BACKGROUND

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] (known under synonyms*P.americanum* (L.) Leeke or *P. typhoides* (Burm) Stapf and C.E. Hubb.), is an important cereal of traditional farming systems in tropical and subtropical Asia and sub-Saharan Africa, It accounts as the sixth most important crop after wheat, rice, maize, barley and sorghum in terms of annual global production (FAO 1992). Pearl millet is the staple food grain with a high nutritive value and is also used as feed, fodder and construction material.Its potential as a source of bio fuel is also being explored (Wu *et al*., 2006). Pearl millet (*Pennisetum glaucum),* commonly known as Bajra in India is an important cereal crop grown mainly in the arid and semi-arid regions of India and has the natural ability to withstand many abiotic stresses as low moisture, high temperature and salinity etc.

Abiotic stresses such as drought, salinity, cold and heat stress pose a major concern for agricultural production. With the changing global climate these environmental stresses are becoming of paramount importance because of their potential adverse impacts on agriculture and food security. The predicted increase of 60 % in cereal production (Rosegrant and Cline, 2003) will not be able to meet the food demand of 8.7 ~ 11.3 billion world population by 2050 (Bengtsson *et al.*, 2006).

Water deficiency is an important abiotic factor which restricts the crop productivity in the semi-arid tropics. Along with this, the altering climatic conditions are also expected to contribute towards drought stresses with enhanced severity in the near future. Consequently, sustainable and equitable worldwide food security is mainly dependent on the development of crop plants with better adaptation to water-limited environments
(Kholova *et al*., 2014).

Pennesitum (Bajra) has ability to tolerate drought because it has some drought resistance mechanism which makes them to grow under drought. Post-flowering drought stress is one of the most important environmental factors reducing the grain yield and yield stability of pearl millet and increasing the incidence of crop failure in dry land production environments (Mahalakshmi *et al*., 1987).Droughtare the major abiotic

stresses that adversely affect plant growth and agricultural productivity. To investigate genes that are involved in response to abiotic stresses in *Pennisetum glaucum*, a comprehensivesurvey of genes induced by drought stresses was done by qPCR. Pearl millet (Pennisetum glaucum), used asforage and grain crop is a stress tolerant species. Here weidentify differentially regulated transcripts in response to drought stress from subtractedcDNA libraries by single-pass sequencing of cDNA clones. To elucidate the molecular basis of drought response, to identify and characterize drought responsive genes in pearl millet, a drought stress (30% PEG 6000) responsive subtractive hybridization library was constructed from 22days old pearl millet seedlings (*Pennisetum glaucum* cv. PPMI741and breeding line (652-657)) subjected to drought stress at room temperature by 30 % PEG 6000 for different time period (30 min, 2hr, 4hr, 8hr, 16hr, 24 hr and 48hr). Plants with high capacity for water retention can better survive drought stress). During 0 to 48 hours of a 30% PEG600 treatment. P. glaucum PPMI741 high relative water content (RWC) and showed a low water loss rate (WLR). RWC values indicating the drought tolerance ability of Pearl millet, drought treated seedlings increased duration of stress. A subtractive cDNA library was constructed from drought stressed 22 day old seedlings. A total of 2,400 EST sequences were clustered andassembled into a collection of 745 unique sequences with 70 contigs and 352 singleton sequences. . A total of 422 unique high quality ESTs identified were submitted to ENA-EMBL databases (Accession no. HG516611-HG517355). The ESTs were classified on the basis of molecular function, biological processes and cellular localization based on Gene Ontology search through RGAP (Rice Genome Annotation Project) BLASTx analysis. More than 25 % of the ESTs showed homology to genes directly involved in abiotic stress response whereas 2% of the ESTs in the SSH library library did not show any homology to genes present in database. By sequencecomparisons the putative functions of many ESTs could beassigned. Genes with stress related functions include thoseinvolved in cellular defense against abiotic stresses andtranscripts for proteins involved in stress response signalingand transcription in addition to ESTs encoding unknownfunctions. These provide new candidate genes forinvestigation to elucidate their role in abiotic stress. The relative mRNA abundance of 10 selected genes, Some genes non validated but these involved in abiotics stress, These

