

A Comparative Study of ESTs Induced under Drought and Salinity Stress in Hyacinth Bean (*Lablab purpureus*)

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Abstract

Abiotic stressors like drought and salinity are major causes for loss of agricultural productivity. Comparison of expressed sequence tags (ESTs) under different abiotic stresses provides insight into underlying mechanism of stress response, and candidate genes to improve tolerance to abiotic and biotic stresses via breeding and transgenic methods. In order to identify and compare stress-specific ESTs from drought and salinity stressed Hyacinth Bean, ten days old seedlings were subjected to respective stresses and RNA was extracted from control and stressed leaves for EST identification. 31 and 12 ESTs, respectively, were characterized from leaves of drought and salinity stressed seedlings of Hyacinth Bean, *Lablab purpureus* by differential display RT-PCR using identical combinations of 48 primers and validated using quantitative RT-PCR. Relative fold expression was higher under salt stress than drought stress. Whereas 19 EST overexpressed under drought, 12 EST were down regulated. Of the 12 EST under salinity, 9 EST were downregulated and 3 EST upregulated. Putative functions predicted from sequence homology indicated that 11 drought specific EST corresponded to metabolic functions, and 4 of them corresponded to transcription regulation. Under salinity, 4 and 2 EST, respectively, corresponded to metabolic and RNA associated functions. Under both stresses, there were ESTs associated with unknown functions, whose characterization may throw light on the regulatory mechanism. Differing number of ESTs differentially expressed under drought and salt stress, and their predicted functionalities, suggested distinct set of response genes involved under these two stresses, despite a good number of physiological players being common. From the predicted functions of ESTs, the paper attempts to explain the possible mechanism of response of Hyacinth Bean to these two stresses.

Keywords

Hyacinth Bean, Drought, Salinity, DDRT-PCR, ESTs

1. Introduction

Growing economy in many ways has led to climatic change by increasing global temperature, thereby rise in sea level resulting in increased salinity of ground-water and arid land [1]. Also, increase in global temperature has increased frequency of drought [2] posing a big challenge for agriculture [3]. Drought and salinity affected soils contain high concentration of ions such as chloride, sodium, calcium and magnesium in soil [4] or are nutrient deficient. Thereby, forcing the use of chemical fertilizers leading to increased cost of crop production environmental pollution. Breeding techniques have been beneficial in creating new cultivars resistant to drought and salinity. Also, understanding the tolerant genotypes response towards stress gives insights into improving the tolerance of crops.

Plant *response* to drought and salinity have been studied in terms of alterations in metabolism [5], detoxification [5], ABA induced response [6], proteome [7], transcriptome [8]. Transcriptome networks [9] and differential gene expression studies with model plants under abiotic stress [10] [11] have provide candidate genes for improving tolerance and productivity. Plant hormones play a role in inducing osmotic effects eliciting overlapping responses under drought and salinity [12]. Transcription factor families like NAC [13], WRKY [14], DERB [15] [16], are reported to be differentially regulated under stress. Specific protein like defensins, SpPKE1 [17], cysteine proteases [18], RING finger protein [19] an E3 ubiquitin ligase [20] and genes modified using RNAi mediated gene silencing [21] are known to increase tolerance towards abiotic stress.

Among food crops, legumes form a major source of protein and contribute to enhancement of soil quality through their nitrogen fixing ability. A Fabaceae member, *Lablab purpureus* L. (Hyacinth Bean) HA4 variety is extensively grown in south India for its pods, and previous reports have indicated its tolerance to drought [22] and salinity [23]. Transcriptomic variations, in root tissue, under drought stress [24] have given insight into the expression pattern. A comparative account of leaf specific transcripts expressed under drought and salinity stress in *Lablab purpureus* L. (Hyacinth Bean) variety HA4, are described herein with the objective of delineating the mechanism of stress response in terms of differentially expressed ESTs.

2. Material and Methods

2.1. Plant Growth, Stress Induction and Total RNA Isolation

The seeds of *L. purpureus* (cv. HA-4) were obtained from National Seed Project, University of Agricultural Sciences, GKVK, Bangalore, India. Seeds were surface

sterilized with 0.1% (w/v) mercuric chloride for 30 s, rinsed immediately with distilled water several times and imbibed overnight in distilled water. The overnight-soaked seeds were sown in plastic trays containing vermiculite and acid-washed sand (1:1 w/w) and irrigated daily with distilled water.

