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Research Article

Transcriptome-based identification of water-deficit stress responsive genes in the tea plant, *Camellia sinensis*

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Abstract A study aimed at identifying putative drought responsive genes that confer tolerance to water stress deficit in tea plants was conducted in a 'rain-out shelter' using potted plants. Eighteen months old drought tolerant and susceptible tea cultivars were each separately exposed to water stress or control conditions of 18 or 34% soil moisture content, respectively, for three months. After the treatment period, leaves were harvested from each treatment for isolation of RNA and cDNA synthesis. The cDNA libraries were sequenced on Roche 454 high-throughput pyrosequencing platform to produce 232,853 reads. After quality control, the reads were assembled into 460 long transcripts (contigs). The annotated contigs showed similarity with proteins in the *Arabidopsis thaliana* proteome. Heat shock proteins (*HSP70*), superoxide dismutase (SOD), catalase (*cat*), peroxidase (*PoX*), calmodulin-like protein (*Cam7*) and galactinol synthase (*Gols4*) drought-related genes were shown to be regulated differently in tea plants exposed to water stress. *HSP70* and *SOD* were highly expressed in the drought tolerant cultivar relative to the susceptible cultivar under drought conditions. The genes and pathways identified suggest efficient regulation leading to active adaptation as a basal defense response against water stress deficit by tea. The knowledge generated can be further utilized to better understand molecular mechanisms underlying stress tolerance in tea.

Keywords Water stress, Transcriptome, Pyrosequencing, Gene ontology, *De novo*, Assembly

Introduction

Tea (*Camellia sinensis* L. (O) Kuntze) leaf extracts are among the most widely consumed beverages in the world (Cabrera et al. 2003). Popularity of this beverage is ascribed to its aroma, pleasant taste and medicinal benefits, the latter conferred by its potent antioxidant activity (Frei and Higdon 2003; Lin et al. 2003; Karori et al. 2007). In several African and Asian countries including Kenya, tea is an economically important crop contributing significantly to foreign exchange earnings and rural development (Wachira and Ronno 2004). Tea plants grow as an evergreen bush that can attain a height of up to 15 m in the wild but the commercial crop is maintained at 0.6 ~ 1.0 m high to facilitate harvesting of the leaves. Cultivated tea is diploid (2n=2x=30) (Bezbaruah 1971; Kondo 1977) with a relatively large genome size of about 4.0 GB (Tanaka and Taniguchi 2006) though it forms a stable polyploid series (Wachira and Nge'tich 1999).

Tea is largely grown under rain-fed conditions where it flourishes in conditions characterized by well distributed rainfall of 1150 ~ 1400 mm per year (Carr 1972). With climate change, most of the tea growing areas in the world are increasingly getting prone to water stress with drought now associated with 14 ~ 20% reduction in yield (Ngetich et al. 2001) and 6 ~ 19% plant mortality (Cheruiyot et al. 2007) in Kenya. The tea plant naturally responds to drought at the physiological (Shakeel et

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al. 2011; Maritim et al. 2015), biochemical (Chaves et al. 2003; Xu et al. 2009; Cheruiyot et al 2007; Maritim et al. 2015) and molecular levels (Bartels and Sunkar 2005; Shinozaki et al. 2003). Since plant responses are controlled by the genome, many studies in plants are now increasingly focusing on molecular response to stress (Mohammad and Lin 2010). To better understand the mechanisms involved in molecular responses to water stress, genes responsible for such responses must be characterized. Studies have shown that several classes of genes including those responsible for regulation, signalling and cellular adaptation are induced in plants in response to water deficit (Mohammad and Lin 2010). The genetic basis of drought tolerance in *C. sinensis* is however poorly understood with limited amount of data available in the databases. Three (3) drought responsive expressed sequence tags (ESTs) from tea leaf tissues have been reported using the differential display technique (Sharma and Kumar 2005). In addition, 572 ESTs from young roots of drought tolerant tea cultivars have been identified using the suppression subtractive hybridization (SSH) technique (Das et al. 2012). Muoki et al. (2012), also reported ESTs responsible for cell rescue, defense, cellular transport, metabolism, energy, protein synthesis, cell cycle and DNA processing, signal transduction, transcription and biogenesis of cellular components in drought stressed tea. In their study, Muoki et al. (2012) established that chaperones and defence related genes, traumatin like proteins, chitinase and heat shock proteins, were more expressed in the leaf tissues of drought tolerant cultivars than in the susceptible ones. Indeed, sequencing of cDNA library clones and generation and analysis of ESTs provides a rapid, low cost and efficient way to identify functional genes in tea. Generally, 7,084 drought related ESTs in tea, one genome survey sequence, 158 sequences reads archives (SRA), and 38,789 proteins have been deposited in the Gene Bank. However, much more needs to be done to fully elucidate the molecular basis of drought tolerance in tea. A better understanding of the genetic basis of drought tolerance in tea is essential in enhancing the efficiency and effectiveness of tea breeding programs. The present study was therefore designed to identify water deficit stress-responsive gene(s) in two tea cultivars widely grown in Kenya and to facilitate future research and development of drought tolerant tea cultivars.

