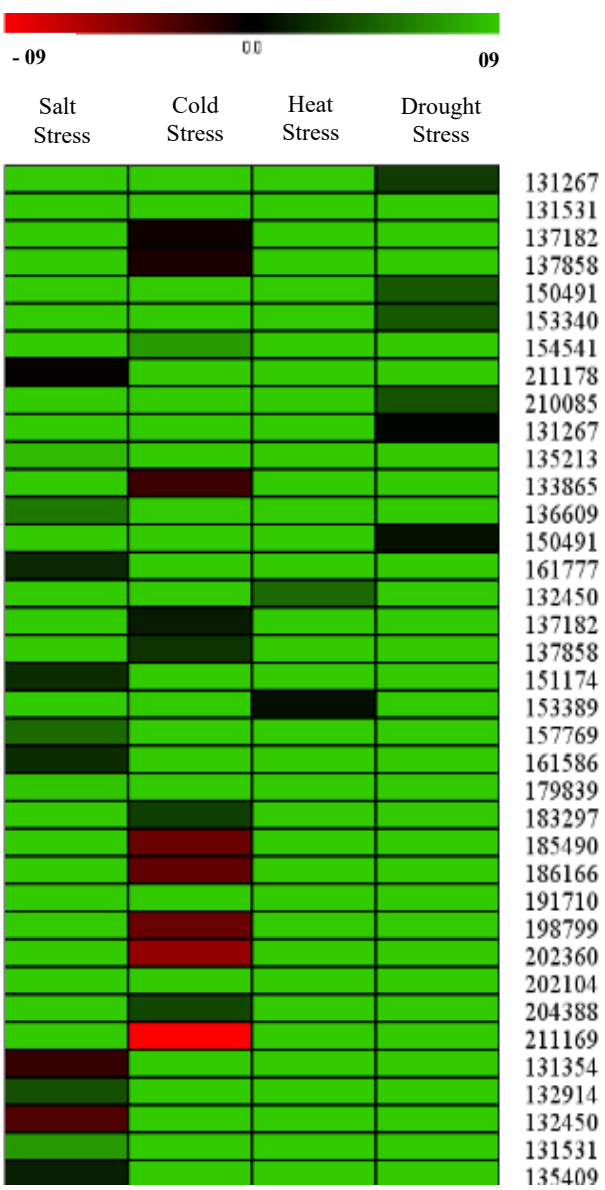

Fig. 2a. MEV analyses for the up-regulated genes showing gene expression under three or four abiotic stresses among salt, cold, heat and drought stresses respectively.

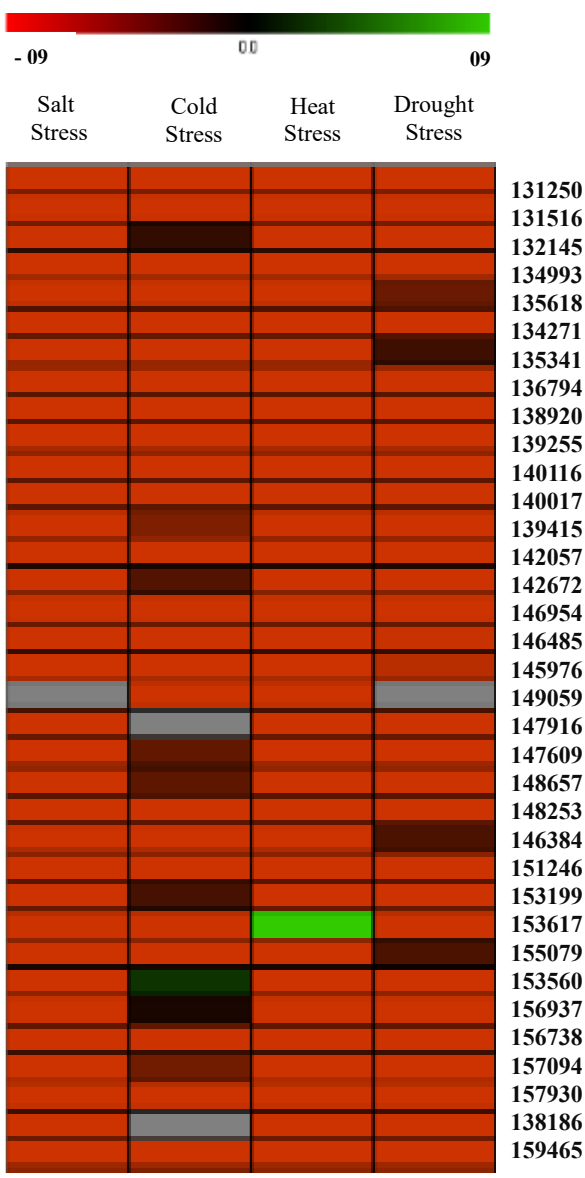

Fig. 2b. MEV analyses for the down-regulated genes showing gene expression under three or four abiotic stresses among salt, cold, heat and drought stresses respectively.

