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IN SILICO IDENTIFICATION AND CHARACTERIZATION OF CUMULATIVE ABIOTIC STRESS RESPONDING GENES IN POTATO (*SOLANUM TUBEROSUM* L.)

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Abstract

Potato (*Solanum tuberosum*) is the world's fourth largest food crop, following rice, wheat, and maize. In potato abiotic stresses commonly reduce both yield and quality. The understanding of plant responses to abiotic stresses at transcriptome level provides a foundation for the identification of stress responding genes. The cumulative abiotic stresses genes, responding to three or more than three abiotic stresses, will be a good source of candidate genes for engineering and developing the abiotic stress resistant food crops. In potato various studies have been made for the analysis of gene expression under different abiotic stress conditions, using DNA microarray technology. These available microarray data under abiotic stresses like; salt, cold, heat and drought were analyzed by applying the bioinformatic tools. Total 217 cumulative abiotic stresses responding genes were identified and characterized at different stressed stages after the study of 64896 ESTs. The abiotic stresses responsive genes were characterized on the basis of their function and validated using *Arabidopsis thaliana* as reference species.

Introduction

Potato (*Solanum tuberosum*) is a key member of the family Solanaceae. It is the world's fourth largest food crop, following rice, wheat and maize (Evers *et al.,* 2007). The potato spread from its centre of origin in the high Andes of South America to other parts of the globe (Messer, 2000). Pakistan is the seventh largest potato producing country in the world (Humera & Iqbal, 2010). There are about five-thousand potato varieties worldwide and the major species grown is *Solanum tuberosum*. Potato is one of the most important crops in terms of its use in human food and the starch industry (Fabeiro *et al.,* 2001; Abbas *et al*., 2011). It is a key player of the global sustainable food system, producing more food energy on less land with low cost of cultivation. It is the most important tuber crop in terms of production, accounting for about 45% of the total world production of all tuber crops (Shewry, 2003).

Abiotic stresses like drought, salinity, low and high temperature have adverse effects on plant growth and productivity (Shamim *et al.,* 2009; Urano *et al.,* 2010). In potato abiotic stresses commonly reduce both yield and quality (Waterer *et al.,* 2010). Potato due to its bare and shallow root system is very susceptible to abiotic stresses. All such stresses considerably reduced tuber yield (Jefferies & Mackerron, 2008).

To cope with these highly variable environmental stresses plants have remarkable abilities (Kreps *et al.,* 2002). The survival of plants depends on the rapid regulation of gene expression in order to adapt their physiology to abiotic stresses (Floris *et al.,* 2009**)**. Furthermore, biotic and abiotic stresses can induce the expression of both distinct and overlapping sets of genes (Cheong *et al.,* 2002). The identification and analysis of genes exhibiting large expression responses to abiotic stresses provides functional basis of multiple stress tolerance in plant species (William, 2006). Plants have developed processes to overcome biotic and abiotic stresses by up- or down-regulating a number of genes or proteins, which are believed to have a role in different defense mechanisms (Cushman & Bohnert, 2000; Hu *et al.,* 2006).

DNA microarray a recent high-throughput gene expression technology have been extensively used in gene expression analysis in plants (Ma *et al.,* 2005; Oono *et al.,* 2006; Mantri *et al.,* 2007; Fernandez *et al.,* 2008). In potato various studies have been made for the analysis of gene expression under different abiotic stress conditions by using DNA microarray technology (Rensink *et al.,* 2005).

In silico identification and characterization of the genes in various organisms under different conditions got importance due to growing data in the data bases (Aceituno *et al.,* 2008). In this research, total 217 cumulative abiotic stress responding genes, responding to at least three abiotic stresses among heat, drought, cold and salinity, in potato were identified by analyzing the microarray data from Gene Expression databases at National Centre for Biotechnology Information (GEO-NCBI**) (**Barrett *et al*., 2005) using the bioinformatic tools. Out of these 217 cumulative abiotic stress responding genes, 38% genes are up-regulated and 62% are down-regulated. Their functional characterization categorized them in growth, transcription, biotic & abiotic stresses and miscellaneous. These are the excellent potential candidate genes for engineering and developing the abiotic stress resistant food crops.

Materials and Methods

Microarray data mining: The current study is based on the comparative analysis of the microarray data through various bioinformatics' tools. For such studies, it is mandatory to use uniform microarray platform for which a number of series and samples should be available. So, the first step is the microarray data mining to meet the criteria.

Potato is fortunately one of the top plants whose extensive microarray data is publically available at GEO-NCBI (www.ncbi.nlm.nih.gov/geo/). Total 15 platforms, 103 series and 2628 samples were found for potato microarray data. All these potato microarray data were mined for abiotic stresses (salinity, cold, heat and drought) and finally two platforms having eight series and 39 samples were selected for the downstream studies and

analysis. The two platforms are GPL 1901 and GPL 1902, were used for studies. The platform GLP 1902 is the continuation of platform GPL1901. Both platforms have total 64896 printed probes. All the relevant data belonging to these platforms, series and samples were downloaded and saved.

Experimental design: The experimental design is a very crucial step for the microarray data analysis (Joshua *et al.*,2011). So for the current study a comprehensive, concise and logical experimental design was developed, to achieve the best set of abiotic stress responding genes, as shown in Fig. 1. Briefly all the data was clustered into five stages on the basis of their stress period. These stages were Early-1 (E-1), Early-2 (E-2), Middle-1 (Mid-1), Middle-2 (Mid-2) and Final (F). In the E-1 the stress period were 6 hour, 4 hour, 6 hour and 1 day for salt, cold, heat and drought stresses respectively. Similarly the rest of stages were ranged from 12-96 hour and 3-10 days stressed periods for the four abiotic stresses.

