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Identification of Differentially Expressed Gene Profiles in Young Roots of Tea [*Camellia sinensis* (L.) O. Kuntze] Subjected to Drought Stress Using Suppression Subtractive Hybridization

Akan Das · Sudripta Das · Tapan Kumar Mondal

Abstract Drought is an important abiotic stress that limits the production of tea in different regions of the world. Young roots of tea are responsible for nutrient and water uptake; hence, they are the first tissues to perceive drought stress. In this study, a forward suppression subtractive hybridization library was constructed from the tender roots of drought-tolerant tea (*Camellia sinensis* (L.) O. Kuntze) cultivar (TV-23) subjected to 21 days of drought stress. A total of 572 quality expressed sequence tags were generated by sequencing of 1,052 random clones which have resulted to 246 unigenes comprising 54 contigs and 192 singlets. The unigenes were assigned to various functional categories, i.e. cellular components, biological processes and molecular functions as defined for the *Arabidopsis* proteome. There were 13.04% of differentially regulated genes that have been associated to various stresses. A total of 123 putative drought-responsive genes were identified which include candidate genes of ubiquitin-proteasome, glutathione metabolism and sugar metabolism pathways and several transcription factors. In order to determine the possible expression, 10 genes associated to drought-responsive pathways were further analysed by reverse transcription polymerase chain reaction. This study provides a basis for studying the drought tolerance mechanism of this important commercial crop which will also be a valuable resource for the functional genomics study of woody plants in future.

Abbreviations

ESTs	Expressed sequence tags
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
SSH	Suppression subtractive hybridization
HSPs	Heat shock proteins

A. Das · T. K. Mondal
Biotechnology Laboratory, Faculty of Horticulture,
Uttar Banga Krishi Viswavidyalaya,
Cooch Behar, West Bengal 785165, India

S. Das
Biotechnology Laboratory, Tocklai Experimental Station,
Tea Research Association,
Jorhat, Assam 785008, India

Present Address:
A. Das
ICAR Research Complex for NEH Region, Centre for Biotechnology,
Umiam, Barapani 793103 Meghalaya, India

Present Address:
T. K. Mondal (✉)
National Research Centre on DNA Fingerprinting,
National Bureau of Plant Genetic Resources,
Pusa, New Delhi 110012, India
e-mail: mondaltk@yahoo.com

Introduction

Tea [*Camellia sinensis* (L.) O. Kuntze] is one of the most important plantation crops grown worldwide for the production of popular non-alcoholic beverage called 'tea'. Since most of the world's tea-growing areas are prone to drought, the tea plant is often subjected to water-deficit stress (Mondal 2008). Drought is the most important recurrent limiting factor of tea cultivation in India and other tea-growing countries

which incurred around 40% of crop loss (Handique 1992). It severely affects the growth, crop yield and several biochemical processes of tea plantations (Zhu 2002). Although conventional breeding and propagation techniques contributed significantly to varietal improvement for the last several decades, yet due to the limitations of conventional breeding and lack of a distinct mutant, application of biotechnology becomes an alternative approach (Mondal et al. 2004).

Plant adaptations to harsh environmental conditions include synthesis of compatible solutes, exportation and compartmentalisation of ions and scavenging of reactive oxygen species (ROS) (Zhu 2002). These adaptations involve the expression of cascade of genes and interactions among their gene products (Shinozaki and Yamaguchi-Shinozaki 2007). There is very little information available on drought stress responses of tea plant at the molecular level which was basically studied in leaves. It has been reported that there are three drought-modulated expressed sequence tags (ESTs), namely *dr1*, *dr2* and *dr3* using differential display of mRNA (Sharma and Kumar 2005). The crop is being studied at the genomics level for secondary metabolites (Park et al. 2004), low temperature stress (Wang et al. 2009), transcriptome profiling (Shi et al. 2011), identification of conserved miRNAs (Das and Mondal 2010) and so on. However, no information exists on the drought stress responses of roots. Particularly in tea plants, young roots of current year growth play an important role for water and nutrient uptake (Konwar 2004). Since the roots are the primary site involved in the perception of drought stress, as such they are assumed to have a crucial role to trigger drought tolerance mechanism. The transcriptional changes can result in successful adaptations leading to stress tolerance by regulating gene expression and signal transduction in the stress response (regulatory proteins) or by directly protecting the plant against drought stress (functional proteins) (Shinozaki and Yamaguchi-Shinozaki 2007).

Recent efforts have demonstrated that analysis of ESTs is an appropriate strategy for identifying genes involved in specific biological functions in model plants (Yamamoto and Sasaki 1997) and even in non-model plants in which genomic data are not available (Xuxia et al. 2011; Xu et al. 2011b; Yu et al. 2011). The suppression subtractive hybridization (SSH) technique enables specific cloning of ESTs representing genes that are differentially expressed in different mRNA populations and isolates genes without prior knowledge of their sequence or identity (Diatchenko et al. 1996; Jin et al. 2011). Moreover, the availability of databases of known genes and proteins and gene ontology (GO) annotation provides an opportunity to predict the functions of newly isolated putative gene sequences (Ashburner et al. 2000). The present study was conducted for the identification of gene profiles in roots of tea plants under drought stress by analysing SSH cDNA library. Bioinformatics

analyses of the ESTs allowed us to detect a wide spectrum of gene expression in tea roots that enabled the plant to withstand drought conditions. The differentially regulated genes in tea roots are potential candidate genes for engineering drought-tolerant crops and for revealing the molecular mechanisms of adaptation of plantation crops to water-deficit stress. Furthermore, possible expression patterns of superoxide dismutase (*SOD*), glutathione reductase (*GR*), glutathione S-transferase (*GST*), trehalose-6-phosphate synthase (*TPS*), calmodulin-like protein (*CML*), f-box protein, cullin, arginine decarboxylase (*ADC*), mitogen-activated protein kinase (*MAPK*) and S phase kinase-associated protein 1 (*Skp-1*) genes were confirmed under drought stress.

Materials and Methods

Plant Materials, Drought Stress Induction and Sampling

Two-year-old vegetatively propagated well-rooted tea plantlets (~36-in. height) of TV-23 cultivar (drought-tolerant) (Konwar 2004; Das 2012) were planted in earthen pots (12-in. diameter) under controlled greenhouse conditions (at a light intensity of 300 μmol m^{-2} s^{-1} and 25±2°C with relative humidity of 65–70%) at Cooch Behar, West Bengal, India. Drought sensitivities of the cultivar were confirmed by breeders and commercially established on the basis of yield stability during drought periods over many years. Initially, the plantlets were watered regularly for 2 months to establish new growth, and subsequently, water was withheld in experimental ones for drought stress induction. Soil moisture content (SMC), relative water content (RWC) and physiological status of the plants were evaluated in every 2/3-day interval from the day of withholding water according to Black (1965) and Barr and Weatherley (1992) and using a Photosynthesis System (LCpro+, ADC, UK), respectively. On the 21st day of stress induction, tender roots were collected for the isolation of RNA.

Construction of SSH Library and Sequencing

Total RNA was isolated from collected roots (100 mg) of both drought-induced and well-watered plants (control) using a modified protocol based on sodium dodecyl sulphate-LiCl (Das 2012). Different aliquots of total RNA were pooled in order to extract the mRNA by PolyATtract® mRNA Isolation System (Promega, USA). The mRNA pool was precipitated with 0.1 volume of 3 M ammonium acetate (pH 5.2) and 1.0 volume of isopropanol at −70°C for 3 h. The concentrated mRNAs (4 μg) were used for the synthesis of cDNAs. A forward SSH library was constructed using the cDNAs of drought-induced plant as 'tester' and cDNAs of control plant as 'driver' according to the user manual of

PCR-Select[TM] cDNA subtraction kit (Clontech, USA). The subtracted cDNAs were cloned into pGEM®-T easy vector (Promega, USA) and transformed into electrocompetent DH10β *Escherichia coli* cells (Invitrogen). Plasmid DNAs were purified from the overnight grown transformed *E. coli* cells using the manufacturer's protocol of GenElute plasmid miniprep kit (Sigma-Aldrich). A cycle sequencing PCR was performed using M13 forward primer (3.2 pmol) and Big Dye Terminator v 3.1 (Applied Biosystems) taking the recombinant plasmids as template. Single-pass sequencing was conducted at the 5′ end of cDNA clones with Genetic Analyzer 3130*xl* (Applied Biosystems).