Materials and Methods

Plant materials

The experiment was carried out in a rain-out shelter as described by Maritim et al. (2015). In brief, two cultivars, drought tolerant (cultivar TRFCA SFS150) and drought susceptible (cultivar AHP S15/10) were selected based on their phenotypic traits as earlier established by breeders using yield stability scores during drought periods (Kamunya et al. 2009) and their physiological and biochemical responses to soil water deficit (Maritim et al. 2015). The potted plants were allowed to establish for two months before they were transferred to the rain-out shelter and were arranged according to treatments. For molecular studies, the two extreme soil moisture content (SMC) treatments of 34% v/v (high soil moisture/field capacity) and 18% v/v (low soil moisture) were used in the study.

Sampling and Extraction of RNA from *C. sinensis* leaves

The third and fourth leaves (n = 10) of fresh shoots were randomly selected and separately harvested from each treatment and immediately snap frozen in liquid nitrogen. Total RNA was isolated from each of the frozen (100 mg) and grounded leaf samples using the ZR Plant RNA Miniprep Kit (Zymo Research, Irvine, CA, USA). Subsequently, mRNA was isolated from the total RNA using mRNA Isolation Kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. In all cases, the integrity of extracted RNA was validated by electrophoresis in 1.0% agarose (Sigma-Aldrich Chemie, Gmbh) RNA denaturing gel in 1.4% sodium phosphate with 1 μg/ml ethidium bromide staining for visualization. The concentration of total RNA and mRNA was determined spectroscopically (Sambrook et al. 1989) using a 2000-NanoDrop spectrophotometer (Thermo Fisher Scientific, DE, USA).

Preparation and pyrosequencing of *C. sinensis* cDNA library

The cDNA libraries were synthesized from the isolated mRNA using a cDNA Rapid Library Preparation kit for GS FLX Titanium Series (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. The products were purified to remove fragments less than 50 bp long using Individual Sample Cleanup (ISC) sizing solution. The cDNA libraries were subsequently quantified and assessed for quality using a TBS 380 Fluorometer (Turner Biosystems, USA) and Agilent Bioanalyzer High Sensitivity DNA chip (Agilent Technologies, Germany), respectively. Additionally, clonal amplification of the product was done through emulsion PCR (emPCR) using the emPCR Kit for GS FLX Titanium series (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. The PCR program used comprised: 1 cycle at 94°C for 4 minutes, 50 cycles at 94°C for 30 seconds, 58°C for 4.5 minutes, and 68°C for 30 seconds, followed by a 10°C hold. The library of clonally amplified DNA fragments for each treatment and replicate were subsequently loaded onto a

PicoTiterPlate™ (Roche Applied Science, Mannheim, Germany) and separately sequenced on a half-plate run on a 454 GS FLX Titanium Series sequencer. The emergent data were processed using GS FLX gsRunBrowser version 2.5.3 (Roche Applied Science, Mannheim, Germany) to obtain 454 sequence FASTA files (sff) with quality scores.

Sequence processing and analysis

The raw reads were processed by removing adaptor sequences, redundant reads and those containing more than 10% N (ambiguous bases in reads), and low-quality reads (containing more than 50% bases with Q-value < 20). The quality of the reads data was assessed based on base-calling quality scores using FastQC software version 0.10.1, (Babraham Bioinformatics, UK). The reads were subsequently de novo assembled using Newbler program version 1.03 (Roche Applied Science, Mannheim, Germany). All the assembled contigs longer than 100 bp were annotated by BLAST analysis (Altschul et al. 1997) against similar proteins in the *Arabidopsis thaliana* proteome (https://www.arabidopsis.org/), and BLASTx routine with E-value threshold of10^{-5} in Blast2GO software (Conesa et al. 2005) against NCBI Gene Bank non-redundant (nr) (http://www.ncbi.nlm.nih.gov), and Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg) databases, to determine category and metabolic pathway ontologies of the differentially induced genes between the treatments. Variation in expression of potential genes was achieved by generation of a heat map that clearly distinguished gene expression levels.

Results and Discussion

Quality control and summary statistics of the sequences

The cDNA libraries synthesised from the isolated mRNA produced thick band between 600 and 1200 bp (Fig. 1)

Overall, 232,385 reads were generated from the four cDNA libraries. The read-lengths ranged from 40 -1143 bp and averaged 369 bp. FastQC analysis also revealed that all the four libraries had Phred-like quality scores greater than Q20 level (with an error probability of 0.01) (Fig. 2).

All high-quality reads were deposited in the National Center for Biotechnology Information (NCBI) Short Read Archive (SRA) database under the accession number SRX485271. The preprocessed sequences were assembled into 460 contigs of 100 ~ 2,466 bp with majority of the contigs ranging between 100 ~ 500 bp (Fig. 3). The mean length of the contigs was 250bp with 13 contigs being greater than 1kb. The total number of bases in all the contigs was 115,177 with a GC content of 43.9%.