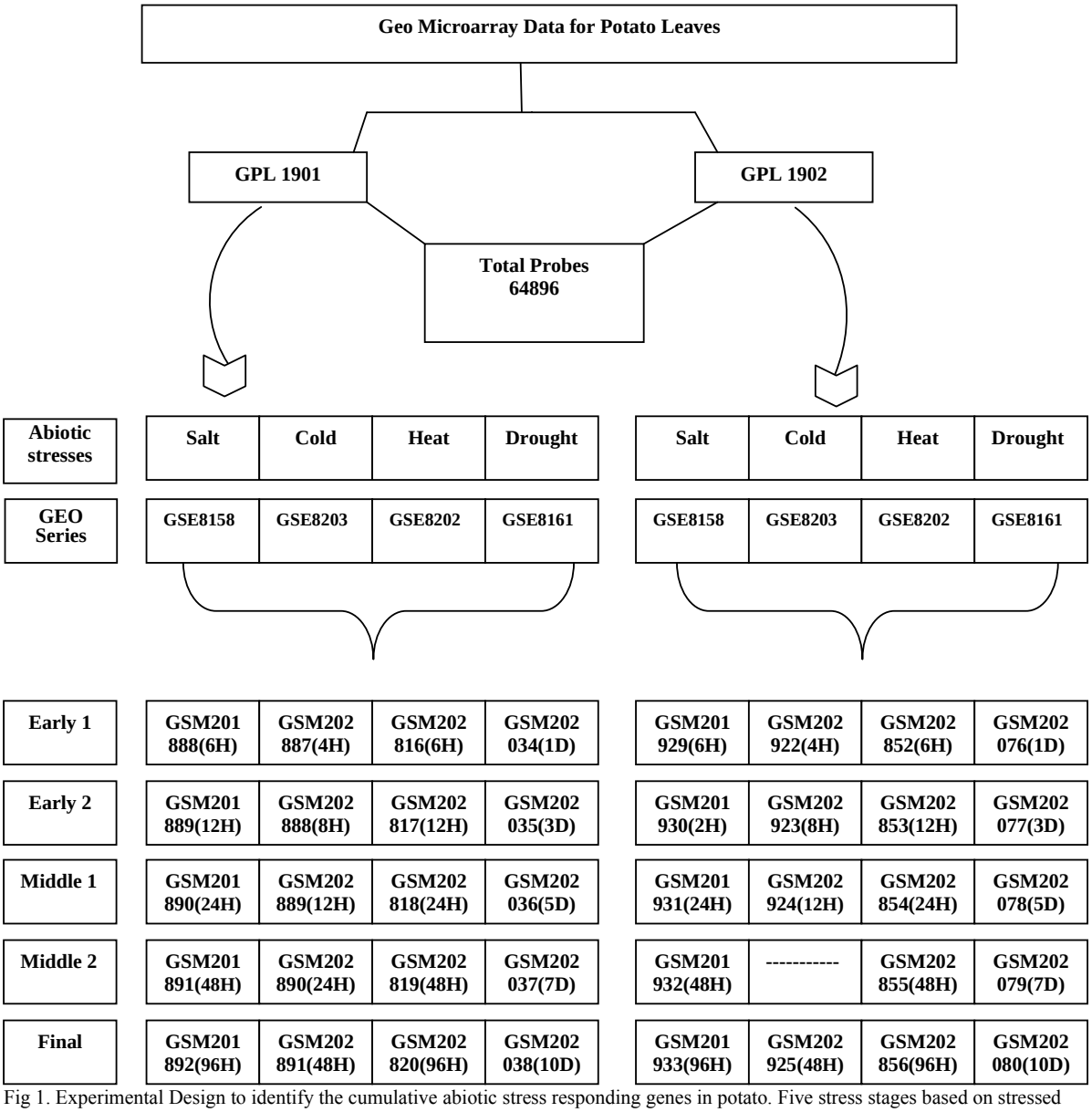

Fig 1. Experimental Design to identify the cumulative abiotic stress responding genes in potato. Five stress stages based on stressed periods were analyzed to find the correlation of cumulative stress responding genes and stressed periods.

Creation of aligned tab-delimited data: The excel sheet was generated for the platform Spot identifiers (IDs). For these IDs, the normalized log2 intensities under abiotic stresses (salt, cold, heat and drought) were entered and aligned. Separate excel sheets were generated for all the five different (E-1&2, Mid-1&2 and Final) stressed stages. Later these aligned platform IDs and their log2 gene expression ratios were saved as tab-delimited files (Liu *et al.*, 2008; Mochida *et al.*, 2009).

Gene expression data analysis: The tab-delimited aligned data was analyzed by MultiExperimental Viewer

(MeV) tool that is skilled of sending and receiving expression and annotation data within the Gaggle framework of bioinformatic applications (Quayum *et al.,* 2008 and Sharif *et al.*,2007), publically available at (www.tm4.org/mev). The MeV is an application algorithm that allows the user to view and analyze the normalized microarray data and identify differentially expressed genes. Briefly, the MeV was run in JavaScript and the created aligned tab-delimited data was loaded in Multiple Array Viewer windows.

Identification of cumulative responding genes: Genes responding under at least three stresses among cold, heat, drought and salt stresses; showing log2 signal intensities ≥/≤ 1.0-fold were identified as cumulative abiotic stressed responding genes and saved. The putative functions of these genes were assigned through information available at platform and using BLASTx and BLASTn tools respectively (Altschul, 1990; Stephen *et al.,* 1997).