Clustering and Functional Annotation

The raw ESTs were cleaned by removing the vector and adaptor sequences present at both 5′ and 3′ ends using Sequence scanner v 3.1 (Applied Biosystems). Trimmed sequences with no more than 178 bp in length were selected for BLASTn and BLASTx analyses against the nonredundant nucleotide and protein sequence databases of NCBI (www.blast.ncbi.nlm.nih.gov/Blast.cgi), respectively at an e-value threshold of $1e^{-05}$. Sequences with no significant hits were considered as novel. Sequences that passed through our set quality parameters were clustered using the dirty data algorithm considering ambiguous base calls for poor matches to exact base with the following criteria: (a) gap optimisation for small inserts and double-called bases through ReAligner algorithm, (b) prefer 3′ gap replacement, (c) minimum overlap of 20 bp and (d) minimum of 85% match using Sequencher 4.1 (Gene Codes Corporation). Both contigs and singletons were altogether defined as unigenes. Further, the unigenes were annotated for various functional properties.

Due to unavailability of a genome sequence for tea, we conducted GO analysis of unigenes against *Arabidopsis* genome by using TAIR 9 transcripts database (www.arabidopsis.org/index.jsp) through WU-BLAST. The corresponding locus hits of unigenes were selected at an e-value threshold of $1e^{-10}$ and further categorised on the basis of GOslim categories of TAIR (www.arabidopsis.org) based on: annotations to terms in GOslim category / total annotations to terms in this ontology$\times 100$.

Identification of putative drought-responsive genes was performed on the basis of BLAST hits in non-redundant databases of NCBI and our knowledge on physiology and biochemistry. Wherever two or more genes were found in a single pathway, the corresponding pathway genes were analysed using the KEGG database (www.genome.jp/kegg). For the comparison of tissue-specific gene expression in roots and leaves, the available ESTs of tea leaves generated under drought stress were downloaded from NCBI (GenBank accession numbers GH623813–GH623574, GH738509–GH738781, by June

2011). The downloaded ESTs were vector-cleaned and passed through the quality parameters ((>100 bp length and e-value< $1e^{-05}$). Finally, sequences that passed through the quality parameters were clustered and used as query for searching the root's homologue on the basis of bit score and identity percentage (minimum of 85%) by standalone BLASTn program of NCBI (Stephen et al. 1997).

RT-PCR Analysis

Total root RNAs isolated from control and drought-induced plants were used for reverse transcription polymerase chain reaction (RT-PCR) analysis. Genomic DNA was removed by DNase I. First-strand cDNA was synthesised from each 1 μg of total RNA sample using MMLV reverse transcriptase (Invitrogen, USA) according to the manufacturer's protocol. The cDNAs were purified using a commercial column (Qiagen). To determine the expression of candidate genes, PCR was performed with 2 μl of the first-strand cDNA template and gene-specific primer pairs. Gene-specific RT-PCR primers were designed with Primer 3.0 (http://frodo.wi.mit.edu/primer3/) according to the EST sequences and were synthesised commercially (Sigma-Aldrich, USA). Tea 26S ribosomal RNA (rRNA) was used as inner control for RT-PCR analysis. All primers for the candidate genes and 26S rRNA are listed in Table 1. General PCR was conducted with the following programme: an initial denaturation at 94°C for 3 min, followed by 24 to 30 cycles with denaturation at 94°C for 30 s, annealing at 61°C (or as required for the specific primer pairs Table 1) for 45 s and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. RT-PCR experiments were repeated three times, and the PCR products (8 μl) were detected by 1% agarose gel in 1× TAE with EtBr and viewed under ultraviolet light.

Results

Response of Tea Plants to Drought Stress Induction

To confirm an appropriate duration of drought stress induction to be applied for stress treatment of plants prior to SSH library construction, SMC, RWC and physiological parameters, i.e. photosynthesis rate and stomatal conductance were monitored till the plants get wilted permanently. The visible wilting symptoms were observed in the plants from the 18th day onwards. As shown in Fig. 1, after 18 days of drought stress induction, SMC and RWC were decreased significantly compared to the control ($P<0.05$), while there was no significant difference between the 24th and 27th day of stress induction. The plants showed severe wilting symptoms from the 24th day onwards. After 27 days of drought stress induction, the plants were getting permanently wilted

Table 1 Primer sequences of subset of ESTs and 26S rRNA for RT-PCR analysis

Accession number	Putative function	Tm (°C)	Primer sequences (5′–3′)
AY283368	26S rRNA	54	F: CACAATGATAGGAAGAGCCGAC
			R: CAAGGGAACGGGCTTGGCAGAATC
GT969323	Arginine decarboxylase	60	F: GCTTTGAATACAGTGATTGTGCTTGAGC
			R: CTAGTCCGCCACCAATATCAATGACTTC
GT969381	Superoxide dismutase	61	F: CAAGAAGGAGATGGTCCAACTACAGTTACC
			R: AACAACAACAGCCCTTCCAATGACG
GT969218	F-box	61	F: GGCAAAATAGAGATTCGATTGCTGTCC
			R: AACAACGAGGCCAGACTTCCAGTTAC
GT969255	S phase kinase protein 1	60	F: GCCATGTATTTCTTCTTACTCAAAGGCC
			R: GTTTGAGGAAATGAGATGTCTTCGTCG
GT969327	Glutathione reductase	60	F: CTACAAACGGTTACTTTCAAACGCAGG
			R: TCTGCCTTCCAGATTTCTTGCAACC
GT969164	Glutathione S-transferase	61	F: AAAGACAATAGAGGAAAGGGGTTTGGTG
			R: CCTCTGTCTCAAATCTCTCTATCCCTTCG
GT969138	Cullin	60	F: TGTTCCGAAGAGTATCTAATGGACTCTCG
			R: CCATGCTAGAATAGAACCCTTGCATTG
GT969228	Calmodulin-like protein	61	F: GAGTTGCACTCGGTGATGAAGAGATTG
			R: TATCCATATGAGTAACACGACAAATCAAGC
GT969159	Trehalose protein synthase	61	F: CTAGCACCGTTGATTGGCAATTTTAGAG
			R: CACACACAAAACAAAATCCGGCG
GT969241	Mitogen-activated protein kinase	60	F: GTAAATAGTTGGATTTGAGCTTACCCG
			R: ATTTGAAACCCAGCAATCTCTTGCTG

and they could not be revived even after sufficient rewatering. Thus, we decided to induce 21 days of drought stress to the plants for constructing the SSH library. On the 21st day of stress induction, SMC and RWC were found as 7% (−1.2 ±0.20 MPa) and 63%, respectively (Fig. 1). Moreover, the photosynthesis rate and stomatal conductance were recorded as 8.73 μmol m^{-2} s^{-1} and 0.42 mmol m^{-2} s^{-1}, respectively.
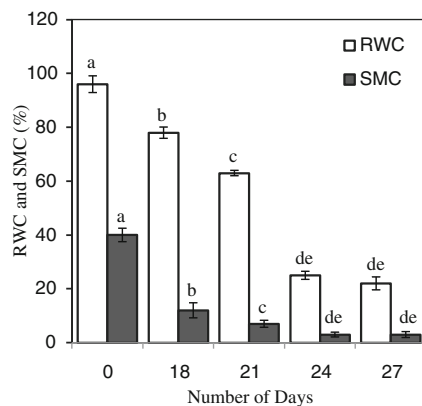

Fig. 1 Relative water content (*RWC*) in leaves and soil moisture content (*SMC*) in 3-day interval of drought induction from 0 to 27 days. Values are means±S.E. based on three replicates. *Means with the same letters are not significantly different at* $P<0.05$