include glutathione peroxidase (EST ID: Plate 6F_B5_Pg_SSH), Adenylate kinases proteins (EST ID: Plate_AF_O3_ Pg_SSH, citrate synthase , chloroplast 30S ribosomal protein S7.A, Zn finger protein, glutathione peroxidase, Adenylate kinases proteins, citrate synthase, chloroplast 30S ribosomal protein, Adenylate kinases proteins, protein phosphatases, phospholipid metabolism, Amino transferase protein, ATP-citrate synthase, Glutamine synthetase, glycine-rich RNA-binding protein (GR-RBP), serine/arginine rich (SR),Metallothionein, Hsp70 proteins, P700 chlorophyll apoprotein A2, photosystem II D2 protein and photosystem II 44 kDa reaction center protein.The differentially expressed ten genes like Abscisic stress ripening protein(ESTS ID: Contig_12-FSSH-Pg), Ascorbate peroxidase (ESTS ID: Contig_23-FSSH-Pg) ,Glyoxalase (ESTS ID: Contig_24-FSSH-Pg), Rab-7(ESTS ID: Singlet_223-FSSH-Pg),Aspartic proteinase Oryzasin (ESTS ID: Contig_37-FSSH-Pg), Ubiquitin-Conjugating enzyme E2-7 (ESTS ID: Contig_27-FSSH-Pg), DnaJ-like protein, Calmodulin-like protein (ESTS ID: Singlet_204-FSSH-Pg), Putative beta-1, 3-glucanase (ESTS ID: Contig_53-FSSH-Pg), and Inosine-5'-monophosphate dehydrogenase(ESTS ID: Contig_11-FSSH-Pg) were selected obtained in the forward subtracted library were randomly selected for validation by real time RT-PCR in *Pennesitum glaucum* under drought stress for different time durations (30 min, 2hrs, 4hrs, 8hrs, 16 hrs 24hrs and 48 hrs).The quantitative up-regulation of the selected genes for their gene expression in response to drought stress clearly showed that the subtractive cDNA libraries constructed in this study were substantially enriched for stress responsive genes (obtained in the subtracted library). These EST sequences are a rich source of stress-related genes and reveal a major part of the stress-response transcriptomes that will provide the foundation for further studies into understanding *Pennisetum g.*adaptability to harsh environmental conditions.The present study provides an insight into the genes differentially expressed in response to drought stress in pearl millet. Further studies on full length cloning of the identified genes and functional validation through over-expression in model plant systems will be useful in the development of improved drought tolerant crops for the future.

Keywords Pearl millet _ Real time PCR _ Abiotic stress _Stress responsive ESTs _ Differential expression.

INTRODUCTION:

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] (known under synonyms: *P.americanum* (L.) Leeke or *P. typhoides* (Burm) Stapf and C.E. Hubb.), is an important cereal of traditional farming systems in tropical and subtropical Asia and sub-Saharan Africa. It accounts as the sixth most important crop after wheat, rice, maize, barley and sorghum in terms of annual global production (FAO 1992). Pearl millet is the staple food grain with a high nutritive value and is also used as a feed, fodder, construction material. Its use as a source of bio fuel is being explored (Wu *et al*., 2006). It is grown on 29 million ha (FAO 2005) in Africa and Indian sub-continent supporting millions of poor rural families mostly in the drought-prone areas where rain fed agriculture is commonly practiced. Pearl millet is the fourth most important cereal crop in India, after rice, wheat and sorghum, which is widely grown in the states of Rajasthan, Maharashtra, Gujarat and Haryana where the food security of the population depends immensely on pearl millet production. India is the largest producer of pearl 66millet in Asia, both in terms of area (about 9 million ha) and 67production (8.3 million tons) with an average productivity of 930 kg/ha during the past 3 years (ICAR-AICPMIP report (2014). The average agricultural area sowed under pearl millet in India is approximately to 9.5 Mha with an average annual grain production of 8.3 M tons (FAO 2005). Pearl millet is known to yield up to 6 t/ha, but due to environmental conditions the common average grain yields are lowered to 800-600 kg/ha (FAO 2005, 2010).

