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GENERATION AND ANALYSIS OF EXPRESSED SEQUENCE TAGS FROM ROOT CDNA LIBRARY OF COTTON (*GOSSYPIUM ARBOREUM*)

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Abstract

Cotton is major fiber crop among agricultural commodities grown worldwide. Prolonged osmotic stress and seasonal water shortage effect plant growth and yields. To explore the drought responsive molecular mechanism in cotton roots, we constructed cDNA library for the identification, functional annotation and expression analysis of potential drought responsive transcripts. mRNA extracted from cotton roots under drought stress was used to construct cDNA library. A total of 711 expressed sequence tags were generated from cDNA library. NCBI BLAST analysis of reported expressed sequence tags showed homology of 27% sequences with *Gossypium* species while 24% sequences showed no sequence similarity in non-redundant nucleotide database. Remaining 49% sequences revealed sequence similarity results with other plant species. Functional annotation was performed for sequenced EST's using *Arabidopsis* Gene ontology database (TAIR). We examined the expression pattern of 08 differentially expressed ESTs using RT- qPCR and validated that all these were up-regulated in roots under osmotic stress, but to different folds. The candidate EST sequences identified in this study will be valuable resource to elucidate stress responsive molecular mechanism and genetic breeding of cotton in future.

Key words: Gene annotation, Homology, Unigenes, Expression

Introduction

Cotton is an important fiber crop grown in more than 30 countries of world with major share from China, India, United States and Pakistan (Riaz *et al.,* 2013). Cotton fiber accounts 90% economic value besides cottonseed and its related products (Campbell & Hinze, 2010). In Pakistan, cotton production is declined by 34% to just 9.68 M bales against the production of 14.4 M bales from previous years because of high temperature and water shortage (Dawn news, 2016). Among abiotic stress factors, drought adversely affects both cotton growth and production (Bradow & Davidonis, 2010; Park *et al.,* 2012; Liu *et al*., 2017). Cotton as glycophyte can endure drought and heat-stress for a specific time period (Parida *et al.,* 2007).

Root is considered a vital organ for constant supply of water and nutrients and also acts as primary site of soil stresses perception. Changes at transcriptional level begin in plants by triggering gene expression and regulation of various abiotic stress mechanism (Shinozaki Yamaguchi-Shinozaki, 2007). Root acts as major connection in soil between plant and multiple biotic and abiotic factors that activate root-to-root chemical messengers which finally regulate stomata and reduces evaporation (Davies & Zhang, 1991; Jia & Zhang, 2008). A few reports on the regulation mechanism at molecular level in cotton orchestrate drought tolerance genes using quantitative characteristic loci (QTL) mapping, drought stressed cDNA libraries (Zhang *et al.,* 2009; Levi *et al.,* 2011) or by gene expression profiling (Payton *et al.,* 2011). There are several approaches for the identification of expressed sequence tags (EST's), but it depends the sequence information retrieval and availability of plant genome being investigated. Identification of EST's can unreveal and explore potential sequences of those plants whose genome sequence is not yet available (Adams *et al.,* 1991).

Expressed sequenced tags (EST's) are partial transcribed sequences of genome and represent gene expression in different tissues in different genotypes depending on development stage (Hatey *et al.,* 1998). ESTs are known to be acquired from mRNA which is cloned into cDNA libraries which are later sequenced to retrieve biological functions. Several attempts have been made to construct different cDNA libraries of plants such as *Lotus japonicus* (Asamizu *et al.,* 2004), *Medicago truncatula* (Cheung *et al.,* 2006), *Gossypium hirsutum* (Park *et al.*, 2012) and *Gossypium arboreum* (Barozai & Husnain, 2012). ESTs reveal the expressed portion of genome, hence they are considered to be most suitable to characterize the functional genes (Yamamoto & Sasaki, 1997; Thanh *et al*., 2011). In this study, we utilized cDNA library construction strategy for the identification and functional characterization of drought induced transcripts from cotton roots. Key understanding of ESTs involved in cotton root drought stress is necessary for plant development that can maintain high yields under water deficit and the cultivation of drought tolerance cotton varieties. Our current study of cotton responses at the root transcriptome level will elaborate the intricate mechanism of multiple gene responses involved in drought stress.