Table 2. Cumulative abiotic stress down-regulated genes in tomato.

Platform ID	Gene Bank Acc. No	Putative function
131250	BQ113481	Oxoglutarate-dept dioxygenase
131516	BQ508767	Proteinase inhibitor
132145	BQ111514	Gamma-aminobutyrate transaminase
134993	BQ112385	Putative cytochrome P450
135618	BQ115143	Ent-kaurenoic acid oxidase
134271	BQ506891	Putative cytochrome P450
135341	BQ515885	Proteinase inhibito
136794	BQ508406	Unknown protein
138920	BQ115169	Acyl CoA reductase-like protein
139258	BQ113584	Putative glucosyltransferase
140116	BQ113907	Putative cytochrome P450
140017	BQ506705	Proton-dependent oligopeptide transport
139415	BQ115763	Zeatin O-glucosyltransferase
142057	BQ504961	Putative cytochrome P450
142672	BQ512999	Hypothetical protein
146954	BQ115734	Putative glucosyltransferase
146485	BQ112919	Proteinase inhibitor
145978	BQ113576	Limb deformity protein
149059	BQ112433	Ovule/fiber cell elongation protein
147916	BQ113197	Sterol 4-alpha-methyl-oxidase
147609	BQ505688	Putative cytochrome P450
148657	BQ115877	Putative cytochrome P450
148253	BQ112080	Ovule/fiber cell elongation protein
148384	BQ113773	Arginine decarboxylase
151246	BQ118369	Delta-7-sterol-C5(6)-desaturase
153199	BQ506747	Cellulose synthase catalytic protein
153617	BQ511530	Protein phosphatase 2C
155079	BQ116958	Hydroxymethylglutaryl coenzyme A synthase
153560	BQ117795	Zeatin O-glucosyltransferase
156937	BQ112361	Gamma-aminobutyrate transaminase
156758	BQ118409	Putative cytochrome P450
157094	BQ115061	Zeatin O-glucosyltransferase
157930	BQ506105	Oxoglutarate-dependent dioxygenase
158186	BQ113446	Glucosyltransferase
159465	BQ507066	Oxoglutarate-dependent dioxygenase
160819	BQ512442	Xyloglucan endo-1 4-beta-D
160759	BQ113053	Oxoglutarate-dependent dioxygenase
161227	BQ111471	Hypothetical protein
163312	BQ505800	Cell elongation protein
160943	BQ115730	Unknown protein
162020	BQ113219	Oxoglutarate-dependent dioxygenase
162637	BQ507218	Putative cytochrome P450
181637	BQ112787	Null
183782	BQ114166	Major ampullate gland dragline silk protein-2
187387	BQ120907	Protein induced upon tuberization
194286	BQ113576	Putative C-4 sterol methyl oxidase
194066	BQ114139	Null
194378	BQ114283	Probable Transcription regulator
198211	BQ112433	Ovule cell elongation protein
197211	BQ111869	Putative GDSL-motif hydrolase
199255	BQ119359	ATP synthase protein I-related protein
202133	BQ511606	Hypothetical protein
201125	BQ114466	Probable permease
205779	BQ116424	Null
209484	BQ113219	Oxoglutarate-dependent dioxygenase
131250	BQ113481	2-oxoglutarate-dependent dioxygenase
131516	BQ508767	Proteinase inhibitor
131933	BQ511035	Protein phosphatase
134090	BQ508578	Unknown protein

Table 2. (Cont'd.).