Pearl millet is well adapted to production systems characterized by low rainfall, low soil fertility, and high temperature, and thus can be grown in areas where other cereal crops, such as rice wheat or maize, would not survive. Its rapid growth rate in favorable conditions, high temperature tolerance and ability to extract mineral nutrition and water from even the least fertile soil make it an ideal crop to be grown in even the harshest agricultural production environments across the world.

Work on biofortification of pearl millet is also progressing with the aim of meeting the nutritional requirements of poor people especially children for whom pearl millet is the only food grain available (Kodkany et al., 2013)

The International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) germplasm collection consists of 21,594 accessions of cultivated pearl millet from 50 different countries, and it is the largest collection of pearl millet germplasm in the world. More than 25 million ha of pearl millet are cultivated in Africa and East Asia where it is a staple grain crop. There is a wide range of variability in the ICRISAT collection, and it includes collections from institutions (10,201), farmers' fields (6,537), commercial markets (1,681), farmer co-ops (1,357), and threshing floors (479) (ICRSAT, 2011).

Under both natural and agricultural conditions plants are often exposed to various environmental Stresses. Drought is one of most important environmental factors inhibiting photosynthesis and decreasing growth and productivity of plants. It is one of the major causes of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Bray *et al.*, 2000; Wang *et al.*, 2003). Under these stress conditions usually a water deficit in plant tissues develops. In the last years effects of water deficit were studied on different levels from physiology to cell metabolism (Shinozaki and Yamaguchi-Shinogzaki, 1997; Chaves *et al.*, 2003).

There are basically three levels of plant drought tolerance/resistance.

- **Drought escape**: The mean of drought escape is avoiding living in stress full times. Some plants have adapted by having rapid growth, maturation, flowering and fruiting. This keeps tissues from being excessively exposed to dehydration e.g. winter wheat.
- **Drought avoidance**: is the ability of plants to maintain relatively high tissue water potential despite a shortage of soil moisture.
- **Drought tolerance**: is the ability to withstand water deficit with low tissue water potential.

- *Pennesitum* (Bajra) has ability to tolerate drought because it has some drought resistance mechanism which makes them to grow under drought. Post-flowering drought stress is one of the most important environmental factors reducing the grain yield and yield stability of pearl millet and increasing the incidence of crop failure in dry land production environments (Mahalakshmi *et al*., 1987). Terminal drought stress (flowering through grain filling) is more damaging to pearl millet productivity than stress at the vegetative or pre-flowering reproductive crop growth stages. This is because pearl millet's asynchronous tillering behavior and rapid growth rate allow it to recover rapidly from intermittent drought stress during these earlier stages of plant development, but provide no advantages under unrelieved terminal drought stress (Bidinger *et al*., 1987; Mahalakshmi *et al*., 1987). Improving the adaptation of pearl millet to terminal drought stress environments is, therefore, a major objective for breeding programmers' aimed at improving both the crop's productivity and its yield stability (Yadav *et al*., 2002; Bidinger and Hash, 2004).

REVIEW OF LITERATURE:

Linzhou Huang, (2013) reported that Arabidopsis *ERECTA* gene (*ER*) in wheat, *TaER*, is considered to be a promising candidate gene for the genetic improvement of water use efficiency (WUE) and drought tolerance in breeding programs. In this study, he isolated two distinct homologues (*TaER1*and *TaER2*) of *TaER*genes in common wheat through in silico screening and PCR-based homologous cloning.

Pruthvi, V., (2012) identified candidate stress responsive genes from adapted crop; he Carried out expression analysis of a few droughts responsive ESTs from Arachishypogaea L. (peanut). The expression patterns of nine AhDR (Arachis hypogea drought responsive) clones were analyzed under drought. Quantitative reverse transcription PCR analysis revealed stress responsive nature of the selected genes.

Krasensky, (2011) discussed the information about metabolic regulation in response

to drought, extreme temperature, and salinity stress is summarized and the signaling events involved in mediating stress-induced metabolic changes are presented. Plants regularly face unfavorable growth conditions, such as drought, salinity, and chilling, freezing, and high temperatures.

Nicky. J. (2012) discussed the plant responses to different stresses are highly complex and involve changes at the transcriptome, cellular, and physiological levels. Recent evidence shows that plants respond to multiple stresses differently from how they do to individual stresses, activating a specific programme of gene expression relating to the exact environmental conditions encountered.

Johnsi Rani, R. (2011) studied salt stress as a major adverse factor can lower leaf water potential, leading to reduced responses, and ultimately lower crop productivity in arid and semi-arid zone. Plant responses to salt stress have much in common. Salt stress reduces the ability of plants to take up water and this quickly causes reductions in growth rate. The initial reduction in shoot growth is probably due to salt effects. If excessive amounts of salt enter into the plant, salt will eventually rise to toxic levels and reduce the photosynthetic leaf area of the plant that cannot sustain growth.

Gill, D. S. (2011) studied the thermo tolerance and good comparison of temperature limits of growth in different plants. Temperature stress due to high temperature is a major problem in agriculture in many areas. Continuously increasing temperature cause an disorder of physiological and biochemical changes in crop plants, which affects many growth process i.e. different phenological stages of the crops and development that may lead to a sharp reduction in grain yield. The detrimental effect of temperature stress can be mitigated by developing crop plants with thermo tolerance using various approaches.

Ram Kumar, G. (2010) reported that it is important to use this genomic information for the identification and isolation of novel and superior alleles of agronomically important genes from crop gene pools to suitably deploy for the development of improved cultivars.

The differentially expressed genes responsible for conferring drought resistance in *Pennisetum glaucum* would be studied as per following objectives.

Objectives:

1. Identification of Genes expressed under drought stress in *Pennisetum glaucum*.
2. Analysis of differential expression of candidate gene(s) identified under drought stress.

Plan of Work:

- Collection of seeds of different cultivars of Pearl millet (*Pennisetum glaucum*)and standardization of seed germination in pots.
- Standardization of RNA isolation from leaf samples of Pearl millet.
- Identification of genes expressed under drought stress by using suppression subtractive hybridization. Subtractive hybridization is a technology that allows for PCR-based amplification of only cDNA fragments that differ between a control and experimental transcripts.
- All ESTs obtained will be cloned and sequenced to identify the candidate genes under drought stress.
- Analysis of all ESTs for homology with drought tolerant genes by nBLAST software.
- Primers will be designed for candidate genes expressed under drought.
- Drought responsive candidate genes identified from stress induced cDNA library will be analyzed for differential expression by Real Time PCR.

METHODOLOGY:

22 old days seedlings were subjected to low moisture stress Relative water content (RWC) was calculated in different time period (30 min,2hrs, 4hrs, 8hrs, 16hrs and 24hrs, 48hrs) after subjecting 22 old days seedlings of *Pearl millet* under 30% PEG6000 treatment.

Suppression subtractive hybridization library is constructed from PEG6000 stressed seedling.Isolation of total RNA from leave tissues of Pearl millet seedling.

First strand cDNA was synthesized SMART cDNA Subtraction with driver (unstressed) and tester (stressed) total RNA .first strand tester and driver cDNA were used for second strand synthesis then add to the first-strand synthesis reaction. The following procedure was performed with each experimental double-stranded (ds) tester and driver cDNA. This step generates shorter, blunt-ended ds cDNA fragments which are optimal for subtraction and required for adaptor ligation. In the following procedure, an excess of driver cDNA was added to each tester cDNA, samples were heat denatured and allowed to anneal. The remaining single stranded (ss) cDNAs (available for the second hybridization) were dramatically enriched for differentially expressed sequences because non-target cDNAs present in the tester and driver cDNA formed hybrids. samples from the first hybridization are mixed together, and fresh denatured driver DNA was added to further enrich for differentially expressed sequences. This step led to the formation of new hybrid molecules which consisted of differentially expressed cDNAs with different adaptors at each end.

Polymerase chain reaction amplification: Differentially expressed c-DNAs were selectively primary and secondary amplification to thermal cycling, In the first amplification, only ds cDNAs with different adaptor sequences on each end were exponentially amplified. In the second amplification, nested PCR was used to further reduce the background and enrich for differentially expressed sequences.