Materials and Methods

Plant material and stress treatment: Cotton variety FDH-786 seeds were obtained from local germplasm center (CCRI, Multan) and delinted with concentrated H_2SO_4. Delinted Seeds were grown in soil mixture containing a similar ratio of peat, sand, soil (1:1:1) in plastic bags under controlled environmental conditions (temp; 25±2°C and relative humidity; 45-50%). For supplement natural radiation, metal halide illumination lamps (400 W) were to provide an irradiance of 1500

µmol m^2 s^{-1}. The volume of pure water added to the pots was calculated periodically to maintain the pots of osmotic stress treatments at 5% gravimetric humidity (GH) and non-stressed (control) treatments at 15% GH. Forty five days old cotton seedlings were subjected to drought stress for fifteen days along control plants as described (Maqbool *et al*., 2007; Jamal *et al*., 2014). Thereafter healthy roots of control and drought stressed plants were harvested, immediately frozen and grinded in liquid N_2 to fine powder.

RNA isolation, cDNA library construction and clones amplification: Total RNA isolation was performed from 1 g of control and drought stressed roots following Jaakola *et al.,* (2001) protocol with little modification. To check the RNA integrity, RNA was analyzed using 0.8% agarose gel electrophoresis and was quantified using spectrophotometer (ND-1000 NanoDrop Technologies, Inc) by means of the nucleic acid in software. RNA was treated with Ambion's DNAfree™ Kit (Thermo Fisher scientific ™, USA) to avoid any contamination and residues of DNA. mRNA extraction and purification was performed using oligotex mRNA mini kit (Qiagen, Valencia USA). To construct the cDNA library, we used CloneMiner™ cDNA Library Construction Kit (USA, Invitrogen). Briefly, first and second stranded cDNA synthesis reactions were performed. Later, the double stranded blunt ended synthesized cDNA was proceeded for attB1 adapter ligation. The adapter ligated cDNA was size fractioned using 0.8% low melt agarose. cDNA size ranging from ≥100 bp - < 1Kb was excised carefully from gel.

The double stranded cDNA was eluted from gel using DNA Gel Extraction Kit (Thermo Fisher scientific ™ USA,) followed by manufacturer instructions. The eluted and quantified cDNA was further used for BP recombinant reaction. cDNA in fractions were later precipitated using 7.5M Ammonium acetate (pH 4.8), Glycogen (20 µg/µl) and 2.5 volume of 100% ethanol. Following BP recombination reaction, 1.5 µl cDNA was added to each aliquot 50 µl thawed ElectroMAX™ DH10B™ T1 phage resistant competent cells (Invitrogen CloneMiner™, USA) following manufacturer protocols. The electrophorated cells were incubated for 1 h at 37ºC at 220 rpm to allow the expression of antibiotic marker. All Incubated cell cultures were pooled into a 15 ml culture tube followed by addition of equal volume of sterile freezing media (60% SOC medium: 40% glycerol). The pooled library aliquots were fractioned and stored at -80ºC.

Restriction digestion was performed to confirm the clones of library. To confirm the presence of inserts, 10µg of isolated plasmid DNA from randomly selected clones was digested by 10U of *BsrGI* enzyme (Fermentas**,** Germany). Overnight incubated reaction was performed at 37ºC in theromocycler. Recombinant plasmids of cDNA library followed were electrophoresed on 0.8% agarose gel for confirmation.

PCR amplification was performed for the confirmation of clones using the M13 sense and antisense universal primers designed against the flanking regions. To perform it, 5 µl of overnight grown culture given heat shock at 95ºC for 5 min and centrifuged at 3,000 rpm for 5 min at 4ºC. Inserts in clones were PCR amplified using 5 µl of diluted culture using *Pfu* DNA Polymerase (recombinant) (Fermentas, Cat# EP0501, USA) following thermocycler profile with initial denaturation at 94ºC 5 min; followed by 35 cycles of denaturation at 95ºC, annealing at 52ºC, and extension at 72ºC and a final extension at 72ºC each for 45 s, 45 s, 60 s and 10 min respectively.