Validation and characterization: For validation and characterization studies *Arabidopsis thaliana* was used as reference organism. The blast algorithm was applied to find the identified cumulative responding genes orthologues in *Arabidopsis thaliana*. For validation, the *Arabidopsis* orthologues were subjected to Genevestigator Response Viewer (Zimmermann *et al*., 2004), to find their expression pattern by analyzing the Log2 ratios (Abiotic stressed/control). For characterization, the gene code names (Atg) of *Arabidopsis* orthologues, were subjected to Gene Ontology (GO) functional categorization at TAIR web site publically available at (http://*Arabidopsis*.org/tools/bulk/go/index.jsp), (Berardini *et al.,* 2004) on the basis of cellular components, molecular functions and biological processes. The genes annotation list and charts were saved.

Results and Discussion

Cumulative abiotic stress responding genes: The *In silico* research through bioinformatics tools is a rational approach to find interesting findings (Barozai *et al.,* 2011a; 2011b; Barozai, 2012). Through another similar attempt, the potato microarray data mining, filtering and analysis have resulted 217 cumulative abiotic stress responding genes from 64896 (Tables 1 & 2). These genes have shown log2 signal intensities ≥/≤ 1.0 fold under at least three stresses among cold, heat, drought and salt stresses as shown in Fig. 2. Out of these 217, 38% genes are observed with up-regulation and 62% with down-regulation responding. It suggests that most potato genes have switched off their expression under abiotic stresses. Similar findings were reported for *Arabidopsis* (Seki *et al.,* 2002).

Table 1. Cumulative abiotic stress up-regulated genes.

Plateform ID	GenBank Acc.	Putative function
151300	BQ121834	Putative stress-induced protein
159619	BQ119739	Putative stress-induced protein
133865	BQ119086	Glucan endo-1 3-beta-glucosidase
144644	BQ505697	Hypothetical protein
155199	BQ120474	Brassinosteroid-regulated protein
163389	BQ121482	Amino acid binding protein
191626	BQ121995	Pathogenesis-related protein
190558	BQ117476	Transcriptional regulator-like protein
211793	BQ515793	1 3-beta-D-glucan glucanohydrolase
134255	BQ118916	Polygalacturonase precursor
136979	BQ515274	Branched-chain amino acid aminotransferase
146663	BQ515588	Endochitinase precursor
159573	BQ515669	Glutathione reductase chloroplast precursor
180166	BQ517641	Zinc finger protein-like lipoxygenase
181614	BQ118714	Conserved hypothetical protein
182864	BQ121778	Anthranilate N-hydroxycinnamoyl
191093	BQ505697	Hypothetical protein
195525	BQ121464	1 3-beta-glucan glucanohydrolase
200141	BQ506567	Induced stolon tip protein
203648	BQ118538	Putative CTP synthasehypothetical protein
210929	BQ120158	Brassinosteroid-regulated protein
131531	BQ514118	Fasciclin-like arabinogalactan-protein
132384	BQ120934	Photosystem I reaction centre chloroplast precursor
134954	BQ113508	Serine protein kinase
139574	BQ121528	Phylloplanin precursor
140362	BQ113511	Phylloplanin precursor
144708	BQ120581	Xyloglucan endotransglycosylase
145797	BQ115515	Xyloglucan endotransglycosylase
148511	BQ519032	DNA-binding protein
155199	BQ120474	Brassinosteroid-regulated protein
157253	BQ504726	Hypothetical protein

Table 1. (Cont'd.).

Plateform ID	GenBank Acc.	Putative function
160798	BQ115016	Putative serine carboxypeptidase
160417	BQ515669	Glutathione reductase chloroplast
162621	BQ120158	Brassinosteroid-regulated protein
131540	BQ120934	Photosystem I reaction centre chloroplast precursor
135592	BQ114653	Thioredoxin chloroplast precursor
134262	BQ516506	Lignin forming anionic peroxidase
136979	BQ515274	Branched-chain amino acid aminotransferase
136792	BQ120916	Hypothetical protein
140349	BQ112312	Chlorophyll A-B binding protein
139518	BQ113511	Phylloplanin precursor
141409	BQ511673	3-methyl-2-oxobutanoate dehydrogenase
139346	BQ516559	Chlorophyll A-B binding protein
141022	BQ517253	Ribosomal protein
142904	BQ508891	ATP binding protein
142929	BQ506305	Hypothetical protein
142637	BQ111567	Photosystem I subunit chloroplast pre-
144093	BQ112730	Chlorophyll a/b-binding protein
146748	BQ119157	Phytochrome-interacting factor 4
146649	BQ512359	Photosystem I protein psaH precursor
145819	BQ515588	Endochitinase precursor
144858	BQ516600	Chlorophyll a/b-binding protein
147796	BQ517431	Putative leucine zipper protein
148435	BQ111877	Chlorophyll A-B binding protein
148029	BQ515024	Lipid transfer protein
148970	BQ117537	5-phosphoribosyl-1-pyrophosphate amidotransfera
150021	BQ113389	Putative leucine zipper protein
148680	BQ518011	Hypothetical protein
147562	BQ516435	Endochitinase precursor
152101	BQ113008	Chlorophyll a/b-binding protein
150257	BQ115483	Putative B-box zinc finger protein
154051	BQ111817	Plastid-lipid associated protein
154052	BQ113763	Delta 9 desaturase-like protein
153340	BQ120474	Brassinosteroid-regulated protein
157253	BQ504726	Hypothetical protein
156943	BQ507658	Hypothetical protein
157969	BQ507336	Chloroplast nucleoid DNA binding protein
158717	BQ519358	Subtilisin-like proteinase
159147	BQ111843	Photosystem I reaction centre chloroplast precursor
158326	BQ516460	Ultraviolet-B-repressible protein
158446	BQ113365	Chlorophyll a/b-binding protein
161097	BQ111722	Chlorophyll a/b-binding protein
161055	BQ514809	Serine protease
181493	BQ112195	Oxygenase activase chloroplastprecursor
182784	BQ117905	Pathogenesis-related protein
183932	BQ509993	Pathogenesis-related protein
182630	BQ121548	Pathogenesis-related protein
185287	BQ515275	Branched-chain amino acid aminotransferase
185529	BQ505673	Histone H1 stress-inducible protein
190184	BQ509994	Multidrug resistance protein
191799	BQ515342	Ss- Beta-galactosidase precursor
197181	BQ515024	Non-specific lipid transfer protein
199508	BQ513172	Putative branched chain alpha-keto acid dehydrogenase
210597	BQ515154	Auxin-induced beta-glucosidase 1
210929	BQ120158	Brassinosteroid-regulated protein
161488	BQ113336	Ultraviolet-B-repressible protein