Analyses of the ESTs and Functional Annotation

In order to identify up-regulated genes during drought stress, a forward SSH library was constructed with 21 days of drought-induced plant cDNAs as the 'tester' set and control plant cDNAs as the 'driver' set. A total of 1,052 bacterial colonies were randomly picked from the library and sequenced. The analysis of cDNA sequences showed that 572 clones were passed through the set quality parameters and found to contain inserts ranging from 178 to 832 bp. The total of 572 up-regulated ESTs assembled into 246 unigenes comprises 54 contigs and 192 singlets. The contigs were composed of 2 to 201 individual ESTs (Fig. 2). Analyses of the SSH library are summarised in Table 2. BLASTx analysis of deduced amino acid sequences was performed to identify any encoded proteins. Using BLASTx, out of 572 ESTs, 248 (43.36%) were identified encoding different enzymes and functional proteins, 207 (36.19%) similar to unknown proteins, 40 (6.99%) similar to predicted proteins, 22 (3.85%) homologue to small heat shock proteins, 21 (3.67%) encoding molecular chaperone and 34 (5.94%) ESTs failed to match any sequences in the public database (Supplementary material 1/ESM1). There were most abundant ESTs for ribosomal protein, cysteine protease and chaperone followed by heat shock proteins (Table 3). There were 14 non-redundant ESTs (5.69%) without significant hits in *Arabidopsis* genome. The nucleotide
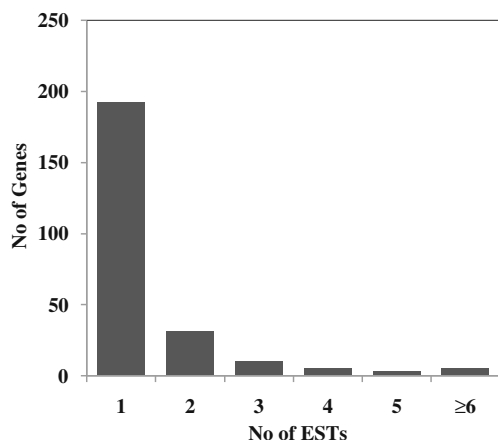
Fig. 2 Distribution and numbers of assembled ESTs

sequence data of our EST collection have been deposited to the GenBank dbEST database under the accession numbers from GT968791 to GT969386, GW316843 to GW317159 and GW315010 to GW315149.

For the comparison of in silico tissue-specific gene expression under drought stress, 665 available drought-responsive ESTs of tea leaf were downloaded from the GenBank dbEST database. After vector trimming and removing of low quality sequences, finally, 321 ESTs were considered for further analysis. The ESTs were clustered into 116 unigenes containing 45 contigs and 71 singlets. Out of 116, there were 12 unigenes found to be homologue with root-specific unigenes (Fig. 3). The homologue unigenes were encoding dehydrin, sugar transporter, histone, unknown proteins as well as predicted proteins (Supplementary material 2/ESM2).

A total of 187 (76%) differentially expressed unigenes could be assigned to three broad functional categories, i.e. cellular components, biological processes and molecular functions as established for the *Arabidopsis* proteome (Fig. 4). In the cellular components category, genes assigned to the 'other intracellular components' category accounted for the largest group (19.65%), followed by 'other cytoplasmic components' associated genes (16.78%), and genes of

Table 2 Summary of SSH library

Total clones sequenced	= 1,052
ESTs taken for analysis	= 572
Number of unigenes	= 246
Number of contigs	= 54
Number of singlets	= 192
Average length of unigenes	= 513 bp
Average length of ESTs	= 429 bp
Average GC content (%) of ESTs	= 43.5
Average GC content (%) of unigenes	= 44.28
Coding GC (%)	= 44.46

'endoplasmic reticulum related' represented least in the group (0.88%). In the biological process category, the largest group was 'other cellular processes' (20.83%) followed by other metabolic process (18.28%), and the signal transduction-associated genes were the least in the group (0.95%). Interestingly, 13.04% of genes represented the 'stress responsive' category, while 11.45% of genes were in the 'abiotic or biotic stimulus' responsive group. In the molecular functions category, the highest percentage was covered by 'other binding' related genes (12.90%) followed by 'transferase activity' related genes (12.20%), and the 'transcription factor' activity-associated genes were the least in the group (2.44%). Based on GO annotation, we found ESTs that are associated to ubiquitin-proteasome degradation pathways, glutathione synthesis and metabolism pathways as well as genes associated to sugar synthesis, transport and metabolism (Table 4; Fig. 5).

Comparison of Tea Root ESTs with Published Abiotic Stress-Responsive Genes

Drought, low temperature and high salinity are common stress conditions that adversely affect plant growth and crop production (Xiong et al. 2002). In our collection of nucleotide sequences, there are ESTs having homology to transcripts known to be associated with these abiotic stress tolerances. In order to identify drought-responsive gene profiles in tea roots, we presented 123 putative drought-responsive genes in Table 4. It includes a diverse set of genes involved in synthesis, metabolism, signalling and transcription (Fig. 5). There are transcripts related to polyamine synthesis (arginine decarboxylase); sugar synthesis, transportation and metabolism (trehalose-6-phosphate synthase, UDP-glucose 4-epimerase, sugar transporter, hexose transporter, glucose-6-phosphate, glyceraldehyde 3-phosphate dehydrogenase, phosphoenolpyruvate carboxykinase); glutathione synthesis and metabolism (γ-glutamyl-cysteine synthetase and glutathione reductase); and molecular chaperone and HSPs (late embryogenesis abundant protein, dehydrin, heat shock protein 70, heat shock protein 80 and mitochondrial heat shock protein). These genes are involved in reducing the initial impact of water-deficit stress. There are also some genes encoding proteins to detoxify the ROS generated by drought stress, such as SOD, peroxidase (POX), thioredoxin peroxidase and probable phospholipid hydroperoxide, GST. Several genes involved in proteasome degrading pathways were also reported such as ubiquitin, f-box protein, skp1, cullin, ring finger protein, enzyme 2 and polyubiquitin. These genes are mostly involved in the SCF complex-mediated protein degradation through ubiquitination. Leaf senescence-associated genes such as ACC oxidase were also found in our SSH library.

Table 3 Contigs that contain more than four ESTs and e-value$<1e^{-10}$

Contig IDs	Homology	No. of ESTs	Organisms	Gene IDs	e-value	Score
Contig1	18S rRNA gene (partial)	201	*Glomus proliferum*	FN547501.1	$9e^{-153}$	549
Contig2	Chaperone, mRNA	22	*Agave tequilana*	DQ515785.1	$4e^{-93}$	351
Contig4	Heat shock protein, mRNA	14	*Camellia sinensis*	EU727315.1	0.0	1141
Contig5	Predicted protein, mRNA	9	*Populus trichocarpa*	XM002305555.1	$2e^{-80}$	307
Contig6	Ubiquitin carrier protein mRNA, predicted	6	*Vitis vinifera*	XM002277413.1	$1e^{-17}$	98.7
Contig7	Aspartate aminotransferase, mRNA	5	*Daucus carota*	M92660.1	$3e^{-101}$	378
Contig9	Heat shock protein, mRNA	5	*Hevea brasiliensis*	AF521007.1	0.0	699
Contig10	60S acidic ribosomal protein PO, mRNA	4	*Euphorbia esula*	AF227622.1	$4e^{-115}$	423
Contig11	Evolutionarily conserved C-terminal region 6, mRNA	4	*Arabidopsis thaliana*	NM_001084699.1	$2e^{-91}$	345
Contig13	Putative remorin protein, mRNA	4	*Ricinus communis*	XM_002529577.1	$2e^{-101}$	378

In addition, the EST set contains some transcripts encoding proteins with signalling functions such as CML and MAPK. The CML functions as transducers of Ca^{2+} signals in plants. It provides a sense to activate MAPKs, a family of ser/thr protein kinases functioning in many signal transduction pathways (Ranty et al. 2006; Xu et al. 2010). In our SSH library, other transcripts homologue to signalling proteins are protein kinase APK1A, chloroplast precursor, NAD+kinase, cyclin-dependent kinase 8 (cdk8) and nucleoside diphosphate kinase. In general, the signalling molecules act by interaction with nuclear transcription factors which induce the expression of a specific set of genes (Sahi et al. 2006).