EST's analysis, functional annotation and Expression analysis of unigenes: Plasmid DNAs were extracted from randomly selected clones using modified alkaline lysis method (Sambrook *et al*., 1998). Sanger sequencing methodology was performed for 711 randomly selected PCR amplified clones by using Big Dye Terminator cycle sequencing kit (ABI PRISM Foster, USA) and forward sequencing primer (5′ GTAAAACGACGGCCAG 3′). Clones were sequenced using Applied Biosystems Sequencer model 3100/3700. After sequencing, vector and adaptor sequences present at both 5′ and 3′ ends of EST's were removed using Phred software (Ewing & Green, 1998; Ewing *et al*., 1998).

Trimmed sequences of 711 with more than 100 bp were considered as unigenes. EST's accession numbers JK757087-JK757798 were submitted to NCBI GenBank. For EST's analysis, NCBI BlastN program was used to search nucleotide similarity corresponding to *Arabidopsis* with a threshold similarity around 75% against our unigene set. Unigenes with >100bp size were analyzed in *NCBI* using BLASTN (non-redundant nucleotide databases) (https://blast.ncbi.nlm.nih.gov)with an E-value threshold < 1.0 e^{-2} against *Arabidopsis* to investigate orthologs. The gene code names (Atg) of *Arabidopsis* orthologs were saved. After similarity search, gene code names (Atg) of *Arabidopsis* were further analyzed for gene ontology to predict the homolog gene functions. For this purpose, gene annotation was performed at TAIR (http://arabidopsis.org/tools/bulk/go/index.jsp), (Berardini *et al*., 2004) to predict the molecular functions, biological processes and cellular components.

RNA extraction from control and stress plant roots was performed following Jaakola *et al*., (2001). cDNA synthesis was performed using cDNA synthesis kit (*Fermentas*, Germany) followed by DNase1 treated 1µg RNA. Real time PCR *iQ*5 (Bio-Rad, USA) was used for the relative gene expression using *IQTM SYBR-Green* super mix (Fermentas, USA). Primers were designed using freely available online software primer 3 ver 4 (http://primer3.ut.ee/) and synthesized using commercial facility (GeneScript, NJ, USA). Cotton Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers were used as reference genes as mentioned in previous studies (Zahur *et al*., 2012). In each reaction, 200 ng cDNA of both control and drought stress plants was used for each biological and technical replicate in triplicate. Real time thermocycler profile was set as: initial denaturation at 95ºC for 3 min followed by 40 cycles of denaturation at 95ºC, annealing at 60ºC, and extension at 72ºC and final extension step at 72ºC each for 30 s, 30 s, 30 s and 10 min respectively. Melting curve analysis of the amplified genes was used to confirm the specificity of amplicons. Real time results were analyzed using iQ5 software (Bio-Rad Ver 1.0) based upon the cycle threshold (CT) values of the different unigenes along reference gene. Primer efficiencies (E) were analyzed for each experimental set

by Miner software (Zhao & Fernald 2005), that identified the starting and ending points of PCR exponential stage from raw fluorescence data, and estimates by establishing a nonlinear regression algorithm without the standard curve. The $2^{-\Delta\Delta CT}$ approach was used to analyze the relative expression of each gene normalized with reference gene (Livak & Schmittgen 2001). Statistical approach was performed using the online available SAS software (https://www.sas.com).

Results

All RNA samples showed an absorbance ratio of A260/A280 ranged from 1.80-1.88 with concentration of 800-1200 ng/µl which confirmed the good quality and suitable quantification of the isolated RNA. Sharp 16s and 23s bands were observed on agarose gel of 1.8 and 5.0 kb molecular masses respectively which is an authenticity of integrated RNA. Double stranded cDNA synthesis was confirmed on low melt agarose gel in the form of smear which authenticated the synthesis of double stranded cDNA. The titer of cDNA library was found to be 1.22×10^6 cfu/ml. cDNA Clones (insert) size was qualified and confirmed using the restriction digestion. Vector presence was confirmed followed by plasmids isolation along with their insert size range. Colony PCR amplification was performed to amplify the cDNA library clones and further PCR products were electrophoresed on agarose gel. Distinct apmlicons bands were observed on agarose gel with their insert size ranging from >100 bp - <1 kb. EST sequences of cotton root cDNA library inserts (sequences) ranged from 100-800 bp with mostly sequence length of 200-300 bp. Most sequences according to their size ranged from 100-200 bp followed by 201-300, 301-400, 401-500, 601-700, 701-800 and 50-600 bp (Fig. 1). A total of 711 cDNA library clones sequences were submitted in *NCBI* (GenBank Accessions #: JK757087-JK757798). The read length of these sequences varied from 100-800 bp (Fig. 1). After vector and adapter sequences removal, most of sequences were in the range of 101-200 bp followed by 201-300 bp.