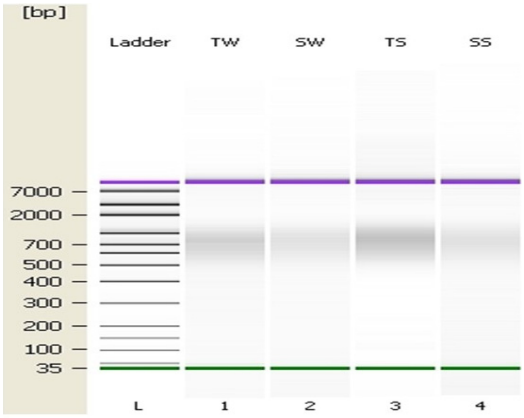


Fig. 1 Gel-like image of the cDNA library samples as run on an Agilent Bioanalyzer High sensitivity DNA chip. The initials; TW = TRFCA SFS150 (Watered), TS = TRFCA SFS150 (stressed), SW= AHP S15/10 (Watered), SS= AHP S15/10 (stressed) are the four libraries synthesised for use in sequencing. The top and bottom distinct band are the upper and lower markers used

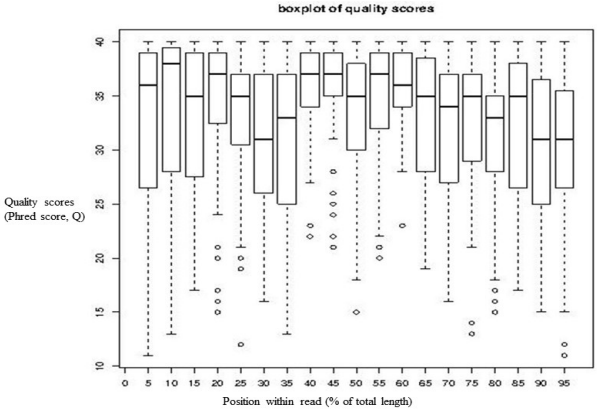


Fig. 2 Box plot showing quality scores of trimmed sequence. The Y-axis shows the quality scores referred to as phred scores (Q) which is equivalent to the probability of errors in a particular base. In the scale used, quality score, Q10, means the probability of an incorrect base call is 1 in 10, Q20 = 1 in 100, Q30 = 1 in 1000. The lowest score was Q25. The X-axis shows the position within the read (0-100% of the total length of read)

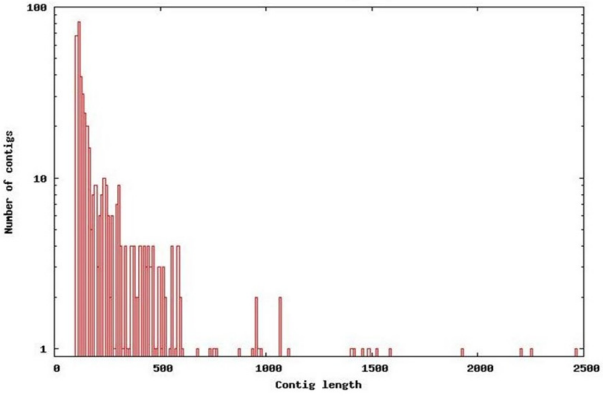


Fig. 3 Size distribution of the contigs generated by de novo assembly of the filtered and trimmed 454 pyrosequence reads

GO and KEGG functional annotation of the tea transcript.

Gene ontology (GO) categorization derived from sequence homology to *A. thaliana* genes revealed three broad categories, 'biological processes', 'cellular component' and 'molecular functions' (Fig. 4). In the biological process category, the 'metabolic processes' related genes were dominant (20%), followed by 'multicellular organismal development' (15%) and 'cellular processes' (12%). About 10% of those in the 'cellular processes' category, represented genes related to 'stimulus response'. The 'secretion' related genes, (0.4%), were the least in this category. This indicates that diverse metabolic processes are active in the *C. sinensis* leaf, and a variety of metabolites are synthesized. The dominance of genes associated with cellular development and response to stimulus is an indication that whereas the plants under high soil moisture content (SMC) are actively growing, the stressed plants induce stimulus response related genes to mediate signaling as a result of exposure to water deficit and other drought related phenomena like heat.

In the 'cellular component category', genes assigned to the 'intracellular region' accounted for the largest group (78%) followed by those of the 'cell part' (2%) whereas genes of the 'extracellular region' were the least (1%). In the 'molecular function' category, the highest percentage was covered by 'binding related genes (43%), followed by the 'catalytic activity' related genes (27%), 'Nucleic acid binding' (10%) and 'structural molecule activity' related genes (10%). The 'signal transduction' (2%) and 'transporter activity' (2%) related genes were the least in this category of genes.

The most dominant biological pathways active in the leaf of *C. sinensis* are presented in Figure 5. Majority of the genes in our study were assigned to 'metabolic pathways' related to energy metabolism/oxidative phosphorylation (53%), photosynthesis (31%), carbohydrate metabolism, and 'nitrogen metabolism' (8%). A significant proportion of the contigs were