In our SSH library, several transcripts were found encoding transcription factors (TFs). Transcription factors are critical regulators of the changes in gene expression and abiotic stress responses. Ethylene-responsive factors (ERFs) belong to the large APETALA2 (AP2)/ERF transcription factor superfamily that is unique to plants (Song et al. 2005). NAC family of transcription factor represents one of the largest families of transcription factor in plants (Hu et al. 2006). The ERF and NAC domain protein factors were found to be induced in our library. The other TFs found in our SSH library were global transcription factor, NAM-like proteins, YIPF1 and arginine-serine-rich factors.
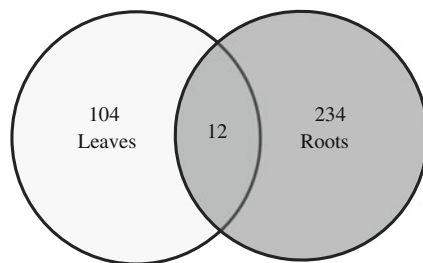

Fig. 3 Venn diagram of drought-induced genes in roots and leaves. The genes were put in three categories: drought induced in roots (total unigenes 246), leaves (total unigenes 116) and in both (total unigenes 12)
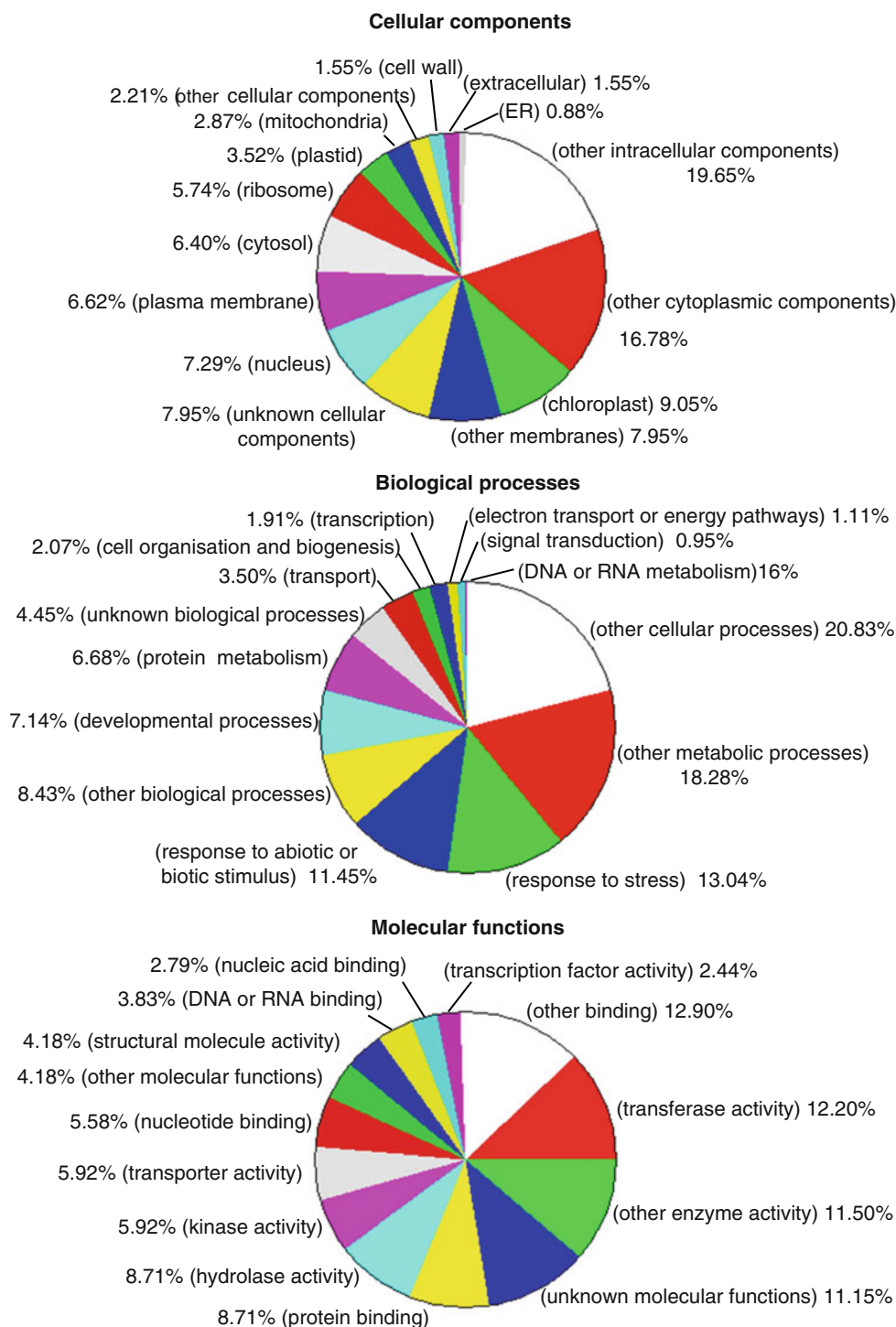
RT-PCR Analysis for the Confirmation of Differential Expressed Genes

In order to determine the validity of the library with respect to drought-induced expression, 10 interested genes from different drought-responsive pathways were analysed by RT-PCR. RNA was isolated from the roots of drought-induced and control plants. The amount of cDNA was adjusted to yield equivalent levels of 26S rRNA amplification. RT-PCR results showed that the expression levels of seven candidate genes were more highly expressed in drought-stressed roots (Fig. 6). The *GST* and *GR*, glutathione synthesis and metabolism pathway-associated genes were found to be up-regulated. The genes involved in trehalose and polyamine synthesis, namely *TPS* and *ADC*, were also found to be up-regulated. The other up-regulated genes include antioxidative enzyme, *SOD*; calcium sensor, *CML*; and regulatory protein, *MAPK*. However, the ubiquitination pathway-associated genes, i.e. *F-box*, *Cullin* and *Skp-1* were found to be down-regulated (Fig. 6). It was concluded that overall, there was a good agreement between the SSH library data and the RT-PCR results.

Discussion

Drought stress is one of the major factors limiting the growth and crop yield of tea plantations in India and other tea-growing countries. Drought is a complex quantitative trait regulated by a large number of genes (Shinozaki and Yamaguchi-Shinozaki 2007). Exploitation and enrichment of drought tolerance-associated genes are very important to accelerate research in this area. Although several studies have been made to explore the transcripts in the leaves of tea plants, no information is available on the expression of genes involved in responses to drought stress in roots. The SSH approach has been proven to be a powerful tool to

Fig. 4 Gene ontology (*GO*) analysis of *C. sinensis* unigenes. The relative frequencies of GO hits for unigenes assigned to the GO functional categories: cellular components, biological processes and molecular functions, as defined for the *Arabidopsis* proteome

Cellular components

1.55% (cell wall)
(extracellular) 1.55%
2.21% (other cellular components)
(ER) 0.88%
2.87% (mitochondria)
(other intracellular components) 19.65%
3.52% (plastid)
5.74% (ribosome)
6.40% (cytosol)
6.62% (plasma membrane)
(other cytoplasmic components) 16.78%
7.29% (nucleus)
7.95% (unknown cellular components)
(chloroplast) 9.05%
(other membranes) 7.95%

Biological processes

1.91% (transcription)
(electron transport or energy pathways) 1.11%
2.07% (cell organisation and biogenesis)
(signal transduction) 0.95%
3.50% (transport)
(DNA or RNA metabolism) 16%
4.45% (unknown biological processes)
(other cellular processes) 20.83%
6.68% (protein metabolism)
7.14% (developmental processes)
8.43% (other biological processes)
(other metabolic processes) 18.28%
(response to abiotic or biotic stimulus) 11.45%
(response to stress) 13.04%

Molecular functions

2.79% (nucleic acid binding)
(transcription factor activity) 2.44%
3.83% (DNA or RNA binding)
(other binding) 12.90%
4.18% (structural molecule activity)
4.18% (other molecular functions)
5.58% (nucleotide binding)
(transferase activity) 12.20%
5.92% (transporter activity)
5.92% (kinase activity)
(other enzyme activity) 11.50%
8.71% (hydrolase activity)
(unknown molecular functions) 11.15%
8.71% (protein binding)

enrich the differentially expressed genes (Diatchenko et al. 1996). This article reports the construction of subtracted cDNA library from root tissues of tea plant under drought stress and the analysis of 572 putative drought-responsive genes. The differential expression of 10 genes of interest upon drought stress was further confirmed by RT-PCR analysis. The study was focussed on generating the gene profiles at the early stages of wilting of the plants due to important roles of early wilting-responsive genes in mediating the effects of drought stress.