The contents were mixed thoroughly by vortexing, and centrifuged briefly. Master Mix (25.0 µl) was added into each of the reaction tubes prepared previously. The reaction mixture was incubated at 75 °C for 5 min in a thermal cycler to extend the adaptors, during the process the samples were not removed from the thermal cycler. The contents were placed on (S1000 Thermo cycler TM, BioRad,USA) for thermal cycling under specific conditions: (94 °C for 25 sec, followed by 27 cycles of 94 °C for 10 sec, 66 °C for 30 sec and 72 °C for 1.5 min). PCR product (8.0 µl) from each tube was analyzed on a 1 % agarose + 10mg/ml EtBr gel. Primary PCR mixture (3.0 µl) was diluted with 27.0 µl of sterile H2O. 1.0 µl of each diluted primary PCR product mixture was added into an appropriately labeled tube.

Ligation: Concentration of purified PCR product was measured by Nano drop (Thermo Scientific) before proceeding to ligation step. Purified PCR product was ligated into pGEM-T Easy vector (Promega, Madison, USA)using T4 DNA ligase (supplied with kit) followed by the protocol described by (Sambrook*et al.*,1989). 3.0µl of purified DNA product was added to 10.0 µl of 2xligation buffer, 1.0µl (50.0ng) of cloning vector and 1.0µl of T4 DNA ligase.

Transformation: 2.5 µl of ligated product was used for transformation of 40 µl of electro competent *E. coli* cells (NEB 10-beta electro competent cells, New England Biolabs). The mixture was taped gently, transferred in to a pre-chilled electroporation cuvette and placed in the Electroporator (Eppendorf Multiorator 30672, USA). Electroporation was perform at 1800 V for 4 ms .After electroporation, 1 ml of SOC medium was added immediately into the cuvette and mixed well by inverting the cuvette. The culture was transferred into new 1.5 ml tube to incubate at 37^{o}C in shaker (200 rpm) for 1 hr. After incubation,100µl of the transformed culture was spread on pre-warmed LA plate supplemented with Ampicillin (100mg/ml) + IPTG (200mg/ml) + X-gal (20mg/ml) and incubated overnight at 37oC.There after incubation only white colonies from the mixture of blue and white were selected from the transformation plate and picked with toothpick. They were streaked in master grid of a new IXA plate, called master plate. The colonies

were labeled by number and the plate was incubated for overnight at 37^0C. The colonies were screened for positive clone by confirmed colony PCR method.

Colony Polymerase chain Reaction: Colony PCR was carried out to confirm the presence and size of insert fragments. T7:5'-TAA TAC GAC TCA CTA TAG G-3' and Sp6:5'-TAT TTA GGT GAC ACT ATA G-3' primers (Promega, USA) were used to amplify the insert fragments from selected clones. The colony PCR was carried out as per the procedure below. Sterile water (18.6 µl) was added to each of 96 well PCR plate. Small amount of cells from randomly clones was transferred into each well. The cells were homogenizes by gentle pipetting and 6.4 µl of master mix solution (10X buffer A (Bangalore Genie), 25 mM MgCl2 (Bangalore Genie), 25 mM dNTPs (Bangalore Genie), primers (Sp_6 and T_7) and Taq polymerase 5U/µl (Bangalore Genie)) as added to each well.

Sequencing and Sequences analysis: Positive Clones from the subtracted cDNA library with insert size of 500bp or more were sequenced by single pass sequencing using the vector specific sequencing primer T7. 5'TAA TAC GAC TCA CTA TAG G by an automated DNA sequencer (sequencing were performed at Macrogen Inc., South Korea, through Sequencher Tech Pvt. Ltd, Ahmedabad, India). The vector and poor sequences were refined by Laser gene DNASTAR version 9 software. After that checked all sequencing reads manually by NCBI VecScreen tool (http://www.ncbi.nlm.nih.gov/Vecscreen). The good quality ESTs which had more than 100 bp in length were assembled into contigs and singlets using SeqMan Pro program. The obtained contigs and singlete were subjected to BLASTn, available in NCBI website (http://www.ncbi.nlm.nih.gov/blast) to identify homology for known ESTs. BLASTx via NCBI (http://www.ncbi.nlm.nih.gov/blast) and BLASTx of RGAP (http://rice.plantbiology.msu.edu/analyses_search_blast.shtml) were performed to determine similarity of these ESTs with known proteins from other cereal crops. Drought stress modulated genes were functionally categorized using the GO (Gene Ontology) IDs available through RGAP BLASTx Locus IDs. These locus IDs were used to assign GO

terms under three main categories viz. Biological process, Cellular component and Molecular function.