The germination was carried out under natural greenhouse conditions; day/night temperature and relative humidity were 30/25°C, and 75/70%, respectively. The average photoperiod was 12 h light/12 h dark. Plants were grown for 10 days before inducing stress. Drought stress was induced by withdrawing water for 6 Days whereas control plants were watered. Salt stress was induced using half strength Hoagland media with 300 mM NaCl, and 150 mM CaCl₂ for 48 h. Control plants were grown in half strength Hoagland media without 300 mM NaCl. After stress exposure leaves were collected from both control and stress plants. The samples were immediately ground to a fine powder in liquid nitrogen and used for total RNA isolation. Total RNA was isolated using Tri-Reagent (Sigma-Aldrich) according to the manufacturer's instructions. The quality of total RNA was observed by electrophoresis on 1.5% Formaldehyde-MOPS gel. The purity was assessed by reading the A260/A280 ratios and A260/A230 ratios using Biomate 3S UV-Visible spectrophotometer (Thermo Scientific).

2.2. Differential Display Reverse Transcription Polymerase Chain Reaction

Differential display was performed according to the method of Liang and Parde [25] with minor modifications. Total RNA was treated with RNase free DNase-I (0.1 U per mg RNA for 30 min) to remove the contaminating genomic DNA. In brief, first strand cDNAs was synthesized using 2 µg of Total RNA and 10 mM oligo dT primer. Initial incubation was at 70°C for 5 min followed by quick chilling on ice. To this, 40 units of Superscript™ II RNase H RT (Thermo Scientific), 20 units RNasin (Thermo Scientific), 0.5 mM dNTPs (Thermo Scientific) and 1X reverse transcriptase reaction buffer (250 mM Tris-HCl, pH 8.3, 250 mM KCl, 20 mM MgCl₂ and 50 mM DTT) were added and made up to final volume of 20 µL.

The reaction was performed in Eppendorf master cycler by incubating at 40°C for 60 min, and terminated by heating at 70°C for 10 min. All cDNA samples were stored at -80°C until further use. A total of three anchor primer and 16 arbitrary primer (Table 1) were used for DDRT-PCR. Amplification was carried out in a final reaction volume of 50 µL containing 1 µL of the cDNA as template, 0.5 U Taq DNA polymerase (Thermo Scientific), 12.5 mM each of specific arbitrary primer and anchor primer with restriction sites of *Bam*HI and *Hind*III, respectively. 0.1 mM dNTPs and 1x Taq DNA polymerase reaction buffer (10 mM Tris-HCl, pH 9.0, 1.5 mM MgCl₂, 50 mM KCl and 0.01% gelatin). The reaction conditions were: an initial denaturation step of 95°C/4min, followed by 35 cycles of 94°C/60s (denaturation), 50°C to 55°C (according to primer combination)/60s

Table 1. List of primer used in DDRT-PCR.

Anchore primers	Sequence
DST-PR/1	AAGCTTTTTTTTTTTTGG
DST-PR/2	AAGCTTTTTTTTTTTTGG
DST-PR/3	AAGCTTTTTTTTTTTTGA
Arbitrary primers	Sequence
DST-PR/9	AAGCTTGATTGCC
DST-PR/15	AAGCTTCCTGGA
DST-PR/17	AAGCTCTFCTGG
DST-PR/18	AAGCTTACGATGC
DST-PR/19	AAGCTTAGCAGCA
DST-PR/22	AAGCTTCCTGCAA
DST-PR/23	GAGGATCCGATTGCC'
DST-PR/24	GAGGATCCCAAGACC
DST-PR/25	CGGGATCCTATTTAT
DST-PR/26	GCGGATCCCGACTGT
DST-PR/27	GTGGATCCGCCTTTA
DST-PR/28	GTGGATCCCTTTGGT
DST-PR/29	CAGGATCCGCACCAT
DST-PR/30	CAGGATCCAGAGGCA
DST-PR/31	CTGGATCCTCATATG
DST-PR/32	CTGGATCCTTGAGGT

for annealing and 72°C/60s for extension and 72°C for 15 min for final extension. Each reaction was run in triplicate, and products obtained were subjected to denaturing 6% urea gel electrophoresis at a voltage of 0.45 V/cm² for 12 h at room temperature. DNA was visualized by staining with ethidium bromide and documented using Alpha Imager gel doc. Differential bands were excised and cloned into pGEM-T-easy vector (Promega) according to manufacturer's instructions. Plasmid expressing the band of interest was sequenced on an automated sequencer (ABI PRISM 3700) using Bio Rad Taq cycle sequencing kit (Amersham Pharmacia Biotech,). Sequence analysis was performed using BLASTn (<http://www.ncbi.nlm.nih.gov/blast>). All of these ESTs were submitted to the dbEST database at NCBI (<http://www.ebi.ac.uk>).

2.3. Quantitative Real Time PCR

qRT-PCR was carried out using iQ SYBR green supermix (Biorad, India) on Biorad iQ5 Multicolor Real Time PCR Detection System. The reaction mix contained 0.5 µL cDNA, 10 µL 2X SYBR green mix, 0.5 µL of 1 µM forward primer and 0.5 µL of 1 µM reverse primer in 20 µL reaction volume. The cycling conditions were: initial denaturation of 95°C/3 min followed by 40 repetitive cycles at

95°C for 20 s for denaturation, 60°C for 30 s for annealing (according to primer combination) and 72°C for 30 s for extension. Melt curve analysis was performed at 55°C - 95°C. β -Actin was used as the endogenous control and analysis was carried out in three replicates. The Cq values were converted to relative quantities using the formula: $2^{-\Delta\Delta Ct}$ [26].

3. Results

DDRT-PCR was performed using 48 combinations of primers. Bands with clear differential expression were excised from polyacrylamide gels, amplified and sequenced. All obtained ESTs were submitted to NCBI EST database. After removing the redundant sequences, 31 differentially expressed ESTs were obtained under drought stress and 12 under salinity stress. All the sequenced fragments were of the expected length as ascertained from their positions in the gel and ranged between 98 - 423 bp for ESTs under drought, and 137 - 311 bp for ESTs under salinity stress. Sequence homology of the expressed fragments expressed under stress ranged between 73% - 93% similarity to sequence in Reference mRNA database. The ESTs showed similarity to sequences from legume family. Quantitative real time PCR was carried out to ascertain expression pattern (Figure 1 and Figure 2). The ESTs from salinity stress showed homology to genes encoding sugar transporter, ion transporter, chromatin remodeling components and RNA splicing components. Expression profile of salinity induced ESTs indicated that 3 ESTs were up regulated and 9 were down regulated. Eight EST corresponded to genes with putative functions (Table 2) and 4 were hypothetical

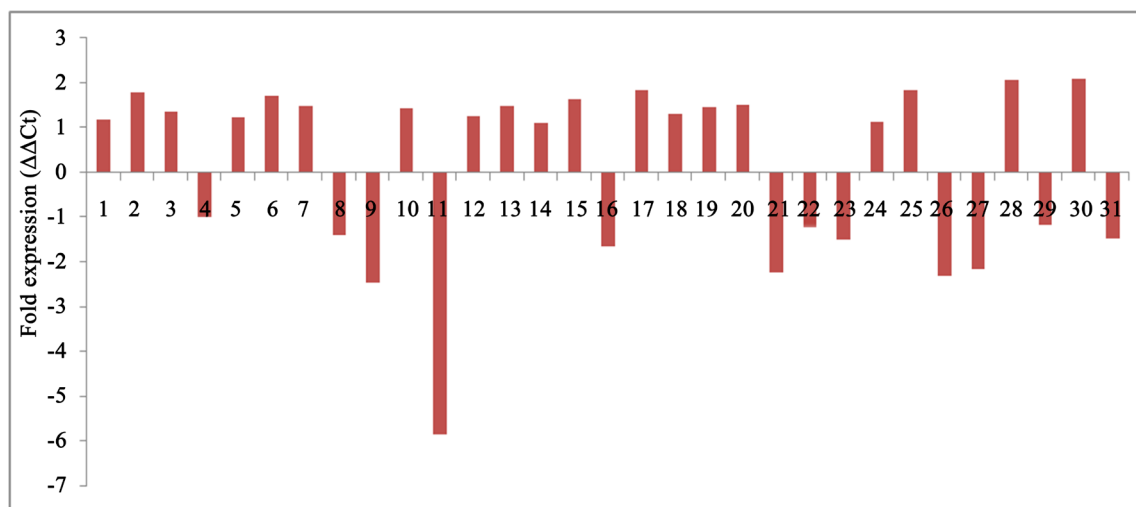


Figure 1. Differential expression of 31 ESTs under drought stress. Actin gene used as control. The ESTs expression below zero are down regulated, above zero are up regulated. Changes in gene expression are calculated using $\Delta\Delta Ct$ method. The plotted values are average of triplicates. (On the X-axis are the EST (Number assigned and their corresponding Genbank accession number are; 1-JZ515981, 2-JZ515982, 3-JZ168400, 4-JZ168401, 5-JZ168402, 6-JZ168403, 7-JZ168404, 8-JZ168405, 9-JZ168406, 10-JZ168407, 11-JZ168408, 12-JZ168409, 13-JZ515983, 14-JZ515984, 15-JZ515985, 16-JZ515986, 17-JZ515987, 18-JZ515988, 19-JZ515989, 20-JZ515990, 21-JZ546398, 22-JZ546399, 23-JZ546400, 24-JZ546401, 25-JZ546402, 26-JZ546404, 27-JZ546405, 28-JZ546406, 29-JZ546407, 30-JZ546408 and 31-JZ546409).

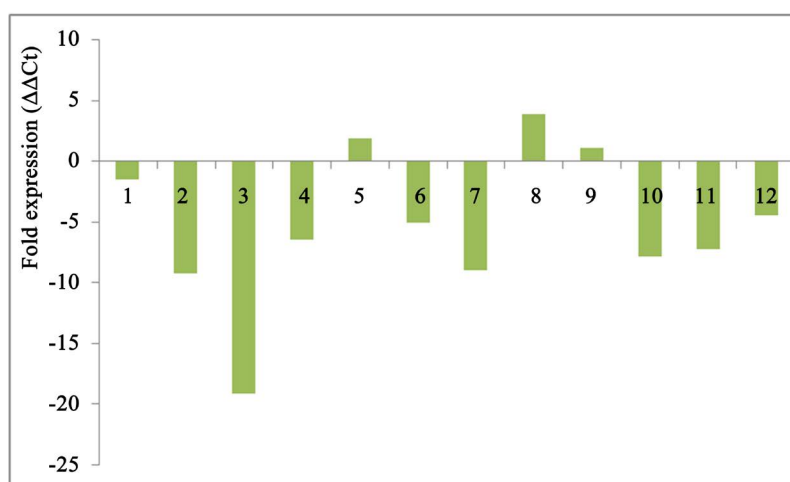


Figure 2. Differential expression of 12 ESTs transcripts under salinity. Actin gene is used as control. The ESTs with fold expression below zero are down regulated, above zero are up regulated. Changes in gene expression are calculated using $\Delta\Delta C_t$ method. The plotted values are average of triplicates. On the X-axis are the EST (Number assigned and their corresponding Genbank accession number are 1-JZ515991, 2-JZ515992, 3-JZ515993, 4-JZ515995, 5-JZ515996, 6-JZ515997, 7-JZ515998, 8-JZ515999, 9-JZ516000, 10-JZ516001, 11-JZ516002 and 12-JZ516003).

Table 2. List of ESTs differentially regulated under drought and salinity stress.

Up/Down regulated.	GenBank accession number	Similar protein	Size (bp)	E value	Condition	Fold change.
Upregulated ESTs						
Metabolism associated	JZ546402	Poly (A) binding protein (PABP) RBP45	360	9e-58	Drought	1.8
	JZ515990	Mitochondrial ATP synthase F1 complex assembly factor2	194	9e-28	Drought	1.5
	JZ515983	carbon catabolite repressor protein 4 like (CCRP)	205	8e-41	Drought	1.474
	JZ168409	cell wall associated hydrolase	127	2e-49	Drought	1.265
	JZ515981	Cytochrome c oxidase (COX)	149	3e-18	Drought	1.17
	JZ546401	Ubiquitin carboxyl-terminal hydrolase	267	1e-80	Drought	1.14
	JZ515984	vesicle associated membrane protein 727 like. VAMP727	387	1e-110	Drought	1.117
	JZ515999	plastidic glucose transporter	234	9e-23	Salinity	2.907
Transcriptional regulators	JZ546408	mediator of RNA polymerase II transcription subunit 26b-like	106	0.17	Drought	2.09
	JZ168404	MYB transcription factor	149	5e-23	Drought	1.47
	JZ515989	Calmodulin-binding Transcription Activator 3 like (CAMTA3)	302	7e-83	Drought	1.453
	JZ515988	nuclear TF-Y subunit A-7 like	204	6e-05	Drought	1.319
Ribosome associated	JZ515982	30S ribosomal subunit protein S7	148	7e-50	Drought	1.8
	JZ168407	Ribosomal protein L22	261	9e-86	Drought	1.443
Down regulated ESTs						
Metabolism associated	JZ546398	clathrin interactor EPSIN 1-like protein	157	1e-25	Drought	2.234
	JZ168405	maturase K	532	1e-151	Drought	1.4
	JZ546400	E3 ubiquitin-protein ligase PUB22-like	161	2e-15	Drought	1.48