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

Plateform ID	GenBank Acc.	Putative function
132094	BQ113481	2-oxoglutarate-dependent dioxygenase
134408	BQ112708	Putative acid phosphatase
138025	BQ119556	bZIP protein
141863	BQ508284	Hypothetical protein
156340	BQ113913	Hypothetical protein
156765	BQ515803	Fructose-1 6-bisphosphatase
157930	BQ506105	2-oxoglutarate-dependent dioxygenase
159148	BQ113794	Putative acid phosphatase
158900	BQ113053	2-oxoglutarate-dependent dioxygenase
162872	BQ510458	Steroid 22-alpha-hydroxylase
161574	BQ513581	Putative acid phosphatase
162020	BQ113219	2-oxoglutarate-dependent dioxygenase
182239	BQ119335	Hypothetical protein
132635	BQ511306	Cysteine protease inhibitor
132360	BQ508767	Proteinase inhibitor
135825	BQ113026	Zinc finger protein
136267	BQ112708	Putative acid phosphatase
134090	BQ508578	Hypothetical protein
136399	BQ117345	Copper ion binding protein
136020	BQ113897	Aspartic protease inhibitor
137181	BQ119556	bZIP protein
139231	BQ113091	Cysteine protease inhib
139493	BQ115415	Hypothetical protein
140034	BQ516541	Putative membrane protein
143736	BQ509878	Putative kunitz-type tuber invertase inhibitor
142430	BQ113430	Putative FRO2 NADPH oxidase
143516	BQ512999	Hypothetical protein
145970	BQ504881	Proteinase inhibitor
144754	BQ516562	Proteinase inhibitor
148020	BQ113673	Proteinase inhibitor
159992	BQ113794	Putative acid phosphatase
163568	BQ115530	Putative miraculin
163068	BQ513259	Xyloglucan endo-transglycosylase
183213	BQ119655	Holotricin-like peptide
182848	BQ117345	Copper ion binding protein, putative
191757	BQ507143	Copper ion binding protein, putative
193062	BQ516562	Proteinase inhibitor
197172	BQ113673	Proteinase inhibitor type II
143741	BQ112142	2-oxoglutarate-dependent dioxygenase
145291	BQ116380	DNA topoisomerase II
153493	BQ115238	Putative chloroplast thiazole biosynthetic protein
162020	BQ113219	2-oxoglutarate-dependent dioxygenase
180601	BQ120131	Predicted protein

Table 2. (Cont'd.).

Plateform ID	GenBank Acc.	Putative function
181570	BQ518334	Actin binding protein
182089	BQ516371	Cinnamoyl-CoA reductase-like protein
179844	BQ113109	S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase
179845	BQ115272	Myb DNA binding protein-like
181092	BQ114467	DNA binding protein
183887	BQ116304,	Binding transcription factor
183484	BQ113897	Aspartic protease inhibitor 10 precursor
186849	BQ112220	Chitin-binding protein
187741	BQ510432	Unknown protein
188462	BQ113590	Proteinase inhibitor
189320	BQ113889	Monoterpene synthase
192907	BQ112467	RE37920phypothetical protein
190673	BQ114556	Putative bZIP DNA-binding protein
193533	BQ115490	Hypothetical protein
193852	BQ512635	Senescence-inducible chloroplast stay-green protein
193445	BQ119611	Pentatricopeptide repeat-containing protein,
194274	BQ114238	S-receptor kinase M4I22.110 precursor
205910	BQ118409	Putative cytochrome P450
206719	BQ505398	Heat shock protein
209321	BQ510459	Steroid 22-alpha-hydroxylase
133559	BQ516102	Unknown protein
133467	BQ511888	Metal ion binding protein
132762	BQ505320	Neutral invertase
135338	BQ509633	Sucrose synthase 2
137181	BQ119556	bZIP protein
136887	BQ514141	Peroxidase prx14 precursor
139887	BQ506977	Neutral invertase
141656	BQ510461	Chloroplast small heat shock protein
143842	BQ514017	Spermidine synthase
143588	BQ117791	Sucrose synthase 2
150056	BQ514414	Resistance protein
151074	BQ113618	Metallothionein-like protein
150469	BQ505871	dnaK-type molecular chaperone hsc70
150266	BQ516602,	Jasmonic acid 2
153617	BQ511530	Protein phosphatase 2C
154796	BQ121692	Pathogen-inducible alpha-dioxygenase
153605	BQ514416	Brassinosteroid receptor kinase precursor
157158	BQ114252	Lipid transfer protein 2
156815	BQ511368	Heat shock protein
157556	BQ513215	G-box-binding protein
161621	BQ119687	Protein phosphatase 2C
131780	BQ517356	Jasmonic acid 2
135338	BQ509633	Sucrose synthase 2

Table 2. (Cont'd.).