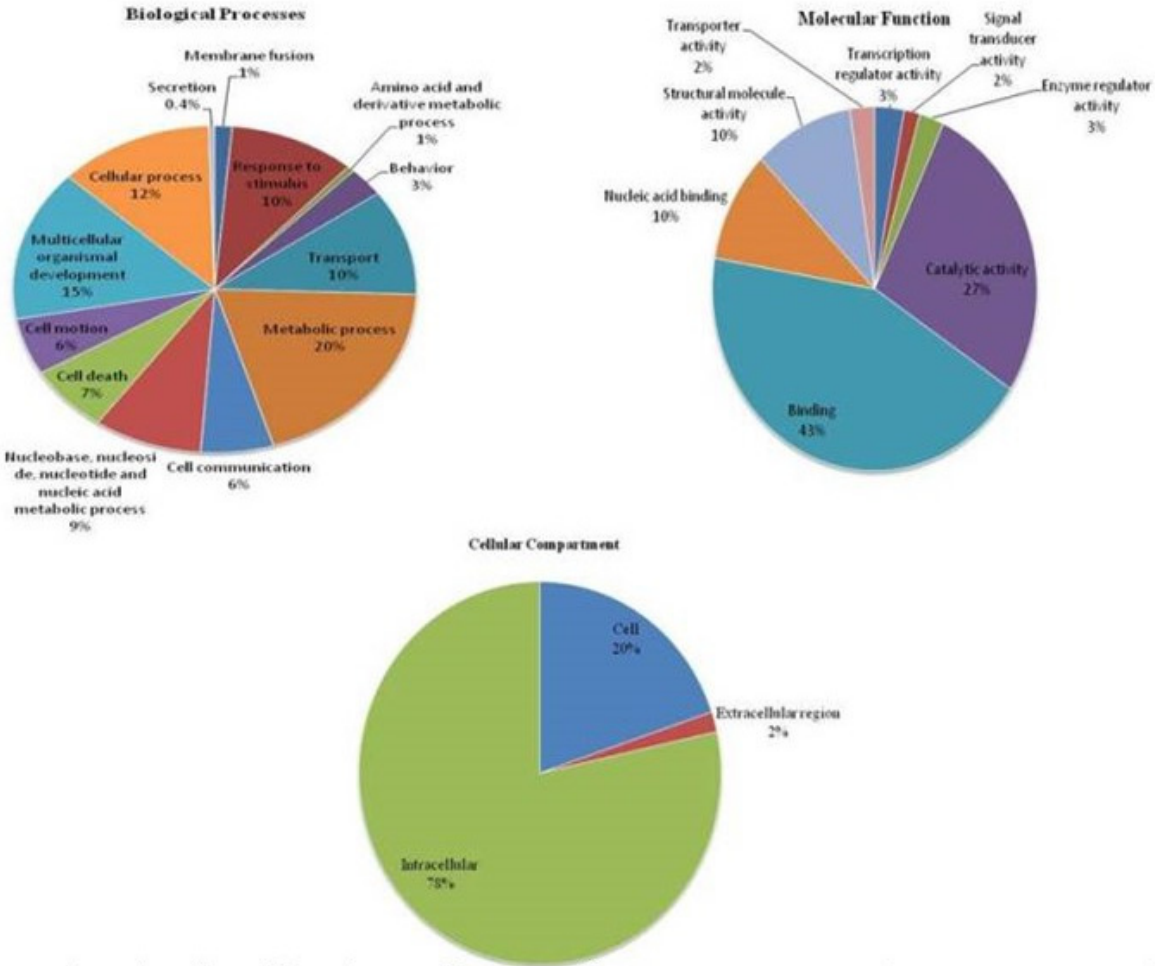


Fig. 4 Gene ontology (GO) classification of *Camellia sinensis* contigs as summarized into Biological processes, Molecular functions and Cellular components. The percentages show the proportion of genes related to various activities within the three main functional categories of genes

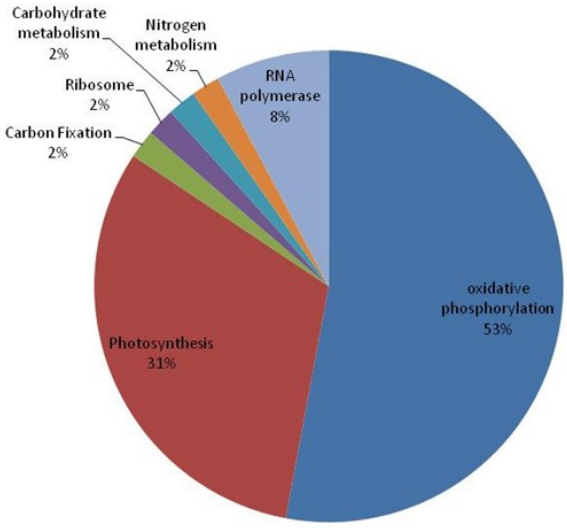


Fig. 5 Biologically active pathways in the leaf transcriptome of tea

related to photosynthetic processes. This can be attributed to the fact that the leaf is the main photosynthetic site in a tea plant. Carbohydrate metabolism and energy metabolism were also represented in the KEGG pathway indicating that many active metabolic processes requiring ATP take place in tea leaf. The leaf acts as the main organ for complex carbohydrate synthesis and energy conversion in plants (Wu et al. 2012).

Water-deficit stress responsive genes in tea

The genes induced by water-deficit as presented in form of a heat map in Figure 6 were classified based on sequence similarity to those in the *Arabidopsis* proteome. Under water stress conditions, the assayed tea plants up-regulated or down-regulated several genes to mitigate against cellular damage. These varied from signaling to defense related genes. The drought sensitive Cultivar, AHP S15/10, showed that genes responsible for defense were down-regulated under low soil moisture content (stressed) conditions. The stressed tea plants showed down-regulation of Heat shock protein related genes (*cpHsc70-1* and *2*), and *Galactinol synthase* related gene (*GolS4*) as compared to the unstressed plant. In this study, the signaling gene '*calmodulin' like protein* (*CAM6*) signal inducer was induced (Fig. 6) in the stressed tea plants. This phenomenon allows the tea plants under stress to transduce calcium ion signals that activate major pathways that convert extracellular signals, such as growth factors, hormones and abiotic stress stimuli into intracellular responses (Munnik and Meijer 2001). *Calmodulin like protein* was expressed at a higher level in the susceptible cultivar, AHP S15/10, as compared to the tolerant cultivar TRFCA SFS150, under water stress conditions. The induction of this gene at various levels in the tea cultivars suggest that it plays a significant role in signal transduction during water stress. Other genes that were minimally expressed under the water stress conditions as compared to control conditions were the *nucleic acid binding protein* and *glyceraldehyde-3-phosphate dehydrogenase*.