Expression analysis of drought responsive genes: Primer DesigningReal-Time polymerase chain reaction experiment was carried out to validate some of the genes differentially expressed in the SSH Library. Specific primer pairs for each of the sequences were designed using the Primer Quest software of Integrated DNA Technology (IDT) and were synthesized by Biochem Life Sciences, New Delhi. Lyophilized form of primers were dissolved in sterile distilled water as recommended by the company to obtain the final concentration of 100 µM (100 Pico mole/µl) primer stock. Working solution of primer 10 µM was prepared from 100 µM stock solution.

Validation of genes by Quantitative Real Time PCR : Validation of genes was performed by quantitative RT-PCR using Roche LightCycler 480TM system (Roche, Germany) equipped with a 96 well plates system and KAPA SYBR® FAST Master Mix reagent (KAPA Biosystems, USA). The RT PCR experiments were performed with three technical replicates with 10 µl containing 4 µl of cDNA (diluted according to initial concentration so as to contain 150 ng cDNA), 1.0 µl of forward and reverse primers, and 5 µl of KAPA SYBR® FAST qPCR Master Mix according to the manufacturer's instructions. The following thermal cycling profile was used for all qRT-PCR.

RESULT:4

Pearl millet cultivar PPMI741 (breeding line, 652-657) seeds were procured from the Genetic Division and germinated in autoclaved pots in Soilrite™ at the National Phytotron of Indian Agricultural Research Institute, New Delhi. Drought stress was induced by 30% PEG 6000 in 22 days old seedling of Pearl millet cultivar PPMI741and breeding line 652-657. Different time periods (0.5, 2, 4, 8, 16, 24, and 48hr) of stress was applied. Fresh weight of the seedling was recorded and then floated in water for 4 hr to gain turgidity. After recording the turgid weight, the samples were dried overnight in oven at 80 °C to constant weight to record dry weight and RWC was estimated.

Suppression subtractive hybridisation (SSH) was successfully employed to obtain these differentially expressed transcripts. The constructing subtractive cDNA libraries are having high throughput cloning of stress-responsive genes that are differentially

expressed in response to drought stress in *Pennisetum glaucum*. We have performed a subtraction protocol, for isolation and study of stress-specific ESTs, that effectively removes the highly abundant housekeeping and non-targeted genes that are equally expressed in both control and stress exposed samples, prior to subtractive cDNA library construction. The representation of various cDNA messages in these subtractive cDNA libraries were not only normalized for their sequence abundance but also enriched for differentially expressed cDNAs in response to various environmental stresses. We randomly selected the recombinant cDNA clones from these subtractive cDNA libraries and established a collection of stress responsive *Pennisetum* ESTs. We have also validated the differential up-regulation of these genes in response to drought stresses, in *Pennisetum* seedlings, by real-time quantitative RT-PCR analysis. The putative functions of these *Pennisetum* ESTs were established based on the homology searches and the presumed biological implications of the products of these differentially expressed genes in relation to the complex networks of stress-adaptive processes in *Pennisetum* are also discussed.

During 0 to 48 hours of a 30% PEG600 treatment. P. glaucum PPMI741 high relative water content (RWC) and showed a low water loss rate (WLR). RWC values indicating the drought tolerance ability of Pearl millet, drought treated seedlings also decreased duration of stress.