Platform ID	Gene Bank Acc. No	Putative function
135341	BQ515885	Proteinase inhibitor
137181	BQ119556	BZIP protein
136794	BQ508406	Unknown protein
138920	BQ115169	Acyl CoA reductase-like protein
139258	BQ113584	Putative glucosyltransferase
139310	BQ113590	Proteinase inhibitor
142897	BQ112142	2 oxoglutarate-dependent dioxygenase
144420	BQ118500	Alanine aminotransferase
142057	BQ504961	Putative cytochrome P450
143516	BQ512999	Hypothetical protein
146954	BQ115734	Putative glucosyltransferase
146485	BQ112919	Proteinase inhibitor
145134	BQ113576	Limb deformity protein
149059	BQ112433	Ovule cell elongation protein
148253	BQ112080	Fiber cell elongation protein
153617	BQ511530	Protein phosphatase 2C
154948	BQ113746	Predicted Protein of unknown function
157930	BQ506105	2-oxoglutarate-dependent dioxygenase
155665	BQ115484	Flavanone 3 beta-hydroxylase
158077	BQ515792	Unknown protein
156622	BQ517167	Unknown protein
162155	BQ506900	Caffeoyl-CoA O-F
163312	BQ505800	Cell elongation protein
162020	BQ113219	2-oxoglutarate-dependent dioxygenase
161718	BQ516525	Maturase K
185022	BQ121281	Ci21A protein
189339	BQ507624	Non-specific lipid transfer protein
193403	BQ115734	Putative glucosyltransferase
194286	BQ113576	C-4 sterol methyl oxidase
194170	BQ114182	Hypothetical protein
197367	BQ112433	Ovule cell elongation protein
197405	BQ112080	Ovule cell elongation protein
201507	BQ506747	Gamma tubulin Cellulose synthase protein
205779	BQ116424	Null
207512	BQ121743	Neoxanthin cleavage enzyme-like protein
131250	BQ113481	2-oxoglutarate-dependent dioxygenase
135907	BQ120597	Bactinecin 11
142897	BQ112142	Oxoglutarate-dependent dioxygenase
143264	BQ118527	Unknown protein
145087	BQ506895	Transformer-SR ribonucleoprotein
147544	BQ507514	Chloroplast heat shock protein
151246	BQ118369	Delta-7-sterol-C5(6)-desaturase
152792	BQ116964	Unknown protein
153617	BQ511530	Protein phosphatase 2C
157930	BQ506105	2-oxoglutarate-dependent dioxygenase
157233	BQ515792	Unknown protein
161885	BQ511710	Homeotic protein
162224	BQ513259	Xyloglucan endo-transglycosylase
179558	BQ113481	2-oxoglutarate-dependent dioxygenase
180649	BQ112195	Ribulose bisphosphate carboxylase/activase
180654	BQ121900	Zinc finger protein
183935	BQ516200	Beta ketoacylACP synthase II
182239	BQ119335	Unknown protein
187618	BQ113590	Proteinase inhibitor
187663	BQ510121	Unknown protein
188272	BQ121780	Putative LIM domain containing protein
189117	BQ506639	Type-A response regulator
191205	BQ112142	2-oxoglutarate-dependent dioxygenase
191868	BQ113714	Galactokinase like protein

Table 2. (Cont'd.).

Platform ID	Gene Bank Acc. No	Putative function
190682	BQ516342	LOC402856 protein
192532	BQ114556	bZIP transcription factor BZI-2
194239	BQ506895	Putative transformer-SR ribonucleoprotein
194066	BQ114139	Null
194274	BQ114237	S-receptor kinase M4I22.110 precursor
194378	BQ114283	Probable transcription regulator
197136	BQ118484	Nam-like protein
196547	BE341199	Potato ubiquitin
197367	BQ112433	Ovule cell elongation protein
197405	BQ112080	Ovule cell elongation protein
199554	BQ118369	Delta-7-sterol-C5(6)-desaturase
198447	BQ115033	Putative transformer-SR ribonucleoprotein
200546	BQ510195	Unknown protein
201507	BQ506747	Gamma tubulin Cellulose synthase protein
203696	BQ518761	Caffeoyl-CoA O-methyltransferase
202723	BQ111910	Paired box protein
205779	BQ116424	Null
205066	BQ118409	Putative cytochrome P450
204379	BQ506105	2-oxoglutarate-dependent dioxygenase
206961	BQ111497	Unknown protein
207208	BQ113053	2-oxoglutarate-dependent dioxygenase
211422	BQ118449	Unknown protein
209263	BQ115036	Unknown Protein
209484	BQ113219	2-oxoglutarate-dependent dioxygenase
210110	BQ117701	Null
147544	BQ507514	Chloroplast heat shock protein
147569	BQ505006	Small heat shock protein
150469	BQ505871	dnaK-type molecular chaperone hsc70-3
137181	BQ119556	Unknown protein
145087	BQ506895	Putative transformer-SR ribonucleoprotein
149605	BQ112770	Cysteine protease precursor
149926	BQ514700	S-adenosylmethionine decarboxylase
147569	BQ505006	Small heat shock protein
151998	BQ115033	Putative transformer-SR ribonucleoprotein
152792	BQ116964	Unknown pro.
181614	BQ118714	Unknown pro.
182466	BQ516461	Polyphenol oxidase chloroplast precursor
183068	BQ113887	Protein kinase homolog
186169	BQ511515	Heat shock pro.
185373	BQ505576	Unknown pro.
190478	BQ113380	Unknown protein
192394	BQ509142	DNA-binding protein
191052	BQ117791	Sucrose synthase 2
192532	BQ114556	bZIP transcription factor BZI-2
194066	BQ114139	Null
198569	BQ505787	Unknown protein
198777	BQ505871	dnaK-type molecular chaperone hsc70-3
203026	BQ121873	Ras-related protein
202724	BQ114475	Adenosylhomocysteinase
203833	BQ505147	Alkylated DNA repair protein
204336	BQ113351	Unknown
204062	BQ112894	Cytochrome b
205922	BQ115105	Hypothetical protein
206728	BQ113088	Putative transcription factor
207665	BQ116001	Unknown protein
207182	BQ113355	2-on-2 hemoglobin
209229	BQ505772	Unknown protein
209640	BQ113316	Putative E2 ubiquitin-conjugating enzyme
210058	BQ117718	Alanine aminotransferase