Transcripts showing homology to *galactinol synthase* (*Gols*) were down-regulated in the water stressed susceptible cultivar AHP S15/10, suggesting that drought adaptation or tolerance

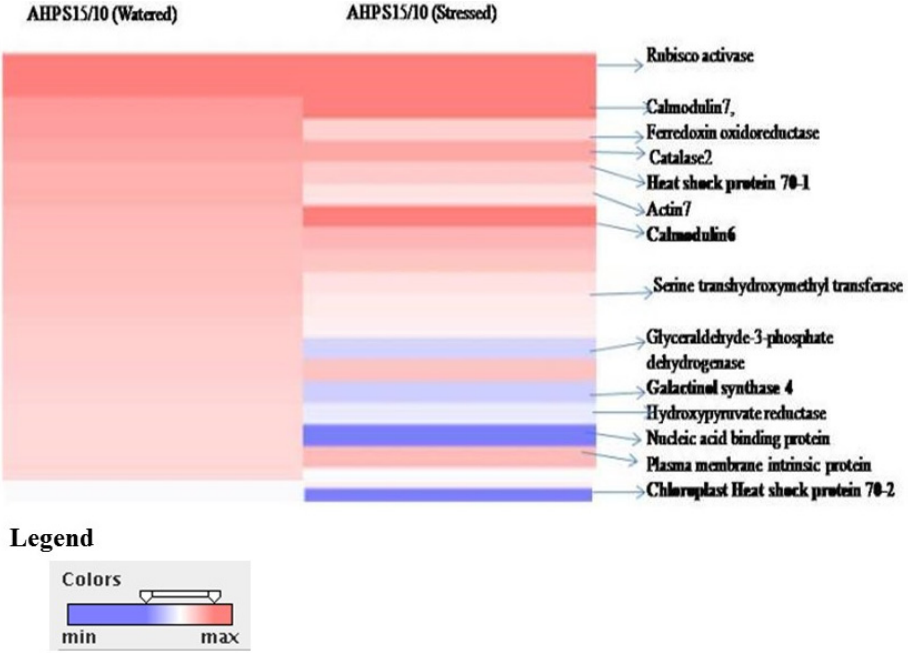


Fig. 6 Heat map of expression pattern of genes in the drought susceptible cultivar (AHP S15/10) with response to water deficit

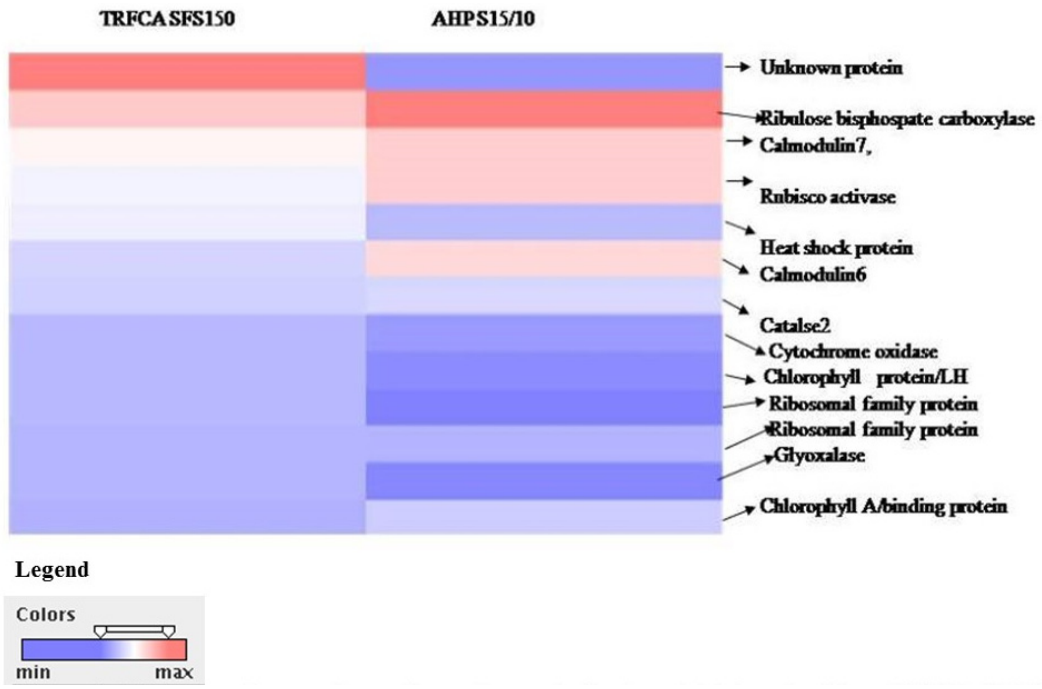


Fig. 7 Heat map of expression pattern of genes in the drought tolerant cultivar TRFCA SFS150 and susceptible AHP S15/10 in response to water deficit