Plateform ID	GenBank Acc.	Putative function
135176	BQ113897	Aspartic protease inhibitor 10
137181	BQ119556	bZIP protein
138223	BQ507400	Putative non-specific lipid transfer protein
138121	BQ511652	F20P5.25 protein/polypeptide with a gag-like domain
137315	BQ510678	Calmodulin binding / protein binding
138051	BQ119290	Mitochondrial small heat shock protein
141695	BQ511497	Heat shock protein
141072	BQ513303	Chloroplast small heat shock protein
142744	BQ117791	Sucrose synthase 2
142672	BQ512999	Hypothetical protein
142675	BQ519042	Heat shock protein
150545	BQ119612	Sodium proton exchange
153228	BQ512490	Sodium proton exchange
153886	BQ121198	DS2 protein
157825	BQ504750	Zinc finger ankyrin protein
156815	BQ511368	Heat shock protein
160270	BQ505398	Heat shock protein
161521	BQ511171	Calmodulin binding / protein binding
160939	BQ518432	Putative photosystem I antenna protein
184068	BQ113834	Chitin-binding protein
183086	BQ507958	Zinc finger protein
183588	BQ113936	Gamma-aminobutyrate transaminase
185290	BQ112826	12-oxophytodienoate reductase
186228	BQ117868	Glycine hydroxymethyltransferase
186953	BQ112111	Flavonoid 3-glucosyl transferase
187162	BQ114891	Hypothetical protein
185515	BQ119290	Mitochondrial small heat shock protein
185623	BQ510678	Hypothetical protein /calmodulin binding / protein binding
186767	BQ511881	Hypothetical protein
187436	BQ115232	Succinic semialdehyde reductase isofom2
187682	BQ113028	Hydroquinone glucosyltransferase
187801	BQ115415	Hypothetical protein
189255	BQ112583,	Envelope glycoprotein
191210	BQ121991	Hypothetical protein isoform 2
192571	BQ504557	Tyrosine kinase
193395	BQ506895	Putative transformer-SR ribonucleoprotein
194170	BQ114182	Acid phosphatase 1
196328	BQ113673	Proteinase inhibitor type II
198853	BQ504663	Na H-antiportor/sodium proton exchanger
206089	BQ112361	Gamma-aminobutyrate transaminase
206072	BQ513297	Chloroplast small heat shock protein
206719	BQ505398	Heat shock protein
209067	BQ113053	2-oxoglutarate-dependent dioxygenase
209829	BQ511171	Hypothetical protein /calmodulin binding / protein binding
210178	BQ508030	Hypothetical protein

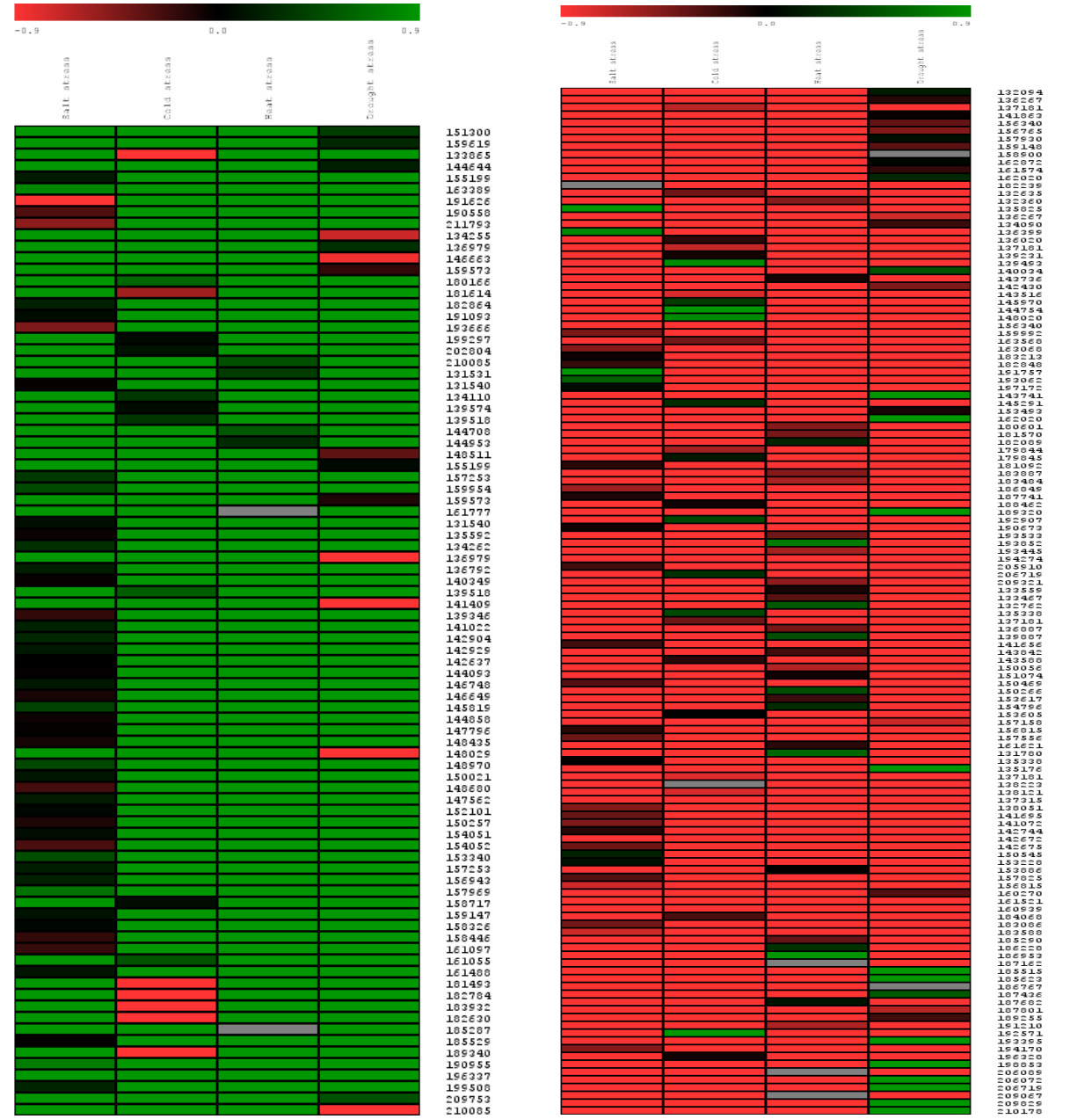

Fig. 2. MEV analyses for the up-regulated (left) and down-regulated (right) genes. The responding genes have been shown their response at least under three abiotic stresses among drought, cold, heat and salt stresses respectively.