Drought stress imposes osmotic stress, oxidative stress and ionic stress to plants. There are cascades of gene networks involved in stress perception, signal transduction, transcriptional control and scavenging of ROS (Zhu 2002). A number of genes get up-regulated or down-regulated during drought by increasing or decreasing the levels of

Table 4 Drought-responsive ESTs in roots of tea plant

GenBank IDs	Homology	*Arabidopsis* gene loci	Length	e-value
GT969327	Glutathione reductase [*Picrorhiza kurrooa*](ACF93236.1)	AT3G24170.1	571	$1.00e^{-70}$
GT969133	Thioredoxin h1 [*Glycine max*] (ABV71991.1)	AT3G51030.1	501	$2.00e^{-48}$
GT969341	Glucose-6-phosphate [*Pisum sativum*] (AAC08525.1)	AT5G54800.1	587	$1.00e^{-82}$
GT969343	Dehydrin [*Camellia sinensis*] (ACJ65691.1)	AT3G50980.1	239	$5.00e^{-15}$
GT969344	Vacuolar H+-ATPase C subunit [*Plantago major*] (CAH58637.1)	AT4G34720.1	697	$2.00e^{-47}$
GT969349	Predicted H+-pyrophosphatase [*Vitis vinifera*] (XP_002282358.1)	AT1G15690.1	376	$5.00e^{-30}$
GT969164	Glutathione S-transferase 5 [*Vitis vinifera*] (ABW34390.1)	AT2G30860.1	737	$4.00e^{-54}$
GT969354	UDP-glucose 4-epimerase, putative [*Ricinus communis*] (XP_002529901.1)	AT2G34850.1	407	$4.00e^{-19}$
GT969365	Peroxidase a [*Eucommia ulmoides*] (AAU04879.1)	AT3G21770.1	717	$1.00e^{-74}$
GT969366	8-Amino-7-oxononanoate synthase-like protein [*Arabidopsis thaliana*] (CAB85568.1)	AT5G04620.2	737	$2.00e^{-11}$
GT969159	Trehalose-6-phosphate synthase [*Solanum lycopersicum*] (BAF98176.1)	AT1G70290.1	352	$6.00e^{-14}$
GT969176	ACC oxidase [*Camellia sinensis*] (ABI33224.1)	AT1G62380.1	517	$9.00e^{-77}$
GT969368	GTP-binding protein [*Helianthus annuus*] (AAM12880.1)	AT5G55190.1	488	$6.00e^{-22}$
GT969369	Amine oxidase (copper-containing) precursor, putative [*Ricinus communis*] (XP_002509596.1)	AT4G12280.1	503	$1.00e^{-31}$
GT969308	Cell differentiation protein rcd1, putative [*Ricinus communis*] (XP_002529459.1)	AT3G20800.1	721	$1.00e^{-82}$
GT969157	Ubiquitin ligase-like protein [*Oryza sativa*] (ABS18744.1)	AT4G05320.2	688	$1.00e^{-19}$
GT969381	Cu/Zn superoxide dismutase [*Camellia sinensis*] (AAU08173.1)	AT1G08830.1	348	$4.00e^{-50}$
GT969070	Heat shock protein 83 [*Ipomoea nil*] (P51819.1)	AT5G52640.1	611	$4.00e^{-82}$
GT969378	Mitochondrial small heat shock protein [*Solanum lycopersicum*] (BAA32547.1)	AT4G25200.1	480	$1.00e^{-37}$
GT969311	Protein transporter [*Zea mays*] (ACG24610.1)	AT4G26670.1	392	$4.00e^{-19}$
GT969386	ormdl, putative [*Ricinus communis*] (XP_002523307.1)	AT5G42000.1	619	$5.00e^{-77}$
GT969372	Late embryogenesis abundant protein 5 [*Nicotiana tabacum*] (AAC06242.1)	AT1G02820.1	655	$1.00e^{-19}$
GT969363	Putative ethylene-responsive element-binding protein 2 [*Gossypium hirsutum*] AAX68525.1)	AT3G16770.1	693	$3.00e^{-51}$
GT969361	NAC domain protein [*Populus trichocarpa*] (XP 002297860.1)	AT2G33480.2	509	$7.00e^{-10}$
GT969329	Chitinase [*Camellia sinensis*] (ACX42261.1)	AT3G12500.1	681	$3.00e^{-112}$
GT969191	Benzoquinone reductase [*Gossypium hirsutum*] (ABN12321.1)	AT4G27270.1	542	$2.00e^{-83}$
GT969274	Ubiquitin-conjugating enzyme [*Hyacinthus orientalis*] (AAT08675.1)	AT1G14400.1	395	$1.00e^{-50}$
GT969166	Profilin-3 [*Hevea brasiliensis*] (Q9M7N0.1)	AT2G19760.1	665	$2.00e^{-60}$
GT969278	Nam-like protein [*Camellia sinensis*] (ACH87170.1)	AT1G77450.1	449	$7.00e^{-39}$
GT969253	Heat shock protein 70 [*Nicotiana tabacum*] (CAA44820.1)	AT1G16030.1	620	$3.00e^{-87}$
GT969292	Sugar transporter [*Citrus unshiu*] (AAN86062.1)	AT1G67300.1	589	$4.00e^{-69}$
GT969312	Glycosyl transferase family 17 protein [*Arabidopsis thaliana*] (NP_189391.1)	AT1G12990.1	668	$7.00e^{-79}$
GT969323	Arginine decarboxylase [*Humulus lupulus*] (ACX56223.1|)	AT4G34710.1	748	$4.00e^{-92}$
GT969254	Adenosine kinase [*Arabidopsis thaliana*] (NP_181262.1)	AT2G37250.1	627	$3.00e^{-49}$
GT969196	Potassium channel beta, putative [*Ricinus communis*] (XP_002518142.1)	AT1G04690.1	705	$1.00e^{-65}$
GT969079	Remorin, C-terminal region [*Medicago truncatula*] (ABN08208.1|)	AT2G41870.1	337	$1.00e^{-16}$
GT969087	L-Aspartate [*Arabidopsis thaliana*] (NP_196713.1)	AT5G11520.1	539	$1.00e^{-33}$
GT969206	ATP-binding protein, putative [*Ricinus communis*] (XP_002531014.1)	AT5G16590.1	391	$3.00e^{-22}$
GT969062	Metallothionein-like protein [*Camellia sinensis*] (ABD97882.1|)	AT1G07600.1	397	$2.00e^{-10}$
GT969219	Hexose transporter 1 [*Solanum lycopersicum*] (ACX47459.1)	AT1G11260.1	510	$5.00e^{-32}$
GT969220	Metal ion-binding protein, putative [*Ricinus communis*] (XP_002534419.1)	AT4G38580.1	682	$3.00e^{-67}$
GT969121	Predicted similar to putative ankyrin-repeat protein [*Vitis vinifera*] (XP_002283498.1|)	AT4G35450.2	544	$6.00e^{-60}$
GT969167	Purple acid phosphatase 1 [*Solanum tuberosum*] (AAT37529.1)	AT2G01880.1	518	$2.00e^{-67}$
GT969225	1-deoxy-D-Xylulose 5-phosphate synthase [*Salvia miltiorrhiza*] (ACF21004.1)	AT4G15560.1	424	$3.00e^{-43}$
GT969021	Zinc finger protein [*Camellia sinensis*] (ABI31653.1)	AT1G51200.2	395	$2.00e^{-32}$
GT969228	Calmodulin-related protein [*Arabidopsis thaliana*] (AAM67124.1)	AT1G66400.1	302	$6.00e^{-17}$

Table 4 (continued)