There were 2400 clones obtained, clone sequences randomly base 1344, high quality stress responsive ESTs were identified and assembled into a collection of 422 unique sequences with 70 contigs and 352 singleton sequences. A total of 422 unique high quality ESTs identified were submitted to NCBI,ENA-EMBL Database, Accession number HG516611-HG-517355. The ESTs were classified on the basis of molecular function, biological processes and cellular localization based on Gene Ontology search through RGAP (Rice Genome Annotation Project) BLASTx analysis. More than 25 % of the ESTs showed homology to genes directly involved in abiotic stress response whereas 2% of the ESTs in the SSH library library did not show any homology to genes present in database. This result was corroborated by the presence of many genes expressed in drought responsive SSH library. Some genes non validated but theses involved in directly and indirectly abiotics stress, These include glutathione peroxidase (EST ID: Plate 6F_B5_Pg_SSH), Adenylate kinases proteins (EST ID: Plate_6F_O3_ Pg_SSH), citrate synthase(EST ID: Plate_7F_O7_ Pg_SSH) and chloroplast 30S ribosomal protein

S7(EST ID: Contige_33_ Pg_SSH), Adenylate kinases proteins (EST ID: Plate_1F_O3_ Pg_SSH), phosphatases (EST ID: Plate_6F_G2_ Pg_SSH), phospholipid metabolism (EST ID: Plate_7F_E12_ Pg_SSH), Amino transferase protein (EST ID: Plate_3F_G11_ Pg_SSH), ATP-citrate synthase (EST ID: Plate_contegi_50_ Pg_SSH), Glutamine synthetase EST ID: Plate_9F_G10_ Pg_SSH), RAD23 DNA repair proteins(EST ID: Plate_5F_B11_ Pg_SSH) ,Zinc finger protein (EST ID: Plate_6F_E11_ Pg_SSH) , Hsp70 proteins(EST ID: Plate_contegi_45_ Pg_SSH) ,metallothionein(EST ID: Plate_8F_F2_ Pg_SSH), serine/arginine rich (SR) (EST ID: Plate_contegi_26_ Pg_SSH) , glycine-rich RNA-binding protein (EST ID: Plate_2F_D9_ Pg_SSH) , P700 chlorophyll apoprotein A2 (EST ID: Contige _6_Pg_FSSH), photosystem II D2 protein (EST ID: Plate_7F_G6_Pg_FSSH) and Photosystem II 44 kDa reaction center protein (EST ID: 5F_B2_Pg_FSSH). The differentially expressed ten genes like like Abscisic stress ripening protein(EST ID: Contig_12_ Pg _FSSH), Ascorbate peroxidase (EST ID: Contig_23_Pg_FSSH), Glyoxalase (EST ID: Contig_24_Pg_FSSH), Rab7(EST ID: Singlet_223_Pg_FSSH), Aspartic proteinase Oryzasin (EST ID: Contig_37_Pg_FSSH-P), Ubiquitin-Conjugating enzyme E2-7 (EST ID: Contig_27_ Pg_FSSH), DnaJ-like protein (EST ID:4F_E10_Pg_FSSH), Calmodulin-like protein (EST ID: Singlet_204_Pg_FSSH) ,Putative beta-1,3-glucanase (EST ID: Contig_53_Pg_FSSH), and Inosine-5'-monophosphate dehydrogenase(EST ID: Contig_11_Pg_FSSH) were selected obtained in the subtracted library were randomly selected for validation by real time RT-PCR in *Pennesitum glaucum* under drought stress for different time durations (30 min, 2hrs, 4hrs, 8hrs, 16 hrs 24hrs and 48 hrs).The quantitative up-regulation of the selected genes for their gene expression in response to drought stress clearly showed that the subtractive cDNA libraries constructed in this study were substantially enriched for stress responsive genes. The present study provides an insight into the genes differentially expressed in response to drought stress in pearl millet.

The expression of 10 selected genes obtained in the forward subtracted library) were validated by qRT-PCR analysis at various durations drought stress. The quantitative gene expression results clearly indicate that the SSH library contains genes which have been differentially expressed during drought stress.

Future scope: The differentially expressed genes with higher expression levels are identified, their function in response to drought stress can be validated by cloning the full length genes and identifying the up-stream promoter elements .The full length sequences of heat responsive genes identified in this study can be completely cloned using 5' and 3' Rapid Amplification of cDNA ends (RACE) technique. These can then be functionally validated through over-expression in model plant systems via transgenic technology. This study provides essential information to further understand and gain helpful insights into the genetic basis of differential adaptation of plants to adverse environments.