The 38% up-regulated genes are the most important set of potential candidate genes for abiotic stress resistant. Some of the key genes are stress-induced protein, Brassinosteroid-regulated protein, binding protein, pathogenesis-related protein, transcription factors, photosynthetic proteins and transporter proteins. Seki *et al.,* (2002) reported the up-regulation for almost all of these genes under drought, cold and salinity. Later Kagale *et al.,* (2007) conferred Brassinosteroid tolerance in *Arabidopsis thaliana* and *Brassica napus* to abiotic stresses especially drought and cold stresses. The transcription factor is an important family of proteins and many researcher groups showed their involvement in abiotic stresses resistance (Seki *et al.,* 2002; Rabbani *et al.,* 2003; Kazuo & Shinozaki, 2009; Barozai & Husnain, 2011c). We also found the same family members; Zinc finger protein, leucin zipper protein, DNA binding protein, transcriptional regulator-like protein, Putative B-box zinc finger protein and bZIP protein, showing up-regulation against three abiotic stresses (cold, heat, drought & salt). Photosynthesis is a crucial requirement in plant physiology, which involves the trapping of solar energy. Chlorophyll a/b-binding proteins (CABs) are the key players of photosynthesis. Recently, Dittami *et al.,* (2010), also suggested its role in plant stress tolerance. The current study is also resulted many members of

chlorophyll a/b binding protein as up-regulated genes cumulative stresses. Recently Qin *et al.,* (2011), and Rodrigues *et al.,* (2011), have been reported lipid transfer proteins up-regulation to salt and water stresses in plants. Our study also confirmed the three members of lipid transfer proteins as up-regulated genes against cumulative responses.

The 62% down-regulated genes also serve as potential resistant gene candidates against cumulative abiotic stresses. We found transcription factors, protein inhibitors, metabolic-related proteins, binding proteins, cell kinases and hypothetical proteins in the list of down-regulated genes. Seki *et al.,* (2002), have been reported almost all these protein classes as down regulated genes in *Arabidopsis* under drought, cold and salt stresses.

Responding genes and stress stages: The comparative analysis of up and down-regulated genes under five stress stages have shown a defensive to offensive strategic approach as shown in Fig. 3. The more down-regulated genes are observed than the up-regulated at E-1, E-2, M-1 and M-2 stressed stages. It means from early to mid-stressed stages, the potato plants adopted defensive approach to switch off their gene expression. As the stresses prolonged to final stage, the potato plants turned to offensive response. These findings are in agreements with the earlier studies (Urano *et al.,* 2010).

The up-regulated genes distribution under five stressed stages showed that maximum genes (57%) have responded to cold- heat-drought; followed by salt-heat-drought (19%), salt-cold-heat (16%), salt-cold-drought (7%) and salt-cold-heat-drought (1%). Similarly, the down- regulated genes dispersal under five stressed stages is observed with almost equal genes (25-26%) responding to cold- heat-drought, salt-cold-heat and salt-cold-drought respectively; followed by salt-heat-drought (20%) and salt-cold-heat-drought (4%). Similar findings were given by a number of researchers (Seki *et al.,* 2002; William *et al.,* 2006).