GenBank IDs	Homology	*Arabidopsis* gene loci	Length	e-value
GT969229	Mitochondrial malate dehydrogenase [*Solanum lycopersicum*] (AAU29198.1)	AT3G15020.1	625	$2.00e^{-73}$
GT969120	Ozone-responsive stress-related protein, putative [*Arabidopsis thaliana*] (NP 171625.1)	AT1G01170.1	362	$2.00e^{-19}$
GT969241	Mitogen-activated protein kinase [*Citrus sinensis*] (ABM67698.1)	AT3G45640.1	717	$7.00e^{-75}$
GT969245	Lactoylglutathione lyase, putative [*Ricinus communis*] (XP_002518470.1)	AT1G11840.2	536	$1.00e^{-91}$
GT969137	Phosphate high response [*Phaseolus vulgaris*] (ACD13206.1|)	AT2G01060.2	696	$3.00e^{-57}$
GT969248	Glyceraldehyde 3-phosphate dehydrogenase [*Daucus carota*] (AAR84410.2)	AT3G04120.1	359	$2.00e^{-09}$
GT969216	Thioredoxin peroxidase [*Capsicum annuum*] (AAL35363.2)	AT1G65970.1	307	$600e^{-20}$
GT969214	o-Methyltransferase, putative [*Ricinus communis*] (XP_002514167.1)	AT4G26220.1	595	$6.00e^{-67}$
GT969190	CAXIP1 protein [*Arabidopsis thaliana*] (AAO19647.1)	AT3G54900.1	549	$2.00e^{-35}$
GT969184	AP-2 complex subunit beta-1, putative [*Ricinus communis*] (XP_002523245.1)	AT4G23460.1	583	$2.00e^{-44}$
GT969202	ATPase activator/ chaperone binding [*Arabidopsis thaliana*] (NP_200619.1)	AT5G58110.1	682	$2.00e^{-53}$
GT969208	Nucleolysin tia-1, putative [*Ricinus communis*] (XP_002529199.1)	AT3G14100.1	484	$3.00e^{-69}$
GT969135	Arginine/serine-rich splicing factor, putative, expressed [*Oryza sativa*] (ABF96333.1)	AT5G64200.1	511	$2.00e^{-14}$
GT969163	Selenium binding [*Arabidopsis thaliana*] (NP_190314.2)	AT3G47300.1	464	$5.00e^{-19}$
GT969217	NADK2; NAD+kinase/calmodulin binding [*Arabidopsis thaliana*] (NP_564145.1)	AT1G21640.1	446	$300e^{-14}$
GT969048	Galactolipase/phospholipase [*Arabidopsis thaliana*] (NP_176378.4|)	AT1G61850.1	375	$5.00e^{-26}$
GT969192	CMP-sialic acid transporter, putative [*Ricinus communis*] (XP_002523445.1)	AT4G35335.1	501	$200e^{-48}$
GT969189	Cytochrome c oxidase subunit 3 [*Carica papaya*] (YP_002608210.1)	AT2G07687.1	263	$4.00e^{-20}$
GT969255	skp1 [*Medicago sativa*] (AAD34458.1)	AT1G20140.1	573	$7.00e^{-13}$
GT969199	ATVAMP726 [*Arabidopsis thaliana*] (NP_171968.1)	AT1G04760.1	178	$2.00e^{-15}$
GT969256	Nuclear protein skip, putative [*Ricinus communis*] (XP_002530607.1|)	AT1G77180.1	603	$4.00e^{-74}$
GT969270	AAA-type ATPase family protein [*Arabidopsis thaliana*] (NP_192327.3)	AT4G04180.1	487	$2.00e^{-22}$
GT969200	U1snRNP-specific protein, U1A [*Solanum tuberosum*] (CAA90282.1)	AT2G47580.1	368	$200e^{-40}$
GT969161	Global transcription factor group [*Populus trichocarpa*] (XP_002313759.1)	AT4G08350.1	832	$2.00e^{-118}$
GT969275	Phosphatidylinositol synthase, putative [*Ricinus communis*] (XP_002533188.1)	AT1G68000.1	629	$5.00e^{-26}$
GT969221	Nucleotide-binding protein, putative [*Ricinus communis*] (XP_002526531.1)	AT2G20330.1	371	$9.00e^{-18}$
GT969282	Mago nashi-like protein 1 [*Physalis pubescens*] (ABQ11262.1)	AT1G02140.1	595	$2.00e^{-24}$
GT969306	Translation initiation factor, putative [*Ricinus communis*] (XP_002514839.1)	AT5G36230.1	709	$3.00e^{-82}$
GT969307	Nucleoside diphosphate kinase [*Nicotiana tabacum*] (Q56E62.1)	AT4G09320.1	678	$2.00e^{-72}$
GT969291	Steroid-binding protein, putative [*Ricinus communis*] (XP_002510808.1)	AT3G48890.1	351	$1.00e^{-19}$
GT969350	Inosine monophosphate dehydrogenase [*Vigna unguiculata*] (AAO40253.1)	AT1G16350.1	586	$2.00e^{-40}$
GT969218	F-box family protein [*Populus trichocarpa*] (XP_002308717.1)	AT2G02310.1	507	$4.00e^{-05}$
GT969296	Cytidylyltransferase family [*Arabidopsis thaliana*] (NP_175708.2)	AT1G53000.1	707	$5.00e^{-71}$
GT969127	Histone H3.2 [*Arabidopsis thaliana*] (NP_001078516.1)	AT4G40030.2	664	$2.00e^{-70}$
GT969320	Nudix hydrolase, putative [*Ricinus communis*] (XP_002512761.1)	AT1G79690.1	741	$6.00e^{-78}$
GT969301	GID1-5 [*Gossypium hirsutum*] (ACN86360.1)	AT5G27320.1	424	$1.00e^{-29}$
GT969305	Radical-induced cell death 1 [*Arabidopsis thaliana*] (NP_564391.1)	AT2G35510.1	646	$2.00e^{-17}$
GT969309	Protein YIPF1, putative [*Ricinus communis*] (XP_002512371.1)	AT5G27490.1	762	$4.00e^{-77}$
GT969223	RNA-dependent RNA polymerase [*Populus trichocarpa*] (XP_002308662.1)	AT3G49500.1	554	$6.00e^{-15}$
GT969314	Predicted similar to putative isopropylmalate synthase [*Vitis vinifera*] (XP_002268942.1)	AT1G74040.1	497	$5.00e^{-21}$
GT969315	Glycosyltransferase UGT72B11 [*Hieracium pilosella*] (ACB56923.1)	AT4G01070.1	680	$4.00e^{-14}$
GT969321	Non-canonical ubiquitin conjugating enzyme, putative [*Ricinus communis*] (XP_002519212.1)	AT3G17000.1	562	$4.00e^{-76}$
GT969175	Calcium-binding EF hand family protein [*Arabidopsis thaliana*] (NP_173582.2)	AT1G21630.1	366	$2.00e^{-32}$
GT968799	Interferon-induced guanylate-binding protein, putative [*Ricinus communis*] (XP_002509420.1)	AT5G46070.1	509	$6.00e^{-07}$
GT969155	Transport protein particle (TRAPP) component Bet3 family protein [*Arabidopsis thaliana*] (NP 187151.1)	AT3G05000.1	523	$9.00e^{-30}$

Table 4 (continued)

GenBank IDs	Homology	*Arabidopsis* gene loci	Length	e-value
GT969335	gamma-Glutamylcysteine synthetase [*Zinnia elegans*] (BAD27390.1)	AT4G23100.1	422	$2.00e^{-73}$
GT969339	Salicylic acid-binding protein 2 [*Nicotiana tabacum*] (gb|AAR87711.1|)	AT2G23620.1	492	$4.00e^{-51}$
GT969234	Protein kinase APK1A, chloroplast precursor, putative [*Ricinus communis*] (XP_002531440.1)	AT2G28930.2	463	$9.00e^{-70}$
GT969338	FUS-interacting serine-arginine-rich protein 1, putative [*Ricinus communis*] (XP_002520437.1)	AT1G07350.1	608	$9.00e^{-37}$
GT969303	delta DNA polymerase, putative [*Ricinus communis*] (XP_002524033.1)	AT1G09815.1	445	$7.00e^{-21}$
GT969284	Leucine-rich repeat family protein [*Glycine max*] (ACM89476.1)	AT1G07650.1	699	$5.00e^{-38}$
GT969280	Light-inducible protein ATLS1 [*Elaeis guineensis*] (ACF06473.1)	AT5G01650.1	512	$7.00e^{-19}$
GT969147	Alkaline alpha-galactosidase [*Pisum sativum*] (ABR19752.1)	AT3G57520.3	382	$1.00e^{-65}$
GT969360	Putative oligouridylate-binding protein [*Prunus dulcis*] (ABR13303.1)	AT3G14100.1	684	$6.00e^{-78}$
GT969091	Light-harvesting complex II protein Lhcb5 [*Populus trichocarpa*] (XP_002329192.1)	AT4G10340.1	708	$4.00e^{-65}$
GT969364	ADP-ribosylation factor [*Elaeis guineensis*] (ACF06579.1)	AT3G62290.1	390	$1.00e^{-32}$
GT969233	SGT1 [*Solanum tuberosum*] (AAU04979.1)	AT4G11260.1	582	$6.00e^{-35}$
GT969156	Trafficking protein particle complex subunit 6b, putative [*Ricinus communis*] (XP_002512289.1)	AT3G05000.1	522	$9.00e^{-30}$
GT969374	cdk8, putative [*Ricinus communis*] (XP_002518859.1|)	AT5G63610.1	355	$6.00e^{-12}$
GT969224	2-Oxoglutarate dehydrogenase, E1 subunit-like protein [*Arabidopsis thaliana*] (CAB75899.1)	AT3G55410.1	687	$1.00e^{-48}$
GT969031	Cysteine proteinase [*Elaeis guineensis*] (ABR19827.1)	AT1G47128.1	690	$1.00e^{-46}$
GT969139	Cullin, putative [*Ricinus communis*] (XP_002522416.1)	AT1G69670.1	505	$2.00e^{-18}$
GT969108	AtPH1-like protein [*Arabidopsis thaliana*] (AAM61053.1)	AT5G05710.1	573	$9.00e^{-46}$
GT969100	Membrane-anchored ubiquitin-fold protein 2 [*Arabidopsis thaliana*] (NP 568315.1)	AT5G15460.2	534	$4.00e^{-36}$
GT969152	ER phosphatidate phosphatase [*Ricinus communis*] (XP_002526289.1)	AT1G15080.1	570	$3.00e^{-52}$
GT969097	Starch phosphorylase L-2 [*Solanum tuberosum*] (P53535.1)	AT3G29320.1	518	$8.00e^{-39}$
GT969076	ECT7 (evolutionarily conserved C-terminal region 6) [*Arabidopsis thaliana*] (NP_001078168.1)	AT3G17330.1	706	$3.00e^{-73}$
GT969330	PRT1 [*Arabidopsis thaliana*] (AAM64697.1)	AT3G24800.1	367	$8.00e^{-24}$
GT969286	Transcription initiation factor IIB [*Glycine max*] (P48513.1)	AT3G10330.1	566	$3.00e^{-43}$
GT969181	ATP-binding cassette sub-family f member 2 [*Oryza sativa indica* group)] (ABR25685.1)	AT5G60790.1	440	$1.00e^{-31}$
GT969119	40S ribosomal protein S19 (RPS19C) [*Arabidopsis thaliana*] (NP_200925.1)	AT5G61170.1	604	$4.00e^{-27}$
GT969334	Phospholipase D alpha [*Vitis vinifera*] (ABC59316.1)	AT3G15730.1	666	$5.00e^{-82}$
GT969072	60S acidic ribosomal protein PO [*Euphorbia esula*] (AAF34767.1)	AT2G40010.1	382	$7.00e^{-21}$
GT969336	Ubiquitin-associated (UBA)/TS-N domain-containing protein [*Arabidopsis thaliana*] (NP_180260.1)	AT2G26920.1	685	$7.00e^{-26}$
GT969242	Histone deacetylase complex subunit SAP18, putative [*Ricinus communis*] (XP_002511764.1)	AT2G45640.1	346	$4.00e^{-29}$
GT969237	ATP binding [*Arabidopsis thaliana*] (NP_172382.4)	AT1G09080.1	407	$8.00e^{-09}$
GT969073	60S Acidic ribosomal protein P0, putative [*Ricinus communis*] (XP_002526873.1)	AT2G40010.1	382	$2.00e^{-21}$