BlastN analysis was performed for all sequences using NCBI against non-redundant nucleotide database. Around 24% (170 sequences) sequences did not show any similarity, 27% (195 sequences) homology with *Gossypium* sequences, 8% (55 sequences) with *Populus trichochorpa* spp, 6% (46 sequences) with *Oryza sativa*, 5% (34 sequences) with *Zea Mays*, 4% (30 sequences) with *Glycine Max*, 3% (19 sequences) with *Medicago* and *Nicotiana* spp, 2% (15 sequences) with *A. thaliana* and *Ricinus* spp, 2% (13 sequences) with *Atriplex* and 14% (100 sequences) revealed similarity with other plant species (Fig. 2).

A total 711 unigenes of *Gossypium* to orthologs of *Arabidopsis* assigned to gene annotation (GO) i.e.,' cellular components', 'biological processes', and 'molecular functions'. In the molecular components category, maximum proportion by 'kinase activity' associated genes (11.18%) followed by 'other binding' (11.05%), 'transferase activity' (10.13%), 'hydrolase activity' (9.07%), 'DNA binding' (8.68%), 'protein binding' (8.28%), 'other enzyme activity' (7.63%), 'nucleotide binding' (7.36%), 'transporter activity' (6.05%), 'transcriptional related' (5.78%), 'nucleic acid binding'(1.97%), 'other molecular functions' (1.44%) and 'nucleotide receptor binding' (0.92%) (Fig. 3A). In the cellular components category, the principal contribution reinforced by 'other intracellular components' (19.01%), followed by 'other cytoplasmic components' (13.04%), 'chloroplast' (9.67%), 'other membranes' (9.33%), 'nucleus' (9%) 'Other cellular components' (8.21%), 'unknown cellular components' (6.19%) 'plasma membrane' (5.84%), 'cytosol' (5.73%), 'plastid' (4.27%), 'mitochondria' (2.81%), 'ribosomes' (2.25%), 'golgi apparatus' (1.68%), 'cell wall' (1.12%) and 'endoplasmic reticulum organelles' (0.90%) (Fig. 3B). In the biological significant proportion of sequences was related to 'other cellular processes' (25.41%), followed by 'other metabolic processes' (17.42%), 'response to stress' (8.42%), 'protein metabolism' (7.11%), 'response to abiotic or biotic stimulus' (7.68%), 'other biological processes' (6.68%) 'developmental processes' (5.51%), 'unknown biological processes' (5.59%), 'transport' (4.93%), 'transcription DNA dependent' (4.21%) 'signal transduction' (2.83%), 'cell organization and biogenesis' (2.68%), 'DNA/RNA metabolism' (1.08%) and 'electron transport or energy pathways' (0.43%) (Fig. 3C).

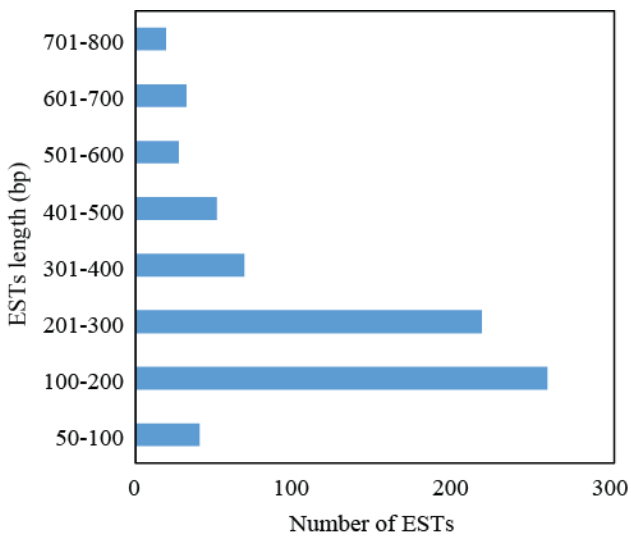

Fig. 1. EST's classification based upon their sizes.