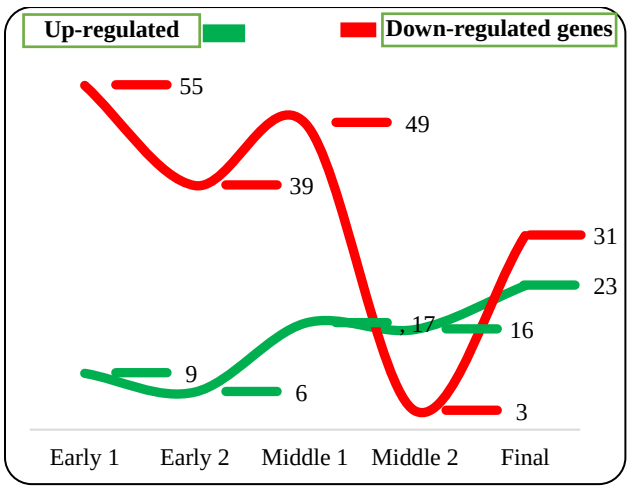

Fig. 3. The cumulative abiotic stress responding genes in tomato showed defensive to offensive strategic approach.

Validation studies: Validation studies of identified genes is performed through model plant *Arabidopsis thaliana* as reference organism (Barozai *et al*., 2008; Barozai & Wahid, 2012). Out of identified genes 64% genes were validated through *Arabidopsis thaliana* gene ontologies. Among validated genes 66% are down-regulated and 34% are up-regulated genes (Fig. 4). Similar outcomes were reported in various other plant species (Mantri *et al*., 2007; Urano *et al*., 2010).

Characterization studies: The characterization studies were performed through homology search. Nucleotide, protein non-redundant and EST databases were used against BLAST algorithms. All the identified genes showed homology in all these databases suggesting the well-known persona.

The *Arabidopsis* orthologs of identified genes were further classified on the basis of biological processes, molecular functions and cellular components through Gene-Ontology (GO) annotation.

The Gene Ontology biological process annotation revealed that most of cumulative abiotic stress responding genes were engaged in cellular processes followed by metabolisms processes, stress response stimulus, protein metabolism, biogenesis, developmental processes, transport, signal transduction, energy processes, other biological processes and DNA or RNA metabolism (Fig. 5a).

The Gene Ontology categorization for molecular function disclosed that the most of the identified genes were involved in DNA, RNA and protein binding followed by transferase activity, hydrolase activity, transporter activity, nucleotide binding, kinase activity and unknown molecular functions (Fig. 5b).

The Gene Ontology annotations for cellular components revealed that majority of abiotic stress resulted genes were related to intracellular components, cytoplasmic components followed by other membranes, chloroplast, nucleus, plastid, extracellular, plasma membrane, cytosol, endoplasmic reticulum, cell wall, golgi apparatus, mitochondria and ribosome (Fig. 5c). Barozai & Wahid, (2012) almost found similar findings in potato.

Fig. 4. In validation studies the tomato genes MEV analysis (left) showed same gene expression patterns as reported for *Arabidopsis* (right) validating our results.