several metabolites and proteins to fight against cellular damages. Under drought stress, genes encoding key enzymes regulating biosynthesis of compatible solutes such as amino acids (e.g. proline) and amines (e.g. polyamines) and a variety of sugars and sugar alcohols (e.g. trehalose, galactinol) play important roles to reduce the damage to plants. Several of these genes introduced into transgenic plants could demonstratively enhance drought stress tolerance (Zhang et al. 2004; Umezawa et al. 2006). In the present study, genes of trehalose (trehalose-6-phosphate synthase), polyamine synthesis (arginine decarboxylase), sugar transporters (hexose transporter, sugar transporter) and several genes of glycolysis pathway (glucose-6-phosphate, glyceraldehyde 3-phosphate dehydrogenase, phosphoenolpyruvate carboxykinase) were induced under drought stress. The trehalose-6-phosphate synthase gene was reported for conferring drought tolerance in cotton (Kosmas et al. 2006). An arginine decarboxylase gene
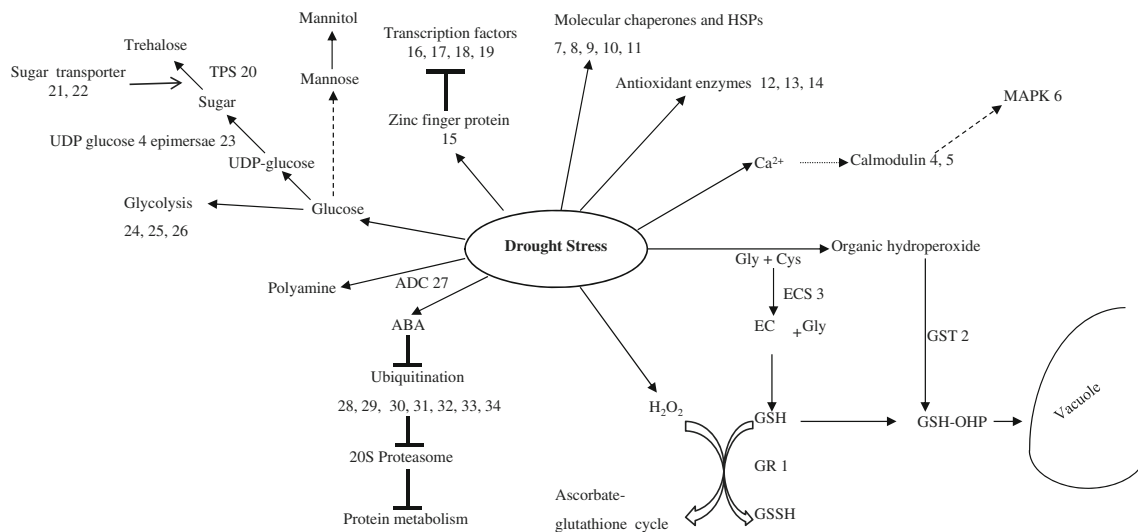
Fig. 5 Scheme of known major metabolic pathways representing drought-induced ESTs: 1=GR (GT969327), 2=GST (GT969164), 3= ECS (GT969335), 4=CML (GT969228), 5=calcium-binding EF hand family protein (GT969175), 6=MAPK (GT969241), 7=LEA (GT969372), 8=dehydrin (GT969343), 9=HSP 83 (GT969070), 10= Mt HSP (GT969378), 11=HSP 70 (GT969253), 12=SOD (GT969381), 13=POX (GT969365), 14=thioredoxin peroxidase (GT969216), 15= zinc finger protein (GT969021), 16=transcription initiation factor IIB (GT969286), 17=global transcription factor group (GT969161), 18=

ethylene-responsive element-binding protein (GT969363), 19=NAM-like protein (GT969278), 20=TPS (GT969159), 21=sugar transporter (GT969292), 22=hexose transporter (GT969219), 23=UDP-glucose 4-epimerase (GT969354), 24=glucose-6-phosphate (GT969341), 25= glyceraldehyde 3-phosphate dehydrogenase (GT969248), 26=phospho-enolpyruvate carboxykinase (GT969259), 27=ADC (GT969323), 28= ubiquitin (GT969157), 29=F-box (GT969218), 30=skp1 (GT969255), 31=cullin (GT969138 and GT969139), 32=ring finger (GT969226), 33=E2 (GT969274), 34=polyubiquitin (GT969158)
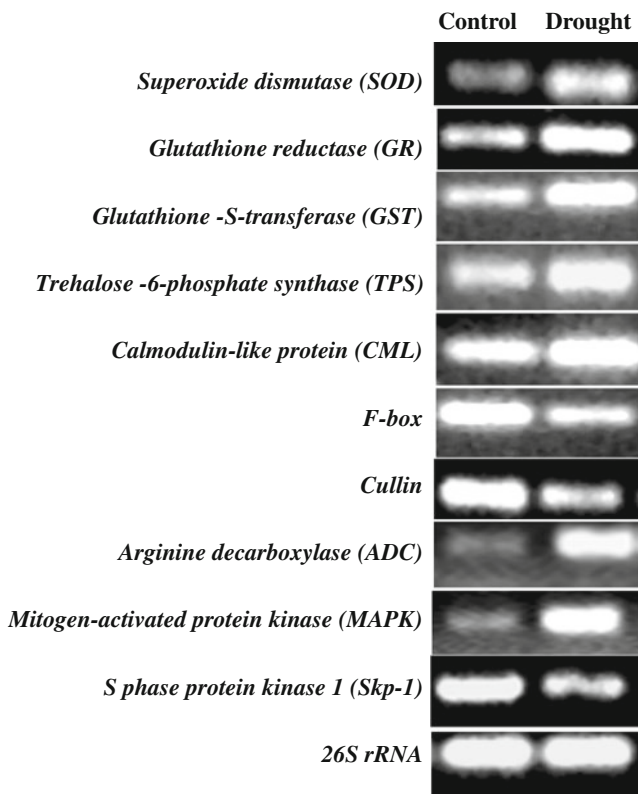
Fig. 6 Verification of SSH library results by RT-PCR analysis. The plants were drought-induced for 21 days. 26S rRNA used as loading control

(*PtADC*) from *Poncirus trifoliata* demonstrated abiotic stress tolerance and promotes primary root growth in *Arabidopsis* (Wang et al. 2010). Prior analysis also demonstrated that drought stress induced the up-regulation of genes encoding enzymes involved in the glycolytic pathway in tobacco and foxtail millet (Rizhsky et al. 2002; Zhang et al. 2007). One of the possible reasons for the up-regulation of genes involved in glycolysis may be to generate more ATP, which is the primary source of energy for cellular metabolism.