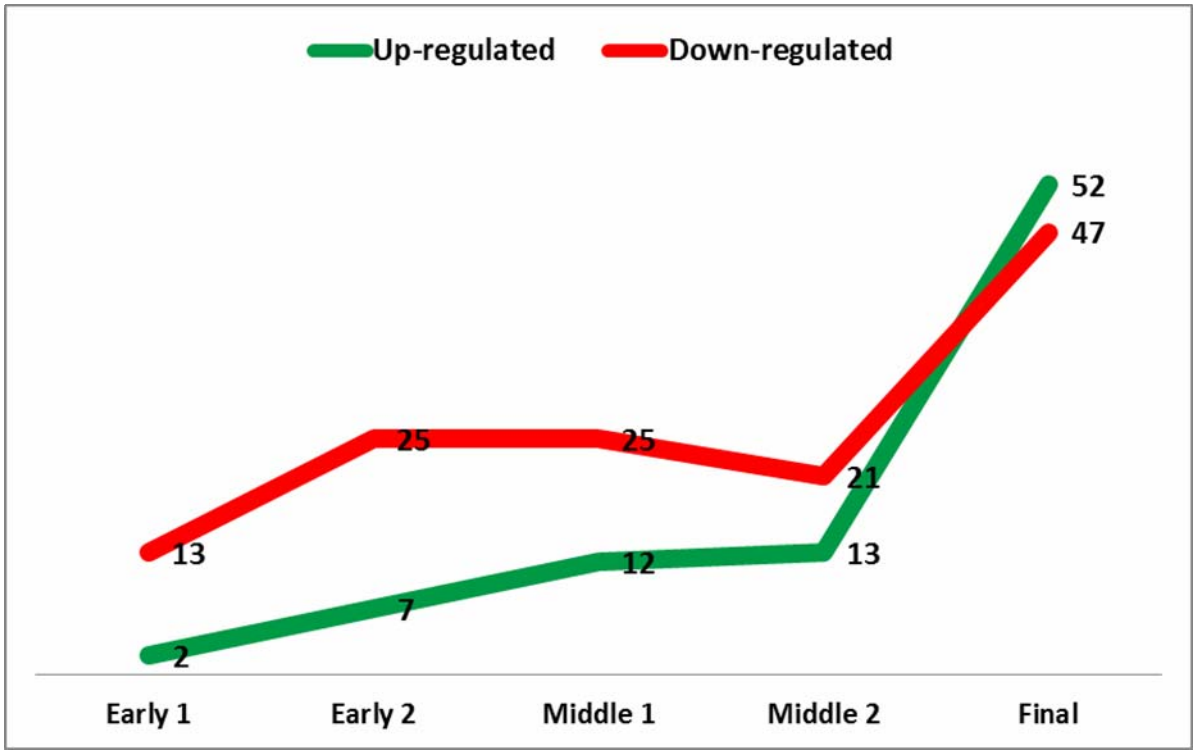

Fig. 3. The defensive to offensive strategic approach of cumulative abiotic stress responding genes in potato.

Validation studies: To validate the potato cumulative stress responding genes, the model plant *Arabidopsis thaliana* is used as reference organism. Many researchers used this plant as reference in their studies (Barozai *et al.,* 2008). Our identified 60% potato cumulative stress responding genes were validated through *Arabidopsis thaliana* gene ontologies (Fig. 4). Out of validated genes 57% are down-regulated and 43% are up-regulated responding genes. Same findings were given in different plant species (Mantri *et al.,* 2007; Urano *et al.,* 2010).

Characterization studies: For the characterization homology studies were conducted. All the cumulative abiotic stress responding gene sequences were subjected to homology search using BLAST algorithms against the nucleotide, protein non-redundant (nr) and EST databases. All the cumulative abiotic stress responding genes showed homology in the three databases, suggesting the well-known persona.

The *Arabidopsis* orthologs of cumulative abiotic stress responding genes were further categorized on the basis of molecular functions, cellular components and biological processes through Gene-Ontology (GO) annotation. The GO molecular function revealed that the majority of the cumulative abiotic stresses responding genes were engaged in nucleic acid & protein binding

followed by unknown molecular function, hydrolase activity, other enzymatic activity, miscellaneous, transferase and kinase activity (Fig. 5a). The GO categorization for cellular components is revealed that most cumulative abiotic stress responding genes were involved in Cytoplasmic organelles followed by cellular components, other intracellular components, chloroplast, membrane, cell wall, nucleus and extracellular (Fig. 5b). The GO biological process annotation categorized the greater part of cumulative abiotic stress responding genes in metabolisms followed by cellular processes, biotic & abiotic stresses, unknown biological processes, growth, and transcription (Fig. 5c). Zhang *et al.,* (2009) reported almost similar results in the cotton.