Continued

	JZ546405	MLP-like protein 28-like	230	3e-09	Drought	2.158
	JZ515992	5'-adenylyl sulphate reductase	192	2e-37	Salinity	9.25
	JZ515991	cell wall associated hydrolase	272	2e-73	Salinity	1.48
	JZ515993	nitrate transporter 1.5 like	294	9e-62	Salinity	1.8
Chloroplast associated	JZ546399	short chain dehydrogenase TIC32	98	4e-25	Drought	1.21
	JZ168408	chlorophyll a/b binding protein	196	8e-11	Drought	5.85
	JZ546409	Photosystem II CP47	308	3e-116	Drought	1.474
	JZ516002	chlorophyllide-b reductase	329	1e-31	Salinity	7.6
RNA associated	JZ168401	rRNA-processing protein EBP2	128	2e-07	Drought	1
	JZ515998	pre-mRNA-splicing factor CWC22	179	5e-18	Salinity	8.9
	JZ515997	pentapeptide repeat protein family	269	2e-72	Salinity	1.839
Chromatin associated protein	JZ516003	SSRP1 of FACT chromatin remodeling complex	137	3e-43	Salinity	4.438

proteins. Thirty-one ESTs characterized under drought stress exhibited homology to transcription factors, chlorophyll, ribosome and RNA polymerase associated proteins, enzymes involved in protein degradation and general metabolism. Expression profile of ESTs indicated that 19 ESTs were up regulated and 12 down regulated. While 21 of the EST corresponded to genes with putative functions (**Table 2**) 10 of them corresponded to hypothetical proteins.

4. Discussion

Lablab purpureus has been reported to exhibit quantitative and qualitative variations in biochemical markers of stress responses under salinity and drought stress [22] [23]. These variations indicate distinctive set of players involved in stress perception and response biochemistry. This is also corroborated by distinct set of differentially expressed ESTs characterized under drought and salinity stress using DDRT-PCR, employing a common set of 48 primers. The distinction was also clear from the fact that 31 ESTs were characterized under drought stress, while only 12 ESTs were characterized under salinity stress relative to their respective controls. Annotation of ESTs, suggested different functionalities involved in drought and salt stress response in *Lablab purpureus*. Even among 12 ESTs differentially expressed under salinity stress, none of them were observed to be similar to those identified under drought stress (**Table 2**). Broadly the annotated ESTs, from both drought and salt stress, were associated with metabolism, photosynthesis, energy production and post transcriptional regulation, such as alteration in mRNA levels.

Metabolic adjustment is crucial to cope with abiotic stress. These changes may involve selective regulation of genes associated with the maintenance of metabolite levels. While synthesis and storage of starch in plastids takes place during the day, under applied drought, the increase in demand for energy and reduced

photosynthetic ability induces the breakdown of starch [27]. The breakdown product, Glucose-6-phosphate, is transported across the plastidic membrane via the Glucose-6-phosphate transporter [27]. Upregulation of EST homologous to Plastidic Glucose Transporter suggested breakdown of starch under salinity stress. A homologue of this transporter has been reported to be upregulated in *Arabidopsis thaliana* during increased sink demand [28] [29] [30].