Plants have evolved a complex signalling network for the perception of and responses to different abiotic stresses. A generic signal transduction pathway starts with signal perception, followed by the generation of second messengers. Second messengers can modulate intracellular Ca^{2+} levels, often initiating a protein phosphorylation cascade that finally targets proteins directly involved in cellular protection or transcription factors controlling specific sets of stress-regulated genes (Xiong et al. 2002). Ca^{2+} signals are deciphered by various Ca^{2+}-binding proteins that convert the signals into a wide variety of biochemical changes. The calmodulin-like proteins (CMLs) are a group of calcium-binding proteins with two to six predicted EF hand motif. There are 50 CML genes identified in *Arabidopsis* genome (Ranty et al. 2006). Recently, a novel calmodulin-like gene, *OsMSR2*, was demonstrated for drought and salt tolerance in *Arabidopsis* (Xu et al. 2011a). A number of plant protein kinases have now also been found to be activated by drought stress (Zhu 2002). MAPK was serine/

threonine protein kinase, which was known to be one of the major pathways by which extracellular signals such as growth factors, hormones and abiotic stress stimuli were transduced into intracellular responses in plants (Munnik and Meijer 2001). An enhanced tolerance to drought and salt stress was reported by overexpressing a MAPK gene, *CsNMAPK*, in tobacco plant (Xu et al. 2010). In our SSH library, *CLM*, *MAPK*, protein kinase *APK1A*, NAD+kinase, *cdk8* and nucleoside diphosphate kinase were induced indicating that these genes may play important roles in transduction of signalling during drought stress in tea plants. Furthermore, the CLM and MAPK genes were also found to be up-regulated.

The ATPases, water channel proteins and ion transporters play a crucial role in maintaining ion homeostasis under drought stress. The proton electrochemical gradient formed by the vacuolar ATPase provides the primary driving force for the transport of numerous ions and metabolites against their electrochemical gradients which is the fundamental requirement of many cellular processes, such as osmoregulation, signal transduction and metabolic regulation (Santos 2006). Late embryogenesis abundant (LEA) proteins are involved in protecting from damage caused by environmental stresses, especially drought (Goyal et al. 2005). Heat-shock proteins (Hsps) or chaperones such as Hsp70 and Hsp90 are responsible for protein folding, assembly, translocation and degradation in many normal cellular processes; stabilise proteins and membranes; and are actively involved in protein refolding under stress conditions including drought (Wang et al. 2004; Liu et al. 2011). Metallothionein, a superfamily of low molecular weight proteins that are involved in metal detoxification and scavenging of oxygen-free radicals, can decrease injury caused due to drought stress (Talame et al. 2007). Phospholipase D and its products, phosphatidic acid, exert their effect by functioning in signal transduction cascades and by influencing the biophysical state of lipid membranes. It has been implicated in multiple plant stress responses in plants including drought (Bargmann and Munnik 2006). In our SSH library, vacuolar ATPase, dehydrin, *LEA*, Hsp70, Hsp90, metallothionein and phospholipase D were obtained indicating the roles of these genes in maintaining ion homeostasis in tea plants under drought stress.

Plants have developed an efficient antioxidative system comprising enzymatic and non-enzymatic components to fight against ROS during stress. The *SOD* is a well-studied antioxidative enzyme providing the first line of cellular defence against oxidative stress by early scavenging of superoxide radicals and then converting them to hydrogen peroxide (Chatzidimitriadou et al. 2009). The peroxidases catalyse the conversion of hydrogen peroxide to water using ascorbate, glutathione or thioredoxin as substrate (Rossel et al. 2006). In our SSH library, SOD, peroxidase and thioredoxin peroxidase were obtained. Expression of *SOD* demonstrated higher

drought tolerance and shoot regeneration in transgenic pepper (Chatzidimitriadou et al. 2009). Glutathione (GSH) is an important non-enzymatic antioxidant which reduces the active oxygen radicals generated due to stress (Shao et al. 2008). Glutathione is synthesised from its constituent amino acids in an adenosine triphosphate (ATP)-dependent, two step reaction catalysed by the enzymes γ-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase. Glutathione reductase (GR) mediates the reduction of GSSG to GSH by using NADPH as an electron donor, and thus a highly reduced state of GSH/GSSG ratio is maintained during stress (Carvalho and Contour-Ansel 2008). Overexpression of *GR* for conferring drought tolerance was reported earlier in cowpea and common bean (Carvalho and Contour-Ansel 2008; Torres-Franklin et al. 2008). Glutathione S-transferase (GST) detoxifies organic hydroperoxides generated under dehydration stress and removed by covalently linking glutathione to a hydrophobic substrate, forming less reactive and more polar glutathione S-conjugate (Neuefeind et al. 1997). Overexpression of the *GST* gene under drought condition was demonstrated in transgenic tobacco plant transformed with a *GsGST* from wild soybean (Ji et al. 2010). The up-regulation of *SOD*, *GR* and *GST* genes in the present investigation corroborated with the earlier reports cited above and suggested the role of enzymatic antioxidants in drought tolerance mechanism in tea plants.

TFs have also been proven quite useful in improving stress tolerance in transgenic plants, through influencing expression of a number of stress-associated target genes (Shinozaki and Yamaguchi-Shinozaki 2007). There are many TFs that have been successfully demonstrated to play key regulatory roles in drought tolerance. Three *Arabidopsis* NAC genes, ANAC019, ANAC055 and ANAC072, were shown to bind to the promoter region of ERD1 which was characterised as a stress-responsive gene and overexpression of these three genes in *Arabidopsis* resulted in enhanced tolerance to drought stress (Tran et al. 2004). Transgenic tobacco plants expressing SodERF3 of sugarcane under the transcriptional control of constitutive *CaMV* 35S promoter showed remarkably enhanced drought tolerance (Trujillo et al. 2009). Overexpression of a NAM, ATAF, and CUC (NAC) transcription factor was reported for the enhancement of drought resistance and salt tolerance in rice (Hu et al. 2006). In our SSH library, the presence of several transcripts of TFs indicates their possible role in drought tolerance mechanism of tea plants.

The response to drought stress involves not only the up-regulation of genes, but also the down-regulation of functional genes. For the last several years, much research has been focussed on how the relevant genes are up-regulated during drought stress. There were 79 genes found to be down-regulated during drought in *Arabidopsis* (Seki et al. 2002). As such, there were 37 genes found to be down-regulated during drought in foxtail millet (Zhang et al.

2007). In our SSH library, 10 unigenes of proteasome degrading pathways were induced which include ubiquitin conjugating enzyme (E2), ubiquitin, polyubiquitin, ubiquitin ligase (E3), f-box, skp1, cullin, ubiquitin specific protease, 26S proteasome and zinc finger protein. Possible expression of three of them, i.e. f-box, cullin and skp1, was validated and found to be down-regulated. These three genes are the components of the SCF complex of E3 ligase. Out of all the ubiquitin ligases, SCF class of E3 ligases has been thoroughly studied in plants (Ciechanover et al. 2000). However, whether these genes are false positive in our SSH library or play an important role for drought tolerance by down-regulating their expression in tea plants need to be further confirmed. It was reported that transgenic tobacco with wheat (*Triticum aestivum*) ubiquitin gene (*Ta-Ub2*) demonstrated to be an effective strategy for enhancing drought tolerance (Guo et al. 2009).

The conclusion of this study is that, in addition to the expression of several well-characterised drought-responsive genes, a high-level constitutive expression of molecular chaperone and heat shock proteins, transcription factor and proteinase inhibitor seems to be a major factor in rendering tea plant resistant to drought. This study is the first global analysis of transcripts in tea under drought stress. The challenge remains to demonstrate which (if any) of these genes has the potential to improve the drought tolerance of plants, or alternatively, if they can be used to develop useful markers for drought resistance in the tea-breeding program. The ESTs identified in this study should provide a useful genomic resource for biologists and plant breeders in developing new strategies for improving drought-tolerant tea cultivar.

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