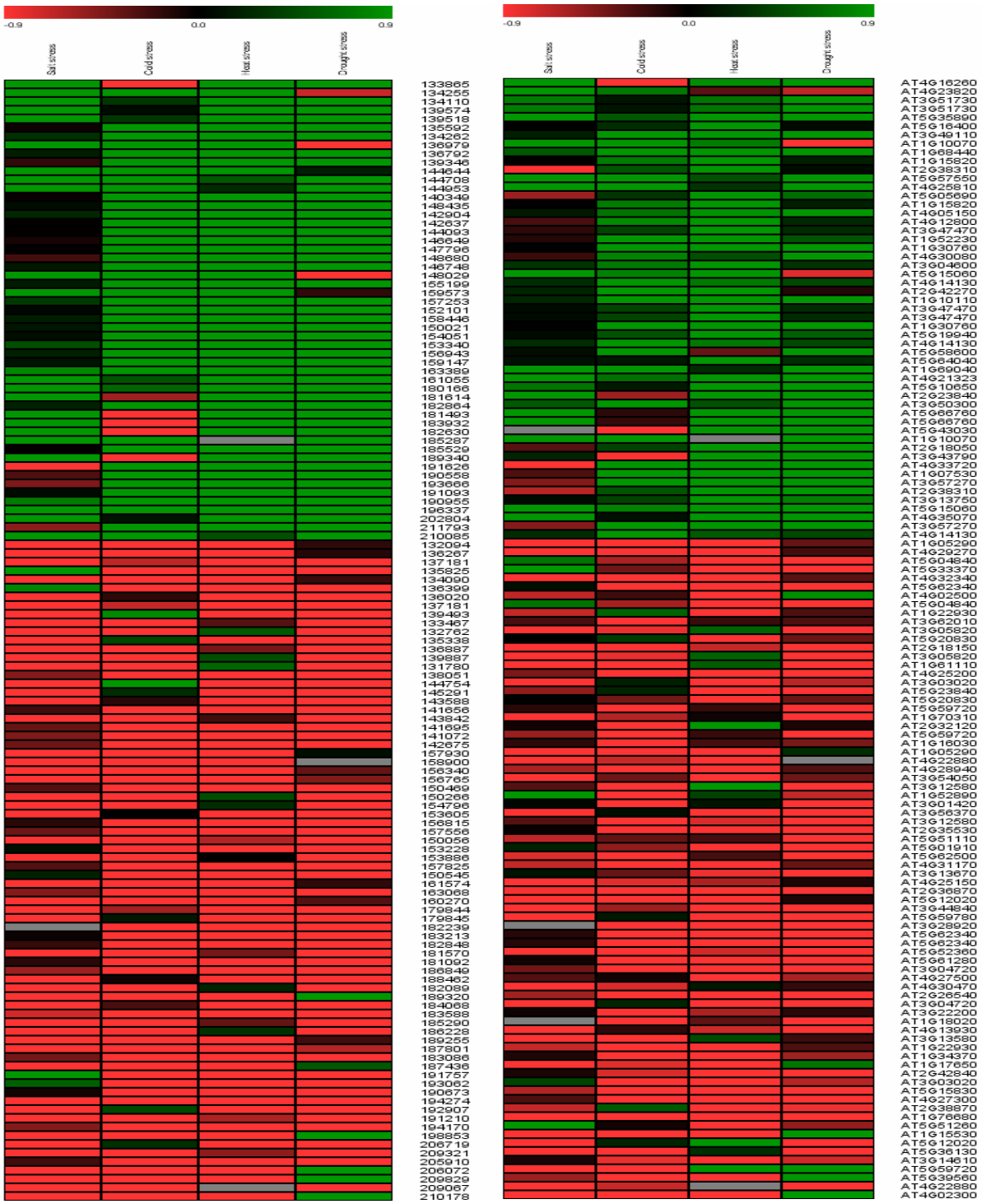

Fig. 4. The cumulative stress responding genes validation studies. The potato genes MEV analysis (left) showed similar expression patterns as reported for *Arabidopsis* (right) under salt, cold, heat and drought stresses, confirming and validating our results.

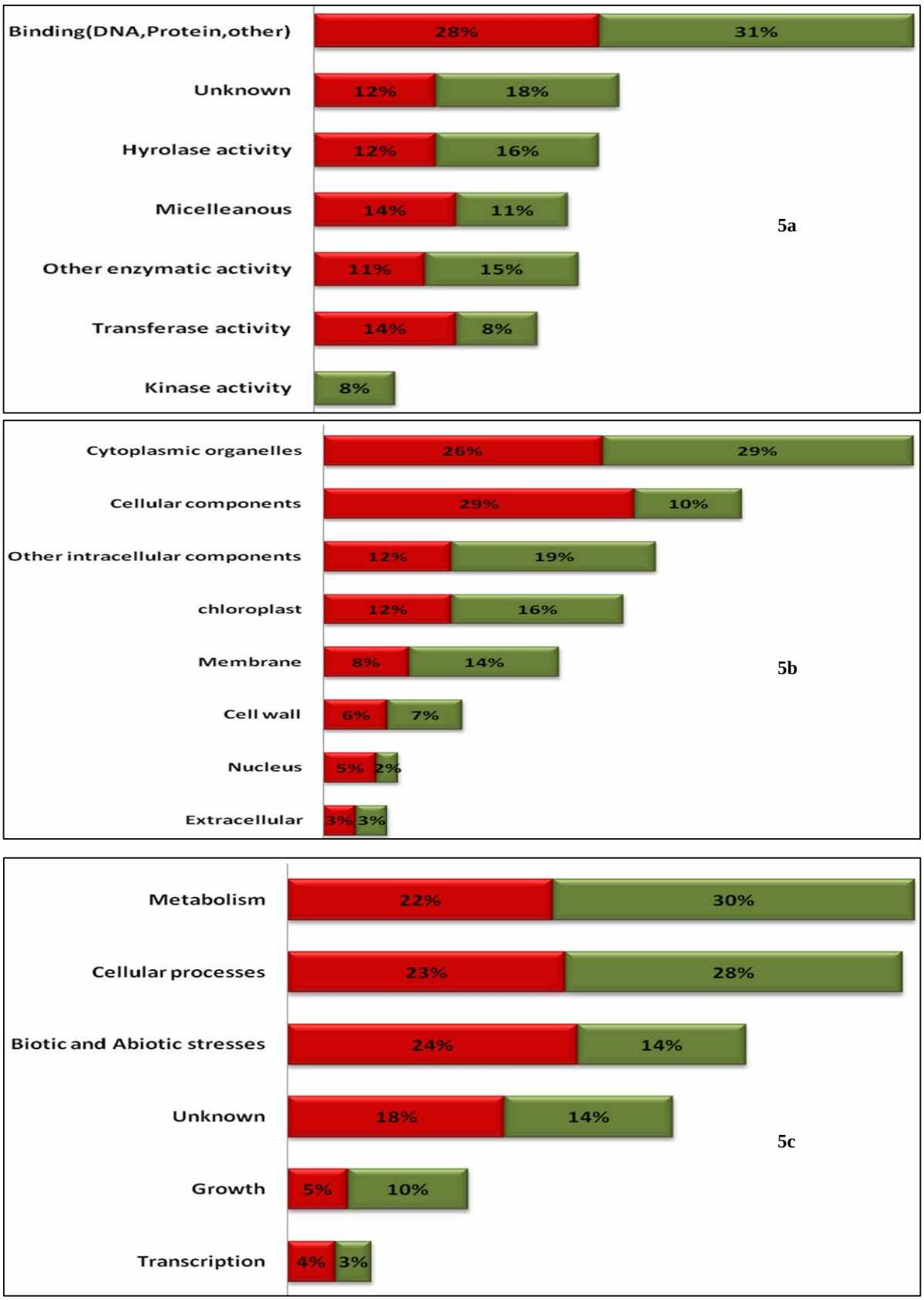

Fig. 5. The potato cumulative stress responding genes characterization by Gene Ontology (GO) studies. The bar charts showed the distribution of cumulative stress responding genes (red, representing down-regulated and green up-regulated genes) among three principal GO categories; Molecular functions (5a),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

Conclusion

Our work suggested that *In silico* analysis is a valid strategy for discovering differentially expressed genes. This type of analysis will provides a valuable resource of information regarding a gene responding program under abiotic stresses. It is also resulted 217 cumulative abiotic responding genes against at least three stresses among drought, heat, cold and salt. These genes are the strong potential resource for the engineering and development of the abiotic stress resistant crops.

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