DECLARATION

I hereby declare that the thesis entitled "**Identification and Characterization of Genes Differentially Expressed under drought Stress in Pearl millet *(Pennisetum glaucum (L.) R. Br.)***" submitted by me, for the award of the degree of Doctor of Philosophy to Department of Biotechnology, Faculty of Biological Engineering, Shobhit University, Modipuram, Meerut (UP) is a record of bonafide work carried out by me under the supervision of **Dr. Jayanand,** Associate Professor**,** Faculty of Biological Engineering**,** Shobhit University, Meerut and co-supervision of **Dr. Jasdeep C. Padaria,** Principal Scientist**,** NRCPB, IARI**,** New Delhi.

I further declare that the work reported in this thesis has not been submitted and will not be submitted, either in part or in full, for the award of any other degree or diploma in this institute or any other institute or university.

Dated:

MINAKSHI CHOUDHARY
Faculty of Biological Engineering
Shobhit University, Meerut (UP)

THIS WORK
IS DEDICATED
TO
MY BELOVED PARENTS
AND
MY HUSBAND

ACKNOWLEDGMENTS

This work is the realization of meticulous guidance, support and help of some special dignitaries whom I would like to acknowledge through this platform. First and foremost I express my sincere gratitude to ***Kunwar Shekhar Vijendra,*** *Hon'ble Chancellor, Shobhit University, Meerut, for strengthening the foundation of this great institution and being the guiding light that has illuminated our academic path throughout this journey.*

I would like to pay my utmost gratitude to ***Prof. (Dr.) D.V. Rai****, Hon'ble Vice Chancellor, Shobhit University, for his scrupulous guidance and supervision that paved way for the smooth completion of my degree.*

I am highly indebted to my supervisor ***Dr. Jayanand****, Associate Professor, Faculty of Biological Engineering Shobhit University, Meerut, for his valuable guidance, expedient advice and encouragement during the course of this study. He is the key person without whose help, care and affection my long cherished dream would not have been fulfilled.*

Further, my sincere gratitude and thanks goes to my external co-supervisor, ***Dr. Jasdeep C. Padaria****, Principal Scientist, Advanced Center for National Research Center on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi for her valuable suggestions, and constant encouragement throughout the period of my research work. I am all the more grateful to her for selecting me as Research Fellow in her project entitled "National initiative on climate Resilient Agriculture" funded by ICAR, Govt. of India, and giving me an opportunity, funds and facilities to carry out the research work in her lab.*

I take this opportunity to thank ***Dr. T. R. Sharma, Director,*** *Department of National Research Center on Plant Biotechnology, IARI, New Delhi, for providing me the required facilities.*

I am highly obliged to ***Dr. C. Tara Satayawati****, Department of genetics, Indian agriculture research institute, New Delhi, for providing me required seed material.*

*I am highly beholden to **Dr. Rajendra** National Facility of Phytotron, Indian Agriculture Research Institute, New Delhi, who helped me in maintaining plants in glass house and for his generous cooperation.*

*I convey my special recognition to **Dr. Rekha Dixit and Dr. Joyti Sharma,** who set the base of my degree by their meticulous deliberations, preaching and motivationin the right direction towards the conclusion of this task.*

*I owe my heartfelt gratitude to my lab mates**, Dr. Raj Kumar , Dr. Avijit Tarafdar, Dr. Deepesh bhatt, Ramachandra, Koushik, Showkat, Radha, Harinder, Meenal , Hartish, Arul, Rahul and Priyanka** for their help, support and solving my research problems like anything and my special thanks to our lab attendant **Satish bhaiya** for any kind of help regarding lab works. My special thanks to my friend, **Anvesha Sinha** for her moral support and espousal throughout the compilation of this work.*

*Diction is not enough to express my gratitude and affection to my beloved parents **Smt. Mamta Choudhary and Sri Kulveer Singh Choudhary** and my mother & father in laws **Smt. Mithlesh Tomar** and **Sri Pritam Singh Tomar** for being the pillars of strength and source of blessings. Without consecration this Ph.D. work would not have been realized.*

*I lovingly acknowledge the espousal, affection, and consistent support of the back-bone of my life, my loveable husband, **Mr. Vikas Tomar.** It was the fervor of his encouragement and love that strengthened me at every step to move ahead towards attaining this milestone of my academic career and my delightful thanks to **my cute baby Kabir,** who gave me his significant time to complete this research work.*

*Last but not the least, I convey my heartiest thanks to my sisters **Apeksha, Renu** and **Swati,** for their constant encouragement, inspiration and guidance in all the way, and to my brothers **Sudhir, Kamal and Atul** for their constant concern towards my wellbeing.*

*My study needs special acknowledgement to the **ICAR**, for providing financial assistance in the form of Research Fellowship during the course of my study. I also take this opportunity to thank the **IARI** for giving an excellent opportunity to learn from this prestigious institute.*

At last I extend my deep feeling to almighty God because of their blessing I achieve something in my life and became able to present effort.