of this cultivar to water stress conditions is limited, a trait that had been determined earlier through morphological studies. *Galactinol synthase* has been established as an osmo-protectant in plants (Nishizawa et al. 2008). Galactinol is induced in plants subjected to drought such as *Cucumis melo* (Volk et al. 2003), *Coffea arabica* L. and *Arabidopsis thaliana* (Taji et al. 2002). The induction of *Gols* in these species confers some level of drought tolerance (Gupta et al. 2012). The expression of *galactinol synthase* gene in the drought tolerant cultivar TRFCA SFS150 corroborated the role of this gene in defense against stress

Comparative expression of potential genes in the stressed tolerant and susceptible cultivars showed various genes expressed and or repressed (Fig. 7). The *CAM7* and *CAM6* signal genes were induced in the susceptible cultivar under water stress conditions. Other than *calmodulin like proteins*, *calcium dependent protein kinase* signalling gene (*CDPK*) was also up-regulated in the water stressed plants. The transcript related to defence against effects of drought like heat shock protein was also induced in the tolerant cultivar TRFCA SFS150 relative to the susceptible cultivar AHP S15/10. Other notably expressed genes were *rubisco activase* and the *ribulose bisphosphate carboxylase* both of which are related to photosynthetic processes. Individual analysis on transcripts from the four libraries showed that transcripts related to *Calatase (Cat2), Peroxidase family protein (PRXR1),* and *Superoxide dismutase* (*SOD2*) were also expressed by the test tea cultivars. The tolerant cultivar TRFCA SFS150, expressed all the three antioxidant molecules whereas the susceptible cultivar AHP S15/10, only expressed *catalase* and *peroxidases*. The levels of expression differed between the two cultivars.

Another category of transcripts that showed homology with heat shock proteins (*HSP70-1*), were induced in the test cultivars under water stress conditions. Heat shock proteins serve as intra-cellular chaperones for other proteins and are also involved in plant stress response (Gupta et al. 2012). *HSPs* are involved in protection by controlling protein folding and protection of macromolecules and membranes from dehydration during drought (Das et al. 2012). Genes encoding *HSPs* have been reported to be up-regulated in drought tolerant Indian tea cultivars subjected to water stress (Muoki et al. 2012). The results generated from this study, corroborated this observation; with the drought tolerant cultivar TRFCA SFS150, showing higher levels of *HSP70-1* when compared with the susceptible cultivar AHP S15/10. This implies that the up-regulation of heat shock proteins, *HSP70-1,* confers drought tolerance. Similar observations have been reported in *Pinus* (Heath et al. 2002) and *Apple* (Wisniewki et al. 2008). On the other hand, the inability of cultivar AHP S15/10 to withstand drought can be attributed to the low level of expression of heat shock proteins in its leaf tissues. Such an observation has been reported in *Populus euphratica* (Bogeat-Tribo et al. 2007).

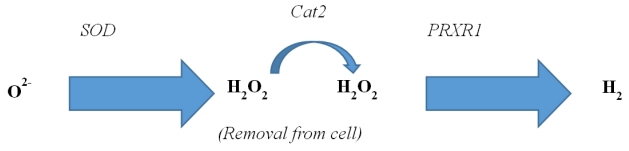
Transcripts showing homology with reactive oxygen scavengers such as peroxidase family protein (*PRXR1*), catalase

Table 1 Showing variation in expression profiles of responsive genes in two different cultivars

Identified genes	TRFCA SFS150 (Tolerant)	AHP S15/10 (Susceptible)
Calatase *(Cat2)*	+	+
Peroxidase family protein *(PRXR1)*	+	+
Superoxide dismutase *(SOD2)*	+	-
Heat shock proteins *HSP70-1*	+	-
Galactinol, synthase, *GOls4*	+	-

(*Cat2*) and superoxide dismutase (*SOD*) were also induced in the assayed tea cultivars in this study. The catalase and peroxidase protein genes were expressed in the drought tolerant cultivar TRFCA SFS150 and the susceptible AHP S15/10 when exposed to water stress conditions. However *SOD* was only expressed in the drought tolerant cultivar under water stress conditions. The accumulation of antioxidant molecules is attributed to their role in scavenging for reactive oxygen species, which damage the photosynthetic machinery in plants (Das et al. 2012). A summarized table of up and down regulated genes is presented in Table 1;

Accumulation of antioxidant molecules such as superoxide dismutase acts as the first line of cellular defense against oxidative stress by catalyzing the dismutation of O_2^- to H_2O_2. The catalases and peroxidases on the other hand catalyse the removal (Chaves et al. 2003) and conversion of H_2O_2 into water (Rossel et al. 2006), respectively as presented below.



Schematic presentation of Enzyme dependent defense against oxidative stress in plants: Where *SOD* is superoxide dismutase, *PRXR1* is peroxidase family protein while *Cat2 is the* catalase enzyme

The existence of a balance between *SOD* and other H_2O_2 scavenging enzymes is crucial in maintaining a steady level of oxidant molecules. Expression of *SOD* has been shown to confer tolerance and enhance shoot regeneration in transgenic pepper under water stress conditions (Chatzidimitriadou et al. 2009). The upregulation of *SOD* in the drought tolerant cultivar TRFCA SFS150 suggests that this antioxidant molecule plays a role in regulating response of tea to drought. The absence of upregulation of *SOD* in the susceptible cultivar AHP S15/10 on the other hand is the probable contributor to its susceptibility to water deficit.