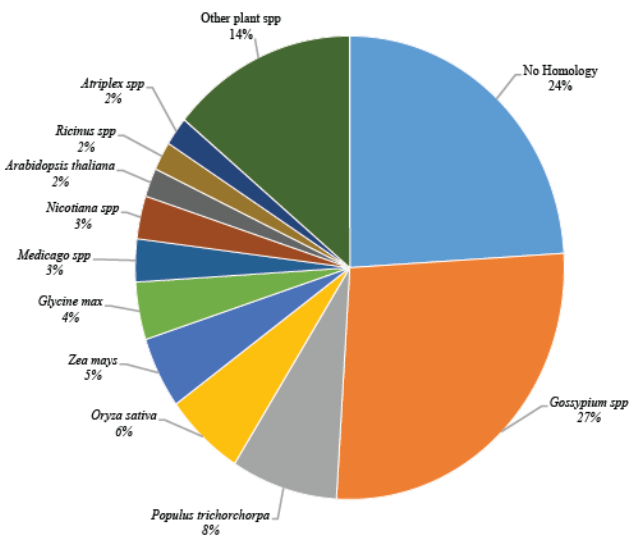

Fig. 2. Non redundant nucleotide based similarity.

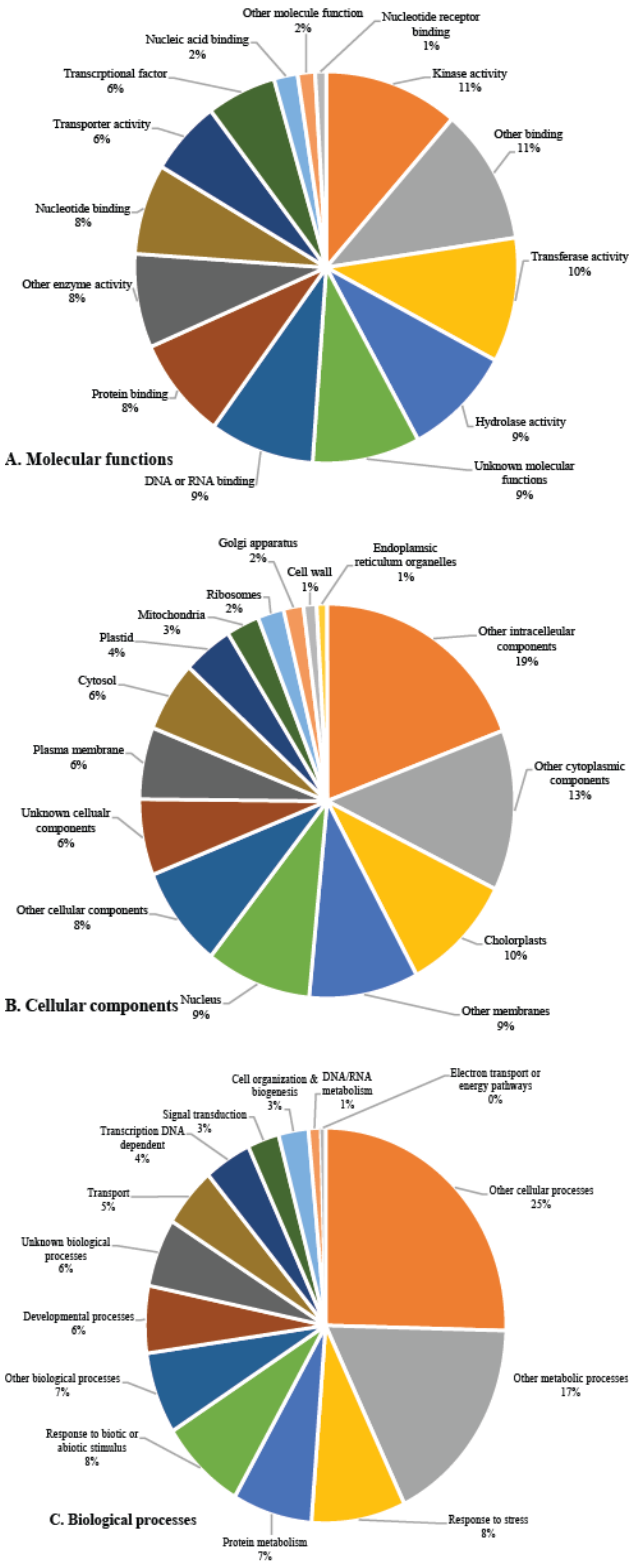

Fig. 3. Functional annotation of drought stress responsive transcripts in cotton roots from cDNA library. Classification of ESTs based on (A) Molecular functions, (B) Cellular components, (C) Biological processes.