Transcript abundance of several genes encoding mitochondrial proteins are known to be upregulated in response to stress [31]. An EST homologous to cytochrome-c oxidase (COX) was differentially regulated under drought stress with 1.17-fold over expression. COX expression has been shown to be associated with thermotolerance as observed in *Arabidopsis thaliana* [32] [33] and monocots such as maize [34]. Therefore, upregulated EST suggested may also contribute to drought tolerance of *Lablab purpureus*, also *Lablab* is also known for thermotolerance which has been shown through biochemical studies [35] COX may also play a role. Another EST showing homology to F1 complex assembly factor 2-like of F0F1 ATP synthase known to help in the stabilization of ATPase complex and facilitate the formation of ATP [36] [37], was upregulated under drought, is in conformity with increased demand for ATP during stress.

Catabolite repression is a general mechanism utilized by prokaryotes and lower eukaryotes to regulate carbon and nitrogen metabolism. Plant homologs are involved in catabolite regulation of various metabolites [38] [39]. Upregulation of EST homologues to CCRP suggested a vital role for its mRNA turnover during drought stress [33]. Plants respond to environmental cues by altering rigidity of the cell wall. Cell wall hydrolases have been shown to be crucial in cell wall dynamics [40] [41]. While an EST homologue to cell wall hydrolase was upregulated under drought, another homologue was down regulated under salinity stress. While upregulation of hydrolases has been reported to have protective effects under drought stress [42] their down regulation has been found to be effective under salinity stress [43].

Cell volume is modulated by alteration in plasma membrane size which is due to dynamics of endocytic vesicles. Endocytosis in plants can be mediated by formation of clathrin-dependent vesicles or clathrin-independent vesicles. EPSIN (Eps15 interactor) has been identified as a key protein under clathrin-mediated endocytosis in flowering plants [44]. In *Lablab purpureus*, the down-regulation of EPSIN homologue indicated the role of clathrin independent endocytosis under drought stress. Another vesicle associated EST showed homology to VAMP727, which is associated with ARA6 a QSNARE [45] [46] [47], was differentially expressed under drought stress. Overexpression under drought stress but not under salt stress, suggested different set of inducers under drought and salinity in *Lablab*. Dehydration leads to protein aggregation and damage [48] [49]. One of the key enzymes in protein turnover, ubiquitin carboxy-terminal hydrolase, catalyses hydrolysis of ubiquitin polymers to monomers and release of ubiquitin from tagged protein [50]. An EST showing homology to this gene was

upregulated under drought as observed in *Brassica napus* [51]. E3 ligase is a key protein which transfers the ubiquitin onto target protein, an EST corresponding to PUB22 U-box-containing E3 ligase is down-regulated under drought. First identified in *Arabidopsis* [52], PUB22 expression was shown to have negative influence on water stress response [52]. The increased activity of hydrolase and reduced E3 ligase indicated the reduction in protein turnover under drought in *L. purpureus*.

Survival of plants under stress is critically dependent on storage and utilization of assimilated elements such as Sulphur and Nitrogen [53]. EST homologous to Adenosine-5'-phosphosulfate-kinase (APSK), involved in sulphur metabolism, and nitrate transporter (NRT 1.5) 1.5 like were down regulated under salinity stress. Reduced APSK would result in reduced 3'-phosphoadenosine 5'-phosphosulfate (PAPS), a principal sulphur donor in metabolism of sulphur containing compounds [54] [55]. Reduction in NRT 1.5 like would cause non mobilization of nitrate stores, with reduction in plant growth [56]. Similar downregulation of NRT homologue has been reported for *Arabidopsis thaliana* [57] under cadmium stress, suggesting the possibility of similar molecular signals prevailing under salinity and heavy metal stress.

Stability and/or turnover of mRNA is one of the factors influencing gene expression under normal and stressed conditions [58]. Differential expression of a homologue of poly A binding protein RBP45 under drought stress suggested its role in stability of mRNA under drought [59]. But a similar response was not observed under salt stress when identical set of primers were employed for DDRT-PCR, although physiological response to dehydration by drought and salinity have been reported to be similar.

Transcription factors play key role in regulation of gene expression [60]. ESTs corresponding to MYB was upregulated under drought stress. MYB transcription factors are known to be involved in cold tolerance [61], stress response [62], ABA induced drought tolerance mechanism [63]. Another EST homologue to NF-Y A7 like has shown to be important in embryonic development, plastid biogenesis, abiotic stress tolerance [64] [65]. Upregulation of MYB and NF-Y A7 like members strongly indicate priming of transcription regulation under drought stress in *Lablab purpureus*.