The identified genes in this study are potential targets for developing DNA based markers associated with water deficit response in tea. Use of such molecular markers in breeding and selection can help in identification of traits of interest at early stages of the breeding cycle and hence reduce the breeding period (Shalini et al. 2007). The advantage of this approach is that molecular markers are not influenced by environmental factors and the developmental stage of the plant and therefore can be selected for at any stage of the plants phenology and in any environment. They can also be used to screen for resistance to a stress condition in the absence of the stress factor (Mphangwe et al. 2013). DNA-based molecular markers have been exploited in breeding programmes of various crops. Tea has however not benefited much from this biotechnological advancement. Initially, this approach was considered less applicable to tea because of the limited genetic information that was available in the public domain. Good progress has, however, been made on development of genetic linkage maps and identification of molecular markers associated with various agronomic traits (Hackett et al. 2000; Mphangwe et al. 2013) including work on quantitative trait loci associated with yield (Kamunya et al. 2010) and genetic diversity of tea germplasm (Wachira et al. 1995). However, the molecular markers that have been identified in tea this far are probably still too few considering the big tea genome and therefore necessitate more research work on molecular markers. Development of such markers will help in the identification of drought resistant/tolerant tea cultivars at the early stages of breeding. Using conventional tea breeding approaches, an elite tea variety can take up to 23 years to be developed but with the use of molecular marker techniques, there is likelihood that this period can be reduced by about 10 years.

The present study used only the Assam variety of tea. Further studies therefore need to be carried out to compare the responses of the Cambod and Chinery varieties of tea.

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Data Archiving Statement

All high-quality reads were deposited in the National Center for Biotechnology Information (NCBI) Short Read Archive (SRA) database under the accession number SRX485271

References

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search program. Nucleic Acids Research 25:3389-3402

Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. Critical Reviews in Plant Science 24:23-58

Bezbaruah HP (1971) Cytological investigation in the family Theacea - I. Chromosome numbers in some Camellia species and allied genera. Carylogia 24:421-426

Bogeat-Tribo L, Brosche M, Renaut J, Jouve L, Thiec DL, Fayyaz P, Vinocur B, Witters E, laukens K, Teichmann T, Altman A, Hausman JF, Polle A, Kangasjarvi J, Dreyer E (2007) Gradual soil depletion results in reversible changes of gene expression, protein profiles, ecophysiology and growth perfomance in *Popolus euphratica,* a poplar growing in arid regions. Plant Physiology 143:876-892

Cabrera C, Gimenez R, Lopez MC (2003) Determination of tea components with antioxidant activity. Agricultural and Food Chemistry 51:4427-4435

Carr MKV (1972) The climatic requirements of tea. A review. Experimental Agriculture 8:1-14

Chatzidimitriadou K, Nianiou-Obeedat I, Madesis P, Perl-Treves R, Tsaftari A (2009) Expression of SOD transgene in pepper confer stress tolerance and improve shoot regeneration. Electronic Journal of Biotechnology 12:1-9

Chavez MM, Maroco JP, Pereira JS (2003) Understanding plant response to drought-from genes to the whole plant. Functional Plant Biology 30:239-264

Cheruiyot EK, Mumera LM, Ng'etich WK, Hassanali A, Wachira F (2007) Polyphenols as potential indicators for drought tolerance in Tea (*Camellia sinensis* L. O kuntze). Biosciences Biotechnology Biochemistry 71:2190-2197

Cheruiyot EK, Mumera LM, Ngetich WK, Hassanali A, Wachira FN (2008) Threshold soil water content for growth of tea *(Camellia sinensis (L.) O. Kuntze)*. Tea 29:30-38

Conesa Götz S, Garcia-Gomez JM, Terol J, Talon MR (2005) Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21: 3674-3676

Das A, Das S, Mondal TK (2012) Identification of differentially expressed gene profiles in young roots of tea (*Camellia sinensis* (L.) O. Kuntze) subjected to drought stress using suppression subtractive hybridization. Plant Molecular Biology 30:1088-1101

Frei B, Higdon JV (2003) Antioxidant activity of tea polyphenols *in vivo*: Evidence from animal studies. Journal of Nutrition 133:3275S-3284S

Gupta S, Bharalee R, Bhorali P, Agarwal N, Ahmed P, Saikia H, Borchetia S, Kalita MS, Handique AK, Das S (2012) Identification of drought tolerant progenies in tea by gene expression analysis. Functional Intergrative Genomics 12:543-563

Hackett CA, Wachira FN, Paul S, Powell W, Waugh R (2000) Construction of a genetic linkage map for *Camellia sinensis* (tea). Heredity 85:346-355

Heath LS, Ramakrishnam N, Sederoff RR, Whetten RW, Chevone BL, Struble CA, Jouenne VY, Chen D, Zyl L, Green R (2002) Studying the functional genomics of the stress responses in Loblolly pine with the expression microarray experiment management system. Comprehensive Functional Genomics 3:226-243