For the validation of EST's expression pattern under osmotic stress in cotton root tissues, RT-qPCR was used for 08 random selected EST's. with an E value < $2E^{-3}$ (Table 1). Real time PCR validated an over expression of Raffinose syntahse *RS2* (JK757654), Zinc finger domain containing protein (JK757674), Glycine rich protein (JK757725), Leucine rich repeats- receptor like kinase (JK757682), Tonoplast intrinsic protein (JK757706), Nucleolar factor (JK757717), Microtubules (dynein) (JK757657) and Chloride ion channel / Cation chloride co-transporters (JK757671) (Fig. 4).

Discussion

Root is an essential organ which perceives soil related factors and produces the principal layer of protection by eliciting a few metabolic progressions. The present findings are novel attempt to provoke root EST's which may be valuable for utilization of novel genes in future. It is imperative to isolate high quality RNA while constructing cDNA libraries. Similarly, the integrity of mRNA is of prime significance for downstream applications (Yadav *et al*., 2014). cDNA library clones amplification is of critical significance because of less titer and clones viability. A cDNA library is said to be well constructed having primary titer of 2.56×10^6 PFU mL^{-1} and final titer after amplification 10^{10} PFU mL^{-1} (Ling *et al*., 2012). The titer of the original and amplified library of the cotton roots were 1.22×10^6 PFU mL^{-1} and 10^8 PFU mL^{-1} respectively. Earlier studies reported that titer of cDNA library of root 1.6×10^6 PFU mL^{-1} (Zhao *et al*., 2013) which support our results. The data generated by EST's of randomly selected transcripts isolated under specific stress factors provide a chance to explore gene function and their category besides their different physiological progressions in plants against different stresses (Wong *et al*., 2005).Genomic approaches may help in understanding the physiological events at cellular and molecular level by dissecting the genetic resources of plants (Chaves *et al*., 2003). Current study revealed 76 % EST's homology with already non redundant nucleotides in NCBI GenBank while remaining 24 % EST's had no similarity and hence it may be considered of significance to explore the gene function in future.

We found that *G. arboreum* EST JK757654 showed up regulation in response to drought. The EST (JK757654) has similarity with members of the *A. thaliana* inhibition protein homolog (At3g57520), involved in osmoprotectants. Ortholog At3g57520 encodes raffinose synthase 2 (*RS2*) activated by galactinol synthase is involved in oxidative stress. Increase of galactinol and raffinose at intracellular level in the plants are correlated with increased tolerance to abiotic factors. Raffinose synthase is an active signaling molecule during seed desiccation and abiotic responses viz pathogen attack and wounding (Couée *et al*., 2006; Kim *et al*., 2008; Song *et al*., 2016) and accumulate in vegetative tissues in response to abiotic stresses (Hincha *et al*., 2005). Raffinose being as antioxidant, membrane stabilizing and predicted signaling roles, hence are considered as key factors during stress responses eliciting their role in scavenging of ROS by protecting the plant cells during osmotic stress (Kim *et al*., 2008; Nishizawa *et al*., 2008a, 2008b).

Table 1. Up-regulated abiotic stress responsive expressed sequenced tags (EST's), their predictive homology and an E-value less than $2E^{-3}$.

EST (Accession number)	Homology (predicted function)	Plant species	Gene IDs	Identity (%)	Score	E-value
JK757674	Zinc finger domain	*G. raimondii*	XM_012587865.1	100	105	$3.00E^{-20}$
JK757725	Glycine rich	*A. thaliana*	CP002687.1	77	117	$4.00E^{-4}$
JK757682	Leucine rich repeat-receptor like kinase	*A. thaliana*	CP002684.1	82	113	$2.00E^{-3}$
JK757706	Tonoplast intrinsic protein (aquaporin)	*A. thaliana*	NM_100713.2	89	37.4	$2.00E^{-5}$
JK757717	Nuclear factor	*A. thaliana*	BT011854.1	97	50	$6.00E^{-5}$
JK757657	Dynenin	*C. sinensis*	HM003277.1	100	55.4	$2.00E^{-5}$
JK757671	Chloride ion channel (cation cl^- co-transporters)	*O. sativa*	AB069968.1	86	107	$8.00E^{-21}$

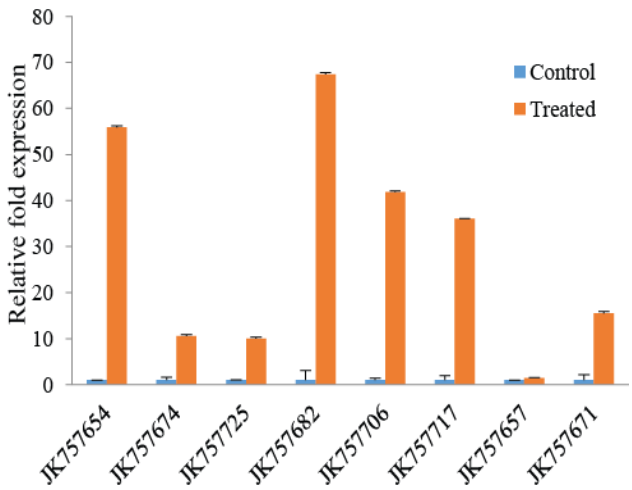

Fig. 4. Relative expression of different EST's in roots of *G.arboreum* as shown by RT-qPCR analysis using each three biological and three technical replicates; JK757654-raffinose synthase 2 (*RS2*), JK757674-zinc finger domain containing protein, JK757725-glycine rich protein, JK757682- leucine-rich repeat receptor-like kinases, JK757706-tonoplast intrinsic protein, JK757717- nuclear factor, JK757657-plant microtubules (dynein), JK757671-chloride ion channel / cation chloride co-transporters.

We identified that the *G. arboreum* EST JK757674 was up-regulated under drought stress. The EST JK757674 exhibited significant similarity with *A. thaliana* putative homolog (AT5G10370) ortholog AT5G10370 zinc finger (ZF) domain containing protein. ZF are involved in biotic and abiotic stresses (Zahur *et al*., 2012; Shi *et al*., 2014, Butt *et al*., 2017). Zinc finger family members have been shown to play diverse roles in the hormone signal transduction and drought stress response (Kiełbowicz-Matuk, 2012; Baldoni *et al*., 2015). Transcription factors bind to these cis-acting elements in DNA binding region near promoter regions of target genes and cooperatively activate transcription and gene regulation (Abe *et al*., 2003). ABA signal perception leads to induction of transcription factors and their product bind to promoter domain and thereby regulate it (Chen *et al*., 2010). ZF transcription factors are involved in the integration and development of many organs and tissues, such as seed and root maturation (Feurtado *et al*., 2011) floral development (Wu *et al*., 2008), secondary metabolism and cell wall structure (Al-Ghazi *et al*., 2009).

In this study the *G. arboreum* EST JK757725 showed an increased expression under drought stress. EST JK757725 is identified as homolog of *A. thaliana* (AT5G01010). Ortholog AT5G01010 is considered to function as glycine rich protein. Many glycine-rich proteins (GRPs) are involved in transcriptional regulation, signaling, protein interaction, seed germination, plant development and stress responses, but the function, signaling and importance of some GRPs in stress responses is still unknown in plants (Bocca *et al*., 2005; Mangeon *et al*., 2010). GRP mRNA expression is triggered by abiotic factors and its up-regulation is controlled by CAMV-35s promoter in plants (Long *et al*., 2013). EST JK757725 showed an over expression in root tissue under water deficit and this can be attributed to role of GRP's in seed germination and seedling growth of plants under abiotic stresses. GRP's encompass putative RNA-binding motif and a glycine-rich domain. These proteins domains are conserved in higher plant. GRPs domains play role during abiotic factors and ABA signaling, while the latter function is yet unknown completely (Yang *et al*., 2014; Rodríguez-Hernández *et al*., 2014).

Several biologists identified the role of Leucine-rich Repeat Receptor-Like Kinases (LRR-RLK) in the protection of plants under different abiotic factors. We found that *G. arboreum* EST JK757682 is recognized as homolog of *A. thaliana* (AT4G00231) receptor like kinases. EST JK757682 displayed up-regulated expression under drought stress. LRR-RLK family is involved in stress and developmental pathways (Dievart *et al*., 2016). These are activated during defense/resistance against plant pathogens (Morillo & Tax, 2006; De Smet *et al*., 2009, Gish & Clark, 2011; Kemmerling *et al*., 2011; Antolín-Llovera, 2014). LRR has been reported in plants involved in cell division and elongation in shoot and root (Nakamura *et al*., 2006), root outer layer cell development (Suzaki *et al*., 2004). It also negatively regulates polar transport and thus affects root architecture and auxin accumulation in roots (Zou *et al*., 2014).