Another EST homologous to transcriptional activator CAMTA3, a major regulator in biotic defence [66] [67] and regulator of rapid stress response element [68] was differentially expressed under drought. Considering the fact that CAMTA3 mutants accumulate greater DNA damage [69], its upregulation in *Lablab purpureus* suggested its protective role under drought stress. As most of the gene regulation by transcription factors are mediated by mediator complexes, up and down regulation of mediators [70] [71] [72] [73] would have an impact on the extent of regulation by a given transcription factor [74]. Upregulation of homologue of Med26 under drought suggested its positive effect in drought tolerance.

Post transcriptional regulation via siRNA and miRNA have been shown to contribute to stress response either positively, or negatively [75] [76]. Down regulation of a homologue of Maturase K under drought stress, which is known to be involved in splicing group II introns, in relation to plant development indicated non processing of the transcripts [77] [78] and has protective role in *Lablab purpureus*. As part of post transcriptional regulation of gene expression, modification of mRNA also plays a key role. Pentatricopeptide repeat proteins (PPR) are known to be associated with mRNA splicing and processing components of organelles, and overexpression of a PPR gene in *A. thaliana* has been shown to contribute to tolerance to salinity stress [79] [80] [81]. Down regulation of an EST homologous to pentapeptide repeat protein family (PPRP family) and pre-mRNA-splicing factor (CWC22) under salinity, indicate reduced stability of mRNAs under salt stress.

Abiotic stress responses are also known to be epigenetically regulated [82]. A homologue of SSRP1, which is known to associate with FACT (Facilitates chromatin transcription complex) complex [83] [84], required for transcription elongation by RNA polymerase II, was down regulated under salinity. This indicated significant role for FACT mediated epigenetic regulation under salt stress.

Environmental stress has been shown to influence ribosomal synthesis [85]. Two EST homologous of ribosomal subunits, chloroplast encoded S7 and the nucleus encoded L22, were upregulated suggesting the dynamics of ribosome under drought stress. A similar observation with upregulation of S7 has been observed in Tomato [86]. An EST homologous to rRNA-processing protein EBP2 mRNA [87] [88] showed down regulation under drought. In *Arabidopsis* this protein expression is altered in *salt hypersensitive mutant 9 (sahy9)* mutant under salinity stress [89]. The down regulation of predicted EBP2 in *Lablab purpureus* might slowdown the progression of mitosis and effect salinity induced ribosomal protein alteration in biogenesis.

Drought and salinity stress are known to affect the photosynthetic capacity, which is linked to chlorophyll content [90]. Two ESTs one homologous to chlorophyll a/b binding protein, and another showing homology to Photosystem II CP47, a component of Light harvesting complex component was down regulated under drought stress, suggesting reduced photosynthetic capacity in *Lablab purpureus*. Declined photosynthetic activity due to down regulation of LHCB protein family has been shown in *Arabidopsis* [91] and pepper [92].

Downregulation of salt stress specific differentially expressed EST, homologous to chlorophyll reductase in *Lablab*, which indicated delay in senescence [93] [94] under salt stress as observed in stay green phenotypes of *Oryza sativa* [95]. Another EST homologous to TIC32, a short chain dehydrogenase, which is part of Toc-Tic complex involved in regulation of protein import [96] in response to calcium sensing was down regulated under drought. A similar down regulation has been observed in *O. sativa* under drought stress [97]. Apart from these known homologs, there were ESTs matching the unknown function giving

a scope for identification of new roles.

Previous studies have shown that *Lablab purpureus* exhibits more or less similar pattern of biochemical parameters such as antioxidants and antioxidant enzymes under drought and salt stress. However, the foregoing discussion shows a contrasting response in terms of relative abundance of transcripts under these two conditions. Further, the transcripts identified under these two conditions were altogether different despite identical set of primers employed in DDRT-PCR method. Compared to other study on root transcriptome, only one EST (MYB) showed similarity. Therefore, it can be concluded that, at transcription level, distinct signalling molecules and elicitors are employed by the plants under drought and salinity stress. Nevertheless, the observed levels of transcripts may not always indicate the expected levels of translation products, as the stability of mRNAs and translational regulation further add a layer of regulation to stress. A clear picture may require exhaustive transcript profiling and study of epigenetic regulation to compare the molecular events that distinguish response of the plant to drought and salt stress.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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