Conclusion

The present study indicates that *In-silico* analysis is an appropriate strategy to find out the cumulative abiotic stress responding genes. This kind of research will provide a precious resource of information about gene responding program under abiotic stresses. The study resulted 248 cumulative abiotic responding genes against at least three stresses among salt, cold, heat and drought, which are strong potential resource for the production of the abiotic stress resistant crops in future.

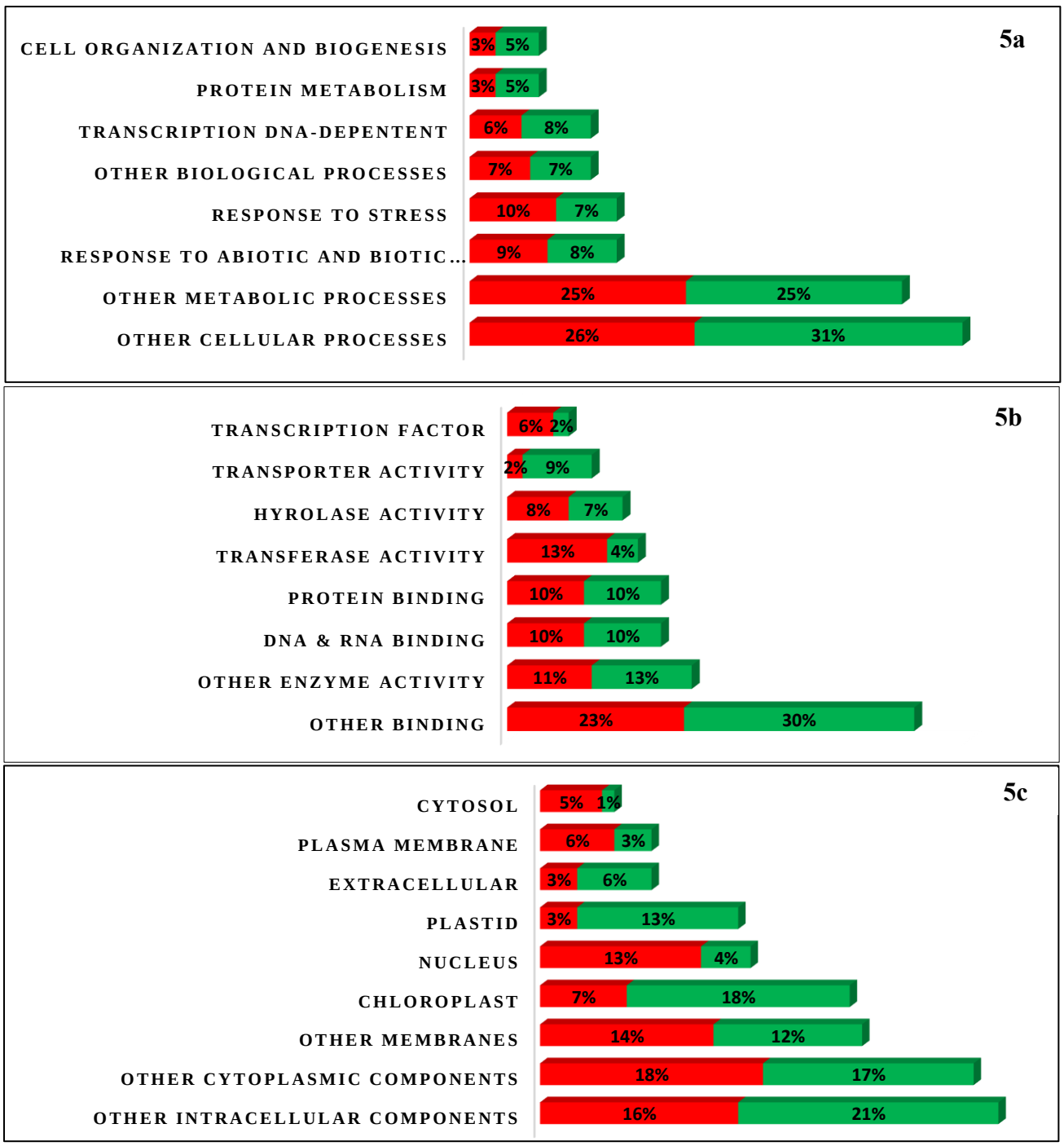

Fig. 5. In Gene Ontology (GO) characterization studies the bar charts showed the distribution of down-regulated (red) and up-regulated genes (green) among three principal GO categories; Biological processes (5a), Molecular functions (5b) and Cellular components (5c).

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