In our results, the *G. arboreum* EST JK757706 showed significant homology with tonoplast intrinsic protein (TIP). EST JK757706 is considered as homolog of *A. thaliana* (AT1G08400). TIP is family of protein (AQP's) found in plasma and vacuolar membranes act as a water channel and functions in water homeostasis

(Alexandersson *et al*., 2005). AQP's are involved in water uptake, flow across cell membrane for proper plant growth and development. EST JK757706 showed an over expression under osmotic stress. Under osmotic stress, AQPs and its isoforms like TIP activity altered by down regulation of anti-sense RNA, which may finally lower protoplast osmotic permeability, to increased dimension of root systems and or higher susceptibility to water deficit and osmotic stress (Siefritz *et al*., 2002). AQP's regulating gene expression may alter depending upon hormone, tissue and abiotic stress (Tyerman *et al*., 2002). Earlier studies also revealed elevated expression of intrinsic proteins (IP) genes in aerial parts and roots in response to abiotic factors and abscisic acid (ABA) treatment (Jang *et al*., 2004; Zhang *et al*., 2017).

In our findings, the *G. arboreum* EST JK757717 revealed homology with *Arabidopsis* ortholog (AT3G57000). EST JK757717 showed an up-regulation in response to drought stress. Nuclear factor (NF) is a ubiquitous transcription factor containing subunits. These Conserved domains along subunits are involved in protein-protein interface and nucleic acids binding (Ni *et al*., 2013). Several members of the NF family play role in development and responses to adverse environmental abiotic factors (Ben-Naim *et al*., 2006; Nelson *et al*., 2007; Li *et al*., 2008; Zhao *et al*., 2009; Na *et al*., 2017). Several members of NF gene family are regulated by microRNA through an intricate mechanism at both transcriptional and post-transcriptional level (Li *et al*., 2008). NF gene family tolerate the osmotic stress tolerance through up-regulation of genes involved in ABA pathway, signaling and the proteins that lead to stress protection (Ni *et al*., 2013). In plants, no complete NF-Y complex has been reported so far and the biological function of NF still remains unclear (Nelson *et al*., 2007).

We found that the *G. arboreum* EST JK757657 was homolog of *Arabidopsis* (AT1G29830), a microtubule (dyenin protein). Plant microtubules (PMT's), dynein have major role in in cell division and expansion, sensory transmission particularly pertinent to membrane stress and its derivatives, such as cold and water stress (Nick, 2013; Guan *et al*., 2015). MT's has been significantly known for their roles in sensing of osmotic stress (Wang *et al*., 2011). The EST JK757657 having up regulated response may be the potential EST to cope the water stress.

The EST JK757671 was found to *Arabidopsis* homolog (AT3G25010), as chloride ion channel. In plants, turgor and osmotic regulation processes occur in vacuole. Chloride (Cl^-) being as co-transporter function in development of plants (Broadley *et al*., 2012; Birni & Masmoudi 2012; Franco-Navarro *et al.,* 2016). Cl^- uptake and its thermodynamics involving passive channel transport occurs at endomembrane because of presence of plant chloride channels in this zone. Cation chloride co-transporters (CCCs) also encode the potential Cl^- transporters in plants (Henderson *et al*., 2015). EST JK757671 exhibited an over expression under water deficit. Previous studies also reported the role of cation Cl^- co-transporters (AtCCCs) in *A. thaliana*, differentially regulated in root and shoot tissues. These co-transporters mediate the coordinated unidirectional movement of K^+, Na^+, and Cl^- and have been supposed to contribute in the transport of Cl^- distantly. (Henderson *et al*., 2015).

Conclusions

In conclusion, current study illustrated the genomic resources of roots, which elaborate their drought responsive specificity. These genomic resources identified in this study with key role in molecular mechanism of drought stress can be further applied by biologist to develop useful stress tolerant markers for cotton resistant breeding.

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