Kamunya SM, Wachira FN, Pathak RS, Muoki RC, Wanyoko JK, Ronno WK, Sharma RK (2009) Quantitative genetic parameters in tea (*Camellia sinensis* (L.) O. Kuntze): I Combining abilities for yield, drought tolerance and quality traits. African Journal of Science 3:93-101

Kamunya SM, Wachira FN, Pathak RS, Korir R, Sharma V, Kumar R, Bhardwaj P, Chalo R, Ahuja PS, Sharma RK (2010) Genomic mapping and testing for quantitative trait loci in tea (*Camellia sinensis* (L.) O. Kuntze). Tree Genetics and Genomes 6: 915-926

Karori SM, Wachira FN, Wanyoko JK, Ngure RM (2007) Antioxidant capacity of different types of tea products. African Journal of Biotechnology 6:2287-2296

Kondo K (1977) Chromosome numbers in the genus Camellia. Biotropica 9:86-94

Lin YS, Tsai YJ, Tsay JS, Lin JK (2003) Factors affecting the levels of tea polyphenols and caffeine in tea leave. Journal of Agricultural Food Chemistry 51:1864-1873

Maritim TK, Kamunya SM, Mireji P, Mwendia V, Muoki RC, Cheruiyot EK and Wachira FN (2015) Physiological and biochemical responses of tea (*Camellia sinensis* L. O Kuntze) to water-deficit stress. Journal of Horticultural Science & Biotechnology 90(4):395-400

Mohammad IA, Lin TP (2010) Molecular analysis of dehydration in plants. Plant Science 1:21-25

Mphangwe NI, Vorster J, Steyn M, Nyirenda HE, Taylor NJ, Apostolides Z (2013) Screening of tea (*Camellia sinensis*) for traits associated Molecular markers. Applied Biochemical Biotechnology 171:437-449

Munnik T, Meijer H (2001) Osmotic stress activate distinct lipid and MAPK signalling pathway in plants. FEBS Lett 498

Muoki RC, Paul A, Kumar S (2012) A shared response of thaumatin like protein, chitinase and late embryogenesis abundant protein to environmental stresses in tea (*Camellia sinensis* (L.) O. Kuntze). Functional Intergrative Genomics 12(3):565-571

Ngetich WK, Stephen W, Othieno CO (2001) Responses of tea to environment in Kenya. III. Yield and yield distribution. Experimental Agriculture 37:361-372

Nishizawa A, Yabuta Y, Shigeoka S (2008) Galactinol and raffinose constitute a novel function to protect plants from oxidative

damage. Plant Physiology 147:1251-1263

Rossel JB, Water PB, Hendrickson L, Chow WS, Poole A, Mullineaux PM, Pogson BJ (2006) A mutation affecting ascorbate peroxidase 2 gene expression reveals a link between responses to high light and drought tolerance. Plant Cell Environment 29: 269-281

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York, USA

Shakeel AA, Xiao-yu X, Long-chang W, Muhammad FS, Chen ML (2011) Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research 6:2026-2032

Shalini KV, Manjunatha S, Lebrun P, Berger A, Baudouin L, Pirany N, Ranganath RM, Prasad TD (2007) Identification of molecular markers associated with mite resistance in Coconut (*Cocos nucifera* L.). Genome 50:35-42

Sharma P, Kumar A (2005) Differential display-mediated identification of three drought-responsive expressed sequence tags in tea (*Camellia sinensis* (L.) O. Kuntze). Journal of Bioscience 30:231-235

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Current Opinion in Plant Biology 6:410-417

Taji T, Ohsumi C, Luchi S, Seki M, Kasuga M, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K (2002) Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. Plant Journal 29: 417-426

Tanaka J, Taniguchi F (2006) Estimation of the genome size of tea (*Camellia sinensis*), Camellia (*C. japonica*), and their interspecific hybrids by flow cytometry. Journal of the Remote Sensing Society of Japan 101:1-7

Volk GM, Haritatos EE, Turgeon R (2003) Galactinol synthase gene expression in melon. American Society of Horticultural Science 128:8-15

Wachira FN, Ngetich WK (1999) Dry matter production and partition in diploid, triploid and tetraploid tea. Journal of Horticultural Science and Biotechnology 74:507-512

Wachira FN, Ronno W (2004) Current research on tea in Kenya. In:. *Proceedings of 2004 International Conference on O-Cha (Tea) Science*; Shizouka, Japan, 59-65

Wachira FN, Waugh R, Hackett C, Powell W (1995) Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. Genome 38:201-210

Wisniewki M, Bassett C, Norelli J, Macarisin D, Artlip T, Gasic K, Korban S. (2008) Expressed sequence tag analysis of the response of apple (*Malus x domestica* Royal Gala) to low temperature and water deficit. Physiology Plant 133:298-317

Wu H, Chen D, Jiaxian L, Yu B, Qiao X, Huang H, He Y (2012) *De novo* characterization of leaf transcriptome using 454 sequencing and development of EST-SSR markers in tea, (*Camelia sinensis*). Plant Molecular Biology Report 31(3):524-538

Xu ZZ, Zhou GS, Shimizu H (2009) Effects of soil drought with nocturnal warming on leaf stomatal traits and mesophyll cell ultrastructure of a perennial grass. Crop Science 49:1843-1851