Dated: MINAKSHI CHOUDHARY

ABSTRACT

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 deteriorating global climatic conditions, these environmental stresses are emerging as the prime predicaments in agriculture, due to their potential adverse impacts on food productivity and security. The expected enhancement in the cereal production of around 60% (Rosegrant and Cline, 2003) will not be sufficient to meet the food demand of 8.7 ~ 11.3 billion world population by 2050 (Bengtsson *et al.*, 2006).Drought is one of the main environmental constraints to agricultural productivity worldwide. Many efforts have been made to elucidate the mechanisms of drought tolerance in plants through molecular and genomics approaches, and a number of genes that respond to drought stress at the transcriptional level have been reported.

Pennesitum (Bajra) has ability to tolerate drought owing to its several drought resistance mechanism which aids them in growing under drought. Post-flowering drought stress is one of the most important environmental factors that reduce the grain yield and yield stability of pearl millet and increase the incidence of crop failure in dry land production environments (Mahalakshmi*et al*., 1987).Drought are 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 comprehensive survey of genes induced by drought stresses was done by qPCR. Here we identified differentially

regulated transcripts in response to drought stress from subtracted cDNA 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 periods (30 min, 2hr, 4hr, 8hr, 16hr, 24hr and 48hr). Plants with high water retention capacity can better survive the drought stress. During 0 to 48 hours of a 30% PEG600 treatment, *P. glaucum* PPMI741 exhibited high relative water content (RWC) and showed a low water loss rate (WLR). RWC values indicate the drought tolerance ability of Pearl millet in drought treated seedlings which increased with the 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 and assembled 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 did not show any homology to genes present in database. By sequence comparisons, the putative functions of many ESTs could be assigned. Genes with stress related functions include those involved in cellular defense against abiotic stresses and transcripts for proteins involved in stress response signaling and transcription in addition to ESTs encoding unknown functions. These provide new candidate genes for investigation to elucidate their role in abiotic stress. The relative mRNA abundance of 10 selected genes was non-validated but these were found to be involved in abiotic stresses. 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 S7A, 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 apoproteinA2, photosystem II D2 protein and photosystem II 44 kDa reaction center protein. The differentially expressed ten genes viz. 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 obtained in the forward subtracted library and 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 aimed towards understanding *Pennisetum glaucum* 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 in the future.

Keywords Abiotic stress, Differential expression, Pearl millet, Real time PCR, Stress responsive ESTs

TABLE OF CONTENTS

LIST OF FIGURES

LIST OFTABLES

LIST OF ABBREVIATIONS

Abbreviation	Full Forms
ABA	Abscisic acid
APX	Ascorbic peroxidase
ASR	Abscisic stress ripening protein
BLAST	Basic Local Alignment Search Tool
Bp	Base pair
CAT	Catalase
c-DNA	Complementary DNA
c-DNA-AFLP	c-DNA-amplification fragment
CDS	Coding sequence
COS	Conserved orthologous sequences,
DEPC	Diethylpyrocarbonate
DNA	De-oxy ribonucleic acid
DnaJ	DnaJ-like protein
dNTP	Deoxynucleotide triphosphates
ds-RNA	Double-stranded RNA
EDTA	Ethylene diamine tetraacitic acid
EST	Expressed sequence tag
EtBr	Ethidium bromide
GO	Gene Ontology
GR	Glutathione reductase
GS	Growth Stages
GXB	Glyoxalase
HRC	High-resolution cross
HSP70	Heat-shock proteins
IARI	Indian Agricultural Research Institute
ICRISAT	International Crop Research Institute for Semi-Arid Tropics
IMD	Inosine-5’ mono phosphate dehydrogenase
IPTG	Isopropyl β-D-thio-glactoside

IXA	IPTG, X-gal and ampicilin
LA	Luria agar
LB	Luria broth
LD-PCR.	Longer distance polymerase chain reaction
MCS	Multiple cloning sites
MOPS	Morpholinopropane-1-sulfonic acid
mRNA	Messenger RNA
MS	Murashige and Skoog
NaCl	Sodium chloride
NCBI	National centre for Biotechnology
Nt	Nucleotide
OD	Optical density
PBG	Putative beta-1, 3-glucanase
PCI	Phenol: Chloroform: Isoamyl alcohol
PCR	Polymerase chain reaction
PEG	Poly ethylene glycol
QTL	Quantitative trait loci
RGAP	Rice Genome Annotation Project
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
RT	Room temperature
RT-PCR	Reverse transcriptase- polymerase Chain reaction
RWC	Relative water content
SDS	Sodium dodecyl sulphate
SDW	Sterile distilled water
SOD	Superoxide dismutase
SSH	Suppression subtractive hybridization
SsRNA	Single stranded RNA
TAE	Tris-acetate-EDTA
UNEP	United Nations Environment Program
VPD	High vapor pressure deficit
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside