DECIPHERING THE DIFFERENTIALLY EXPRESSED GENES IN FOXTAIL MILLET (*Setaria italica* L.) IN RESPONSE TO WATER STRESS

THESIS

Submitted to
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
in partial fulfilment of the requirements
for the Degree of

DOCTOR OF PHILOSOPHY
IN
AGRICULTURE
(AGRICULTURAL BIOTECHNOLOGY)

By
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Enrolment Number- KK-2559 2016

DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the thesis entitled **"DECIPHERING THE DIFFERENTIALLY EXPRESSED GENES IN FOXTAIL MILLET (*Setaria italica* L.) IN RESPONSE TO WATER STRESS"** or part thereof has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis / publication of any University or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged.

Place: Akola (Gawai Dipti Chandrabhan)

Date: / / 2016 Enrolment No. KK-2559

CERTIFICATE

This is to certify that thesis entitled **"DECIPHERING THE DIFFERENTIALLY EXPRESSED GENES IN FOXTAIL MILLET (*Setaria italica* L.) IN RESPONSE TO WATER STRESS"** submitted in partial fulfillment of the requirement for the degree of "**Doctor of Philosophy in Agricultural (Agricultural Biotechnology)**" of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by Gawai Dipti Chandrabhan under my guidance and supervision.

The subject of the thesis has been approved by the Student's Advisory Committee.

Place:
Date: 30 /06/ 2016

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Chairman,
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THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE
INCLUDING EXTERNAL EXAMINER (AFTER VIVA-VOCE)

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Place: Akola
Date:

Gawai Dipti Chandrabhan

Table of Content

(D) List of Tables

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(G) Abbreviations

∞	:	Infinity
%	:	Percentage
@	:	At the rate of
~	:	Nearly about
<	:	Less than
Δ	:	Delta
°C	:	Degree Celsius
µg	:	Micro grams
µl	:	Micro liters
µM	:	Micro molar
A	:	Absorbance
AA	:	Amino acid
ABA	:	Abscisic acid
ABF	:	ABA-binding factor
ABI3	:	Abscisic acid insensitive 3
ABREs	:	ABA-responsive elements
AFLP	:	Amplification Fragment Length Polymorphism
ANOVA	:	Analysis of variance
AP	:	Andhra Pradesh
AP2	:	Apetala2
APS	:	Ammonium persulfate
AQP	:	Aquaporin
AREB	:	ABA-responsive element binding protein
ARF	:	Auxin response factor
ARFs	:	ADP-ribosylation factors
ASR	:	ABA-water stress ripening induced
ATAF	:	Arabidopsis transcription activation factor
AtHK1	:	*Arabidopsis thaliana* Histidine Kinase
ATP	:	Adenosine 5’-triphosphate
AUX	:	Auxin
BC	:	Before Christ

bHLH	:	Basic helix–loop– helix
BLAST	:	Basic Local Alignment Search Tool
bp	:	Base pair
bZIP	:	Basic-domain leucine zipper
C	:	Carbon
Ca^{2+}	:	Calcium
$CaCl_2$	:	Calcium chloride
CaM	:	Calmodulin
CAMTA	:	Calmodulin-Binding Transcription Activators
CAT	:	Catalase
CBFs	:	C-repeat binding factor
CBL	:	Calcineurin B-like proteins
CC	:	Chlorophyll content
CCI	:	Chlorophyll Content Index
CD	:	Critical Difference
cDNA	:	Complementary DNA
CDPK	:	Calcium dependant protein kinases
CDS	:	Coding sequence
CF	:	Crude fiber
CHO	:	Carbohydrates
CIPK	:	CBL-interacting protein kinases
cm	:	Centimeter
CNGC	:	Cyclic nucleotide gated channels
CO_2	:	Carbon dioxide
CRD	:	Completely Randomized Design
CRTs	:	C-repeat
CTAB	:	Cetyl trimethyl ammonium bromide
CUC2	:	Cup shaped cotyledon
CV	:	Critical Variance
d	:	Days
dATP	:	Deoxyadenosine triphosphate
dCTP	:	Deoxycytidine triphosphate
DDRT-PCR	:	Differential-display reverse transcription PCR
DEPC	:	Diethyl pyrocarbonate
dGTP	:	Deoxyguanosine triphosphate

DMSI	:	Dry matter stress tolerance index
DNA	:	Deoxyribonucleic acid
dNTPs	:	Deoxy Nucleotide Triphosphate
Dr. PDKV	:	Dr. Panjabrao Deshmukh Krishi Vidyapeeth
DREBs	:	DRE binding protein
DREs	:	Dehydration responsive element
DsPTP	:	Dual-specificity protein Tyr phosphatase
DTT	:	Dithiothreitol
dTTP	:	Deoxythymidine triphosphate
DW	:	Dry weight
e.g.	:	*exempli gratia* (for example)
EAR	:	ERF-associated amphiphilic repression
EBI	:	European Bioinformatics Institute
EDTA	:	Ethylenediaminetetra acetic acid
EF1α	:	Elongation factor 1 α
EIN3	:	Ethylene insensitive 3
erd	:	Early responsive to dehydration
ERE	:	Ethylene responsive element
EREBP	:	Ethylene responsive element binding protein
EST	:	Expressed Sequence Tag
et al.	:	*et alia* (and others)
EtBr	:	Ethidium Bromide
etc.	:	*et cetera*
EU	:	Enzyme unit
F	:	Forward primer
FmMDb	:	Foxtail millet Marker Database
FmMiRNADb	:	Foxtail millet miRNA Database
FmTEMDb	:	Foxtail millet Transposable Elements-based Marker Database
FmTFDb	:	Foxtail millet Transcription Factor Database
FW	:	Fresh weight
g	:	Grams
G6PDH	:	6 Phosphate dehydrogenase
GDH	:	Glutamate dehydrogenase
GI	:	Glycemic index

GlyBet	:	Glycine betaine
GRAS	:	Gibberellin-acid insensitive (GAI), repressor of GA1 (RGA) and scarecrow (SCR)
GR	:	Glutathione reductase
GS	:	glutamine synthetase
GSDS	:	Gene structure display server
GSI	:	germination stress tolerance index
h	:	Hours
H_2O_2	:	Hydrogen peroxide
H_2SO_4	:	Sulphuric acid
H_3BO_3	:	Boric acid
HAP	:	Hemi-activated protein
HCI	:	Hydrochloric acid
$HgCl_2$	:	Mercuric chloride
HMG-box	:	High-Mobility Group
HNO_3	:	Nitric acid
HP	:	Himachal Pradesh
HSP	:	Heat shock proteins
i.e.	:	*id est* (that is)
IAA	:	Indole acetic acid
IDVs	:	Intensity derived values
ILP	:	Intron length polymorphic
ISSR	:	Inter simple sequence repeats
K	:	Potassium
$K_2Cr_2O_7$	:	Potassium dichromate
K_2HPO_4	:	Potassium phosphate dibasic
KA	:	Karnataka
kb	:	Kilobase pair
kca	:	Kilo calories
KCl	:	Potassium chloride
kDa	:	Kilo Dalton
kg	:	Kilo grams
KH_2PO_4	:	Potassium phosphate monobasic
KNO_2	:	Potassium Nitrite
KNO_3	:	Potassium Nitrate

KOH	:	Potassium hydroxide
LEA	:	Late embryogenesis abundant
LRR/RLK	:	Leucine-rich repeat receptor like kinase
Ltd.	:	Limited
LWP	:	Leaf water potential
LZ	:	Leucine zipper
M	:	Molar
MADS box	:	MCM1, AG, DEFA and SRF domains
MAPK	:	Mitogen-activated protein kinase
MDA	:	Malondialdehyde
mg	:	Milli gram
$MgCl_2$	:	Magnesium chloride
$MgSO_4$	:	Magnesium sulphate
MH	:	Maharashtra
min	:	Minutes
MIP	:	Major intrinsic proteins
ml	:	Milli liter
mM	:	Milli molar
mm	:	Milli meter
MOPS	:	3-[N-Morpholino-Propane Sulphonic Acid]
MP	:	Madhya Pradesh
MPa	:	Mega Pascal
MYB	:	Myeloblastosis oncogene
MYC	:	Myelocytomatosis oncogene
N	:	Nitrogen
Na_2Cl_3	:	Sodium hypochlorite
Na_2HPO_4	:	Disodium phosphate
$Na_3C_6H_5O_7$	:	Trisodium citrate
NAC	:	NAM, ATAF and CUC
NaCl	:	Sodium Chloride
NADP	:	Nicotinamide Adenine Dinucleotide Phosphate
NaH_2PO_4	:	Monosodium phosphate
NAM	:	No apical meristem
NaOAc	:	Sodium acetate
NaOH	:	Sodium hydroxide

NBPGR : National Bureau of Plant Genetic Resources
NBT : Nitro blue tetrazolium
NCBI : National centre for biotechnology information
NF-Y : Nuclear factor family
ng : Nano gram
NIP : Nodulin- 26–like intrinsic membrane proteins (NIPs),
NIPGR : National Institute of Plant Genetic Resources
NJ : Neighbour-joining
nm : Nano meter
NO : Nitric oxide
no. : Numbers
NO_2 : Nitrite
NPA : Aspargine- Proline- Alanine
O_2 : Oxygen
OD : Optical density
OH : Hydroxyl radicals
ORF : Open Reading Frame
P : Phosphorylation/ Phosphate group
p : Probability
P5C : Pyrroline-5-carboxylate
PCR : Polymerase Chain Reaction
PEG : Polyethylene Glycol
Pfu : *Pyrococcus furiosus*
pH : Potential of Hydrogen
PI : Promptness index
PIP : Plasma membrane intrinsic protein
PK : Protein kinases
PLC : Phospholipases C
PLD : Phospholipases D
PLEs : Phospholipid-cleaving enzymes
PMSF : Phenyl methyl sulphonyl fluoride
*P*n : Net photosynthetic rates
POD : Peroxidase
PP2C : Protein Ser/Thr phosphatase 2C
PPases : phosphoprotein (serine/threonine) phosphatases

PROSA	:	Protein structure analysis
PtdOH	:	Phosphatidic acid
PTP	:	Protein Tyr phosphatases
qRT-PCR	:	Quantitative Reverse transcription polymerase chain reaction
QTL	:	Quantitative trait loci
R	:	Reverse primer
RAPD	:	Random amplified Polymorphic DNA
RARS	:	Regional Agricultural Research Station
RBOH	:	Respiratory burst oxidase homolog
rd	:	Responsive to dehydration
RFLP	:	Restriction Fragment Length Polymorphism
RGR	:	Argenine-Glycine- Argenine
RGRP	:	Argenine-Glycine- Argenine-Proline
RLSI	:	Root length stress tolerance index
RO	:	Alkoxy radicals
ROS	:	Reactive oxygen species
rpm	:	Rotations per minute
RT	:	Reverse transcriptase
RT-PCR	:	Reverse transcription polymerase chain reaction
RuBisCo	:	Ribulose-1,5-bisphosphate carboxylase oxygenase
RWC	:	Relative water content
S	:	Stressed
s	:	Seconds
SAGE	:	Serial analysis of gene expression
SAZ	:	SUPERMAN (SUP) family of plant-specific zinc-finger genes
SE(m)±	:	Standard error of means
Ser	:	Serine
Si	:	*Setaria italica*
SIP	:	Small intrinsic proteins
SLSI	:	Shoot length stress tolerance index
SMART	:	Simple Modular Architecture Research Tool
SNP	:	Single Nucleotide Polymorphism
SOD	:	Superoxide dismutase
sp	:	Species

SPMR : SPAD chlorophyll meter reading
SRAP : Sequence Related Amplified Polymorphism
SRL : Sisco Research Lab
SSH : Suppression subtractive hybridization
SSR : Simple Sequence Repeat
STI : Stress tolerance index
STMS : Sequence Tagged Microsatellite
STP : Signal transduction pathways
STS : Sequence Tagged Site
T_a : Annealing temperature
Taq : *Thermus aquaticus*
TBE : Tris Borate EDTA
TDFs : Transcript-derived fragments
TEMED : N,N,N',N'-Tetramethylethylenediamine
TFs : Transcription factors
Thr : Threonine
TIP : Tonoplast intrinsic proteins
Tm : Melting temperature
TM : Trans membrane
TRAP : Target Region Amplification Polymorphism
Tris : Tris Hydroxymethyl aminomethane
Tris-HCl : Tris Hydrochloride
TSS : Transcriptional start site
TW : Turgid weight
Tyr : Tyrosine
U : Unit
UAS : Upstream acting region
US : Unstressed
UV : Ultra Violet
V : Volt
v/v : Volume per volume
viz. : *Videlicet* (namely)
W : Watt
w/v : Weight by volume
WD40 : Trp-Asp proteis

WRKY	:	Tryptophan (W),Arginine (R), Lysine (K), and Tyrosine (Y)
x	:	Fold concentration
ZFHD	:	Zinc finger homeo domain
ZnO	:	Zinc oxide
α	:	Alpha
β	:	Beta
µ	:	Micro
µmol	:	Micro moles
g^{-1}	:	Per gram
h^{-1}	:	Per hour
$^1O_2$	:	Singlet oxygen
O^{-2}	:	Anion radicals
3’	:	3 prime end
5’	:	5 prime end

(H) Glossary

Term		Definition
3′-untranslated region	:	The untranslated region of an mRNA downstream of the termination codon.
5′-untranslated region	:	The untranslated region of an mRNA upstream of the initiation codon.
Abiotic stress	:	The negative impact of non-living factors on the living organisms in a specific environment.
Accession number	:	A unique identifier that is assigned by the curator when an accession is entered into a gene bank.
Accessions	:	A distinct, uniquely identifiable sample of seeds representing a cultivar, breeding line or a population, which is maintained in storage for conservation and use.
Agarose gel electrophoresis	:	Agarose gel used to separate DNA molecules between 100 bp and 50 kb in length.
Allele	:	One of two or more alternative forms of a gene.
Amino acid	:	One of the monomeric units of a protein molecule
Amino terminus	:	The end of a polypeptide that has a free amino group.
Amplicon	:	A piece of DNA or RNA that is the product of amplification formed using polymerase chain reactions (PCR)
Analysis of variance	:	A statistical procedure that splits the total variation into different components.
Annealing	:	Attachment of an oligonucleotide primer to a DNA or RNA template.
Aquaporins	:	Are integral membrane proteins from a larger family of major intrinsic proteins that form pores in the membrane of biological cells, mainly facilitating transport of water between cells.
Base pair	:	The hydrogen-bonded structure formed by two complementary nucleotides.
Bioinformatics	:	The use of computer methods in studies of genomes.
C terminus		The end of a polypeptide that has a free carboxyl group.
Candidate genes	:	A gene believed to be related to a particular trait or condition.
***cis*-regulatory elements**	:	Cis-regulatory elements (CREs) are regions of non-coding DNA which regulate the transcription of nearby genes.
Clone	:	A group of cells that contain the same recombinant DNA molecule.

Codon	:	A triplet of nucleotides coding for a single amino acid.
Comparative genomics	:	A research strategy that uses information obtained from the study of one genome to make inferences about the map positions and functions of genes in a second genome
Complementary DNA (cDNA)	:	A double-stranded DNA copy of an mRNA molecule.
Denaturation	:	Breakdown by chemical or physical means of the non-covalent interactions, such as hydrogen bonding, that maintain the secondary and higher levels of structure of proteins and nucleic acids.
Detoxification	:	The process of removing toxic ROS generated during stress.
DNA	:	Deoxyribonucleic acid, one of the two forms of nucleic acid in living cells; the genetic material for all cellular life forms and many viruses.
DNA marker	:	A DNA sequence that exists as two or more readily distinguished versions and which can therefore be used to mark a map position on a genetic, physical or integrated genome map.
DNA sequencing	:	The technique for determining the order of nucleotides in a DNA molecule.
DNA-binding motif	:	The part of a DNA-binding protein that makes contact with the double helix.
Downstream	:	Towards the 3′ end of a polynucleotide.
Drought	:	A period of below-average precipitation in a given region, resulting in prolonged shortages in its water supply, whether atmospheric, surface water or ground water.
Functional analysis		The area of genome research devoted to identifying the functions of unknown genes.
Gene	:	A DNA segment containing biological information and hence coding for an RNA and/or polypeptide molecule.
Gene expression	:	The series of events by which the biological information carried by a gene is released and made available to the cell.
Genome	:	The entire genetic complement of a living organism.
Genome-wide analysis	:	Is an examination of **a** genome-wide set of genetic variants associated with a particular trait.
Genome-wide repeat	:	A sequence that recurs at many dispersed positions within a genome.
Genotype	:	A description of the genetic composition of an organism.
Germplasm	:	A collection of genotype of an organism.
Glycemic index	:	A figure representing the relative ability of a carbohydrate food to increase the level of glucose in

the blood.

Homeostasis	:	The tendency of an organism to seek and maintain a condition of balance or equilibrium within its internal environment, even when faced with external changes.
Homology searching	:	A technique in which genes with sequences similar to that of an unknown gene are sought, the objective being to gain an insight into the function of the unknown gene.
Housekeeping protein	:	A protein that is continually expressed in all or at least most cells of a multicellular organism.
Initiation codon	:	The codon, usually but not exclusively 5′–AUG–3′, found at the start of the coding region of a gene.
Initiation of transcription	:	The assembly upstream of a gene of the complex of proteins that will subsequently copy the gene into RNA.
Intron	:	A non-coding region within a discontinuous gene.
Leaf water potential	:	Water potential is the measure of potential energy in water and drives the movement of water through plants, designated by ψ
Messenger RNA (mRNA)	:	The transcript of a protein-coding gene.
Model organism	:	An organism which is relatively easy to study and hence can be used to obtain information that is relevant to the biology of a second organism that is more difficult to study.
Multigene family	:	A group of genes, clustered or dispersed, with related nucleotide sequences.
N terminus	:	The end of a polypeptide that has a free amino group.
Open reading frame (ORF)	:	Is the part of a reading frame that has the potential to be translated. An ORF is a continuous stretch of codons that do not contain a stop codon (usually UAA, UAG or UGA).
Osmoprotection	:	The upregulation of compatible solutes (osmolytes) that function primarily to maintain cell turgor, but are also involved in antioxidation and chaperoning through direct stabilization of membranes and/or proteins
Osmotic potential	:	The potential of water molecules to move from a hypotonic solution (more water, less solutes) to a hypertonic solution (less water, more solutes) across a semi permeable membrane.
Oxidative stress	:	Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage

Phenotype	:	The observable characteristics displayed by a cell or organism.
Photosynthetic capability	:	A measure of the maximum rate at which leaves are able to fix carbon during photosynthesis.
Primer	:	A short oligonucleotide that is attached to a single-stranded DNA molecule in order to provide a start point for strand synthesis.
Promoter	:	The nucleotide sequence, upstream of a gene, to which RNA polymerase binds in order to initiate transcription.
Reactive oxygen species	:	Chemically reactive chemical species containing oxygen.
Reading frame	:	A series of triplet codons in a DNA sequence.
Relative water content	:	Measurement of leaf hydration status relative to its water holding capacity at full turgidity.
Reverse complement	:	The reverse complement of a DNA sequence is formed by reversing the letters, interchanging A and T and interchanging C and G.
RNA	:	Ribonucleic acid, one of the two forms of nucleic acid in living cells; the genetic material for some viruses.
Semi-quantitative PCR	:	Use of an internal standard to monitor each reaction and allow comparisons between different reactions to be made.
Sequence alignment	:	A way of arranging the sequences of DNA, RNA, or protein to identify regions of similarity that may be a consequence of functional, structural, or evolutionary relationships between the sequences.
Susceptible	:	The inability of a plant variety to restrict the growth and/or development of a specific condition.
Tertiary structure	:	The structure resulting from folding the secondary structural units of a polypeptide.
The Ramachandran plot	:	A way to visualize energetically allowed regions for backbone dihedral angles ψ against φ of amino acid residues in protein structure.
Tolerant	:	Tendency of a plant able to endure specified conditions or treatment.
Transcript	:	An RNA copy of a gene.
Transcript profiling	:	The measurement of the activity (the expression) of many of genes at once, to create a global picture of cellular function.
Transcription	:	The synthesis of an RNA copy of a gene.
Transcription factors (TFs)	:	Proteins that bind specific sequences of DNA *(cis* regulatory sequences) in the promoter regions of various genes and thus are capable of activating and/or repressing transcription of many secondary responsive genes

Transcription initiation	:	The assembly, upstream of a gene, of the complex of proteins that will subsequently copy the gene into RNA.
Transpiration		The loss of water by evaporation in terrestrial plants, especially through the stomata; accompanied by a corresponding uptake from the roots
Turgor	:	The state of turgidity and resulting rigidity of cells or tissues, typically due to the absorption of fluid.
Upstream promoter element	:	Components of a eukaryotic promoter that lie upstream of the position where the initiation complex is assembled.
Variance	:	A measure of variability which is the average of the square of deviation of the observations from the mean of a sample drawn from a population.
Water deficit/stress	:	The incomplete saturation with water of plant cells, res ulting from an intensive water loss by the plant that is not replenished by absorption of water from the soil.
α-helix	:	One of the commonest secondary structural conformations taken up by segments of polypeptides.
β-sheet	:	One of the commonest secondary structural conformations taken up by segments of polypeptides

(I) Thesis Abstract

a)	**Title of the thesis**	**:**	"Deciphering the differentially expressed genes in Foxtail millet (*Setaria italica* L.) in response to water stress"
b)	**Name of the student (Full)**	**:**	Gawai Dipti Chandrabhan
c)	**Name and Address of the major advisor**	**:**	Dr. S.J. Gahukar Associate professor, Biotechnology centre, Dr. PDKV, Akola (MS) 444104.
d)	**Degree to be awarded**	**:**	Ph.D. (Agriculture) Agricultural Biotechnology
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f)	**Major subject**	**:**	Molecular Biology and Biotechnology
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i)	**Signature of the student**	**:**	
j)	**Signature and name of forwarding authority**	**:**	

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ABSTRACT

The present investigation entitled "Deciphering the differentially expressed genes in Foxtail millet (*Setaria italica* L.) in response to water stress" was carried out at the Biotechnology Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during 2013-2016.

In the present study, sixty two accessions of foxtail millet (*Setaria italica* L.) were investigated for physiological, biochemical and molecular responses for water stress. Water stress was imposed under laboratory conditions by using PEG-6000 and by withholding water and performances of various genotypes were monitored against a control. Seedling traits such as germination percentage, root length, shoot length and seedling dry weight, various physiological parameters like relative water content (RWC), leaf water potential (LWP) and chlorophyll content (CC) were studied in unstressed and stressed experiments. The results showed that water deficit stress has exerted negative effect on all the physiological parameters considered. Based on these observations, most drought tolerant and susceptible accessions were selected. IC97087, IC97189, IC120159 and IC120239 were recorded as the most drought tolerant accessions, whereas, IC97109, IC120234, IC120346 and Lepakshi were recorded as susceptible accessions.

The selected core set of accessions were then screened for their water stress tolerance on the basis of some primary metabolites like proline, total carbohydrates, starch and antioxidant enzymes like superoxide dismutase, peroxidase, catalase and glutathione reductase. Osmolytes viz. proline and carbohydrates increased with increasing drought stress. The proline content in unstressed tolerant plant is found to be much higher as compared to that in unstressed susceptible plants suggesting its important role in drought tolerance of plants. Photosynthetic pigment decreased with increasing drought stress. Activities of antioxidant enzymes increased with drought stress in most of the accessions. The activity of SOD was found to be highest in IC120239 which was found to be a tolerant accession in prior physiological screening. The activity of GR was high in all tolerant accession than in susceptible accessions. Catalase and POD activity was found to be highest in Prasad. Drought stress preferentially enhanced the activities of enzymatic antioxidants and accumulation of osmolytes.

Further, gene expression studies were carried out using the two contrasting accessions IC97189 (tolerant) and IC97109 (susceptible), for deciphering the transcriptional regulation of abiotic stress-related genes.

cDNA SRAP analysis showed a relatively higher number of genes expressed in IC97189. The differential gene expression of drought responsible transcriptional factors like DREB1, DREB2, Aquaporins and C_2H_2 was studied in selected drought tolerant and susceptible plants each one showing considerable difference gene expression. Genome–wide investigation of plant aquaporin protein from foxtail millet was carried out to show that aquaporins comprise of MIP superfamily with conserved motif. Expression profiling of PIPs showed differences in gene expression pattern. Further an attempt was made to characterise full length sequence of PIP 2;7 to isolate a 770bp long DNA stretch out of the 857 base pair CDS having high homology to a UNPRIDICTED PIP2;7 gene in *Setaria italica* L. with an identity of 99%. Theoretical model of the tertiary structure shows that it has a highly conserved containing the NPA motif which was further validated by Ramachandran plot.

Analysis for *cis* regulatory elements identified more than 250 *cis*-regulatory elements present in the promoter region of PIP2;7 aquaporin gene in foxtail millet which revealed the presence of a number of abiotic stress responsive acting elements such as, ABRE binding site, MYB binding site, AP2/ERF binding site, LEA-5 binding site, NF-YB/A/C binding site, Trihelix binding site, EIN3:EIL binding site etc. Promoter analysis also revealed the presence of binding site for a sequence-specific DNA-binding domain (designated CG-1) present in calmodulin-binding transcription activators (CAMTAs) in the upstream region of PIP2;7 aquaporin. This domain could bind DNA directly and activate transcription, or interact with other transcription factors, not through DNA binding, thus acting as a co-activator of transcription. This might be the reason for high expression of PIP2;7 aquaporin.

Applying different physiological and biochemical tests to appreciate drought tolerance in plant leads to faster selection methods. Therefore, these characters can be used as an indirect selection criterion for screening drought tolerance plant materials which will lead to new cultivars with high yield potential and high yield stability that in turn will result in superior performance in dry environments. Hence, this research can provide documentation for breeding/selection of higher drought resistant foxtail millet in arid regions and

acquisition of good information for future molecular research. The physio-biochemically screened accessions can further prove to be useful in studies involving transcriptome changes to fetch out the molecular mechanism underneath of its drought adaptation.

Accomplishments of these goals permit better understanding of molecular and physiological mechanisms utilized by plants during adverse growth conditions and define the biomarkers of tolerance against stresses. The research provides a possible point of integrating various molecular and biological pathways with water stress regulated gene expression.

The study can prove helpful to the farmers in selecting foxtail millet cultivars for unaffected yields in diverse agronomic conditions. These findings provide insight for further investigation of CG-1 domain in plant aquaporins in opening new perspectives for improving drought tolerance which could eventually lead to better crop production. The identified aquaporin could be explored in development of tolerant lines by MAS using PIP 2;7 as a functional marker and also in transgenic development in other related crops.

CHAPTER I
INTRODUCTION

1.1 Background

The present day fast changing world is challenged by two main problems – the growing population with ever increasing demand for various crop productions and global climatic change with environmental deterioration. The later has generated all pervasive abiotic stress which affects every crop to varied extent with possibility of drastic reduction in growth and crop productivity. Worldwide, it has been estimated that approximately 70% of yield reduction is the direct result of abiotic stresses (Acquaah, 2007). The magnitude of these effects depends on its impact on the plant physiological, biochemical, as well as molecular biological processes and the ability of plant to adapt to drought stress (Bulbotko, 1973; Atkinson et al*.,* 2000; Massonnet et al*.*, 2007).

Plants have evolved different adaptive strategies to alleviate the adverse affects of harsh environments by altering their molecular and cellular functions, as well as their physiological and biochemical pathways. Plants adjust their physiology so as to cope with a period of water deficit (drought). It is reported that high relative water content reveals a resistant mechanism to drought (Ritchie, 1990). Deficiency of water in plant tissues leads to stomatal closure resulting in lower CO_2 intake ultimately affecting photosynthesis (Lawlor and Cornic, 2002; Lawlor and Tezara, 2009; Chaves et al., 2009). The reduction in photosynthesis or CO_2 fixation reduces the regeneration of $NADP^+$ which results in leakage of electron to O_2 and subsequent production of ROS (Smirnoff, 1993; Biehler and Fock 1996; Sgherri et al., 1996).The scavenging mechanisms either through anti-oxidative enzymes or anti-oxidant secondary metabolites help the plants to keep the levels of active oxygen species under control (Sharma and Dubey, 2005).These responses originate from changes in the gene expression and subsequent action of their gene products. Earlier studies have identified several transcription factors, such as AP2/EREBP (apetala2/ethylene responsive element binding protein), bZIP (basic leucine zipper), NAC {(no apical meristem (NAM), ATAF1-2, cup

shaped cotyledon (CUC2)}, MYB, MYC, WRKY and their associated signalling network regulating dynamic co-expression of several stress responsive genes together to alleviate stress induced cellular damage.

Understanding the physiological and biochemical responses to drought is essential for a holistic perception of plant resistance mechanisms to water-limited conditions. Hence, knowledge of plants response to cope with abiotic stress like drought at physiological, biochemical and molecular level is of paramount importance leading to identification of relevant genes for mitigating the drought stress losses in major crops. *Arabidopsis* has been initially explored for many of the genes known to be involved in stress tolerance but it is impossible to elucidate all the genes in a single model plant due to wide genetic diversity in crop plants. Being an elite drought-tolerant crop, foxtail millet is thought to be an excellent experimental model in studying abiotic stress tolerance system (Lata et al., 2013) and for biofuel research (Warnasooriya and Brutnell, 2014; Muthamilarasan and Prasad, 2015). The crop has a small genome (1 C ~ 515 Mb; 2*n* = 2*x* = 18), low amount of repetitive DNA, a highly conserved genome structure relative to the ancestral grass lineage, inbreeding nature and short life cycle (Devos et al., 1998; Jayaraman et al., 2008; Doust et al., 2009; Li and Brutnell, 2011; Zhang et al., 2012).

Foxtail millet (*Setaria italica* L. Beauv) is one of the oldest cultivated millet crops serving as food grain in Asia and as forage/fodder in America, Australia and Africa ranking second in the world's total production of millets after pearl millet. India ranks second after China in the world in small millet production with Tamilnadu and Andhra Pradesh as the leading producers.

To gain a better understanding of the molecular responses of this crop to dehydration stress, various methods based on transcript profiling can be used for the analysis for differentially expressed genes. The identification, manipulation and comprehension of gene expression patterns would play an important part in unlocking the mysteries of drought responses and adaptation.

1.2 Importance and need of the study

Understanding drought tolerance at molecular level particularly identification of relevant genes in foxtail millet is likely to pave the ways for

mitigating the drought stress losses in major crops. The adaptation of foxtail millet to low water conditions has been ascribed to its relatively small leaf area, the cell arrangement in its epidermis, its thick cell walls, and its ability to form a dense root system (Li, 1997). Also, the water use efficiency of foxtail millet has been shown to be higher than that of maize, wheat, and sorghum (Gu et al., 1987). However, the molecular mechanism underneath of its drought adaptation is still not clear. The genome size of foxtail millet is very much similar to that of rice and is also one of the smallest among the Panicoid grasses. Another major advantage being the self-compatibility of plants and that they can be transformed by *Agrobacterium*-mediated method (Brutnell et al., 2010). An extensive germplasm collection of the crop is available, providing opportunities to study the various biological processes and to fetch out the molecular mechanism underneath its tolerance.

All these characteristics make the plant an ideal choice to explore candidate genes involved in drought tolerance. Unfortunately, the crop has remained neglected with little or no importance in today's world and has hence lagged behind in genetic and molecular studies.

1.3 Objectives of the study

The purpose of this study was to identify gene(s) that could be associated with drought-tolerance in foxtail millet by deciphering the differences in gene expression between drought tolerant and susceptible accessions. Hence, the present investigation was carried out with the following objectives:

- Screening and evaluation of foxtail millet accessions for dehydration tolerance under controlled conditions
- Biochemical screening for presence of water stress responsive metabolites
- To identify differentially expressed genes in foxtail millet during water stress by cDNA-SRAP and gene specific amplification
- To study the gene expression pattern in the screened water stress tolerant and susceptible foxtail millet accessions

1.4 Hypothesis or assumption

Plant adaptation to environmental stresses is controlled by cascades of molecular networks resulting in a combination of metabolic, physiological and

morphological changes. As many biological processes in plants are regulated at the level of transcription, understanding of transcription factors is an important step towards deciphering the plant responses to environmental conditions. The role for AP2/ERF transcription factors such as CBF/DREB subfamily members has been extensively studied and the CBF/ DREB-pathway in response to abiotic stress have been well established (Agarwal et al., 2007). The bZIP, MYC and MYB families are involved in ABA signaling and its gene activation (Abe et al., 2003). Some zinc finger proteins are involved in responses to environmental stresses (Mukhopadhyay et al., 2004; Sakamoto et al., 2004). The role of few members of the plant specific NAC transcription factor family in providing stress tolerance is well documented (Tran et al., 2004; Hu et al., 2006). A combination of molecular, genomic and genetic analysis is presently used to elucidate the complex system that regulates the response of gene expression to abiotic stresses.

The biological differences among the genotypes used, plant growth conditions, stress treatment conditions and their detection methodologies may result in variation in extent of stress adaptive mechanism. The tolerant species may express some novel stress responsive genes. Hence, comparison of gene expression profiles between contrasting genotypes can provide much information in understanding the spatial and temporal patterns of gene expression required for abiotic stress tolerance.

1.5 Scope and limitations of the study

In the past few decades, several extreme weather disasters have partially or completely damaged regional crop production all over the world. Vidarbha region (jurisdiction of the University) of the Maharashtra state has also been witnessing harsh exposure to drought stress in recent years. Worldwide, it has been estimated that approximately 70% of yield reduction is the direct result of abiotic stresses (Acquaah, 2007). This involvement of abiotic stresses in yield losses cannot be overlooked completely; hence this has generated the need for screening highly tolerant crop varieties or accessions for further exploration of molecular or genetic basis underneath their tolerance.

The goal, however, is probably a difficult task due to the complexity of field conditions and the diversity of drought tolerance strategies developed by the plants. While, some genes are induced by osmotic stress in general, others are modulated only by other environmental stresses. In order to obtain a complete picture of a plant's response to stress, it would be ideal to study the expression profiles of genes in its genome which is now possible for foxtail millet because of availability of its genome sequence.

Advances in plant genomics have offered an opportunity to identify regulatory genes and networks that control important traits. Where variation is identified in candidates, either in the gene structure or at expression levels, the gene(s) can be transferred and tested in non-adapted or cultivated germplasm for assessment. In this way, the variation will not only be used to validate candidate genes for stress tolerance but also to provide a tool for allele discovery. Desirable alleles can be transferred by molecular breeding (transgenic approach) as well as conventional breeding and selection.

The identified tolerant accessions can prove helpful to the breeders in selecting foxtail millet cultivars for unaffected yields in diverse agronomic conditions or may be exploited for commercial cultivation to sustain the global climatic change and environmental deterioration. High throughput evaluation at physiological, biochemical and molecular level may provide closer insights of its tolerance ability. Studies on various physiological and biochemical parameters for screening the tolerant accessions can help in building a selection marker for easy identification of tolerant foxtail millet accessions from a large number of wild collections. The present study can provide clues in identifying candidate genes for further functional analysis to delineate their precise role in abiotic stress response. As key genes are identified, efficiency increases and opportunities for genetic engineering are realized. This is a fundamental aspect of research into abiotic-stress tolerance, and discoveries of abiotic stress-tolerance genes, which is explored in the present study.

CHAPTER II

REVIEW OF LITERATURE

2.1 Foxtail millet

Foxtail millet (*Setaria italica* L.) is an important food and fodder grain crop in arid and semi-arid regions of Asia and Africa. The genus *Setaria* belongs to the tribe Paniceae, subfamily Panicoideae and family Poaceae in the grass family. There are about 125 species widely distributed in warm and temperate parts of the world. It contains crop, wild and weed species with different breeding systems, life cycles and ploidy levels (Benabdelmouna et al., 2001).

2.1.1 Taxonomy

Cultivated foxtail millet was recognized by Linnaeus (1753) as *Panicum italicum*. Variants within the species were later combined into *S. italica* (foxtail millet) by Beauvois (1812), who also transferred the weedy *P. viride* L. (green foxtail) to *Setaria*. Based on the comparative morphology of the foxtail millet accessions, Prasada Rao et al. (1986) recognized three races of foxtail millet: (1) race *moharia* from Europe, Southeast Asia, Afghanistan and Pakistan; (2) race *maxima*, common in eastern China, Georgia (Eurasia), Japan, Korea, Nepal and Northern India; and (3) race *indica*, found in the remaining parts of the India and Sri Lanka. A new system of classification recognizing four races, i.e., *maxima, moharia, indica* and *nana,* of foxtail millet is proposed by Li et al. (1995).

Classification

Kingdom	:	Plantae
(unranked)	:	Angiosperms
Group	:	Monocots
(unranked)	:	Commelinids
Order	:	Poales
Family	:	Poaceae
Subfamily	:	Panicoideae
Genus	:	*Setaria*
Species	:	*italica*
Botanical name	:	*Setaria italica* L.

2.1.2 Foxtail millet gene pool

Observations drawn from inter-specific hybridization and hybrid pollen fertility suggest that the genus *Setaria* is organized into three gene pools. The primary gene pool is composed of diploid species (2n=2x=18) *S. italica* and its putative wild ancestor *S. viridis* (Harlan and de Wet, 1971). A secondary gene pool contains *S. adhaerans* (2n=2x=18) and the two allotetraploids S. *verticillata* and *S. faberii* (2n=4x=36) (Li et al., 1942; Benabdelmouna et al., 2001). The tertiary gene pool contains *S. glauca* (or *S. pumila*, 4x to 8x) in addition to many other wild species (Zangre et al., 1992).

2.1.3 Origin, evolution and distribution of *Setaria italica*

Setaria italica is one of the oldest crops in the world; cultivation probably began 5900 BC in Gansu Province, Northwestern China (Barton et al., 2009). The geographical origin of foxtail millet based on cytological studies indicated that *S. italica* was first domesticated in an area ranging from Afghanistan to India. Afterwards, it dispersed both eastward and westward from there (Sakamoto, 1987). According to Vavilov (1926), the principal center of diversity for foxtail millet is East Asia, including China and Japan. Several hypotheses concerning the origin and domestication of foxtail millet have been proposed (Vavilov, 1926; de Wet et al., 1979; Kawase and Sakamoto, 1987) but the multiple domestication hypothesis (de Wet et al., 1979; Li et al., 1995) is widely accepted where Li et al. (1995) suggested three centers of origin, i.e., China, Europe and Afghanistan-Lebanon.

2.1.4 Morphology of foxtail millet

Foxtail millet is an annual grass with slim, vertical, leafy stems which can reach a height of 120-200 cm (4-7 feet). The inflorescence is a spike with short side branches bearing spikelets and bristles. Each spikelet consists of a pair of glumes that embrace two minute flowers; the lower one sterile and the upper one is bisexual, with three stamens and a long oval smooth ovary with two long styles, which terminate in a brush like stigma (Hector, 1936). One to three bristles develop at the base of each spikelet (Vinall, 1924). Anthesis in foxtail millet generally takes place near midnight and in the morning but varies significantly with environment (Malm and Rachie, 1971). The seed head is a dense, hairy panicle and 5- 30 cm long. The seeds are small, around 2 mm in

diameter. Seed color varies greatly between varieties and range from pale yellow, through to orange, red, brown and black (Figure 2.1).

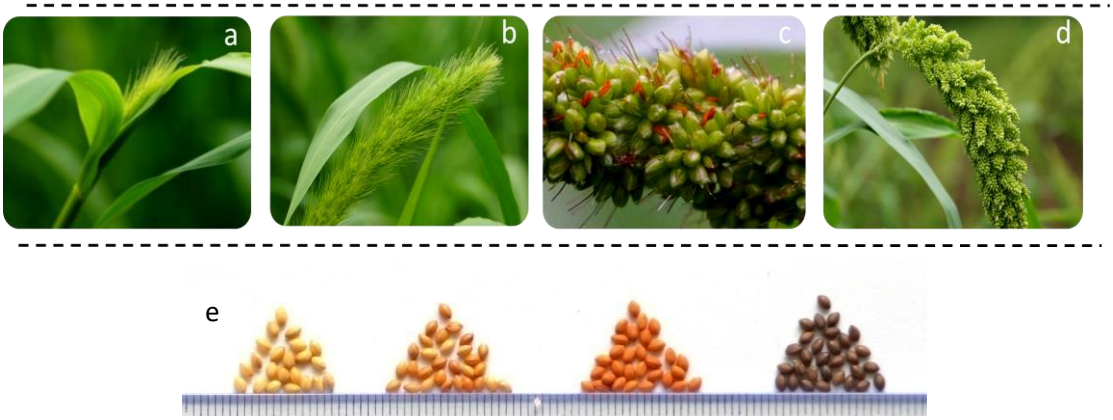


Figure 2.1 Morphological attributes of foxtail millet (a) Emergence of panicle (b) highly bristled panicle (c) inflorescence showing anthesis (d) fully developed panicle (e) variation in grain color

2.1.5 Nutritional importance of foxtail millet

Foxtail millet has many nutritious and medical values. It is non-glutinous, like buckwheat and quinoa, and it is not an acid forming food, so it is soothing and easy to digest. The nutrient composition of foxtail millet is presented in Table 2.1. In fact, it is considered to be one of the least allergenic and most digestible grains available and it is a warming grain (Prashant et al., 2005; Xue et al., 2008). The millet bran is used as animal feed in China extensively (En et al., 2008).

The protein in foxtail millet is known to be deficient in lysine, and its amino acid scores are comparable to that of Maize. It is relatively high in leucine and methionine. The starch in some foxtail millet varieties contain 100% amylopectin, and the starches contained in foxtail, proso and barnyard millets are more digestible than maize starch, they release sugars slowly and thus have a low glycemic index. The total ash content of foxtail millet is good and is much higher than the more commonly consumed cereal grains including sorghum, however de-hulling of the grain, like in other millets, causes considerable nutrient losses.

Table 2.1 Nutrient composition of foxtail millet (100 g) $^{-1}$

Cereal	Energy(kcal)	CHO(g)	Protein(g)	Fat(g)	CF(g)	Iron(mg)	GI
Foxtail millet	331	60.9	12.3	4.3	8.0	2.8	52.0
Little millet	341	67.0	7.7	4.7	7.6	9.3	52.1
Finger millet	328	72.0	7.3	1.3	3.6	3.9	60.9
Rice	362	76.0	7.9	2.7	1.0	1.8	76.0

Values are at 12% moisture level; Abbreviations: CHO, carbohydrates; CF, crude fiber. (Source: Hulse et al., 1980).

2.1.6 Genetic diversity of *Setaria italica* L.

Analysis of genetic relationships in crop species is an important component of crop improvement program, since it provides information about genetic diversity of the crop species, which is a basic tool for crop improvement. Surveys of *S. italica* have revealed a germplasm rich with phenotypic variation. Reddy et al., (2006) compared 1535 *S. italica* accessions collected from 26 countries at the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) to identify large variations in plant height, flowering time, inflorescence architecture, and seed morphology. The estimated rate of outcrossing varies from 0.3% to as high as 4%, suggesting ample opportunity for gene flow between *Setaria* species, particularly from crop to weedy species (Wang et al., 2010). Various types of data have been used to analyze the genetic diversity in foxtail millet including pedigree, morphological, biochemical analysis (Li et al., 1995 and 1996; Murugan and Nirmalakumari, 2006; Nirmalakumari and Vetriventhan, 2010); isoenzymes and seed proteins analysis (Jusuf and Pernes, 1985) and molecular marker data (Schontz and Rether, 1998, 1999; Fukunaga et al., 2002; Fukunaga and Kato, 2002; Van et al., 2008; Jia et al., 2009).

2.2 Abiotic stress with special reference to drought

Environmental factors such as dehydration, cold, salinity and heat impede the productivity of the plants and prevent them from reaching their full genetic potential. Hence modern agricultural crop production relies on the growth of a few of the world's plant species selected for their superior qualities and suitability as food, animal feed, fibre or industrial end uses. Nevertheless, abiotic stresses remain the greatest constraint to crop production with approximately 70% of yield reduction (Acquaah 2007). Drought-induced loss in crop yield probably exceeds losses from all other causes, since both the severity and duration of the stress are critical. Drought stresses reduces leaf size, stem extension and root proliferation, disturbs plant water relations and reduces water-use efficiency. CO_2 assimilation by leaves is reduced mainly by stomatal closure, membrane damage and disturbed activity of various enzymes, especially those of CO_2 fixation and adenosine triphosphate synthesis. Enhanced metabolite flux through the photorespiratory pathway increases the oxidative load on the tissues as both processes generate

reactive oxygen species. Injury caused by reactive oxygen species to biological macromolecules under drought stress is among the major deterrents to growth. Plants display a range of mechanisms to withstand drought stress as individual cells and also synergistically as a whole organism by activating cell signaling pathways and other cellular responses.

2.2.1 Responses of plants to drought

Drought (non-availability of water for crop growth) and water deficit (insufficient plant water status) are variable, complex, and recurring features in most parts of the world. The response of drought range from morphological to molecular levels and are evident at all phenological stages of plant growth, which can be studied by identification of traits that are related to drought tolerance. When insufficient water is available, plant water status is disrupted, which causes imbalances in osmotic and ionic homeostasis, loss of cell turgidity, and damage to functional and structural cellular proteins and membranes. Consequently, water-stressed plants wilt, lose photosynthetic capacity, and are unable to sequester assimilates into the appropriate plant organs. Severe drought conditions result in reduced yield and plant death. Elucidation of plant drought tolerance and response mechanisms has been compounded by the variable levels and forms of drought. An account of various drought stress responses and their extent is elaborated below and depicted in Figure 2.2.

2.2.1.1 Crop growth and yield

The first and foremost effect of drought is impaired germination and poor stand establishment (Harris et al., 2002). Drought stress has been reported to severely reduce germination and seedling stand (Kaya et al., 2006). Similarly, a reduction in germination potential, hypocotyls length, and root and shoot length have been reported in several crops (Zeid and Shedeed 2006; Manikavelu et al. 2006; Baloch et al. 2012). Foxtail millet has been proved a tolerant crop on the basis of its germination traits (Heidari, 2012). High throughput assessment and screening of drought tolerant foxtail millet genotypes from 17313 accessions was carried out in China by measuring the rate of seedling survival under multiple drought stress treatments. More than two hundred lines were classified into the highest category of drought tolerance, including the reference genome cultivar, Yugu 1 (Li, 1997b).

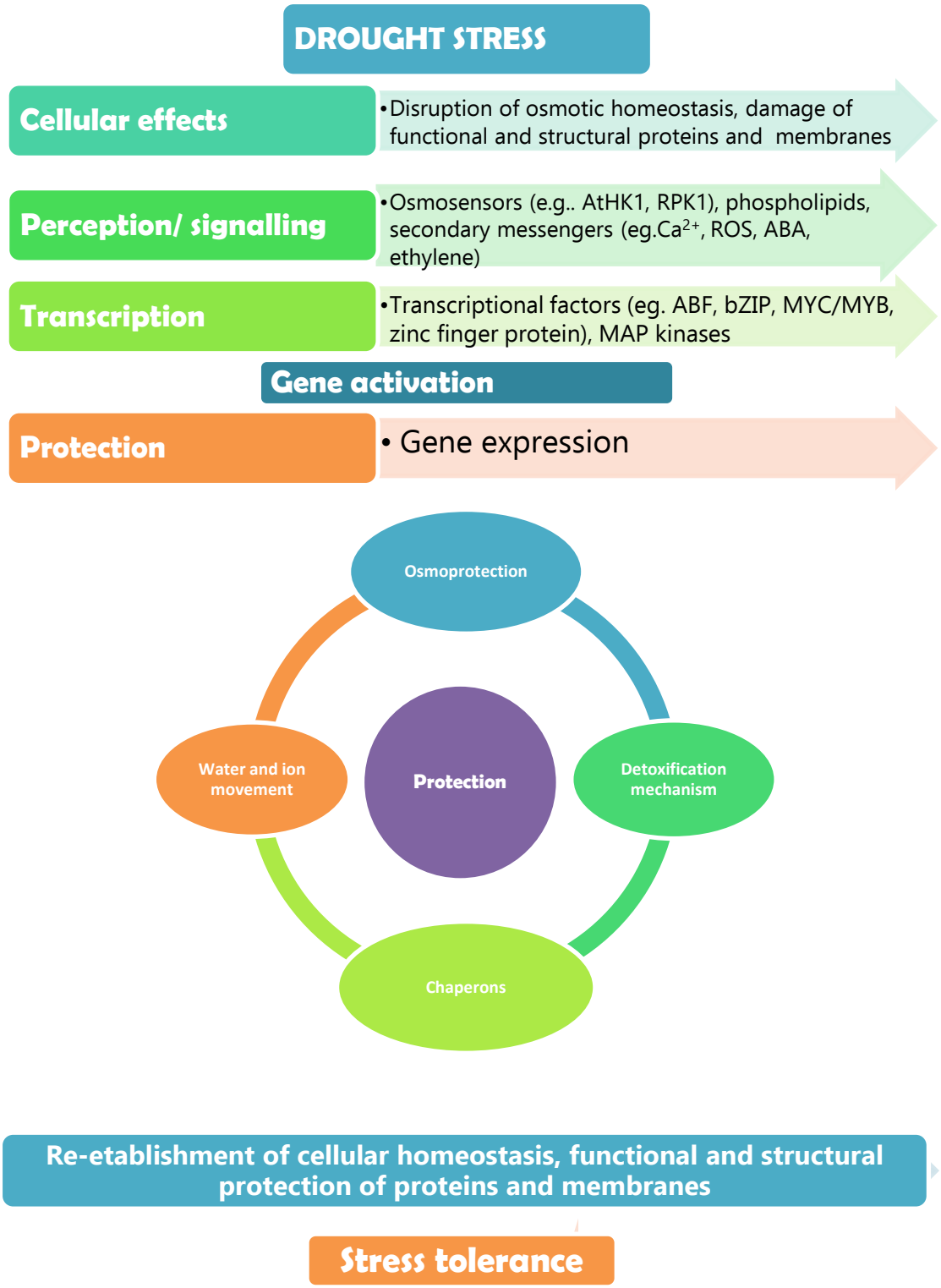


Figure 2.2 Plant responses to drought stress (modified from Vinocur and Altman, 2005; Beck et al., 2007).

Li (1997[b]) categorized a total of 17,799 foxtail millet accessions into five grades of drought tolerance using seedling survival following repeated drought stress. Karyudi and Fletcher, (2002) measured the osmoregulative capacity of 11 accessions of *Setaria italica* L. and found that four accessions of *S. italica* (108042, 108463, 108541 and 108564) demonstrated high osmoregulative capacity. The extent of osmoregulative capacity was associated with osmotic potential at full turgor and the rate of decline in osmotic potential as leaf water potential declined. Screening for drought tolerance using polyethylene glycol (PEG-6000) and mannitol was developed in China and relative water content and germination rates were identified as markers of drought tolerance during seedling stage (Zhang et al., 2005; Zhu et al., 2008). Polyethylene glycol (PEG-6000) was used to generate osmotic stress for screening for drought tolerance during germination in foxtail millet (Zhu et al., 2008). PEG-6000 was used as stress inducer to select drought tolerant cultivar at seedling stage (Lata et al., 2011[a]).

In addition, among the physiological processes, the most sensitive process to get affected by water deficit is the cell growth (Farooq and Azam, 2006). Growth is accomplished through cell division, cell enlargement and differentiation, and involves genetic, physiological, ecological and morphological events and their complex interactions. The quality and quantity of plant growth depend on these events, which are affected by water deficit. The decreased turgor is the major cause of inhibition of plant growth under stress conditions (Ashraf, 1994). It limits cell growth while cell elongation of higher plants is inhibited by interrupted water flow from xylem to the surrounding elongating cells (Nonami, 1998; Taiz and Zeiger, 2006). A cumulative effect of reduction in growth-related traits, viz. plant height, leaf area, number of leaves per plant, cob length, shoot fresh and dry weight due to drought stress was reported in maize (Kamara et al., 2003). Reduced shoot length, photosynthetic pigments and increased root length, compatible solute accumulation is a drought adaptive mechanism in foxtail millet (Paul and Panneerselvam, 2013). Zinc, if not available, causes physiological stress to the plant and has an important role in protecting plant cells against reactive oxygen species. Davoody and colleagues, (2013) investigated the effects of zinc oxide (ZnO) nanoparticles application on foxtail millet in water stress

condition. A declined peduncle length, stomatal conductivity, germination percentage and grain yield was seen in stress conditions.

Prevailing drought reduces plant growth and development, leading to hampered flower production and grain filling and thus smaller and fewer grains. A reduction in grain filling occurs due to a reduction in the assimilate partitioning and activities of sucrose and starch synthesis enzymes. For water stress, severity, duration and timing of stress, as well as responses of plants after stress removal, and interaction between stress and other factors are extremely important (Plaut, 2003). For instance, water stress applied at pre-anthesis reduced time to anthesis, while at postanthesis it shortened the grain-filling period in triticale genotypes (Estrada-Campuzano et al., 2008).

Decline in the rate of grain growth resulted from reduced sucrose synthase activity, while cessation of growth resulted from inactivation of adenosine diphosphate-glucose-pyrophosphorylase in the water-stressed wheat (Ahmadi and Baker, 2001). Drought at flowering stage commonly results in barrenness. A major cause of this, though not the only one, was a reduction in assimilate flux to the developing ear below some threshold level necessary to sustain optimal grain growth (Yadav et al., 2004). Post-anthesis drought stress is detrimental to grain yield regardless of the stress severity (Samarah, 2005). Water deficit during pollination increased the frequency of kernel abortion in maize (*Zea mays*). Under water stress, diminished grain set and kernel growth in wheat and a decreased rate of endosperm cell division was associated with elevated levels of abs*cis*ic acid in maize (Morgan, 1990; Ober et al., 1991). Disruption in leaf gas exchange properties limit the size of source and sink, impairs phloem loading, nutrient uptake, and dry matter partitioning in plants, thus severely declining yield traits (Farooq et al., 2009). Alleviation of leaf senescence played an important role in promoting grain filling and enhancing the grain yield and quality (Dai et al., 2012). Recently, Kahrizsangi and Zargani (2013) evaluated the yield and water use in typical varieties of Pishahang common millet and Boston foxtail millet varieties to show that the grain weight in both types of millet is not affected by tillage method.

These evidences indicate that dehydration stress directly impairs the growth and yield of several crop plants. Mechanisms that could prevent turgor loss and osmotic stress involve minimizing stomatal and cuticular water loss via fewer stomata and thicker cuticle.

2.2.1.2 Water relations

Relative water content, leaf water potential, stomatal resistance, rate of transpiration, leaf temperature and canopy temperature are important characteristics that influence plant water relations. Leaf relative water content (RWC) has also been proposed as a more important indicator of water status than other water potential parameters under drought stress conditions (Carter and Patterson, 1985; Dhanda and Sethi, 2002) as RWC referring to its relation with cell volume, accurately can indicate the balance between absorbed water by plant and consumed through transpiration. It is reported that high relative water content is a resistant mechanism to drought, and that high relative water content is the result of more osmotic regulation or less elasticity of tissue cell wall (Ritchie, 1990). Exposure of the plants to drought stress substantially decreases the leaf water potential, relative water content and transpiration rate, with a concomitant increase in leaf temperature (Siddique et al., 2001). Water-stressed wheat, rice, and foxtail millet plants had lower RWC as compared to the control plants (Siddique et al. 2001; Lata et al. 2011[a]).

The ratio between dry matter produced and water consumed is termed as water-use efficiency (WUE) at the whole-plant level (Monclus et al., 2006). Abbate et al. (2004) concluded that under limited supply, water-use efficiency of wheat was greater than in well-watered conditions and correlated the high water-use efficiency with stomatal closure to reduce the transpiration. Several morphological and physiological adaptations are associated with increased WUE in *S. italica*, including small leaf area, thickening of the cell walls, and ability to form a dense root system (Li, 1997[a]). In fact, although components of plant water relations are affected by reduced availability of water, stomatal opening and closing is more strongly affected. Moreover, change in leaf temperature may be an important factor in controlling leaf water status under drought stress. Lata et al. (2011[a]) correlated the better maintenance of RWC

and higher membrane stability (with lower EL and LP values) with the amount of PEG exposure under stress.

2.2.1.3 Photosynthesis and chlorophyll content

Chlorophyll content of leaf is one of the indicators for photosynthetic capability of plant tissues (Wright et al., 1994; Nageswara et al., 2001) and is widely used by many researchers. A major effect of drought is reduction in photosynthesis, which arises by a decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence and associated reduction in food production (Lawlor and Cornic, 2002; Wahid and Rasul, 2005). With a decrease in RWC and LWP, the foliar photosynthetic rate in higher plants is also observed to decline. When stomatal and non-stomatal limitations to photosynthesis are compared, the former can be quite small. This implies that other processes besides CO_2 uptake are being damaged. The role of drought-induced stomatal closure, which limits CO_2 uptake by leaves, is very important. In such events, restricted CO_2 availability could possibly lead to increased susceptibility to photo-damage (Cornic and Massacci, 1996).

Drought stress produces changes in photosynthetic pigments and components (Anjum et al., 2003), damaged photosynthetic apparatus (Fu and Huang, 2001) and diminished activities of Calvin cycle enzymes, which are important causes of reduced crop yield (Monakhova and Chernyadèv, 2002). Changes in the net photosynthetic rates (*P*n) and the contents of chlorophyll in foxtail millet have been studied under drought stress (Dai et al., 2012). Severe water stress has also been reported to decline the activity of the most important photosynthetic enzyme RuBisCO, and thus limiting photosynthesis (Bota et al., 2004). Chlorophyll content is one of the major factors affecting photosynthetic capacity. However, some contrasting results showing no-change in chlorophyll content in plants under drought stress has been observed in different plant species and its intensity depends on stress rate and duration (Rensburg and Kruger, 1994; Kyparissis et al., 1995; Jagtap et al., 1998).

Another important effect that inhibits the growth and photosynthetic abilities of plants is the loss of balance between the production of reactive oxygen species and the antioxidant defense (Fu and Huang, 2001; Reddy et

al., 2004), causing accumulation of reactive oxygen species which induces oxidative stress in proteins, membrane lipids and other cellular components.

2.2.1.4 Oxidative damage

Exposure of plants to environmental stresses like drought leads to the generation of reactive oxygen species, including superoxide anion radicals (O^{-2}), hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), alkoxy radicals (RO) and singlet oxygen ($^1O_2$) (Munné-Bosch and Penuelas, 2003). Reactive oxygen species may react with proteins, lipids and deoxyribonucleic acid, causing oxidative damage and impairing the normal functions of cells (Foyer and Fletcher, 2001). Many cell compartments produce reactive oxygen species; of these, chloroplasts are a potentially important source because excited pigments in thylakoid membranes may interact with O_2 to form strong oxidants such as O^{-2} or $^1O_2$ (Niyogi, 1999; Reddy et al., 2004). Further downstream reactions produce other reactive oxygen species such as H_2O_2 and OH^-. The interaction of O_2 with reduced components of the electron transport chain in mitochondria can lead to reactive oxygen species formation (Möller, 2001), and peroxisomes produce H_2O_2 when glycolate is oxidized into glyoxylic acid during photorespiration (Fazeli et al., 2007).

Overall, the production of reactive oxygen species is linear with the severity of drought stress, which leads to enhanced peroxidation of membrane lipids and degradation of nucleic acids, and both structural and functional proteins. Various organelles including chloroplasts, mitochondria and peroxisomes are the seats as well as first target of reactive oxygen species produced under drought stress causing severe losses. Though there have been a few studies on differential responses of antioxidant compounds in foxtail millet under salinity stress (Sreenivasulu et al., 1999, 2000), no systematic study has been carried out to study dehydration tolerance in foxtail millet. Tolerant cultivars possess wider array of antioxidant machinery with efficient ascorbate glutathione pathway to cope with drought-induced oxidative stress (Lata et al., 2011[b]).

2.3 The genetic basis of drought tolerance

Drought is the most significant environmental stress on world agricultural production (Tuberosa and Salvi, 2006; Cattivelli et al., 2008) and enormous effort is being made by plant scientists to improve crop yields in the

face of decreasing water availability. Plants adapt to drought conditions by tightly regulating specific sets of these genes in response to drought stress signals, which vary depending on factors such as the severity of drought conditions, other environmental factors, and the plant species (Wang et al., 2003). Majority of these responses originate from changes in the gene expression and subsequent action of their gene products. Nonetheless, it is well established that drought tolerance is a complex phenomenon involving the concerted action of many genes (Agarwal et al., 2006; Cattivelli et al., 2008). Expression studies have shown that drought-specific genes can be grouped into three major categories: (1) Genes involved in signal transduction pathways (STPs) and transcriptional control; (2) Genes with membrane and protein protection functions; and (3) Genes assisting with water and ion uptake and transport (Vierling 1991; Ingram and Bartels 1996; Smirnoff, 1998; Shinozaki and Yamaguchi-Shinozaki, 2000).

The area of plant drought tolerance research and improvement encompasses an enormous range of environmental, genetic, metabolic, and physiological considerations. Gene discovery and functional genomics projects have revealed multitudinous mechanisms and gene families, which confer improved productivity and adaptation to abiotic stresses.

2.3.1 Absicisic acid and transcriptional regulation

The plant hormone ABA regulates the plant's adaptive response to environmental stresses such as drought, salinity, and chilling via diverse physiological and developmental processes. ABA has functional roles ranging from seed maturation processes to lateral root development (McCourt and Creelman, 2008; Wasilewska et al., 2008). Under abiotic stress, ABA induces stomatal closure, reduces water loss via transpiration, and induces gene expression (Chandler and Robertson, 1994). The main function of ABA seems to be the regulation of plant water balance and osmotic stress tolerance; hence it is also called as a stress hormone. Various stress signals and ABA may share common elements in the signaling pathway and cross-talk with each other to maintain cellular homeostasis (Thomashow, 1999; Shinozaki and Yamaguchi-Shinozaki, 2000; Finkelstein et al, 2002).

Many of the drought stress response pathways that have been identified to date appear to be under transcriptional regulation and ABA plays

a key role in this process. Transcriptional regulation involves interaction between TFs and specific *cis*-acting elements located within or near the promoter region upstream of expressed genes. Figure 2.3 shows links between responses to dehydration stress at the transcriptional level.

Transcription factors (TFs) are proteins that bind specific sequences of DNA *(cis* regulatory sequences) in the promoter regions of various genes and thus are capable of activating and/or repressing transcription of many secondary responsive genes. It is the programmed and regulated interaction between TFs and genomic DNA that brings genome to its life and defines many of its functional features (Grandori et al., 2000; Kohler et al., 2003).

Gene expression and biochemical studies into ABA synthesis in *Arabidopsis* and some other model plants have largely elucidated the basic ABA biosynthetic pathway (Schwartz et al., 2003). Use of ABA-deficient *(aba)* or ABA-insensitive (*abi) Arabidopsis* mutants have shown that signaling of osmotic stress may be understood in two major pathways: ABA-dependent and ABA-independent (Bray, 1997; Thomashow, 1999; Shinozaki and Yamaguchi-Shinozaki, 2000).

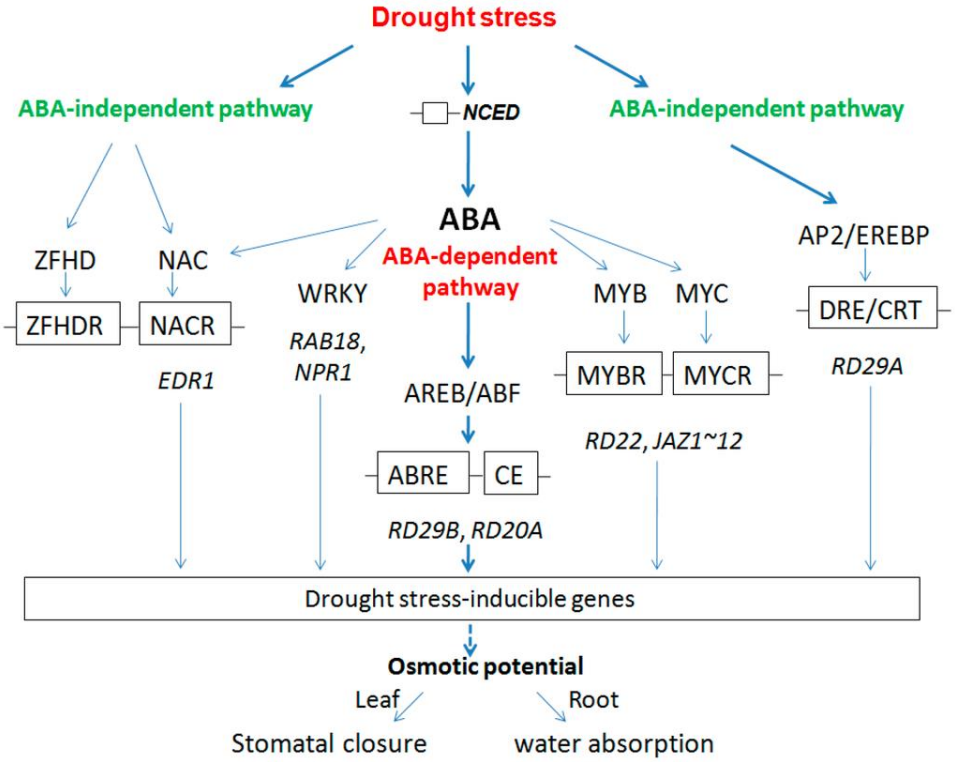


Figure 2.3 Regulatory network of gene expression in response to drought

Specificity and crosstalk of transcriptional regulatory networks of *cis*-acting elements and transcription factors involved in abiotic-stress-responses gene network.

There are two types of ABA-dependent transcription. The “direct” pathway involves *cis*-acting ABA-responsive elements (ABREs), which are directly activated by binding with TFs such as basic-domain leucine zipper (bZIP)-type DNA binding proteins (Shinozaki and Yamaguchi-Shinozaki, 1996; Kobayashi et al., 2008). The ABA-dependent pathway itself has two different routes, which either requires or does not need new protein synthesis (Ingram and Bartels, 1996; Bray, 2002; Shinozaki and Yamaguchi-Shinozaki, 2007). In the route where new protein synthesis is not required, the promoter domain in all ABA-responsive genes contains an ABA-responsive element (ABRE, ACGTGGC). For example, several *rd* (responsive to dehydration) and *erd* (early responsive to dehydration) genes encoding a wide range of proteins are ABA dependent and contain the ABRE motif. In the route where new protein synthesis is required for the ABA induced gene expression, *de novo* synthesis of new proteins is prerequisite. Such genes have no ABREs and its ABA-responsive elements combine with the MYC family transcription factors (TFs). Alternatively, the “indirect” ABA-dependent transcription pathway involves other *cis*-acting elements, such as MYC and MYB. These elements are activated through binding with ABA or drought-inducible TFs, such as basic helix–loop– helix (bHLH)-related protein AtMYC2 and an MYB-related protein, AtMYB2 (Abe et al., 2003).

Some genes are induced by drought stress but are not expressed in response to exogenous ABA applications and these genes are the product of ABA-independent STPs. Non-ABA responsive *cis*-acting elements are known as DREs (dehydration responsive element, TACCGACAT) also referred as CRTs (C-repeat). DRE/CRTs are recognized by their DNA-binding transcription factors DREBs (DRE binding protein) or CBFs (C~repeat binding factor), respectively (Shinozaki and Yamaguchi-Shinozaki, 2000; Valliyodan and Nguyen, 2006). Several ABA deficient mutants namely *aba1, aba2* and *aba3* have been reported for *Arabidopsis* (Koornneef et al., 2002). CBF4 is an apparent homolog of the CBF/DREB1 proteins that is thought to be a critical regulator of gene expression in drought stress signal transduction. The action of CBF4 is thought to be through its binding with CRT/DRE elements in promoter regions of drought- and cold-inducible genes (Haake et al., 2002).

CBF/DREB1 and DREB2, belong to the ethylene responsive element/apetela2 (ERE/AP2) TF family; their expression is induced by cold or drought stress and both activate expression of genes possessing a CRT/DRE *cis*-element (Stockinger et al., 1997; Liu et al., 1998). The CRT/DRE motif also acts as one of the binding sites for the ERF family of TFs (Trujillo et al., 2008). Hence, gene induction by ABA is not an essential condition in the ABA-independent gene expression pathway (Ingram and Bartels, 1996; Bray, 1997). *Arabidopsis* gene RD29A/COR78/LTI78 is one such example (Kreps et al., 2002). It has two regulative elements, of which one is ABA responsive and the other is non-ABA responsive. Thus, the gene is induced in both, an ABA-dependent and an ABA-independent manner.

Other TFs involved in mediation of ABA-dependent and ABA-independent signal transduction and gene expression include NAC, WRKY, RING finger, and zinc-finger TFs (Seki et al., 2003; Zhang et al., 2004; Chen et al., 2006). Zhang and colleagues and Lata and colleagues examined differential gene expression among drought-tolerant and susceptible accessions of *Setaria* by inducing stress and identified significant changes in gene expression of TFs (Zhang et al., 2007; Lata et al., 2010). Nelson et al. (2007) showed that constitutive expression of a TF from the nuclear factor (NF-Y) family, AtNF-YB1, which belongs to the CCAAT-binding TF family which improved performance of *Arabidopsis* under drought conditions. Consequently, an orthologous maize TF gene, ZmNF-YB2, was constitutively expressed in maize.

A comprehensive genome-wide study in foxtail millet (*Setaria italica* L.) and expression profiling of candidate AP2/ERF genes against drought, salt and phytohormones revealed insights into their precise and/or overlapping expression patterns and showed that the genes SiAP2/ERF-069, SiAP2/ERF-103 and SiAP2/ERF-120 may be considered as potential candidate genes for further functional validation (Lata et al., 2014).

Some genes have been shown to suppress expression of drought-response transcription pathways. For example, Jiang et al. (2008) recently characterized SAZ, an *Arabidopsis* gene from the SUPERMAN (SUP) family of plant-specific zinc-finger genes, which encode proteins containing single C_2H_2-type zinc-finger motif with a conserved short amino acid sequence and a

class II ERF-associated amphiphilic repression (EAR) motif-like TF domain at the carboxy-terminal region.

Regulation of gene expression at the level of transcription influences many of the biological processes. Understanding the structure and DNA binding properties of these proteins will help to elucidate how genetic information is utilized for regulation of gene expression under stress. TFs have the characteristic of being modular proteins, generally consisting of a DNA-binding domain, a transcription regulation domain, an oligomerization site and a nuclear localization signal (NLS) (Goff et al., 1992; Washburn et al., 1997). Based on conserved DNA binding domain, they have been classified into several classes, including basic helix-loop-helix (bHLH), zinc finger (ZF), leucine zipper (LZ) or high mobility group (Pabo and Sauer, 1992). Table 2.2 provides a partial list of some of the major families of DNA-binding domains/transcription factors. Of the above, only few TF families are found in plants, including the AP2/EREBP, NAC and WRKY families. This also includes the trihelix DNA binding proteins, auxin response factors (ARFs), Aux/IAA proteins (which interact with the ARF proteins and regulate gene expression) and other smaller families (Riechmann et al., 2000). The activation of stress-responsive genes in plants does not follow a general role concerning which class of TFs activates which class of genes. Individual members of the same family often respond differently to various stress stimuli. On the other hand, the same TF can regulate many genes, as indicated by the significant overlap of the gene expression profiles that are induced in response to different stresses (Seki et al., 2001; Chen and Murata, 2002). A group of genes controlled by a certain type of TF through its specific binding to the *cis*-acting element in the promoter of the target genes is known as a regulon. In the plant response to abiotic stresses, at least four different regulons can be identified; (1) the AREB/ABF (ABA-responsive element binding protein/ ABA-binding factor) regulon; (2) the MYC (myelocytomatosis oncogene)/MYB (myeloblastosis oncogene) regulon; (3) the CBF/DREB regulon, and (4) the NAC (NAM, ATAF and CUC) and ZF~HD (zinc-finger homeodomain) regulon. The first two regulons are ABA dependent, and the last two are ABA independent (Yamaguchi-Shinozaki and Shinozaki, 1994; Nakashima et al, 1997; Choi et al., 2000; Abe et al., 2003).

Table 2.2. Structural features of conserved domains that are used to classify plant transcription factors.

SN	DNA-binding Domain	Domain Architecture
1.	APETALA2/ Ethylene Responsive Element Binding Protein (AP2/EREBP)	A 68 amino acid region with a conserved domain that constitutes a putative amphiphatic alpha-helix.
2.	AT-hook motif	A consensus core sequence R (G/P) RGRP with the RGR region containing the minor groove of A/T-rich DNA.
3.	Auxin response factor (ARF)	A 350 amino acid region with conserved sequence at C-termini of VPI and ABI3
4.	Basic helix-loop-helix (bHLH)	Two amphipathic a-helices with highly conserved basic residues at amino-terminal and several hydrophobic residues at carboxy terminal linked by amino acids forming reverse turns and loops.
5.	Basic region/leucine zipper (bZIP)	Two a-helices held together by hydrophobic interactions between leucine residues, located on one side of each helix. The leucine zipper transcription factors bind DNA as dimers
6.	Helix-turn-helix (Homeo domain)	Approximately 60 amino acid residues producing an N-terminal arm and a three or four a-helices
7.	High Mobility Group	Binds to a 20 bp span of DNA and distorts DNA structure. This motif is also a feature of many structural and non-chromosomal proteins in the nucleus and can mediate bending, wrapping, spacing and coiling of DNA.
8.	HMG-box	L- Shaped domain consisting of three alpha helices with an angle of about 80° between the arms.
9.	MADS box	P-Scaffold factors with minor groove contacts. Approximately 57 amino acid residues that comprise a long alpha helix and two B strands
10.	MYB domain	A basic region with one to three 50 to 53 amino acid imperfect repeats that form the helix-turn helix motifs
11.	NAC domain	A twisted (3-sheet surrounded by a few helical elements.
12.	Trihelix	Basic, acidic and proline/glutamine-rich motif which forms a trihelix DNA binding domain.
13.	WRKY domain	WRKY domain
14.	Zinc finger (ZF)	Finger motif(s) each maintained by cysteine and/or histidine residues organized around a zinc ion. Zinc finger transcription factors must recruit zinc in order to bind to DNA.

(Adapted and modified from Liu et al, 1999).

2.3.1.1 The AREB/ABF regulon

The AREB or ABFs belong to the bZIP (basic leucine zipper) transcription factor family which recognizes the ABRE motif thereby activating ABA-dependent gene expression (Uno et al., 2000). The ABFs/ AREBs are grouped under groupA AtbZIPs (Jakoby et al., 2002), which generally function in ABA signaling during seed maturation as well as under stress conditions (Lata et al., 2011[c]). Reports indicate that these ABFs play a role in diverse stress signaling pathways, viz. drought, cold, heat, salt, and glucose (Kim et al., 2004 ; Fujita et al., 2005).

2.3.1.2 The MYC/MYB regulon

MYC/MYB TFs play active roles in the stress signaling by ABA-dependent pathway and upregulate abiotic stress-responsive genes. *AtMYB2* and *AtMYC2* together act as transcriptional activators in the dehydration and ABA-inducible expression of *RD22* (Urao et al., 1993; Abe et al., 2003). *AtMYB102* assimilates dehydration, salinity, osmotic, ABA, and wound-signaling pathways (Denekamp and Smeekens, 2003). *AtMYB44* confers abiotic stress tolerance by facilitating stomatal closure in an ABA-independent manner (Jung et al., 2008). *AtMyb41* of *Arabidopsis* is transcriptionally regulated under conditions of drought, salinity, drought, and ABA responses. The overexpression of MYB15 in *Arabidopsis* was found to improve drought and salt tolerance (Ding et al., 2009). Increase in the expression levels of AtMYB2, or AtMYC2 independently or together enhanced ABA sensitivity and improved osmotic tolerance (Abe et al., 2003). Expression profiling of candidate MYB genes against abiotic stresses and hormone treatments revealed specific and/or overlapping expression patterns of SiMYBs (Muthamilarasan et al., 2014[a])

2.3.1.3 The CBF/DREB regulon

The CBF/DREB TFs play a significant role in the ABA-independent pathways, inducing the expression of stress-responsive genes. The two main subgroups of DREB subfamily: DREB1 and DREB2 are included in two separate signal transduction pathways under low temperature and dehydration, respectively (Lata and Prasad, 2011). In rice, *OsDREB1A* and *Os-DREB1B* were observed to get induced immediately (within 40 min) after cold exposure, but did not respond to ABA treatment (Dubouzet et al., 2003).

Ca-DREBLP1 from hot pepper was rapidly induced by dehydration and high salinity stresses and to a lesser degree by mechanical wounding (Hong and Kim, 2005). In another study, *Arabidopsis DREB2A* and its homolog *DREB2B* were induced by dehydration and salinity stresses, but not by cold stress and ABA (Liu et al., 1998; Nakashima et al., 2000). In foxtail millet, *SiDREB2* was also evidenced to be upregulated by drought and high salinity treatments (Lata et al., 2011[a]).

2.3.1.1.4 The NAC regulon

The NAC (NAM, ATAF, and CUC) family is one of the plant-specific TF family whose members are involved in plant developmental programs and disease resistance (Lata et al., 2011[c]). Many of these genes were also found to respond to various environmental stresses (Lata et al. 2011[c]; Puranik et al., 2012). *SNAC1*, a stress- responsive NAC is activated primarily in guard cells under dehydration (Hu et al., 2006). *ERD1* (early responsive to dehydration stress 1) promoter analysis revealed that the NAC ZF-HD transcription factors are vital for activation of the ERD1 gene (Tran et al., 2007). *GmNAC2*, *GmNAC3*, and *GmNAC4* were found to be strongly activated by osmotic stress (Pinheiro et al., 2009) OsNAC045 was induced by drought, salinity, low temperature, and ABA in leaves and roots (Zheng et al., 2009). Transcript encoding NAC protein, termed SiNAC was identified from a salt stress subtractive cDNA library of *S. italica* seedling, which is probably a membrane associated transcription factor of the NAC family which mediates various stress responses and developmental processes in foxtail millet (Puranik et al., 2011[a]). Further, genome-wide analysis of NAC TF family members in foxtail millet and its expression profiling during various abiotic stresses and hormonal treatments revealed a stimulus specific and time-dependent responses of these TFs (Puranik et al., 2013).

2.3.1.5 The WRKY regulon

Among TFs, WRKY is the seventh largest TF family. WRKY TFs are characterized by their unique WRKYGQK motif followed by a metal chelating zinc finger motif (CX4−5CX22−23HXHor CX5−8CX25−28HX1−2C) (Eulgem et al., 2000). These WRKY proteins bind to a specific domain called W-box in the promoter region with consensus sequence (C/T)TGAC[T/C], resulting in the expression of downstream target genes (Eulgem et al., 2000). In addition

to W-box, WRKY TFs can also interact with a sugar responsive *cis*-element called SURE and activate transcription of downstream genes (Sun et al., 2003). Putative involvement of *SiWRKY066* and *SiWRKY082* in stress and hormone signaling was suggested by expression profiling of candidate *SiWRKY* genes in foxtail millet (Muthamilarasan et al., 2015).

Recently, Muthamilarasan and colleagues (2014) identified C_2H_2-type zinc finger TFs in foxtail millet (SiC_2H_2) and physically mapped them onto the genome (Muthamilarasan et al., 2014[b]).

2.3.2 Signal sensing, perception, and transduction

Prior to transcriptional activation of genes, drought stress signals are received and messages conveyed to the appropriate components of the downstream pathway (Xiong and Ishitani, 2006). In general, STPs involve perception of stress by specific receptor molecules, which vary in identity, structure, perception, signal relay mechanism, and location within the cell (Xiong and Ishitani, 2006). Plant stress STPs often involves secondary messengers, which may modify signals (often via reversible protein phosphorylation) prior to conveying them from receptor molecules to the activators of the appropriate gene expression pathway (Xiong and Ishitani, 2006). Other molecules may also be involved in stress STPs and the functions of these include recruitment and assembly of signaling complexes, targeting of signaling molecules, and regulation of signaling molecule life span (Xiong and Ishitani, 2006). The major molecules involved in drought stress signal sensing, perception, and transduction include receptor molecules/osmosensors, phospholipid-cleaving enzymes (PLEs), reactive oxygen species (ROS), mitogen-activated protein kinase (MAPK), and Ca^{2+} sensors (Figure 2.4).

2.3.2.1 Receptor molecules/osmosensors

Receptor molecules/osmosensors are the initial stress signal perceivers and they convey the signal to the appropriate molecule to initiate STPs. On the basis of analyses of plants and other species, receptor molecules are thought to include receptor-like kinases, two-component receptors, receptor tyrosine kinases, G-protein-coupled receptors, iontropic channel-related receptors, histidine kinases, and nuclear hormone receptors. Receptor molecules that have been identified in plants include: ROP10, a

small G-protein from the ROP family of Rho GTPases, that negatively regulates ABA response in *Arabidopsis* (Zheng et al., 2002); ATHK1, a putative homolog of the yeast SLN1, which is a functional histidine kinase feeding into the HOG MAPK pathway (Urao et al., 1999; Reiser et al., 2003); NtC7, a receptor-like membrane protein from tobacco (Tamura et al., 2003); and Cre1, a putative cytokinin sensor and histidine kinase from Arabidopsis (Reiser et al., 2003).

The ERECTA gene from *Arabidopsis* is a putative leucine-rich repeat receptor like kinase (LRR/RLK). It was the first gene to be shown to act on the coordination between transpiration and photosynthesis (Masle et al., 2005).

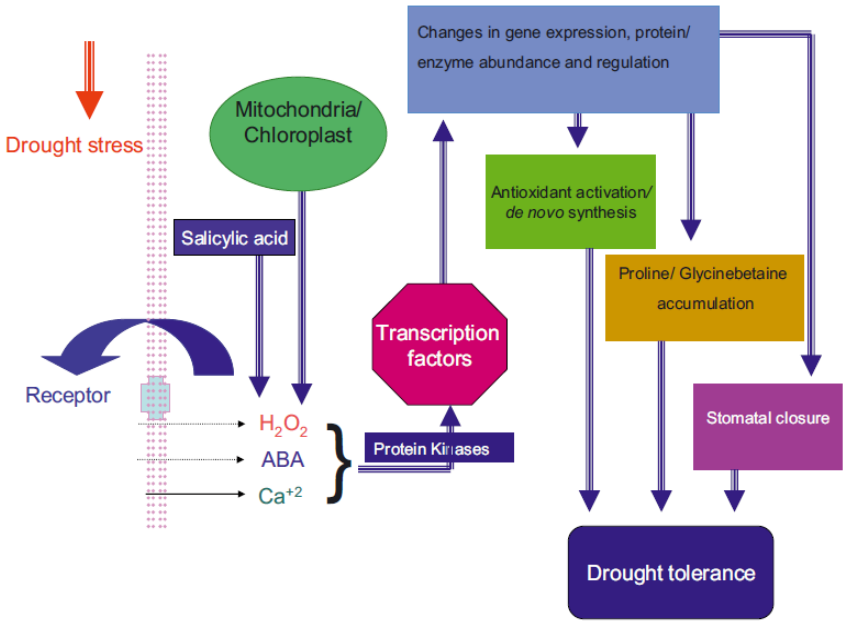


Figure 2.4 Cellular events and signaling cascades in a plant cell responding to drought stress Drought stress is perceived by an unknown mechanism, which then activates the signaling cascades, plausibly by abcissic acid (ABA), hydrogen peroxide (H_2O_2) and calcium (Ca^{+2}). These cascades then activate the synthesis of specific protein kinases which activate more downstream responses such as changes in gene expression. The response to these signaling cascades also results in changes in plant metabolism including activation and synthesis of antioxidants, synthesis and accumulation of osmoprotectants and solutes, and stomatal closure under acute drought stress (Farooq et al., 2009).

2.3.2.2 Phospholipid-cleaving enzymes

PLEs degrade phospholipid membranes, catalyzing the release of lipid and lipid derived secondary messengers (Chapman 1998; Sang et al., 2001). Phospholipases C (PLC) and D (PLD) are both involved in ABA-mediated signal transduction and drought stress tolerance perception in plants.

Phosphatidic acid (PtdOH), a product of the PLC and PLD pathways, is also important in the signaling process (Bartels et al., 2007; Wang et al., 2008).

2.3.2.3 Protein phosphatases

There are two major groups of phosphatases; the phosphoprotein (serine/threonine) phosphatases (PPases) and the protein tyrosine phosphatases (PTPases) that remove the phosphate group from phosphoserine/threonine and phosphotyrosine, respectively. The PPases are further classified into four subgroups (PPl, PP2A, PP2B and PP2C) (Cohen, 1989), whereas, the PTPases are categorized into three subgroups: receptor like PTPases, intracellular PTPases and dual-specificity PTPases (Stone and Dixon, 1994). Several phosphatases, including protein Tyr phosphatases (PTP), dual-specificity protein Tyr phosphatase (DsPTP) and protein Ser/Thr phosphatase 2C (PP2C), were shown to down-regulate MAPKs that are involved in osmotic signaling. The regulatory role of protein phosphatases is be to bring the stress activated MAPKs back to their basal activity level, allowing a new activation of the signaling pathway after a second stimulation.

2.3.2.4 Reactive oxygen species

ROS are generated in plants as photoreaction and cellular oxidation by products under normal conditions and can cause cellular damage under water deficit when they accumulate to toxic levels. Some of these species also have important roles in early stress response through activation of cellular defence mechanisms and mitigation of cellular damage. While plant mechanisms must be in place to detoxify high levels of ROS that occur under drought, low levels of these beneficial ROS must also be maintained. Those ROS known to have important signaling roles in plant stress STPs include nitric oxide (NO) and hydrogen peroxide (H_2O_2).

2.3.2.5 Mitogen-activated protein kinases

MAPKs are enzymes that catalyze reversible phosphorylations, important for relaying signals. They function via cascades, which involve sequential phosphorylation of a kinase by its upstream kinase (Xiong and Ishitani, 2006). The MAP kinases mediate signal transduction from the cell membrane to the nucleus for appropriate cellular recognition (Robinson and Cobb, 1997). The core MAPK cascades are composed of three protein kinases that are activated sequentially by an upstream kinase: MAPKs, MAPK

Kinases (MAPKKs/MKKs) and MAPKK Kinases (MAPKKKs/MEKKs). The MAPKKK upon activation phosphorylates a MAPKK on serine and threonine residues. This dual-specificity MAPKK in turn phosphorylates a MAPK, which is the last component of this cascade on conserved tyrosine and threonine residues. The activated MAPK can then either migrate to the nucleus to activate transcription factor directly, or activate additional signal components to regulate gene expression, cytoskeleton-associated proteins or enzyme activities, or target certain signal proteins for degradation. It has been shown that different MAPK pathways may share common components; yet, activation of one pathway may not necessarily affect another pathway.

Several MAPKs were shown to be activated by abiotic stresses in different plant species. In plants, MAPK cascades participate in auxin and cytokinin signal transduction and cell-cycle regulation. They are also associated with wound and pathogenesis responses as well as in environmental stress signal transduction (Jonak et al., 1999). Recently, the MKK2 pathway was identified in *Arabidopsis* as having involvement in cold and osmotic stress signal transduction. An example of a MAPK having specific involvement in drought and salt stress is the p44MMK4 kinase from alfalfa (*Medicago sativa) (*Jonak et al., 1996).

2.3.2.6 Ca^{2+} sensors

Ca^{2+} sensors are important for coupling extracellular signaling to intercellular responses and comprise calmodulin (CaM) and CaM-related proteins (Sneddon and Fromm, 1998; 2001), calcineurin B-like proteins (CBL; also known as SCaBP/SOS3-like calcium-binding proteins; Kudla et al., 1999), and CDPKs (Harmon et al., 2000). CDPKs have been implicated in signaling pathways in response to stresses such as drought, wounding and cold (Saijo et al., 2000; Chehab et al., 2004). The involvement of CDPKs in stress-induced gene transcription was demonstrated in maize leaf protoplast transient expression system (Sheen, 1996). Ca^{2+} sensors that have been attributed with roles in drought tolerance in plants include the CBL1 gene (Kudla et al., 1999) and the AtCAMBP25 protein (Perruc et al., 2004) from *Arabidopsis*. CBLs seem to be one of the major components in calcium signaling pathways in mediating response to abiotic stress. The non-enzymatic calcineurin B-like (CBL) proteins specifically target a group of

sucrose non-fermenting-related serine/threonine kinases (SnRK3) or SOS2-like protein kinases (PKSs) named CBL-interacting protein kinases (CIPKs), to mediate the sensed calcium signal (Luan et al., 2002; Batistic and Kudla, 2004; Kolukisaoglu et al., 2004).

2.4 Stress-responsive mechanisms in plants

Plant drought resistance mechanisms can be broadly grouped into avoidance or tolerance mechanisms. Plants may also escape drought stress by cutting short their growth duration, and avoid the stress with the maintenance of high tissue water potential either by reducing water loss from plants or improved water uptake, or both. Some plants may reduce their surface area either by leaf shedding or production of smaller leaves. Drought avoidance mechanisms are associated with physiological whole-plant mechanisms such as canopy resistance and leaf area reduction (which decrease radiation adsorption and transpiration), stomatal closure and cuticular wax formation (which reduce water loss), and adjustments to sink-source allocations through altering root depth and density, root hair development, and root hydraulic conductance (Beard and Sifers, 1997; Rivero et al., 2007).

Drought tolerance mechanisms are generally those that occur at the cellular and metabolic level. These mechanisms are primarily involved in turgor maintenance, protoplasmic resistance, and dormancy (Beard and Sifers, 1997). Low-molecular-weight osmolytes, including glycinebetaine, proline and other amino acids, organic acids, and polyols, are crucial to sustain cellular functions under drought. Polyamines and several enzymes act as antioxidants and reduce the adverse effects of water deficit. The stress is first perceived by the receptors present on the membrane of the plant cells, the signal is transduced downstream and this results in the activation of various stress responsive genes. The products of these stress genes ultimately lead to plant adaptation and help the plant to survive and surpass the unfavourable conditions. At molecular levels several drought-responsive genes and transcription factors have been identified, such as the dehydration-responsive element-binding gene, aquaporin, late embryogenesis abundant proteins and dehydrins.

The outcome of stress signal perception, transduction, and transcriptional up or downregulation of genes is the production of molecules with various plant protection, repair, and stabilization functions. These molecules can be broadly grouped into five functional groups: (1) detoxification; (2) chaperoning; (3) late embryogenesis abundant (LEA) protein functions; (4) osmoprotection; and (5) water and ion movement.

2.4.1 Detoxification

To prevent stress injury, cellular ROS need to remain at nontoxic levels under drought stress. The reactive oxygen species in plants are removed by a variety of antioxidant enzymes and/or lipid-soluble and water-soluble scavenging molecules (Hasegawa et al., 2000); the antioxidant enzymes being the most efficient mechanisms against oxidative stress (Farooq et al., 2008). The antioxidant defence system in the plant cell constitutes both enzymatic and non-enzymatic components. Enzymatic components include superoxide dismutase, catalase, peroxidase, ascorbate peroxidase and glutathione reductase. Non-enzymatic components contain cystein, reduced glutathione and ascorbic acid (Gong et al., 2005).

In environmental stress tolerance, such as drought, high activities of antioxidant enzymes and high contents of non-enzymatic constituents are important. Among enzymatic mechanisms, superoxide dismutase plays an important role, and catalyzes the dismutation of two molecules of superoxide into O_2 and H_2O_2; the first step in reactive oxygen species scavenging systems. The transcript of some of the antioxidant genes such as glutathione reductase or ascorbate peroxidase was higher during recovery from a water deficit period and found to play a role in the protection of cellular machinery against damage by reactive oxygen species (Ratnayaka et al., 2003). These metallo-enzymes constitute an important primary line of defense of cells against superoxide free radicals generated under stress conditions. Therefore, increased superoxide dismutase activity is known to confer oxidative stress tolerance (Pan et al., 2006).

Apart from catalase, various peroxidases and peroxiredoxins, four enzymes are involved in the ascorbate-glutathione cycle, a pathway that allows the scavenging of superoxide radicals and H_2O_2. These include ascorbate peroxidase, dehydro ascorbate reductase, mono dehydro

ascorbate reductase and glutathione reductase (Fazeli et al., 2007). Ascorbate peroxidase is a key antioxidant enzyme in plants (Orvar and Ellis, 1997) whilst glutathione reductase has a central role in maintaining the reduced glutathione pool during stress (Pastori et al., 2000). Some proteins, osmolytes, and amphiphilic molecules also have antioxidative functionality (Bowler et al., 1992; Noctor and Foyer, 1998).

Dai and colleagues, (2012) studied the changes in soluble protein, malondialdehyde (MDA), superoxide radical ($O_2^{\cdot-}$), and hydrogen peroxide (H_2O_2), and the activities of superoxide dismutase and catalase in foxtail millet under drought stress. Foxtail millet variety, Jingu32 has improved activities of peroxidase (POD), superoxide dismutase (SOD), 6 phosphate dehydrogenase (G6PDH), glutamine synthetase (GS) and glutamic dehydrogenase (GDH), and has higher absorption ability and conversion efficiency of N, P, K. POD, SOD. Moreover, G6PDH of Jigu32 is more active leading to higher resistance to adversity and aging; also glutamine synthetase (GS) and glutamate dehydrogenase (GDH) of Jigu32 are more active, resulting in higher assimilation and transformation ability of nutrients (Li et al., 2014). Activities of antioxidative enzymes (glutathione reductase and catalase) are found to be higher in tolerant variety, Prasad of foxtail millet (Puranik et al., 2011[b]).

2.4.2 Chaperoning

Chaperone functions involve specific stress-associated proteins, which are responsible for protein synthesis, targeting, maturation and degradation, and function in protein and membrane stabilization, and protein renaturation. Synthesis of stress proteins is a ubiquitous response to cope with prevailing stressful conditions including water deficit. Most of the stress proteins are soluble in water and therefore contribute towards the stress tolerance phenomena by hydration of cellular structures (Wahid, 2007). WD40 proteins are induced by different abiotic stresses which are categorized into 5 distinct sub-families (I–V) in foxtail millet. Preliminary expression profiling of some SiWD40 genes shows that they are influenced by several environmental stimuli, including dehydration, salinity, ABA treatment and cold stress (Mishra et al., 2014)

2.4.2.1 Heat shock proteins

Heat shock proteins belong to a larger group of molecules called chaperones. They have a role in stabilizing other proteins' structure. Low-molecular-weight heat shock proteins are generally produced only in response to environmental stress, particularly high temperature (Wahid, 2007). But many heat shock proteins have been found to be induced by different stresses such as drought, anaerobic conditions and low temperatures (Coca et al., 1994). HSPs, which can be divided into five conserved families, have been shown to have particularly important stress-related chaperone functions in plants (Hendrick and Hartl, 1993; Boston et al., 1996; Hartl 1996; Waters et al., 1996; Torok et al., 2001). Protein denaturation occurs under drought stress because decreased cellular volume increases the likelihood of degradative molecular interactions (Cho and Hong, 2006). HSPs maintain or repair companion protein structure and target incorrectly aggregated and non-native proteins for degradation and removal from cells (Cho and Hong, 2006). They are reported to serve as molecular chaperones that participate in adenosine triphosphate-dependent protein unfolding or assembly/disassembly reactions and prevent protein denaturation during stress (Gorantla et al., 2006).

2.4.3 Late embryogenesis abundant protein functions

LEA proteins are the most abundant stress proteins that are linked to both water and cold stress in plants. LEA proteins are produced in response to dehydration stress and function in water status stabilization, protection of cytosolic structures, ion sequestration, protein renaturation, transport of nuclear targeted proteins, prevention of membrane leakage, and membrane and protein stabilization. LEA and LEA-type genes are found universally in plants. These proteins are active in seeds that contain high ABA levels (Tunnacliffe and Wise, 2007) during the late stages of embryogenesis and are associated with the acquisition of desiccation tolerance under drought, heat, cold, salt, and ABA stress (Sivamani et al., 2000; Bartels et al., 2007). They are also present in the biomass tissue of resurrection plants and are upregulated in many desiccation-sensitive plants in response to drought stress (Bartels et al., 2007). LEA proteins are divided into groups based on conserved sequence motifs (Zhang et al. 2000; Wise, 2003). In plants, at least

seven groups of LEA genes have been identified (Bartels and Salamini, 2001; Ramanjulu and Bartels, 2002; Amara et al. 2014)) of which group 3 and 5 LEA proteins are predicted to play important roles in ion sequestration during cellular dehydration or water deficit (Bray, 1993). Group 1 LEA proteins are predicted to possess enhanced water-binding capability while group 4 LEA proteins may participate in replacing water to protect cellular structures (Bray 1993). Dehydrins are also a group of LEA proteins that are known to accumulate during water deficit stress. However, recent research indicates that additional groups of LEA and LEA-like proteins are still being identified (Park et al., 2003; Wang et al., 2006; March et al., 2007).

Common features of LEA proteins generally include hydrophilicity (Garay-Arroyo et al., 2000; Park et al., 2003), heat stability (Close and Gallagher-Ludeman, 1989; Ceccardi et al., 1994; Houde et al., 1995; Thomashow, 1998, 1999), and transcriptionally regulated and ABA-responsive gene expression (Close and Gallagher-Ludeman, 1989). They play a role in water-deficit tolerance and the possible functions of LEA proteins include binding and replacement of water (Dure, 1993), ion sequestration (Bray, 1993), maintenance of protein and membrane structure (Baker et al., 1998), molecular chaperones (Close, 1996), membrane stabilization (Koag et al., 2003), and nuclear transport of specific molecules (Goday et al., 1994). One class of LEAs, the dehydrins, which have detergent and chaperone-like properties, stabilize membranes, proteins, and cellular compartments (Close, 1996).

2.4.4 Osmoprotection

Osmoprotection involves the upregulation of compatible solutes (osmolytes) that function primarily to maintain cell turgor, but are also involved in antioxidation and chaperoning through direct stabilization of membranes and/or proteins (Yancey et al., 1982; Bohnert and Jensen, 1996; Lee et al., 1997; Hare et al., 1998; McNeil et al., 1999; Diamant et al., 2001). Compatible solutes are low molecular weight, highly soluble compounds that are usually nontoxic at high cellular concentrations. Generally they protect plants from stress through different means such as contribution towards osmotic adjustment, detoxification of reactive oxygen species, stabilization of membranes, and native structures of enzymes and proteins.

The three major groups of compatible solutes are amino acids (such as proline), quaternary amines (glycine betaine (GlyBet), polyamines, and dimethyl sulfonio proprionate), and polyol/sugars (such as mannitol, galactinol, and trehalose; Wang et al., 2003). Many genes involved in the synthesis of these osmoprotectants have been explored for their potential in engineering plant abiotic stress tolerance (Vinocur and Altman, 2005).

As a mechanism, osmotic adjustment has been suggested as an important trait in postponing the dehydration stress in water scarce environments (Morgan, 1990). Of these, proline is one amongst the most important cyto-solutes and its free accumulation is a widespread response of higher plants, algae, animals and bacteria to low water potential (Zhu, 2002; Wahid and Close, 2007). Its synthesis in leaves at low water potential is caused by a combination of increased biosynthesis and slow oxidation in mitochondria. Proline contents were increased under drought stress in pea cultivars (Alexieva et al., 2001). Drought-tolerant petunia (*Petunia hybrida*) varieties were reported to accumulate free proline under drought that acted as an osmoprotectant and induced drought tolerance (Yamada et al., 2005). Islam et al. (2011) reported increased proline content under salt stress in foxtail millet with an increase of 14.7 and 12.6 times more proline under saline and alkaline conditions, respectively as compared to proso millet.

GlyBet and trehalose act as osmoprotectants by stabilizing quaternary structures of proteins and highly ordered states of membranes. Mannitol serves as a free radical scavenger. Proline serves as a storage sink for carbon and nitrogen and a free-radical scavenger. It also stabilizes subcellular structures (membranes and proteins), and buffers cellular redox potential under stress. Many crops lack the ability to synthesize the special osmoprotectants that are naturally accumulated by stress tolerant organisms.

2.4.5 Water and ion movement

Water and ions move through plants via transcellular and intracellular pathways. Aquaporins (major intrinsic proteins; MIPs), facilitate water, glycerol, small molecule, and gas transfer through membranes and, therefore, have a role in water homeostasis (Bartels et al., 2007). Active transport of solutes into the cell and cellular organelles, particularly the vacuole, is another means of cell turgor maintenance as increased solute potential facilitates the

passive movement of water into cells and cellular compartments (Li et al., 2008). In plants, aquaporins are present abundantly in the plasma membrane and in the vacuolar membrane. The structural analysis of aquaporins has revealed the general mechanism of protein-mediated membrane water transport. Nevertheless, it is believed that they can regulate the hydraulic conductivity of membranes and potentiate a ten-to-twenty-fold increase in water permeability (Maurel and Chrispeels, 2001). Phosphorylation (Johansson et al., 1998), calcium and pH (Tournaire- Roux et al., 2003) are important factors modulating aquaporin activity. Recently, efforts have been concentrated on investigating the function and regulation of plasma membrane intrinsic protein aquaporins. The aquaporins play a specific role in controlling transcellular water transport. For instance, they are abundantly expressed in roots where they mediate soil water uptake (Javot and Maurel, 2002) and transgenic plants downregulating one or more prolactin-inducible protein genes had lower root water uptake capacity (Javot et al., 2003).

Attempts have also been made to improve drought tolerance of plants by altering the expression of aquaporins (Aharon et al., 2003; Porcel et al., 2005; Yu et al. 2005; Peng et al., 2006; Jang et al., 2007; Cui et al., 2008; Miyazawa et al., 2008; Zhang et al., 2008). Research into the role of aquaporins in plant drought tolerance has shown that various aquaporins function differently depending on the severity and type of stress. There is also evidence that overexpression of aquaporins in some plants causes them to respond differently to different stresses. For example, Jang et al. (2007) found that *Arabidopsis* and tobacco plants overexpressing *Arabiopsis* PIP's displayed enhanced water flow and improved germination under cold stress, but exhibited rapid water loss, retarded seedling growth, and inferior germination under drought conditions. It is therefore thought that different aquaporin isoforms are associated with different physiological processes and that plants respond to drought conditions either by increasing aquaporin expression, which facilitates water movement (especially into the tonoplast in order to maintain cell-turgor) or downregulating aquaporin expression to avoid excessive water loss (Aharon et al., 2003; Peng et al., 2006). Overexpression of aquaporins has also been implicated in conferring heavy metal tolerance to

transgenic plants by alleviation of metal ion-induced water deficit and oxidative damage caused by metal ions (Zhang et al., 2008).

2.5 Physiological, biochemical, molecular and functional genomics approaches in relation to stress tolerance

The integration of molecular techniques with physiology and biochemistry is a new approach being used to improve the environmental adaptation and yield of field crops. The gene networks that underline plant stress responses can be understood by identifying and characterizing the genes that respond initially as well as when the physiological response to the stress develops.

Biomarkers to identify and characterize the drought tolerant crops have been sought for many decades. These markers fall under three broad categories: morphological, cytogenetic, and biochemical. Morphological markers are based on the traditional botanical descriptions of visible characters and were the first markers to be utilized. Biochemical markers became a popular tool in plant genetics where protein and secondary metabolites of leaves from variety of plants have been examined such as ASR (ABA-water stress ripening induced) protein (Riccardi, 1998), dehydrin (Lopez et al., 2001; Jiang and Huang, 2002), superoxide dismutase (Zang, 2007).

The identification of differentially expressed genes and examination of their patterns of expressions are important to gain information about the functions relevant to processes such as cell differentiation, morphological or metabolic changes (van den Berg et al., 2004). Many scientists have suggested that selection is more convenient and practicable if the plant species possesses distinctive indicators of tolerance at the whole plant, tissue or cellular level (Munns, 2002; Ashraf, 2002).

Molecular markers can be used to identify the genotype of the individual plant and to identify and map the genes affecting complex plant traits such as resistance to biotic or abiotic stresses. The common methods employed for the identification of DNA and cDNA markers are: random amplified polymorphic DNA (RAPDs); simple sequence repeats (SSRs), sequence tagged marker sites (STMS), inter simple sequence repeats (ISSRs), restriction fragment length polymorphism (RFLP); amplified fragment length polymorphism (AFLP) and single nucleotide polymorphisms (SNPs).

The advent of high-throughput sequencing technology has generated abundant information on DNA sequences for the genomes of many plant species. This includes the completion of the draft of the whole genome sequences for the model plant *Arabidopsis thaliana*, in 2000 (The *Arabidopsis* Genome Initiative, 2000) and for rice, one of the most important food crops, in 2002 (Goff et al., 2002; Yu et al., 2002). Since the generation of Foxtail millet genome independently by the Beijing Genomics Institute and the Joint Genomes Institute (Zhang et al., 2012 and Bennetzen et al., 2012) in 2012, many marker systems are now available in the crop facilitating the genetic and genomic studies. The open web resources in foxtail millet developed by NIPGR are presented in Table 2.3.

Multiple mapping populations, fosmid libraries and mutagenized populations have been developed for the genus *Setaria* (some for *S. italica*, others for *S. viridis*). Wang et al. (1998) was the first to report markers in foxtail millet. QTL mapping populations have been published in *Setaria italica* (Devos et al., 1998; Wang et al., 1998).

The first SSR-linkage map of foxtail millet was reported by Jia et al. (2009). Gupta et al. (2011) reported 98 potential intron length polymorphic ILP markers exploiting the EST sequences of dehydration- and salinity stressed suppression subtractive hybridization (SSH) libraries. A total of 447 EST-derived- SSR (eSSR) markers were successfully designed, of which 327 were mapped physically onto nine chromosomes (Kumari et al., 2013).

In addition, the ESTs of other important crop species have been generated, and powerful bioinformatics tools have annotated thousands of sequences as putative functional genes. During the past few decades, advances in molecular genetics have led to the identification of multiple genes or genetic markers associated with genes that affect traits of interest in livestock, including genes for single-gene traits and QTL or genomic regions that affect quantitative traits.

Most of the research in abiotic stress regulation is conducted by a candidate gene approach, i.e. identify candidate genes in heterologous

Table 2.3 Open web resources in foxtail millet developed by NIPGR (New Delhi).

SN	Web resource	Description	URL	Reference
1.	Foxtail millet Marker Database (FmMDb)	A first comprehensive online database for information retrieval, visualization and management of large-scale marker datasets with unrestricted public access. FmMDb provides complete marker information (SSRs, eSSRs and ILPs) to the plant science community.	http://www.nipgr.res.in/foxtail.html	Suresh et al., 2013
2.	Foxtail millet Transcription Factor Database (FmTFDb)	FmTFDb provides free access to the 2297 FmTFs through a set of query interfaces and analysis tools, including the BLAST search, annotation query interfaces, and tools to identify enriched Gene Ontology terms and visualize physical maps.	http://59.163.192.91/FmTFDb	Bonthala et al., 2014
3.	Foxtail millet miRNA Database (FmMiRNADb)	The database encompasses complete information of 355 foxtail millet miRNAs along with a interactive physical map. Further, FmMiRNADb also contains the details of 123 miRNA-based molecular markers for genotyping and molecular breeding of millets, cereals and bioenergy grasses.	http://59.163.192.91/FmMiRNADb	Khan et al., 2014
4.	Foxtail millet Transposable Elements-based Marker Database (FmTEMDb)	The database possess the data of ~30000 foxtail millet transposable elements and 6 different types of markers useful for large-scale genotyping applications in millets, cereals and bioenergy grasses.	http://59.163.192.83/ltrdb/index.html	Yadav et al., 2014

systems and characterize their expression or biochemical function in plants under stress conditions. These studies are sometimes strengthened by altering the expression of genes under various stress conditions to result in phenotypic alterations. Advances and technical developments in genomics, bioinformatics and 'functional genomics' made it possible to address the complexity of stress response on a large scale through genome wide 'expression profiling' (Reymond et al., 2000; Richmond and Somerville, 2000). Scientists are now equipped to perform gene expression analysis to characterize and define the functional roles of all genes - essential, important, and ancillary to the stress response of tolerant genotypes. Obtaining in depth information about these processes necessitates the study of differential patterns of gene expression.

In recent years, researchers have become interested in the use of genomic tools to identify and isolate genes involved in the tolerance of crop plants to various abiotic stress factors. The first step to understand and evaluate such genetically complex responses is to sequence randomly selected cDNA clones or expressed sequence tags (ESTs) from the plants exposed to the environmental stress (Zhang et al., 2001). Analyses of the identities and expression levels of these genes that were high throughout could be conducted with the aid of different molecular biology tools, which has provided a better understanding of the role of the genes to plant stress adaptation and will form basis for effective engineering of the non-model plants for improved stress tolerance. Various molecular techniques have been used for studying differential gene expression in many plant species includes representational difference analysis (RDA), suppression subtractive hybridization (SSH), differential display, differential hybridization, subtractive library construction, serial analysis of gene expression (SAGE), cDNA-RAPD, cDNA-AFLP and cDNA microarrays (Lisitsyn and Wigler, 1993; Velculescu, 1995; Diatchenko, 1996; Schummer, 1997, Kawar et al., 2010) and SRAP (Li and Quiros, 2001).

2.5.1 Sequence-related amplified polymorphism (SRAP)

Sequence-related amplified polymorphism (SRAP) is a PCR-based molecular marker technique developed by Li and Quiros in 2001. This technique has the advantages of being simple, effective, and fast. Analysis

and detection of the fragment length polymorphism with SRAP primers can be carried out with either genomic DNA (SRAP) or cDNA (cDNA-SRAP) as template.

In SRAP technique, the core sequence of the forward primer (CCGG) and the reverse primer (AATT) and the changing annealing temperature ensure the stability of the amplification results (Li et al, 2003; Lu and Wu, 2006; Ma, 2008). More primer combinations can be obtained through replacing the three selective bases at the 3′ ends of the forward and reverse primers. At the same time, as the forward and reverse primers can combine with each other freely, a series of primer combinations can be achieved with only a small number of primers. Not only can this reduce the cost of the primer synthesis but also improve the efficiency of primer use. Since the forward and reverse primers are targeted at relatively conserved exons, introns with large variation, respectively, or promoter and intervening sequence, most SRAP markers are evenly distributed in the whole genome with high-frequency co-dominance. Additionally, while it mainly amplifies the open reading frame (ORF) of the genome, the SRAP technique can increase the correlation between amplicon and phenotype, thus better reflects the phenotypic difference of plant materials at genetic level (Li and Quiros, 2001).

Compared with cDNA-AFLP and DDRT-PCR (Que 2009, 2011), cDNA-SRAP has better repeatability and more amplified fragments, and it can also detect differential gene expression in multiple samples simultaneously with less cost. Contrary to the ESTs acquirement through cDNA library construction and sequencing, cDNA-SRAP can detect expressed genes more uniformly (Li et. al., 2003). Furthermore, as SRAP usually produces high-intensity fragments with few overlaps, the corresponding primers can even be used directly for sequencing of the target fragments (Li and Quiros, 2001).

So far, SRAP has also been successfully applied to research of several crops including cotton, melon, buffalograss, peach, and squash with application in various areas including genetic map construction (Li and Quiros, 2001, Lin et al., 2005], molecular diversity analysis (Ferriol, 2003, Kosman and Leonard, 2007) and comparative genomic study (Li et al., 2003).

The cDNA-SRAP technique has been proved to be suitable for analysis of differential gene expression in several kinds of plants. Li et al. (2003)

conducted SRAP amplification using cDNA template from intraspecific hybrids between cauliflower and cabbage. Lu and Wu (2006) carried out a differential display study on salt-tolerance of *Spartina angelica* using cDNA-SRAP in which a differentially expressed fragment was identified (Lu and Wu, 2006).

Deng et al. (2007) applied cDNA-SRAP in the study of differential gene expression in the restore and maintainer lines of cabbage. Amplification with 30 SRAP primer combinations was performed and two differentially expressed genes were obtained (Deng et al., 2007).

Ma et al. (2008) analyzed differential genes related to seed-coat color in *Brassica napus* L. by cDNA-SRAP technique and a total of 2100 bands were amplified with 996 SRAP primer combinations (Ma et al., 2008) and *Erianthus arundinaceum* (Que et al., 2012).

Zhang and Nakajima (2015) explored and screened SRAP markers associated with main salt tolerance gene in maize while Huang et al. (2015) identified smut-responsive genes in sugarcane using cDNA-SRAP.

Khaled (2014) identified TRAP and SRAP markers linked with yield components under drought stress in wheat (*Triticum aestivum* L.)

2.5.2 Differential gene expression in foxtail millet in response to drought

High expression of PHGPX gene in the tolerant foxtail millet cultivar ‘Prasad’ suggested it’s imperative role in stress-induced defensive reactions (Sreenivasulu et al*.,* 2004).

Veeranagamallaiah et al*.* (2007) developed the expression profiles of glutamine synthetase (GS) and pyrroline-5-carboxylate (P5C) reductase under salinity stress in salt sensitive (cv. Lepakshi) and salt tolerant (cv. Prasad) cultivars of foxtail millet (Veeranagamallaiah et al., 2007)

Zhang et al., (2007) reported the construction of subtracted cDNA libraries from foxtail millet seedlings under dehydration stress and the expression profile analysis showed upregulation od ESTs by dehydration stress (Zhang et al., 2007). Later, Puranik and colleagues constructed two suppression subtractive hybridization cDNA libraries (forward and reverse) in foxtail millet to salinity tolerance to show differential expression of identified unknown genes (Puranik et.al., 2011[b]).

Use of cDNA-AFLP technique was reported by Jayaraman et al., (2008) to compare gene expression profiles of a salt tolerant and a salt-

sensitive cultivar of foxtail millet (*Setaria italica*) and identified early responsive differentially expressed transcripts that accumulated upon salt stress (Jayaraman et. al., 2008).

First report about analysis of differentially expressed transcripts (early- and late- induced) in foxtail millet cv. Prasad after dehydration stress was given by Lata and colleagues (2010). The previously reported as well as unknown genes suggests their function in possible regulation of dehydration adaptation in this crop (Lata et al., 2010).

Expression of *SiOPR1*(12-oxophytodienoic acid reductase1) is in the roots and is not influenced by ABA, NaCl and MeJA treatments throughout the plant suggesting its significant role in drought stress tolerance (Zhang et al. 2007). *DNAj* is thought to be related with drought and heat tolerance in wheat (Wang et al., 2009).

Expression profiling of candidate AP2/ERF genes against drought, salt and phytohormones revealed insights into their pre*cis*e and/or overlapping expression (Lata et al., 2014) and characterized a differentially expressed EST encoding a putative DREB2 gene, SiDREB2 (Lata et al., 2011[a]).

Various unknown genes in response to abiotic stress have been reported in foxtail millet. Zhang et al. (2012) identified 586 genes that were predicted to have roles in stress responses. Qi et al. (2013) analyzed the whole transcriptome of foxtail millet by using the next generation deep sequencing technology and identified a total of 2,824 genes with drought-responsive expression patterns (Qi et al., 2013).

Differentially expressed signaling pathways of up-regulated genes in foxtail millet were studied and significantly up-regulated genes were identified in Yugu-1 in response to rust in foxtail millet (Li et al., 2015). Differential expression behavior of five miRNA-target genes was verified under dehydration stress treatment which highlights the importance of dehydration stress-associated post-transcriptional regulation governed by miRNAs and their targets in a naturally stress-tolerant model crop (Yadav et al., 2015)

Similarly, expression analysis of various TFs like MYB genes (Muthamilarasan et al., 2014[a]), SiNAC genes (Puranik et al., 2013), C_2H_2 type of zinc finger (Muthamilarasan et al., 2014[b]), ADP-ribosylation factors (ARFs) (Muthamilarasan et al., 2016), have been studied in foxtail millet.

CHAPTER III

MATERIAL AND METHODS

The present investigation entitled “Deciphering the differentially expressed genes in Foxtail millet (*Setaria italica* L.) in response to water stress” was conducted during the year 2013-2016 at Biotechnology Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

This chapter elaborates the experimental material and methods adopted during the course of the investigation. The investigation was aimed to explore profiling of drought specific gene/TFs in foxtail millet (*Setaria italica* L.) in response to water stress. Various methodologies and activities that were undertaken during the course of investigation were standard research practices and protocols that are described in literature and were adopted either as such or with minor modifications, wherever required.

The details of the material used and the methods adopted during present investigation are as under,

3.1 Material

3.1.1 Plant material

The experimental material of the present investigation comprised of 62 accessions of foxtail millet (*Setaria italica* L.) procured from National Bureau of Plant Genetic Resources, Regional station, Akola. Accessions with their sources of material are presented in Table 3.1. The screening experiment to determine the most drought tolerant and most drought sensitive foxtail millet accessions was conducted at the Biotechnology Centre, Dr.PDKV, Akola (MH) during the month of September-October 2013.

Table 3.1 List of foxtail millet accessions used in present investigation

SN	Accessions	Source	SN	Accessions	Source
1	IC 28439	Amreli, Gujarat	32	IC120200	Unknown
2	IC41883	Betul, MP	31	IC120201	Unknown
3	IC41898	Akola, MH	34	IC120204	Unknown
4	IC58243	Uttarakhand	35	IC120207	Unknown
5	IC97086	Maharashtra	36	IC120208	Unknown
6	IC97087	Maharashtra	37	IC120210	Unknown
7	IC97109	Maharashtra	38	IC120212	Unknown
8	IC97111	Madhya Pradesh	39	IC120213	Adilabad, AP
9	IC97123	Parbhani, MH	40	IC120221	Bidar, KA
10	IC97172	Maharashtra	41	IC120228	Osmanabad, MH
11	IC97174	Maharashtra	42	IC120239	Unknown
12	IC97175	Unknown	43	IC120251	Sholapur, MH
13	IC97177	Kangra, HP	44	IC120234	Parbhani, MH
14	IC97179	Basti, HP	45	IC120235	Parbhani, MH
15	IC97182	Basti, HP	46	IC120244	Yavatmal, MH
16	IC97189	Maharashtra	47	IC120250	Sholapur, MH
17	IC97295	Sirmour, HP	48	IC120255	Gulbarga, KA
18	IC120148	Gulbarga, KA	49	IC120346	Unknown
19	IC120150	Gulbarga, KA	50	IC120355	Unknown
20	IC120158	Osmanabad, MH	51	IC120394	Unknown
21	IC120159	Parbhani, MH	52	IC120404	Andhra Pradesh
22	IC120160	Buldana, MH	53	IC120406	Andhra Pradesh
23	IC120163	Maharashtra	54	IC120407	Andhra Pradesh
24	IC120164	Unknown	55	IC120408	Andhra Pradesh
25	IC120165	Unknown	56	IC200125	Amravati, MH
26	IC120166	Unknown	57	IC325968	Narmada, Gujarat
27	IC120175	Unknown	58	IC344224	Maharashtra
28	IC120177	Unknown	59	IC344225	Maharashtra
29	IC120179	Unknown	60	IC480117	Uttar Pradesh
30	IC120191	Unknown	61	LEPAKSHI	Variety, RARS, Nandyal
31	IC120192	Unknown	62	PRASAD	Variety, ARS,Ananthpur

3.1.2 General laboratory materials

3.1.2.1 Glasswares and major equipments

All glasswares used for the experimental work were made-up of Borosilicate glass, and disposable plasticware (centrifuge tube, PCR tubes, micro-tips etc.) were from Genaxy, whereas other major equipments and their specifications are listed in Table 3.2.

3.1.2.2 Chemicals and reagents

All the chemicals and reagents were of analytical or molecular biology grade and were obtained from different manufacturing firms/suppliers *viz*., Sigma Aldrich, SD Fine Chem, Sisco Research Lab (SRL), Operon,

Puregene, Genaxy, HiMedia etc. which are presented in Table 3.3 and Table 3.4.

Table 3.2 List of the instruments used during the investigation

SN	Name of the instrument	Source
1	Centrifuge	Eppendorf
2	Thermal cycler	Eppendorf
3	Nanophotometer	IMPLEN
4	Gel documentation unit	Alpha Innotech/ Syngene
5	Electrophoresis unit (horizontal)	Bio-rad
6	Microwave oven	LG
7	Weighing balance	Sartorius, Germany
8	pH meter	Themo Scientific
9	Water bath	WiseCircu, Wisd Instruments
10	Micro-pipettes	Eppendorf
11	Ice maker	SANYO SIM-F140
12	Thermomixer	Eppendorf
13	Magnetic stirrer	Remi
14	Deep freezer (-20°C, -80°C)	Vest Frost/ RS biotech
15	Water distillation unit	Borosil

Table 3.3 List of the kits and enzymes used during the investigation

Molecular biology kits		
SN	**Material**	**Source**
1	First strand cDNA synthesis kit	Agilent,
2	QIAEXII Gel Extraction Kits	Qiagen, Genxany
Enzymes		
1	*Taq* DNA Polymerase	Invitogen, USA
2	*Pfu* DNA Polymerase	Puregene,
3	RNaseA	Fermentas
4	DNasel RNase-free	Fermentas

Table 3.4 List of chemicals and other materials

Category	Material	Source
General purpose chemicals	Isopropanol, Iso-amyl alcohol, Methanol, Chloroform, Formaldehyde, Glycerol, Tris, NaCl, KCl, NaOH, KOH, $CaCl_2$, $MgCl_2$, Potassium acetate, sodium acetate, NaH_2PO_4, Na_2HPO_4, KH_2PO_4, K_2HPO_4 , $MgSO_4$, $K_2Cr_2O_7$, $Na_3C_6H_5O_7$, H_3BO_3, EDTA, Glucose, Sucrose, Acetic acid, HCl, H_2SO_4 , HNO_3 , HCI, Sodium hypochlorite, Mercuric chloride, CTAB, Acrylamide, Bis-Acrylamide, RNase away, MOPS, TEMED, DEPC, Formamide, DTT, PMSF	SRL, Himedia, Puregene
Markers and dNTPs	1 kb DNA ladder, 100 bp DNA ladder, dATP, dCTP, dGTP, dTTP	Fermentas, Genaxy, Puregene.
Dyes	Ethidium Bromide, Xylene cyanol, Bromophenol blue, Coomasie Brilliant Blue, 6X DNA loading dye, 2X RNA loading dye.	HiMedia, SRL, Fermentas, Genaxy, Puregene.
Membrane, filter papers.	Whatman sheet	Genaxy

3.2 Methods adopted

3.2.1 Physiological screening

Screening under laboratory condition was done using PEG-6000 as stress inducer and by water withholding.

3.2.1.1 Laboratory screening using PEG-6000

Polyethylene Glycol with a molecular weight of 6000 (PEG-6000, HiMedia, India) was used as a drought stimulator and water stress levels of zero (unstressed) and -0.3 Mpa were developed by dissolving 0 and 8.6 gm of PEG per 100 ml distilled water (Michel and Kaufmann,1973).

3.2.1.1.1 Surface sterilization

Seeds were surface sterilized with 1 percent Bavistin for 10 minutes followed by 0.1 percent mercuric chloride for one minute and then rinsed three times with distilled water. Fifteen seeds of each genotype were placed in each petriplate containing wet filter paper.

3.2.1.1.2 Experimental Design

The experiment consisting 62 accessions was laid out in a completely randomized design (CRD) with three replications for each experimental unit.

3.2.1.1.3 Procedure

Ten milliliters of the treatment solution was applied daily in each petri plate after washing out the previous solution. Number of seeds germinated was recorded daily upto a period of 14 days. A seed was considered germinated when plumule had emerged to 5 mm. Total germination was expressed as percent of that in the control treatment for each line and then data were analyzed statistically. Shoot and root length, dry weights/seedling were recorded after 14 days of the start of the experiment. Plant dry weights were recorded after drying at 70°C**.**

3.2.1.1.4 Observations for laboratory screening using PEG 6000

The number of seeds germinated was counted and germination percentage was calculated. A seed was considered germinated when both plumule and radicle had emerged to 5 mm.

Promptness index, shoot length stress tolerance index and root length stress tolerance index and dry matter stress tolerance index were calculated using appropriate formulae as below.

3.2.1.4.1 Promptness index

Promptness index is the percentage of seeds that germinate at 2^{nd}, 4^{th}, 6^{th} and 8^{th} day of observation and is indicated by nd2, nd4, nd6 and nd8.

PI= nd2 (1.00) + nd4 (0.75) + nd6 (0.50) + nd8 (0.25)

3.2.1.4.2 Percentage of germination stress tolerance index

Percentage of germination stress tolerance index was calculated using the formula determined as below,

$$GSI(\%) = \frac{\text{Promptness index of stressed seedlings}}{\text{Promptness index of control seedlings}} \text{X100}$$

3.2.1.4.3 Shoot length stress tolerance index

After 14 days seedlings were harvested and their shoot length were measured and shoot length stress tolerance index (SLSI) was recorded as below,

$$SLSI(\%) = \frac{\text{Shoot length of stressed plants}}{\text{Shoot length of control plants}} \text{X100}$$

3.2.1.4.4 Root length stress tolerance index

After 14 days seedlings were harvested and their root length were measured and root length stress tolerance index (RLSI) was recorded as below,

$$RLSI(\%) = \frac{\text{root length of stressed plants}}{\text{root length of control plant}} \text{X100}$$

3.2.1.4.5 Dry matter stress tolerance index

After drying the plant in oven at 70°C for 24 hours, the dry matter stress tolerance index was recorded as below,

$$DMSI(\%) = \frac{\text{Dry matter of stressed plants}}{\text{Dry matter of control plant}} \text{X100}$$

3.2.1.2 Induced water stress experiment

Drought was also induced by withholding water in experimental plants while the control plants were regularly watered. The experiment was conducted in a greenhouse under controlled environmental conditions. Drought was induced after 3 weeks of seedling growth. The physiological measurements were taken from control and experimental water stress plants from 9th day of drought induction.

Based on physiological data, the extent of drought induction was divided into two stages - before wilting and wilting.

3.2.1.3 Physiological measurements for Induced water stress experiment

3.2.1.3.1 Leaf water potential

The leaf water potential was measured using the WP4 Potentio meter. Measurement was taken for three fully expanded leaves per plant.

3.2.1.3.2 Relative water content (RWC)

Relative water content of flag leaf was measured as described by Barrs (1968). Dry weight (DW) was subsequently determined after oven-drying for 2 days and RWC was calculated using the equation,

RWC (%) = [(FW-DW) / (TW-DW)] x 100

3.2.1.3.3 Chlorophyll determination

Chlorophyll content index (relative chlorophyll value) was measured by CCI using an Opti-Sciences CCM-200 at random points. The CCM-200 was calibrated with a blank chamber prior to each series of measurements, as per the manufacturer instructions. Chlorophyll Content Index was expressed as SPMR (SPAD chlorophyll meter reading) and readings measured by the Opti-Sciences CCM-200 is said to correlate positively with 90% acetone extracted Chl *a* content (Biber, 2007).

3.2.1.6 Statistical analysis

The mean values worked out from the measurements recorded on five randomly selected plants for different characters used for statistical analysis. The data collected was analyzed using analysis of variance (ANOVA) technique. IQ Macros in Excel 2007 software package was used for this purpose.

3.2.1.6.1 Mahalanobis D^2 statistics

D^2 analysis was done using Windostat software developed by Indostat services Ltd. Hyderabad was used. The 62 accessions were grouped into clusters as per Tocher method on the basis of physiological data generated following the formula cited by Singh and Chaudhary (1979).

3.2.2 Biochemical screening for presence of drought responsive elements.

Samples for biochemical screening were obtained from the water withholding experiment on 21 day old seedling conducted in a greenhouse under controlled environmental conditions. Before permanent wilting stage i.e. 10^{th} day of imposing water stress was selected for sample collection for biochemical screening.

3.2.2.1 Sample collection, preparation and enzyme extraction

After the experiment, samples were individually collected, labeled in plastic bags and immediately frozen using dry ice and later transferred to (minus) -80°C for storage until further use. For protein and antioxidant enzyme assays, samples were ground to a fine powder with liquid nitrogen and were extracted with Phosphate buffer. The homogenate was centrifuged at 10000 g for 20 min, at 4°C, and the supernatant was used for enzyme activity and protein determinations. Preparations for enzyme extraction and enzyme assay were carried out at 4°C.

3.2.2.2 Protein estimation

Proteins were estimated by using methodology given by Lowry et al., (1951) wherein, 250 mg of fresh leaves were homogenized in 2 ml of 0.1 M phosphate buffer (pH 7.0). The extract was centrifuged at 10000 g for 15 min at 4°C and the supernatant was transferred to the tube containing a mixture of 20 ml acetone and 14 ml of β-merceptoethanol for precipitation of proteins. The samples were stored at 0°C for 5 hr and then centrifuged at 10000 g for 20 min., the supernatant was discarded and the pellet was dissolved in 2.5 ml of NaOH solution. 0.2 ml of this sample was used to prepare the reaction mixture. The intensity of blue color developed was recorded at 660 nm on UV visible spectrophotometer.

3.2.2.3 Enzyme assays

3.2.2.3.1 Activity of superoxide dismutase

SOD activity was estimated by recording the decrease in absorbance of the enzyme as described by Dhindsa et al., (1980). Fresh 500 mg leaves were homogenized in 0.1 M of phosphate buffer (pH 7.5). The extract was centrifuged at 10000 g for 20 min at 4^{o}C and supernatant was used as enzyme source. 3 ml of reaction mixture containing 0.1 ml of 1.5 M Na_2CO_3, 0.2 ml of 200 mM methionine, 0.1 M of 3 mM EDTA, 0.1 ml of 2.25 mM NBT, 1.5 ml of 100 mM potassium phosphate buffer (pH 7.5), 1ml of distilled water and 0.05 ml of enzyme samples. The tube without enzyme was taken as control. Reaction was started by adding 0.1 ml 60 µM riboflavin and placing the tubes below a light source of two 15 W fluorescent lamps for 15 min. The reaction was stopped by switching off the light and covering the tubes with black cloth. Absorbance was recorded at 560 nm. Activity of enzyme was expressed as EU (enzyme unit). One unit of enzyme activity is deduced by amount of enzyme required to result in a 50% inhibition in the rate of nitro blue tetrazolium (NBT) reduction at 560 nm.

3.2.2.3.2 Activity of catalase

The catalase activity was determined according to Luck (1974). 250 mg of leaves were homogenized in 3 ml of 0.1 M phosphate buffer (pH 7.0). The extract was centrifuged at 10000 g for 20 min at 4^{o}C and supernatant was taken as enzyme source. The assay mixture in total volume of 3 ml contained 0.5 ml of 0.2 M phosphate buffer (pH 7), 0.3 ml of (v/v) H_2O_2 and 0.1 ml of

enzyme. The final volume was made 3ml by adding distilled water. The reaction was started by adding enzyme and change in optical density was measured at 240 nm at zero min and 3 min on UV Vis spectrophotometer. Activity of enzyme was expressed as EU (enzyme unit) where one unit of enzyme activity defined as 1 µmol H_2O_2 oxidized min^{-1}.

3.2.2.3.3 Activity of peroxidase

The method proposed by Reddy et al. (1995) was adopted for assaying the activity of peroxidase. A 20% homogenate was prepared in 0.1M phosphate buffer (pH 6.5) from the plant samples, clarified by centrifugation and the supernatant was used for the assay. To 3.0 ml of pyrogallol solution, 0.1ml of the enzyme extract was added and the spectrophotometer was adjusted to read zero at 430 nm. To the test cuvette, 0.5 ml of H_2O_2 was added and mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer. Activity of enzyme was expressed as EU (enzyme unit) where one unit of peroxidase is defined as the change in absorbance/minute at 430nm deduced by the formation 1.0 milligram of purpurogall from pyrogallol.

3.2.2.3.4 Activity of glutathione reductase

The activity of glutathione reductase was determined according to the procedure described by Mavis and Stellwagen (1968)**.** 250 mg of leaves were homogenized in 3 ml of 0.1 M phosphate buffer (pH 7.0). The extract was centrifuged at 10000 g for 20 min at 4˚C and supernatant was taken as enzyme source. The assay mixture in total volume of 3 ml contained 75 mM potassium phosphate, 2.6 Mm EDTA, 1 mM glutathione, 0.09 mM ß-nicotinamide adenine dinucleotide phosphate, reduced form, 0.13 % (w/v) bovine serum albumin and 0.1 ml of enzyme. The change in optical density was measured at 340 nm for 5 minutes on UV Vis spectrophotometer. Activity of enzyme was expressed as EU (enzyme unit). One unit reduces 1.0 µmole of oxidized glutathione per minute at pH 7.6 at 25°C calculated using millimolar extinction coefficient of ß-NADPH at 340 nm.

3.2.2.4 Non enzymatic assays

3.2.2.4.1 Free proline content

Free proline content was estimated by following the method of Bates et al. (1973). Fresh 500 mg of leave samples were homogenized in 5 ml of 3% (w/v) sulphosalicylic acid using mortar and pestle. 2 ml of extract was taken in test tube and to it 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent was added. The reaction mixture was boiled in water bath at 100˚C for 30 min. after cooling the reaction mixture; 4 ml of toluene was added. After thorough mixing, the chromophore containing toluene was separated and absorbance of red color developed was read at 520 nm against toluene blank. Proline accumulation was expressed as µmoles (mg protein) $^{-1}$.

3.2.2.4.2 Total carbohydrates

The phenol sulphuric acid method was used to estimate total carbohydrates (Dubois et al., 1956) where glucose is dehydrated to hydroxymethyl furfural and forms green colored product. The intensity of green color was measured at 490 nm. The amount of total carbohydrate present in the sample solution was calculated by using the standard graph and total carbohydrates were expressed as mg (gFW)$^{-1}$.

3.2.2.4.3 Starch

Estimation of Starch was done by using anthrone reagent as described by Hodge and Hofreiter et al. (1962). The intensity of green colour was measured at 630 nm. The starch content was estimated by multiplying the glucose content in the sample using the standard graph by a factor 0.9 (accounted for the mass of glucose theoretically hydrolyzed from a unit mass of starch). Starch content was expressed as mg (gFW)$^{-1}$.

3.2.2.4.4 Fructose

Fructose was estimated by using the method described by Ashwell (1957) where the hydroxymethy furfural formed from fructose reacts with resorcinol to give red color. The intensity of red the color was measured at 520 nm and the amount of fructose present in the sample was calculated using the standard graph. Fructose content was expressed as mg (gFW)$^{-1}$.

3.2.3 To identify differentially expressed genes in foxtail millet during water stress by cDNA-SRAP and gene specific amplification

Differential gene expression studies were carried out by using two approaches. In the first approach, differential gene expression pattern was studies by using random primers like cDNA SRAP approach.

In the second approach, the drought stress specific genes/TFs (identified earlier in various crops) were analyzed.

3.2.3.1 Stress treatments

21-day-old seedlings were subjected to water with-holding experiments. Before wilting stage (10 days after imposing drought) was finalised for sample collection for RNA extraction.

3.2.3.2 Sample collection:

Seedlings were harvested and frozen immediately in liquid nitrogen and stored at -80˚C for RNA isolation.

3.2.3.3 Total RNA extraction

The RNA isolation was done using a manufacturer's protocol with minor modifications. Total RNA was isolated using TRIzol (Invitrogen, USA) and the concentration was determined using nanophotometer (IMPLEN, Germany). Following extraction, the isolated total RNA was electrophoresed in 1.2 % agarose gel to check for RNA quality and integrity.

3.2.3.4 Denaturing formaldehyde gel for RNA electrophoresis

Total RNA was run on 1.2% denaturing formaldehyde gel. For preparation of gel, 1.2 g agarose was added to 72 ml DEPC treated water and boiled till the agarose dissolved. Once the temperature came down to 55˚C, 18 ml formaldehyde and 10 ml 10X MOPS buffer was added. The contents were mixed by swirling. The molten gel was poured in casting tray with combs already fitted in. Meanwhile, RNA samples were prepared by mixing about 20 pg of total RNA, 2 µl of 10X MOPS buffer, 4 µl of formaldehyde, 1 µl of 10 mg/ml EtBr and 10 ul of formamide. Samples were heat denatured at 65˚C for 10 min and immediately chilled on ice for 2 min. For loading the samples, 2 µl RNA loading dye was added to each RNA sample and gel was run at 60-70 V for 5-6 h in 1X MOPS buffer.

3.2.3.5 First strand cDNA synthesis

The first strand synthesis for all the samples were carried out in duplicates from 200ng RNA using AccuScript High Fidelity 1st Strand cDNA Synthesis Kit, Canada. The reaction contained template RNA, 2.0 µl of AccuScript RT Buffer (10X), oligo (dT) primer, 0.8 µl of dNTP mix (25 mM each dNTP) and RNase-free water to total volume 16.5 µl. The reactions were terminated by placing the tubes in ice. The reaction was Incubated at 65°C for 5 minutes, cooled at room temperature to allow the primers to anneal to the RNA (approximately 5 minutes) and the following components to the reaction, in order, for a final reaction volume of 20 µl: 2 µl of 100 mM DTT, 1 µl of AccuScript RT, 0.5 µl of RNase Block ribonuclease inhibitor (40 U/ µl). The reaction was incubated at 25°C for 10 minutes to extend the primers prior to the 42°C synthesis step. The tubes were placed in a temperature-controlled thermal block at 42°C and incubated for 60 minutes. The reaction was terminated by incubating the reaction at 70ºC for 15 minutes. The completed first-strand cDNA synthesis reaction was kept on ice for use in downstream applications. The reaction was placed at –20°C for long-term storage.

3.2.3.6 Amplification of cDNA using primers

First strand cDNA generated were equalized and used as a template for further amplification studies. Amplification was carried out by using a Thermal cycler.

The reaction was performed by adding following components in order to sterile thin-walled PCR tubes for each PCR amplification reaction: 12.6µl of RNase-free water, 2µl of 10X PCR Buffer, 1.2µl of 50mM $MgSO_4$, 2.0µl of dNTP mix (2mM each dNTP), 0.5µl of upstream primer (0.1 µg/µl), 0.5µl of downstream primer (0.1 µg/µl), 1µl of experimental first-strand cDNA reaction, 0.2µl of *Taq polymerase* (5U/ul). The details are presented in Table 3.5.

Gene specific PCR probability was carried out by using *Taq polymerase* (invitrogen), However, promising samples (differentially expressed amplicons) were again amplified by using high fidelity polymerase, Pfu Ultra DNA polymerase, for sequence characterization.

Table 3.5 PCR reaction mixture for reaction of 20 µl

PCR Component	Concentration	Final concentration	Manufacturer	Volume for 20µl Reaction
Assay buffer	10X	1X	Puregene	2.0 µl
$MgCl_2$	50mM	3mM	Puregene	1.2 µl
dNTP'S	2mM each	0.2mM each	Puregene	2.0 µl
Taq DNA polymerase	5U/1ul	1U	Puregene	0.2 µl
Primer forward	10pmol	5pmol	Eurofins	0.5 µl
Primer Reverse	10pmol	5pmol	Eurofins	0.5 µl
Template cDNA	100ng/ul	100ug/ul	-	1.0 µl
Nuclease Free water	-	-	Puregene	12.6µl
Total	-	-	-	20 µl

3.2.3.7 cDNA-SRAP (Sequence Related Amplified Polymorphism marker profiling)

A set of SRAP markers developed by Li and Quiros (2001) were utilized which consisted of five forward and six reverse primers (Table 3.6). The forward primer set was designed with GC-rich core sequence which targets exonic region in the genome; however the reverse primer set was design to contain AT-rich core sequence which targets promoter and intronic region of gene/genome. Each primer contains a random filter sequence at the 5'end and three variable selective nucleotides at the 3' end (Figure 3.1).

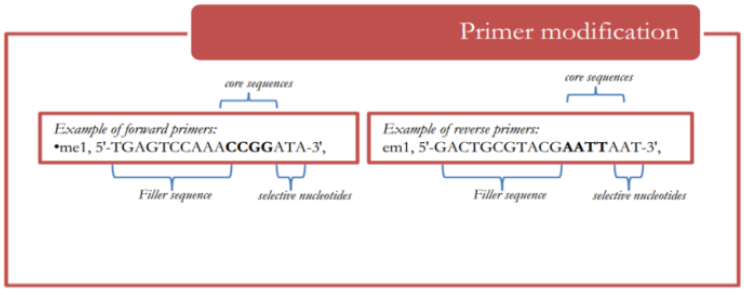


Figure 3.1 Primer modification for SRAP

3.2.3.8 PCR programme for cDNA-SRAP (Sequence Related Amplified Polymorphism marker profiling)

The PCR reaction conditions were, initial denaturation at 94ºC for 5 minutes, 5 cycles of denaturation at 94ºC for 1 minute, annealing at 35ºC for 1 minute and extension at 72ºC for 1 minute, followed by 35 cycles of

denaturation at 94ºC for 1 minute, annealing at 50ºC for 1 minute and extension at 72ºC for 1 minute final extension at 72ºC for 5 minutes and reaction was hold at 4ºC (Figure 3.2) All the PCR products were stored at 4ºC until resolved on 2% agarose prepared in 1x TBE buffer containing 0.5 µg/ml ethidium bromide (EtBr).

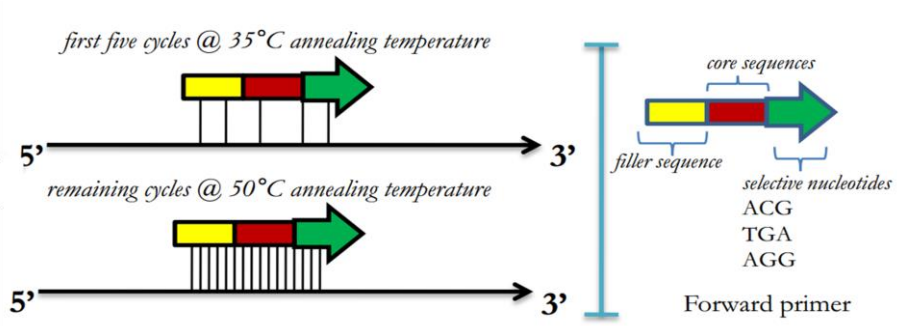


Figure 3.2 PCR program for cDNA-SRAP

Table 3.6 SRAP primer sequences used during the present investigation

SN	SRAP primer	Sequence	Tm
		Forward primers	
1	me1,	5'-TGAGTCCAAACCGGATA-3',	50°C
2	me2,	5'-TGAGTCCAAACCGGAGC-3',	50°C
3	me3,	5'-TGAGTCCAAACCGGAAT-3',	50°C
4	me4,	5'-TGAGTCCAAACCGGACC-3',	50°C
5	me5,	5'-TGAGTCCAAACCGGAAG-3'	50°C
		Reverse primers	
1	em1,	5'-GACTGCGTACGAATTAAT-3',	50°C
2	em2,	5'-GACTGCGTACGAATTTGC-3',	50°C
3	em3	5'-GACTGCGTACGAATTGAC-3',	50°C
4	em4,	5'-GACTGCGTACGAATTTGA-3',	50°C
5	em5	5'-GACTGCGTACGAATTAAC-3',	50°C
6	em6,	5'-GACTGCGTACGAATTGCA-3'.	50°C

3.2.3.9 cDNA-Drought stress specific genes profiling

Gene specific markers for drought stress studied earlier in various crops were utilized which consisted of aquaporin, DREB1, DREB2 and C_2H_2.Reference genes for quantifying gene expression and to ensure proper normalization were utilised in the present study which are mentioned in Table 3.7.

Table 3.7 Drought stress specific gene/TFs primers used during the present investigation

SN	Gene	Sequence		Reference
1	Aquaporin	Forward	CCCGTTCAAGAGCAGGTCTTA	Lata et al., 2010
		Reverse	CCTGTTTGGACTGGCATCTCA	
2	DREB2	Forward	GCCTTGTAGTCATTTGGTGGTTT	Lata et al., 2010
		Reverse	CTCACAACTCCTTTTCTCAAGCT	
3	DREB1	Forward	GGAGCAAGCAGAAACACACA	Nawaz et al., 2014
		Reverse	GCATCGGAAGCCAGAAAAGA	
4	C_2H_2	Forward	ACGACACACCAGTGTCCAAA	In this study
		Reverse	GCTGGTTTGTCTGGTGGGAT	
5	EF-1α	Forward	TGACTGTGCTGTCCTCATCA	Kumar et al., 2013
		Reverse	GTTGCAGCAGCAAATCATCT	
6	TUB α	Forward	TACCAGCCACCATCTGTTGT	Kumar et al., 2013
		Reverse	GGTCGAACTTGTGGTCAATG	

3.2.3.10 PCR programme for cDNA-Drought stress specific genes profiling

Amplifications were performed by a cycles of: 5.00 min at 94°C followed by 39 cycles each of 2 min at 94°C, 2 min at 36-58°C, and 3 min at 72°C, and final extension of 15 min at 72°C (Figure 3.3)

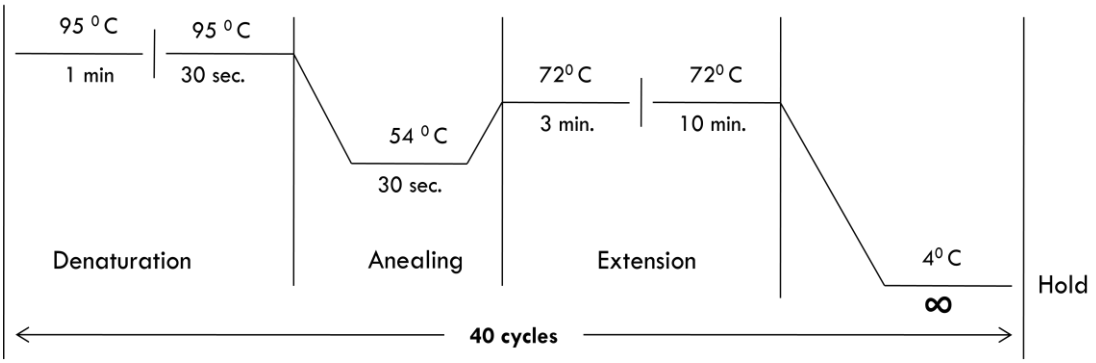


Figure 3.3 Standardised PCR reaction conditions in the present study

3.2.3.11 Gel electrophoretic analysis and gel elution

Separation of amplified fragments was carried out using Bio-rad gel electrophoresis assembly. PCR amplification products were analyzed by agarose gel electrophoresis on 1.5 % agarose gel stained with ethidium bromide solution (0.5 µg/ml). The gel was run in 1X TBE buffer at 70-80 Volts for 45 minutes to 1.5 h. Standard ladders of 100bp and 1kb from

(Fermentas^{TM}) sizes were used and amounts were used according to concentrations given on instruction manual. Gel Doc system (Alpha innotech) was used for further analysis.The computer program AlphaEaseFC (From Alpha Innotech) was used to visualize and analyse the results.

3.2.3.12 Scoring amplicons

Differential analysis on the basis of number of amplicons (present/absent) as well as differences in amplicon intensities to understand differential expression pattern in stressed and unstressed of tolerant and susceptible foxtail millet accessions was done.

3.2.3.13 Generation of IDV

For TDFs those were expressed differentially with change in amplicons intensities were analyzed by densitometric analysis. This analysis based on pixel intensities of bands produced intensity derived values (IDVs) in AlphaEaseFC (Genetic technologies Inc.) image processing software. Generated values were used to compare intensity of gene expression in further studies.

3.2.3.14 Gel elution

Gel elution of amplicons of interest was performed using Qiagen gel elution kit as per the protocol prescribed in technical bulletin.

Procedure

1. The gel slice was excised containing the DNA band of interest with a clean, sharp scalpel. The size of band was minimized by removing excess of agarose.
2. The gel slice was weighed. 1–2 volumes of diffusion buffer to 1 volume of gel were added. (i.e., 100–200 µl for each 100 mg of gel) the gel slice was further incubated at 50°C for 30 min.
3. The sample was then centrifuged for 1 min.
4. The supernatant was removed carefully using a pipette. The supernatant was passed through a disposable plastic column or a syringe containing either a Whatman’s filter or packed, siliconized glass wool to remove any residual gel.
5. The approximate volume of the recovered supernatant was calculated.
6. For DNA fragments >100 bp, 6 volumes of Buffer QX1 to 1 volume of sample was added. The color of the mixture should be yellow.

7. Samples were resuspended in QIAEX II by vortexing for 30 s.
8. 10 µl of QIAEX II was added and mix. The samples were incubated at room temperature (15–25°C) for 10 min., vortex every 2 min to keep QIAEX II in suspension.
9. The samples were centrifuged for 30s and the supernatant was removed.
10. The pellet was washed twice with 500µl of Buffer PE.
11. The pellet was air dried for 10–15 min or until the pellet becomes white.
12. To eluted DNA, 20µl of 10 mM Tris HCl was added, pH 8.5, or H_2O and the pellet was resuspended by vortexing. The pellet was incubated for 5 min at room temperature.
13. The samples were centrifuged for 30s and the supernatant was transferred carefully into a clean tube. The supernatant now contains purified DNA.
14. Steps 13 and 14 was repeated and elutes was combined.

3.2.3.15 Sequence characterization

Sequencing was done through GeneOmBiotech, Pune, India. The sequences are accessed using Bioedit software.

3.3.4 Genome wide investigation of aquaporins in foxtail millet and expression profiling

3.2.4.1 Isolation of aquaporin genes in foxtail millet

To obtain all the aquaporin genes in foxtail millet, BLAST searches were conducted in the Phytozome (http://www.phytozome.net/), and NCBI (http://www.ncbi.nlm.nih.gov/) databases with the rice and Arabidopsis aquaporin proteins as queries. Redundant sequences were removed. Then, the Pfam (http://www.sanger.ac.uk/Software/Pfam/) and SMART (http://smart.embl-heidelberg.de/smart/batch.pl) databases were used to identify the NPA and MIP super family domains of all the candidate proteins. Genes that did not contain the NPA and MIP super family domains were excluded from further analysis.

3.2.4.2 Primer designing for aquaporins

Primers for aquaporins were designed based on the location of MIP superfamily and NPA motif. Primers were designed such that they will amplify the MIP super family along with conserved NPA motif (Figure 3.4) using the NCBI/ pick primer online bioinformatics software. The designed primers are presented in Table 3.8.

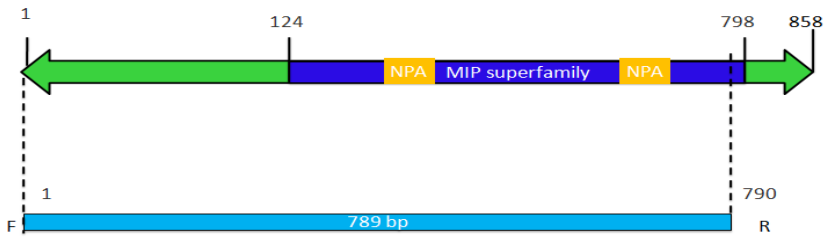


Figure 3.4 Primer designing for PIP 2;7 aquaporin in foxtail millet

CDS sequene of 858 bp, 1 indicates transcription start site(ATG); relative position of MIP family after start site are indicated for targeted primer designing

3.2.4.4 Full sequence characterization of PIP 2;7 auaporin gene

Full gene primer of PIP 2;7 aquaporin was designed to characterize the sequence of its CDS (857bp) using the NCBI/ pick primer online bioinformatics software.

Table 3.8 Primers used for PIP sub-class of aquaporin studies

SN	Gene	Transcript name	Primer pairs	Expected product (bp)
1	PIP 2-5 like	Si036923m	GCCACGTTGCTCTTCGTGTA	720
			GATTCCGGTGTGGTTGTTGTT	
2	PIP1-1	Si010758m	GAAACTGTCGTTGACCAGGG	408
			CCGACCCAGAAGATCCAGTG	
3	PIP1-2	Si017731m	AGTCCAACTCTAAGTGCGCC	557
			CCGACCCAGAAGATCCATCA	
4	PIP2-1	Si030703m	GCTCACCGTCATCGGTTACA	681
			CTGAAGGAGCCGAGGGC	
5	PIP2-1	Si039883m	CACGCTGCTGTTCCTCTACT	606
			ATCCACTGATCCTGCCATGC	
6	PIP2-4	Si017990m	ACGGTCATCGGGTACAAGC	227
			GCGATGATGTAGAGGACCG	
7	PIP1-5	Si017991m	GGGCAAGGAGGAGGATGTTC	416
			GCATCACCATGTAGAAGAGCG	

SN	Gene	Transcript name	Primer pairs	Expected product (bp)
8	PIP2-5	Si010750m	TCTACATCACTGTCGCCACG	484
			GCGAACCCGATGGGAAGAG	
9	PIP1-6	Si007002m	ACAGGTTTCAGGACGACGAG	758
			CGGCCTGTCGTAGATGATGG	
10	PIP2-6	Si030713m	GACTATGTCGACCCACCGC	495
			AAAGGTGCCAATGATCTCAGCG	
11	PIP2-6	Si030712m	AGGTGGATGTGTCCACTCTC	583
			CGGGCGTTACGCTTGGG	
12	PIP2-7	Si030718m	GAGCGAGCTGATGAAGTGGT	509
			GCACCGGGATGAATGAGTCT	

.

Table 3.9 Primer designing for full length PIP 2;7 aquaporin in foxtail millet

SN	Gene	Transcript name	Primer sequence	Expected product length
1	PIP2-7	Si030718m	ACGAAACGCAGGCTGGAATA	790 bp
			CTAGTGAATCCATGGAGGCCG	

3.2.4.5 Bioinformatics studies

3.2.4.5.1 Reverse complement

The sequences synthesize using reverse primers were converted to reverse complement using Bioedit software.

3.2.4.5.2 Homology search

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. (Pearson, 1995) http://blast.ncbi.nlm.nih.gov/Blast.cgi was used for homology searches.

3.2.4.5.3 Pair wise alignment

Clustalw sequence alignment tool available at EBI (European Bioinformatics Institute) was used to align the recovered sequences with the sequences used for primer designing.

3.2.4.5.4 Gene structure analysis of foxtail millet aquaporin genes

The information for aquaporin genes, including chromosomal location, open reading frame (ORF) length, and full length cDNA sequence, were obtained from the foxtail millet sequencing database

(http://www.phytozome.net/). Structures of aquaporin genes were determined by the GSDS tool (http://gsds.cbi.pku.edu.cn/)

3.2.4.5.5 Motif display and phylogenetic analysis of aquaporin proteins

The multiple expectation maximization for the motif elicitation (MEME) utility program was used to display motifs in aquaporin proteins. A phylogenetic tree was constructed in MUSCLE based on the full sequence of the proteins with default parameters and the tree was constructed by the neighbor-joining (NJ) method.

3.2.4.5.6 Prediction Phosphorylation sites

Phosphorylation sites were predicted using NETPhos online web tool.

3.2.4.5.7 Structure prediction

Structure of PIP 2;7 aquaporin was predicted using Phyre2 online tool for protein structure prediction. The structures were sealed in PDB file format for further analysis.

3.2.4.5.8 Structure validation

The Ramachandran plot of the initial and final models were depicted and compared after refinement. In initial model the percent residues in the favored region, allowed region, residues in outlier region were recorded. RAMPAGE online server was used for preparation of Ramchandran plot. Model quality was predicted by Z score analysis. PROSA online tool was used to find the Z-score of the predicted structure (https://prosa.services.came.sbg.ac.at/prosa.php).

3.2.5 Analysis of the promoter regions of aquaporin genes

3.2.5.1 Isolation of genomic DNA from plant tissues

Genomic DNA was isolated from leaves of young foxtail millet by CTAB method as described by Doyle and Doyle (1987). Approximately 10 ml of preheated extraction buffer (2% CTAB (w/v), 1.4 M NaCl, 20 mM EDTA, 0.1 M Tris-Cl, 0.2% β-merceptoethanol (v/v)] was added to 1 g of finely powdered plant tissue and the suspension incubated at 65 °C for 60 min. The lysate was allowed to cool at room temperature and centrifuged at 9000 X g for 20 min. An equal volume of a 24:1 (v/v) solution of chloroform: isoamyl alcohol was added and mixed properly. The aqueous phase was separated by centrifugation at 9000 X g for 10 min. The extraction step was repeated till a clear interphase was obtained following which DNA was precipitated by

addition of 0.6 volume of isopropanol to the aqueous phase and incubated overnight at 4˚C. It was centrifuged at 9000xg 4˚C for 15 min. The pellet was washed with 70% ethanol (v/v) by centrifuging the tube at 9000 X g for 10 min. The pellet was air dried and suspended in 100µl sterile water. For RNase treatment, 5µl (100 ug/ml) RNaseA was added and incubated at 37 ˚C for 45 min. RNase treated DNA was extracted twice with 0.5 volume of 24:1 (v/v) solution of chloroform: isoamyl alcohol followed by precipitation. Equal volume of ice cold absolute ethanol was added and was centrifuged at 4 ˚C at 9000 X g for 20 min. Pellet obtained was washed with cold 70% ethanol (v/v), air dried and dissolved in appropriate amount of double distilled nuclease-free water.

3.2.5.2 Quantitation of nucleic acid

The quality and quantity of nucleic acid was determined by measuring the absorbance at 230nm, 260nm, 280nm and 320nm. The amount was calculated using 1.0 A_{260} = 50 µg/ml for DNA. The purity of nucleic acid was determined by calculating the ratio A260/A280 for each sample.

3.2.5.1 Primer designing

To identify *cis*-elements in the promoter sequences of aquaporin genes showing high expression, 1 kb of foxtail millet genomic DNA sequence upstream of the initiation codon (ATG) were retrieved from Phytozome and the data was used for primer designing (Figure 3.5). The designed primers are presented in Table 3.10.

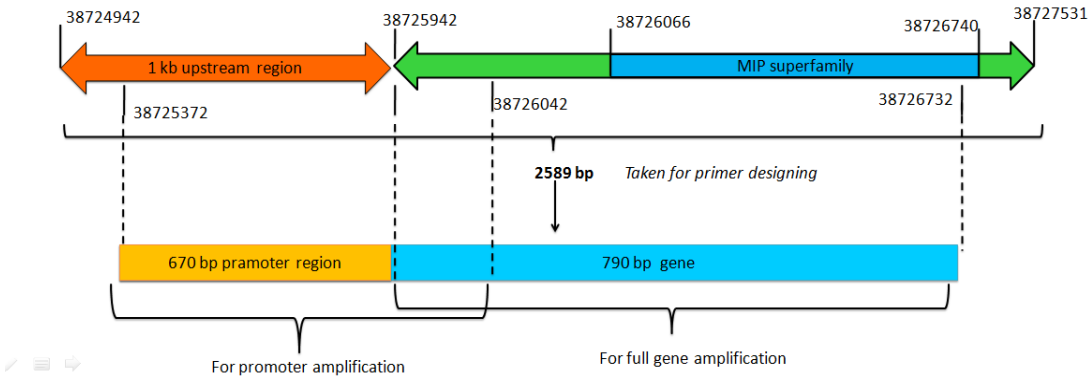


Figure 3.5 Primer designing for promoter region of PIP 2;7 like aquaporins
Relative bp positions on chromosome no. 2 for gene PIP2;7 are indicated here.

Table 3.10 Primer for promoter region of PIP2;7 aquaporin

SN	Primers	Forward Primers	Expected product
1	XPip2.7	CACCTTACCACAAGCGCAAC	670 bp
		GTCGTCGTAGTGTGGGAGAAG	

3.2.5.2 PCR programme, Gel electrophoretic analysis and sequence characterization

PCR, electrophoresis and sequence characterization were carried out as per already discussed procedures under section 3.2.3.10, 3.2.3.11, 3.2.3.14 and 3.2.3.15.

3.2.5.3 Promoter sequence analysis and gene ontology annotation.

The characterised promoter region was analyzed using Plant Promoter Analysis Navigator (http://PlantPAN2.itps.ncku.edu.tw) for identification of *cis*-regulatory elements in the promoters.

CHAPTER IV
RESULTS AND DISCUSSION

The present investigation entitled “Deciphering the differentially expressed genes in Foxtail millet (*Setaria italica* L.) in response to water stress” was carried out at the Biotechnology Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during the year 2013-2016.

Studies of plant responses to abiotic stresses have been of great interest to scientists because of growing water shortages and decreasing soil fertility. Foxtail millet is thought to be an excellent experimental model crop for studying abiotic stress tolerance system. Hence, foxtail millet (*Setaria italica* L.*)* was selected in the present study to investigate the processes at physiological, biochemical level and to fetch out the molecular mechanism underneath its tolerance.

The study was divided into three Phases, Phase I dealt with the physiological screening and evaluation of foxtail millet accession for water stress tolerance under controlled conditions; Phase II dealt with the presence of drought responsive metabolites and Phase III dealt with the differential expression studies for drought responsive genes.

The findings of the investigation are presented in this chapter along with pertinent discussion under the appropriate sub-headings.

4.1 Physiological screening to identify tolerant and susceptible accessions

Several physiological characteristics like RWC and LWP (ψ_L) have been reported as being reliable indicators for the selection of germplasm possessing drought tolerance (Carter and Patterson, 1985; Dhanda and Sethi, 2002). RWC provides a measurement of the ‘water deficit’ of the leaf, and indicates the degree of stress expressed under drought stress. RWC integrates leaf water potential with the effect of osmotic adjustment as a measurement of plant water status. These characteristics also include seed germination and seedling growth in nutrient solutions with low osmotic potential (Richards, 1978; Blum, 1980; Ashraf et al., 1992). One of the most

popular approaches is to use high molecular weight osmotic substances, like polyethylene glycol (PEG), for seed germination (Garg, 2010; Ahmadizadeh et al., 2011; Turkan et al., 2005; Gholamin et al., 2010; Alaei et al., 2010; Al-Karaki et al., 2007; Homayoun et al., 2011).

Illustration for physiological screening to identify tolerant and susceptible accessions among 62 Foxtail millet (*Setaria italica* L.) accessions under study is presented in Plate 4.1.

4.1.1 Effect of water stress on various physiological parameters studied under laboratory screening using PEG-6000

In the present study, 62 foxtail millet (*Setaria italica* L.) accessions were studied for their tolerance to water stress. The results showed that all accessions of foxtail millet have different responses to water stress, indicating that it is feasible to choose the best genotype for resisting drought in controlled conditions.

4.1.1.1 Effect of PEG induced stress (-0.3 MPa) on seed germination

Seedling emergence is one of the stages of growth that is sensitive to water deficit. Therefore, seeds germination is prerequisite for the successful establishment of crop plants. Under semiarid regions, low moisture is limiting factor during germination. Hence, it is critical to understand the seed germination ability of drought tolerant plant species under drought stress and their recovery response when removed from drought condition. Germination is a useful criterion in screening for water stress tolerance (Richards, 1978). As discussed earlier (section 4.1), germination in solution with high osmotic potential is one of the most important laboratory methods suggested for screening drought tolerance of crop plants. Therefore, in the present investigation, PEG 6000 to create a osmotic stress of -0.3 MPa was used for screening drought tolerant accessions which showed significant variation for seed germination. The seed germination decreased under osmotic stress. Out of 62 accessions tested, five genotypes (IC97087, IC97172, IC97174, IC97179, and IC120244) showed maximum of 80% seed germination (Table 4.1). The average germination percentage was 48.38% and 23.65% in unstressed and -0.3 MPa osmotic stress treatments, respectively. Some accessions showed no change in germination percent i.e. zero percent decrease over unstressed condition which shows their tolerant behaviour

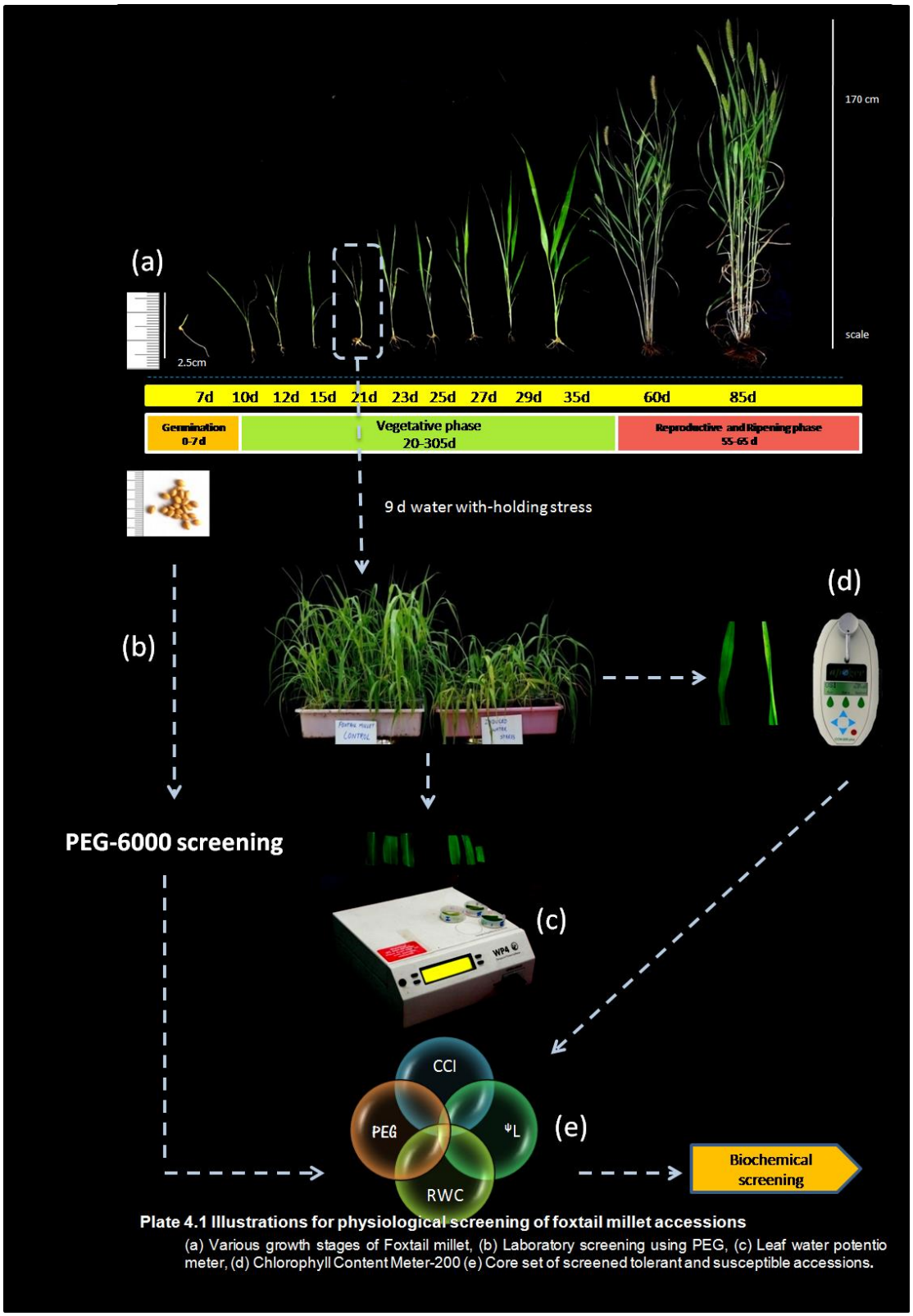


Plate 4.1 Illustrations for physiological screening of foxtail millet accessions

(a) Various growth stages of Foxtail millet, (b) Laboratory screening using PEG, (c) Leaf water potentio meter, (d) Chlorophyll Content Meter-200 (e) Core set of screened tolerant and susceptible accessions.

towards osmotic stress (IC41883, IC41898, IC97086, IC97111, IC97189 IC120159, IC120160, IC120166, IC120192, IC120207, IC120239, IC120250, IC120404, and IC200125). Plate 4.2 depicts the effect of PEG-6000 on seed germination in foxtail millet accession under -0.3 MPa osmotic stress. Highest germination percentage of 73.33% was seen in two genotypes IC97087 and IC97172 in stressed conditions. Prasad, which was earlier characterised as drought tolerant cultivar by Lata et al., (2011[a]) showed only 33.33 % germination with a 50% decrease over unstressed seedlings in -0.3 MPa stress. Five genotypes viz. IC97109, IC120234, IC120346, IC120355 and Lepakshi did not show any germination at -0.3 MPa stress. This inability to germinate indicates their susceptibility to osmotic stresses. In general, germination percentage decreased with PEG-6000 induced osmotic stress. Decline in germination percentage under osmotic stress was reported earlier by Nagarajan and Rane (2000) in spring wheat, Sadasivam et al., (2000) in rice, Manjula et al., (2003) in castor and Lata et al., (2011[a]) in foxtail millet. Such decline in the seed germination may be due to the inability of seed to imbibe water at low osmotic potential (Singh and Africa, 1985). PEG decreases the matrix potential of nutrient solution and makes the water unavailable required for seed emergence, hence can be used drought simulator. In foxtail millet, Zhang and colleagues reported the use of 20% PEG-6000 for screening genotypes for drought tolerance (Zhang et al., 2005).

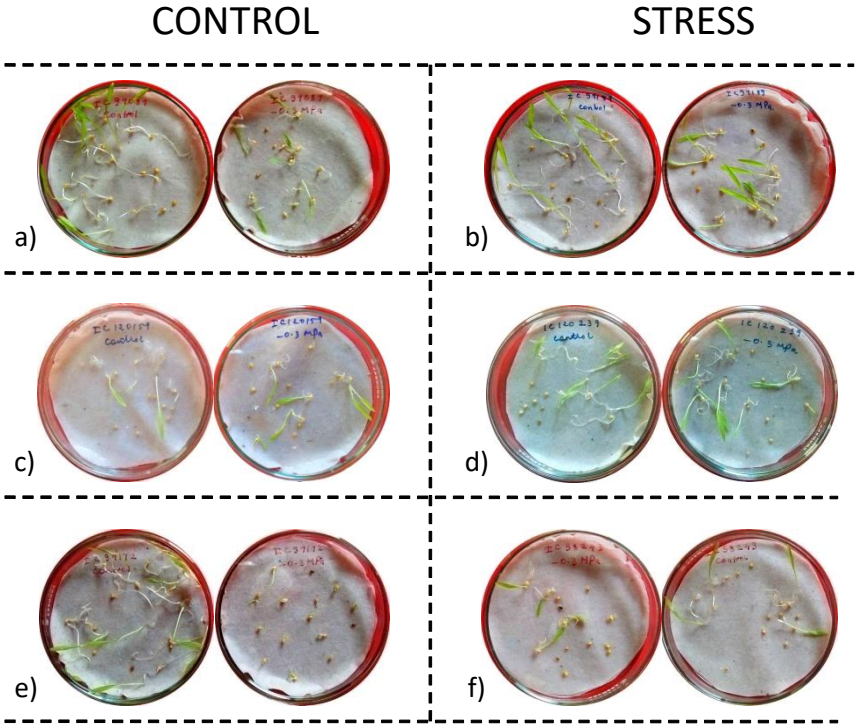
Table 4.1 Effect of PEG induced stress (-0.3MPa) on percent seed germination.

SN	Accessions	Unstressed 0 MPa	Stressed -0.3 MPa	% decrease over unstressed	Seed germination index
1	IC 28439	40.00	20.00	50.00	50.00
2	IC41883	60.00	60.00	0.00	100.00
3	IC41898	60.00	60.00	0.00	100.00
4	IC58243	46.66	26.66	42.86	57.13
5	IC97086	33.33	33.33	0.00	100.00
6	IC97087	86.66	73.33	15.38	84.61
7	IC97109	60.00	0.00	100.00	0.00
8	IC97111	33.33	33.33	0.00	100.00
9	IC97123	46.66	6.67	85.71	14.28
10	IC97172	80.00	73.33	8.33	91.66
11	IC97174	80.00	6.67	91.66	8.33
12	IC97175	40.00	26.66	33.35	66.65
13	IC97177	53.33	40.00	24.99	75.00
14	IC97179	93.33	26.66	71.43	28.56
15	IC97182	66.66	53.33	19.99	80.00
16	IC97189	66.66	66.66	0.00	100.00

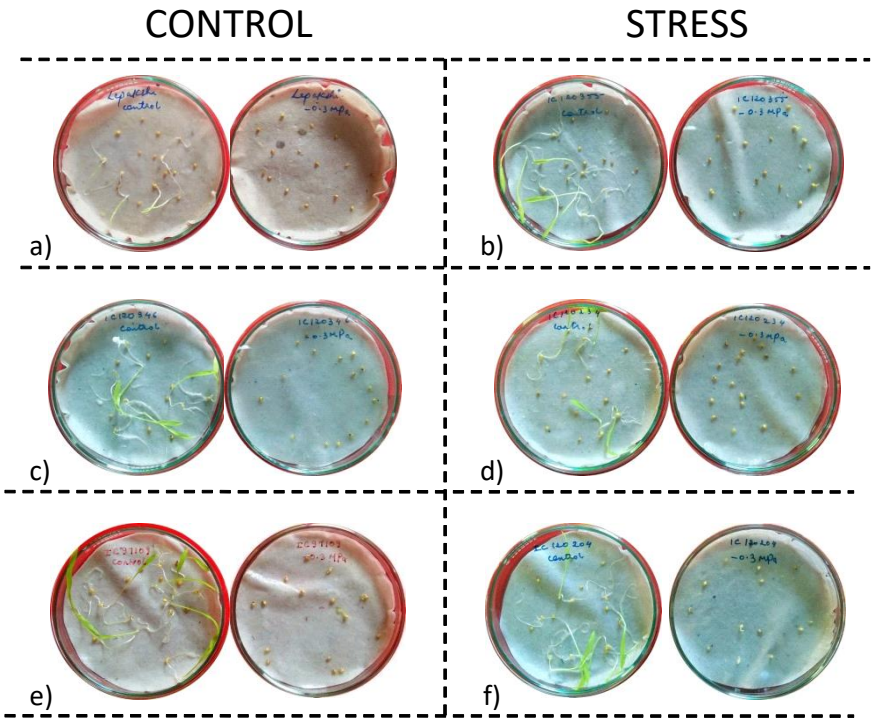
SN	Accessions	0 MPa	-0.3 MPa	% decrease over unstressed	Seed germination index
17	IC97295	33.33	20.00	39.99	60.00
18	IC120148	66.66	6.67	90.00	10.00
19	IC120150	33.33	20.00	39.99	60.00
20	IC120158	53.33	26.66	50.00	49.99
21	IC120159	26.66	26.66	0.00	100.00
22	IC120160	26.66	26.66	0.00	100.00
23	IC120163	53.33	6.67	87.50	12.49
24	IC120164	60.00	20.00	66.66	33.33
25	IC120165	60.00	40.00	33.33	66.66
26	IC120166	26.66	26.66	0.00	100.00
27	IC120175	46.66	26.66	42.86	57.13
28	IC120177	73.33	46.66	36.37	63.62
29	IC120179	33.33	26.66	20.01	79.98
30	IC120191	40.00	26.66	33.35	66.65
31	IC120192	33.33	33.33	0.00	100.00
32	IC120200	26.66	20.00	24.98	75.01
33	IC120201	26.66	6.67	74.99	25.00
34	IC120204	46.66	6.66	85.72	14.27
35	IC120207	46.66	46.66	0.00	100.00
36	IC120208	60.00	6.67	88.89	11.11
37	IC120210	46.66	6.67	85.71	14.28
38	IC120212	66.66	6.67	90.00	10.00
39	IC120213	33.33	20.00	39.99	60.00
40	IC120221	20.00	13.33	33.35	66.65
41	IC120228	33.33	6.67	80.00	20.00
42	IC120239	40.00	40.00	0.00	100.00
43	IC120251	26.66	13.33	50.00	50.00
44	IC120234	26.66	0.00	100.00	0.00
45	IC120235	60.00	13.33	77.78	22.21
46	IC120244	93.33	13.33	85.71	14.28
47	IC120250	13.33	13.33	0.00	100.00
48	IC120255	33.33	13.33	60.00	39.99
49	IC120346	40.00	0.00	100.00	0.00
50	IC120355	46.66	0.00	100.00	0.00
51	IC120394	46.66	20.00	57.13	42.86
52	IC120404	26.66	26.66	0.00	100.00
53	IC120406	53.33	20.00	62.49	37.50
54	IC120407	53.33	33.33	37.50	62.49
55	IC120408	60.00	6.67	88.89	11.11
56	IC200125	26.66	26.66	0.00	100.00
57	IC325968	60.00	6.67	88.89	11.11
58	IC344224	66.66	20.00	69.99	30.00
59	IC344225	46.66	26.66	42.86	57.13
60	IC372606	66.66	13.33	80.00	19.99
61	LEPAKSHI	26.66	0.00	100.00	0.00
62	PRASAD	66.66	33.33	50.00	50.00
Average		**48.38**	**23.65**	**47.39**	**52.60**
SE(m)		0.41	0.62	-	-
CD at (5%)		1.36	1.83	-	-
CV		1.64	4.77	-	

15 seeds per accession/plate

Data represents the means of three independent experiments (n=3) at P<0.05



(A)



(B)

Plate 4.2 Effect of PEG-6000 at -0.3 Mpa on seed germination in foxtail millet (A) Tolerant accessions (a) IC97087 (b) IC97189 (c) IC120159 (d) IC120239 (e) IC97172 (f) IC58243; **(B)** Susceptible accessions (a) Lepakshi (b) IC120355 (c) IC120346 (d) IC120234 (e) IC97109 (f) IC120204

4.1.1.2 Effect of PEG induced stress of -0.3 MPa on promptness index and germination stress tolerance index

The genotypic variation in germination and seedling growth under osmotic stress solely cannot be effectively used for screening crop plants for their tolerance to stress. Bouslama and Schapaugh (1984) and Fernandez (1992) reported that selection based on STI (stress tolerance index) would help to evaluate the higher stress tolerance genotypes with good yield potential. Thus, for identifying healthy and vigorous seed lots capable of germinating under water deficit or under abiotic stress, the germination test of seed may be useful (Ibrahim et al., 2007). With a view of the above fact, for screening the foxtail millet accessions, promptness index of seed germination was calculated. The promptness index was found to be highest in IC97172 (21.5) followed by IC97189, IC41898, and IC41883 which showed PI of 19.5. Lowest PI i.e. zero was observed in 5 genotypes namely IC97109, IC120234, IC120346, IC120355 and Lepakshi. The average PI was found to be 7.1 under -0.3 MPa osmotic stress. By using the promptness indices of stressed and unstressed seedlings; the germination stress tolerance index was calculated at an average of 52.43%. Highest germination stress tolerance index of 100% was recorded in IC120150, IC120159, IC120160, IC120239, IC120404, IC97177, IC41883 and IC97189 whereas the lowest i.e. zero was noted in IC97109, IC120234, IC120346, IC120355, Lepakshi (Table 4.2). Overall, osmotic stress reduced the germination efficiency in foxtail millet. The adverse effects of water shortage on germination and seedling growth had also been well reported in different crops such as wheat (Dhanda et al., 2004), sugar beet (Sadeghian and Yavari, 2004), sorghum (Gill et al., 2002), and sunflower (Mohammad et al., 2002).

4.1.1.3 Effect of PEG induced stress (-0.3MPa) on shoot length (cm) and root length

Measurements of shoot length or rooting depth of seedlings subjected to osmotic stress have been suggested for drought screening (Ahmadizadeh et al., 2011). Shoot length and root length were recorded after 14 days of seedling growth. Various accessions of foxtail millet showed variation in shoot length and root length in response to the PEG induced stress. While comparing the growth performance of genotypes under drought it was

observed that 100% shoot length stress tolerance index was recorded in two accessions namely, IC120164 and IC120213 followed by IC58243 (98.07%), IC120165 (85.71%), IC120255 (85.71%) and IC344224 (85.71%). Lowest SLSI of zero percent was observed in IC97109, IC120234, IC120346, IC120355, Lepakshi and IC97174. Relatively low SLSI was also found in IC97175 with 2.5% tolerance index. The average shoot length stress tolerance index was found to be 45.68% which shows great diversity and all these accessions differed significantly from each other (Table 4.3). The maximum shoot length was reported in IC200125, followed by IC120404, IC120250, IC120208, IC58243 IC120408 and IC120165. Mean shoot lengths were 5.25 cm and 2.31 cm in unstressed and -0.3 MPa osmotic stress respectively which clearly shows decrease over unstressed condition. Grzesiak et al. (1996) also noticed varietal differences in coleoptile length affected by drought simulated by osmotic stress in legume plants. Root length was also found to statistically restrained by drought stress. With increase in the osmotic stress, the root length was interrupted or decreased in most of the accessions. At -0.3 MPa osmotic stress, root induction on was seen only in 9 accessions viz. IC120406, IC97087, IC97182, IC58243 IC120159, IC120160, IC97189, IC120179 and IC120239 with 100% RLSI recorded in IC120179. Mean root lengths were 1.89 cm and 0.24 cm under zero and -0.3 MPa of osmotic stress respectively. The maximum root length was reported in IC120239 (3.5 cm), followed by IC97189 (2.6cm) and IC120160 (2.5 cm) under stress. Increase in the root length was observed in accession IC120239 with a RLSI of 102.94% with a 2.8% increase over unstressed condition. Increase in root length may be due to the diversion of dry matter to the root in search of moisture (Nicholas et al., 1995). Increase in root length as a result of drought stress has also been observed due to higher osmotic adjustment ability of drought tolerant genotypes by Rauf and Sadaqat (2007, 2008), Tahir et al. (2002) and Jaleel et al. (2008). Genotypic ability for high root to shoot length ratio contribute to drought tolerance. The results show that root length can be used for the selection of better performing genotypes under drought environment.

4.1.1.4 Effect of PEG induced stress (-0.3 MPa) on total plant dry matter

A most common damaging effect of low moisture level or low water potential is the decline in fresh biomass and dry matter production as reported by Peschke et al., 1997; Ashraf et al., 1998 and Manabendra and Baruah, 1998. Plant dry weights were recorded after drying the 14 day old seedling at 70°C. The seedling dry matter decreased with the increasing water stress created by PEG-6000 (Table 4.4). However, different accessions showed different responses under stress environment. The average seedling dry matter was 15.2 mg and 7.48 mg in unstressed and -0.3 MPa osmotic stress respectively. The genotype IC97189 showed significant highest total seedling dry matter (23.6 mg) with DMSI of 99.57% followed by IC120207 (99.55%), IC120239 (99.47%), IC372606 (99.44%), IC120407 (99.41%) and IC58243 (99.08%). The average dry matter stress tolerance index was observed at 49.21% under -0.3 MPa osmotic stress. DSI (dry matter stress tolerance index) was suggested as the best tool for measuring the stability of genotypes under stressed conditions compared to non stressed conditions (Clarke et al., 1982). Bruckner and Frohberg (1987) reported DSI as a parameter for measuring genotypic yield potential under drought stress conditions. There are many reports which are in agreement with the present findings indicating that drought stress induced by PEG severely reduced the growth and biomass of the plant. The accessions having genetic potential to maintain the higher growth under stress conditions can be attributed as tolerant accessions. The present study thus reveals the efficiency of PEG-6000 which can be used for screening of genotypes/accessions for drought tolerance under laboratory conditions. Figure 4.1 represents the various physiological parameters studied in foxtail millet accessions under water stress. Data represents the means of three independent experiments ($n = 3$) at $P < 0.05$.

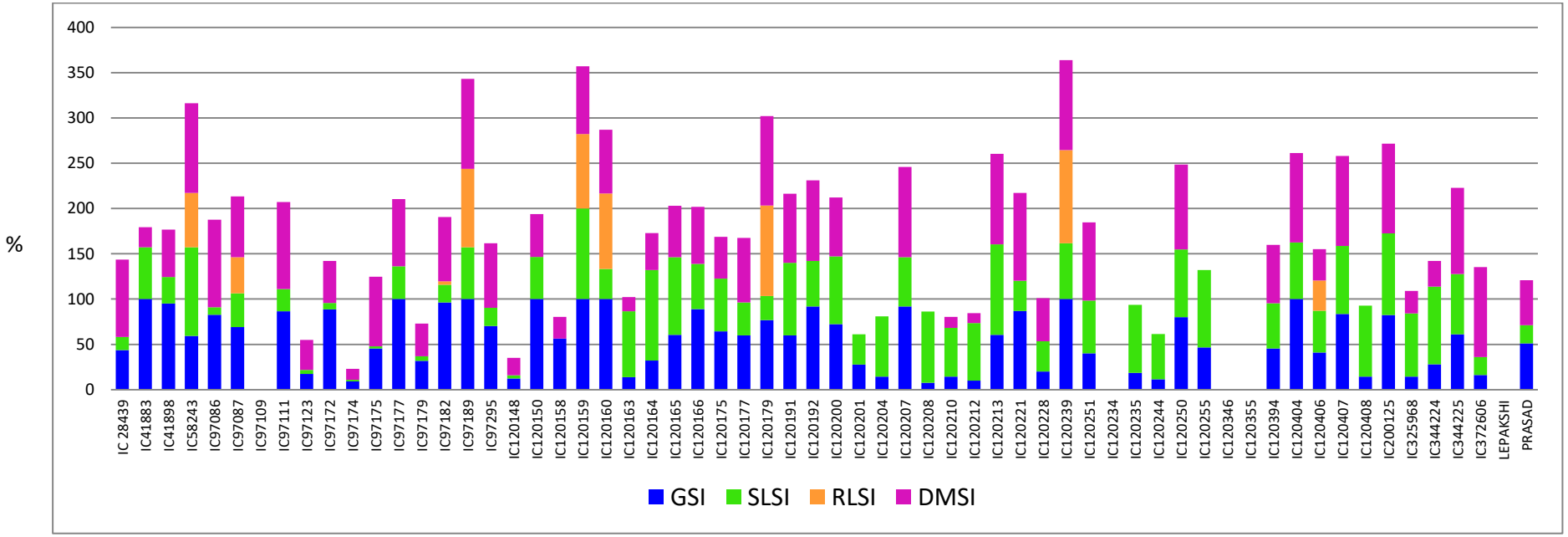


Figure 4.1 Physiological analysis of foxtail millet accessions in response to -0.3 Mpa osmotic stress using PEG-6000.

GSI= Germination stress tolerance index, SLSI= Shoot length stress tolerance index, RLSI= Root length stress tolerance index, DMSI= Dry matter stress tolerance index (Percent values are represented here).

Table 4.2. Effect of PEG induced stress on promptness index and germination stress tolerance index

SN	Accessions	Seed germination (respective day)								Promptness index		GSI
		0 MPa				-0.3 MPa						
		2nd	4th	6th	8th	2nd	4th	6th	8th	0 MPa	-0.3 MPa	
1	IC 28439	1	4	5	6	1	1	2	3	8.00	3.50	43.75
2	IC41883	6	9	9	9	6	9	9	9	19.50	19.50	100.00
3	IC41898	7	9	9	9	6	9	9	9	20.50	19.50	95.12
4	IC58243	3	7	7	7	2	4	4	4	13.50	8.00	59.25
5	IC97086	4	5	5	5	2	5	5	5	11.50	9.50	82.60
6	IC97087	7	10	11	11	4	6	9	11	22.75	15.75	69.23
7	IC97109	8	9	9	9	0	0	0	0	21.50	0.00	0.00
8	IC97111	3	4	4	5	3	5	5	5	9.25	8.00	86.48
9	IC97123	4	7	7	7	1	1	1	1	14.50	2.50	17.24
10	IC97172	9	10	10	11	5	11	11	11	24.25	21.50	88.65
11	IC97174	11	11	11	12	1	1	1	1	27.75	2.50	9.00
12	IC97175	5	5	6	6	2	2	3	4	13.25	6.00	45.28
13	IC97177	2	3	6	8	3	3	5	6	9.25	9.25	100.00
14	IC97179	8	9	12	14	3	3	3	4	24.25	7.75	31.95
15	IC97182	6	9	9	10	7	8	8	8	19.75	19.00	96.20
16	IC97189	7	7	10	10	7	10	10	10	19.75	19.75	100.00
17	IC97295	3	4	4	5	2	3	3	3	9.25	6.50	70.27
18	IC120148	8	8	8	10	1	1	1	1	20.50	2.50	12.19
19	IC120150	2	3	4	5	3	3	3	3	7.50	7.50	100.00
20	IC120158	6	6	7	8	3	4	4	4	16.00	9.00	56.25
21	IC120159	2	2	3	4	3	4	4	4	6.00	6.00	100.00
22	IC120160	2	2	3	4	3	3	4	4	6.00	6.00	100.00
23	IC120163	7	7	8	8	1	1	1	1	18.25	2.50	13.69
24	IC120164	8	8	8	9	2	3	3	3	20.25	6.50	32.09
25	IC120165	8	8	8	9	4	5	6	6	20.25	12.25	60.49
26	IC120166	3	4	4	4	2	4	4	4	9.00	8.00	88.88
27	IC120175	5	5	7	7	3	4	4	4	14.00	9.00	64.28
28	IC120177	11	11	11	11	6	7	7	7	27.50	16.50	60.00
29	IC120179	4	4	5	5	3	3	4	4	10.75	8.25	76.74
30	IC120191	6	6	6	6	3	4	4	4	15.00	9.00	60.00
31	IC120192	5	5	5	5	4	5	5	5	12.50	11.50	92.00
32	IC120200	3	4	4	4	2	3	3	3	9.00	6.50	72.22
33	IC120201	3	4	4	4	1	1	1	1	9.00	2.50	27.70
34	IC120204	7	7	7	7	1	1	1	1	17.50	2.50	14.28
35	IC120207	4	4	7	7	3	4	7	7	12.25	11.25	91.83
36	IC120208	7	8	9	9	0	1	1	1	19.75	1.50	7.59 7
37	IC120210	7	7	7	7	1	1	1	1	17.50	2.50	14.28

SN	Accessions	Seed germination (respective day)								Promptness index		GSI
		0 MPa				-0.3 MPa						
		2nd	4th	6th	8th	2nd	4th	6th	8th	0 MPa	-0.3 MPa	
38	IC120212	10	10	10	10	1	1	1	1	25.00	2.50	10.00
39	IC120213	4	4	5	5	2	3	3	3	10.75	6.50	60.46
40	IC120221	2	2	3	3	2	2	2	2	5.75	5.00	86.95
41	IC120228	5	5	5	5	1	1	1	1	12.50	2.50	20.00
42	IC120239	4	5	6	6	6	6	6	6	12.25	12.25	100.00
43	IC120251	4	4	4	4	1	2	2	2	10.00	4.00	40.00
44	IC120234	4	4	4	4	0	0	0	0	10.00	0.00	0.00
45	IC120235	6	7	8	9	1	1	2	2	17.50	3.25	18.57
46	IC120244	14	14	14	14	1	2	2	2	35.00	4.00	11.42
47	IC120250	2	2	2	2	1	2	2	2	5.00	4.00	80.00
48	IC120255	4	4	5	5	2	2	2	2	10.75	5.00	46.51
49	IC120346	6	6	6	6	0	0	0	0	15.00	0.00	0.00
50	IC120355	5	6	7	7	0	0	0	0	14.75	0.00	0.00
51	IC120394	6	7	7	7	3	3	3	3	16.50	7.50	45.45
52	IC120404	3	3	4	4	4	4	4	4	8.25	8.25	100.00
53	IC120406	7	7	8	8	3	3	3	3	18.25	7.50	41.09
54	IC120407	4	5	5	8	4	4	4	5	12.25	10.25	83.67
55	IC120408	6	7	8	9	1	1	1	1	17.50	2.50	14.28
56	IC200125	4	4	4	4	3	3	4	4	10.00	8.25	82.50
57	IC325968	6	7	8	9	1	1	1	1	17.50	2.50	14.28
58	IC344224	9	9	10	10	2	3	3	3	23.25	6.50	27.95
59	IC344225	5	6	7	7	3	4	4	4	14.75	9.00	61.01
60	IC372606	10	10	10	10	1	2	2	2	25.00	4.00	16.00
61	LEPAKSHI	4	4	4	4	0	0	0	0	10.00	0.00	0.00
62	PRASAD	8	8	9	10	4	4	5	5	21.00	10.75	51.19
Average										**15.41**	**7.42**	**55.17**
SE(m)										0.18	0.43	0.21
CD (5%)										0.5	1.2	0.61
CV										2.07	10.23	0.68

15 seeds per accession/plate

Data represents the means of three independent experiments (n=3) at P<0.05

Table 4.3 Effect of PEG induced stress (-0.3 MPa) on shoot length and root length (cm)

SN	Accession	Shoot length (cm)				Root length (cm)			
		0 MPa	-0.3 MPa	% decrease over unstressed	SLSI	0 MPa	-0.3 MPa	% decrease over unstressed	RLSI
1	IC 28439	3.50	0.50	85.71	14.28	6.10	0.00	100.00	0.00
2	IC41883	3.50	2.00	42.85	57.14	4.20	0.00	100.00	0.00
3	IC41898	5.10	1.50	70.58	29.41	3.50	0.00	100.00	0.00
4	IC58243	5.20	5.10	1.92	98.07	2.50	1.50	40.00	60.00
5	IC97086	6.00	0.50	91.66	8.33	4.00	0.00	100.00	0.00
6	IC97087	5.40	2.00	62.96	37.03	2.50	1.00	60.00	40.00
7	IC97109	5.30	0.00	100.00	0.00	4.10	0.00	100.00	0.00
8	IC97111	6.10	1.50	75.40	24.59	3.50	0.00	100.00	0.00
9	IC97123	4.30	0.20	95.34 2	4.65	3.00	0.00	100.00	0.00
10	IC97172	7.10	0.50	92.95	7.04	3.70	0.00	100.00	0.00
11	IC97174	5.30	0.10	98.11	1.88	3.60	0.00	100.00	0.00
12	IC97175	4.00	0.10	97.50	2.50	2.30	0.00	100.00	0.00
13	IC97177	5.50	2.00	63.63	36.36	3.80	0.00	100.00	0.00
14	IC97179	6.30	0.30	95.23	4.76	2.30	0.00	100.00	0.00
15	IC97182	5.10	1.00	80.39	19.60	2.50	0.10	96.00	4.00
16	IC97189	3.50	2.00	42.85	57.14	3.00	2.60	13.33	86.66
17	IC97295	3.00	0.60	80.00	20.00	2.50	0.00	100.00	0.00
18	IC120148	8.00	0.30	96.25	3.75	3.10	0.00	100.00	0.00
19	IC120150	4.30	2.00	53.48	46.51	0.20	0.00	100.00	0.00
20	IC120158	7.10	0.30	95.77	4.22 1	3.50	0.00	100.00	0.00
21	IC120159	3.50	3.50	0.00	100.00	1.70	1.40	17.64	82.30
22	IC120160	6.00	2.00	66.66	33.33	3.00	2.50	16.66	83.33
23	IC120163	5.50	4.00	27.27	72.72	1.00	0.00	100.00	0.00
24	IC120164	4.00	4.00	0.00	100.00	0.50	0.00	100.00	0.00
25	IC120165	7.00	6.00	14.28	85.71	1.50	0.00	100.00	0.00
26	IC120166	4.00	2.00	50.00	50.00	2.00	0.00	100.00	0.00
27	IC120175	6.00	3.50	41.66	58.33	1.50	0.00	100.00	0.00
28	IC120177	5.50	2.00	63.63	36.36	3.00	0.00	100.00	0.00
29	IC120179	7.50	2.00	73.33	26.66	1.50	1.50	0.00	100.00
30	IC120191	5.00	4.00	20.00	80.00	2.50	0.00	100.00	0.00
31	IC120192	4.00	2.00	50.00	50.00	1.00	0.00	100.00	0.00
32	IC120200	2.00	1.50	25.00	75.00	0.50	0.00	100.00	0.00
33	IC120201	4.50	1.50	66.66	33.33	0.00	0.00	-	0.00
34	IC120204	6.00	4.00	33.33	66.66	0.00	0.00	-	0.00

SN	Accessions	Shoot length (cm)				Root length (cm)			
		0 MPa	-0.3 MPa	% decrease over unstressed	SLSI	0 MPa	-0.3 MPa	% decrease over unstressed	RLSI
35	IC120207	5.50	3.00	45.45	54.54	2.00	0.00	100.00	0.00
36	IC120208	7.00	5.50	21.42	78.57	0.00	0.00	-	0.00
37	IC120210	6.50	3.50	46.15	53.84	0.50	0.00	100.00	0.00
38	IC120212	5.50	3.50	36.36	63.63	1.00	0.00	100.00	0.00
39	IC120213	4.00	4.00	0.00	100.00	3.00	0.00	100.00	0.00
40	IC120221	6.00	2.00	66.66	33.33	0.50	0.00	100.00	0.00
41	IC120228	6.00	2.00	66.66	33.33	0.50	0.00	100.00	0.00
42	IC120239	6.50	4.00	38.46	61.53	3.40	3.50	**-2.94**	**102.94**
43	IC120251	6.00	3.50	41.66	58.33	1.00	0.00	100.00	0.00
44	IC120234	5.50	0.00	100.00	0.00	2.00	0.00	100.00	0.00
45	IC120235	4.00	3.00	25.00	75.00	0.00	0.00	-	0.00
46	IC120244	5.00	2.50	50.00	50.00	0.00	0.00	-	0.00
47	IC120250	6.00	4.50	25.00	75.00	2.00	0.00	100.00	0.00
48	IC120255	3.50	3.00	14.28	85.71	0.00	0.00	-	0.00
49	IC120346	4.50	0.00	100.00	0.00	0.00	0.00	-	0.00
50	IC120355	6.50	0.00	100.00	0.00	0.30	0.00	100.00	0.00
51	IC120394	7.00	3.50	50.00	50.00	2.00	0.00	100.00	0.00
52	IC120404	8.00	5.00	37.50	62.50	2.50	0.00	100.00	0.00
53	IC120406	6.50	3.00	53.84	46.15	3.00	1.00	66.66	33.33
54	IC120407	4.00	3.00	25.00	75.00	2.50	0.00	100.00	0.00
55	IC120408	7.00	5.50	21.42	78.57	0.00	0.00	-	0.00
56	IC200125	5.00	4.50	10.00	90.00	3.50	0.00	100.00	0.00
57	IC325968	5.00	3.50	30.00	70.00	2.00	0.00	100.00	0.00
58	IC344224	3.50	3.00	14.28	85.71	0.50	0.00	100.00	0.00
59	IC344225	4.50	3.00	33.33	66.66	1.00	0.00	100.00	0.00
60	IC372606	3.00	0.60	80.00	20.00	0.00	0.00	-	0.00
61	LEPAKSHI	8.00	0.00	100.00	0.00	0.00	0.00	-	0.00
62	PRASAD	2.50	0.50	80.00	20.00	0.50	0.00	100.00	0.00
Average		**5.25**	**2.31**	**2.94**	**45.68**	**1.9**	**0.24**	**1.65**	**12.69**
SE(M)		0.08	0.02	**-**	**-**	0.08	**0.02**	**-**	**-**
CD (5%)		0.24	0.05	-	-	0.23	0.05	-	-
CV		2.9	1.4	-	-	7.85	14.49	-	-

15 seeds per accession/plate

Data represents the means of three independent experiments (n=3) at P<0.05

Table 4.4 Effect of PEG induced stress (-0.3MPa) on total plant dry matter (mg)

SN	Accessions	Total plant dry matter (g)			
		Unstressed 0 MPa	Stressed -0.3 MPa	% decrease over unstressed	DMSI
1	IC 28439	4.90	4.20	14.28	85.71
2	IC41883	24.10	5.40	77.59	22.40
3	IC41898	17.20	9.00	47.67	52.32
4	IC58243	10.90	10.80	0.91	99.08
5	IC97086	6.30	6.10	3.17	96.82
6	IC97087	26.10	17.50	32.95	67.04
7	IC97109	17.90	0.00	100.00	0.00
8	IC97111	5.10	4.90	3.92	96.07
9	IC97123	10.30	3.40	66.99	33.00
10	IC97172	22.40	10.40	53.57	46.42
11	IC97174	26.00	3.10	88.07	11.92
12	IC97175	5.60	4.30	23.21	76.78
13	IC97177	12.40	9.20	25.80	74.19
14	IC97179	15.70	5.70	63.69	36.30
15	IC97182	19.20	13.60	29.16	70.83
16	IC97189	23.70	23.60	0.42	99.57
17	IC97295	4.90	3.50	28.57	71.42
18	IC120148	16.30	3.10	80.98	19.01
19	IC120150	9.10	4.30	52.74	47.25
20	IC120158	21.60	5.20	75.92	24.07
21	IC120159	11.90	8.90	25.21	74.78
22	IC120160	13.20	9.30	29.54	70.45
23	IC120163	15.90	2.50	84.27	15.72
24	IC120164	12.30	5.00	59.34	40.65
25	IC120165	23.40	13.30	43.16	56.83
26	IC120166	14.10	8.90	36.87	63.12
27	IC120175	17.50	8.10	53.71	46.28
28	IC120177	26.70	19.00	28.83	71.16
29	IC120179	8.20	8.10	1.22	98.78
30	IC120191	10.60	8.10	23.58	76.41
31	IC120192	11.00	9.80	10.90	89.09
32	IC120200	8.00	5.20	35.00	65.00
33	IC120201	6.50	0.00	100.00	0.00
34	IC120204	15.40	0.00	100.00	0.00
35	IC120207	22.30	22.20	0.44	99.55
36	IC120208	17.20	0.00	100.00	0.00
37	IC120210	17.20	2.10	87.79	12.20
SN	Accessions	Total plant dry matter (g)			

		Unstressed 0 MPa	Stressed -0.3 MPa	% decrease over unstressed	DMSI
38	IC120212	23.70	2.60	89.02	10.97
39	IC120213	9.10	9.10	0.00	100.00
40	IC120221	6.70	6.50	2.98	97.01
41	IC120228	8.20	3.90	52.43	47.56
42	IC120239	19.00	18.90	0.52	99.47
43	IC120251	8.90	7.70	13.48	86.51
44	IC120234	28.10	0.00	100.00	0.00
45	IC120235	23.50	0.00	100.00	0.00
46	IC120244	10.60	0.00	100.00	0.00
47	IC120250	11.00	10.30	6.36	93.63
48	IC120255	10.20	0.00	100.00	0.00
49	IC120346	10.40	0.00	100.00	0.00
50	IC120355	12.90	0.00	100.00	0.00
51	IC120394	15.70	10.10	35.66	64.33
52	IC120404	23.70	23.40	1.26	98.73
53	IC120406	20.00	6.90	65.50	34.50
54	IC120407	17.00	16.90	0.58	99.41
55	IC120408	14.80	0.00	100.00	0.00
56	IC200125	11.90	11.80	0.84	99.15
57	IC325968	16.90	4.20	75.14	24.85
58	IC344224	17.60	5.00	71.59	28.40
59	IC344225	18.90	18.00	4.76	95.23
60	IC372606	18.00	17.90	0.55	99.44
61	LEPAKSHI	8.10	0.00	100.00	0.00
62	PRASAD	26.40	13.10	50.37	49.62
Average		**15.2**	**7.48**	**50.71**	**49.21**
SE(m)		0.12	0.08	-	-
CD (5%)		0.36	0.22	-	-
CV		1.47	1.87	-	-

15 seeds per accession/plate
Data represents the means of three independent experiments (n=3) at P<0.05

4.1.1.5 Clustering of accessions based on the parameters from laboratory screening using D^2 analysis

The difference in the response to water stress by foxtail millet accessions was measured by employing D^2 statistic. Based on the physiological screening data, all the accessions were grouped into nine clusters, signaling the different responses of foxtail millet accessions to drought stress. Cluster II had the highest number of accessions (23) followed

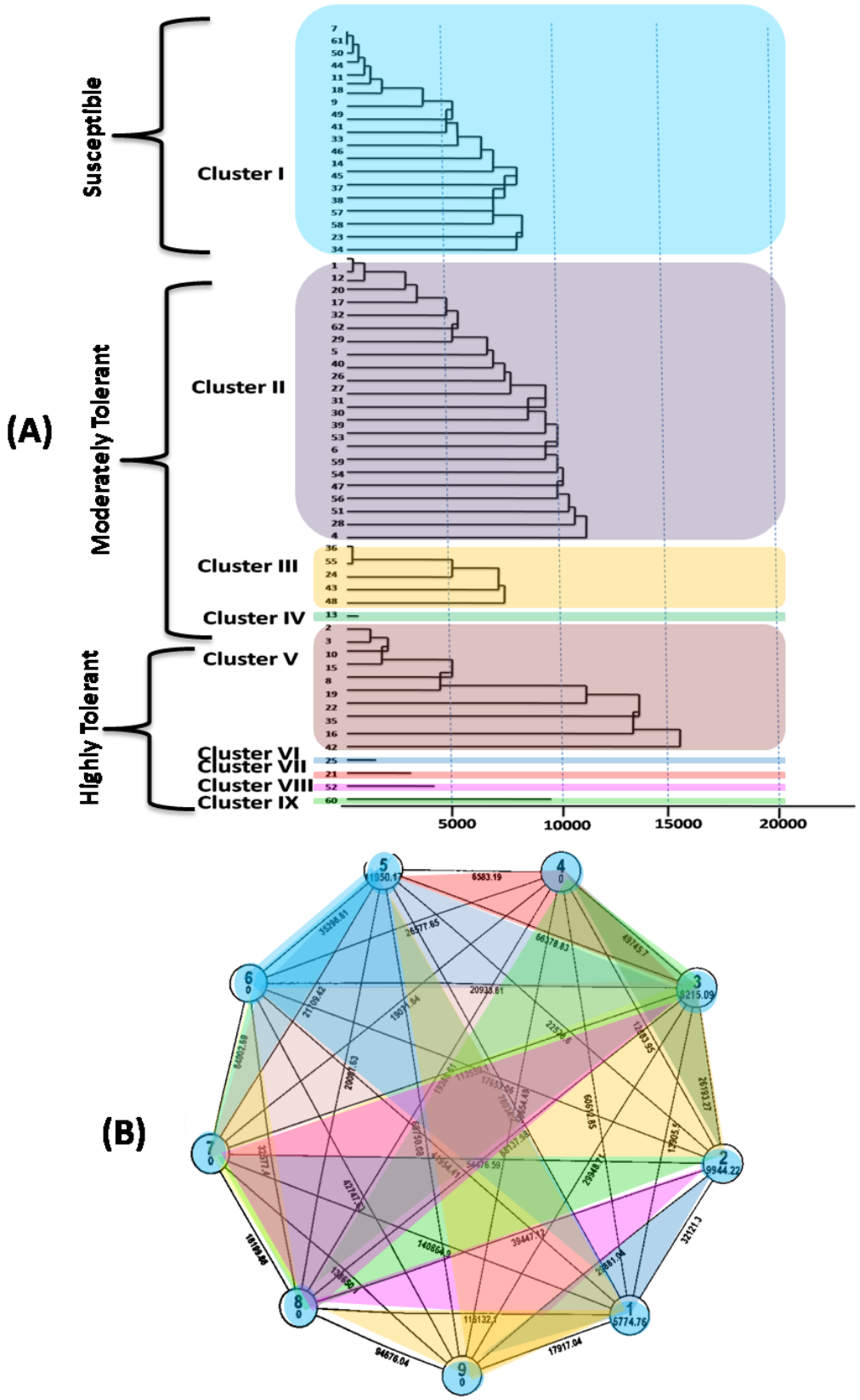


Plate4.3 Clustering of accessions based on the parameters from physiological screening using D^2 analysis (A) Dedrogram grouping 62 accessions of Foxtail millet accessions into nine clusters based on *Tocher* method. Clustering done on the basis of physiological data generated by subjecting the 21 day seedling to dehydration stress of 9 days, (B) Cluster diagram depicting inter and intra-cluster Mahalanobis Euclidean distance between nine clusters of 62 foxtail millet accessions.

by cluster I (19), cluster V (10) and cluster III (5). The cluster IV, VI, VII, VIII and IX were monogenotypic. Clustering of accessions based on the physiological screening under laboratory conditions using PEG-6000 is presented in Plate 4.3 The intra-cluster distances indicate the divergence among the accessions within the clusters and inter-cluster distances indicates diversity between clusters (Table 4.5). The maximum intra-cluster distance was recorded within cluster v (11950.17) followed by cluster II (9944.22) and cluster I (6774.76). The maximum inter-cluster distance is observed between cluster I and VII (140864) followed by cluster VII and IX (138650.04), I and VIII (116132.10) and III and VII (113552.10). The minimum Mahalanobis Euclidean inter-cluster distance was observed between cluster IV and V (6583.19) followed by cluster IV and II (12883.95). These results suggest maximum divergence between accessions of cluster I with accessions of cluster VII, indicating difference in their responsesiveness to water stress. In general, less intra-cluster distance than inter cluster distance suggested homogenous and heterogeneous nature of the genotypes within and between the clusters, respectively (Pawar et al., 2013).

In the present investigation, susceptible accessions were grouped in cluster I while tolerant accessions were grouped in cluster V, VI, VII, VIII and IX. Mahalanobis D^2 statistic was found to be a useful tool in assessing the relative contribution of different parameters in foxtail millet accessions under water stress and the information obtained from inter-cluster distances may be used to select genetically superior accessions for breeding drought tolerant lines.

The contribution of each character towards the difference in response to water stress in foxtail millet is presented in Table 4.6. The seed germination stress tolerance index (65.68 %) contributed highest. Thus, seed germination may be given high emphasis while screening large number of accessions for their drought tolerance ability. This is contradictory with Ashraf et al. (1992) who concluded that under moisture deficit, germination is not the standard for predicting plants drought tolerance. Other characters like shoot length stress tolerance index (20.47%), dry matter stress tolerance index (10.58 %) and promptness index stress tolerance index (3.23 %) contributed very little. The response of genotypes may be different to different factors, which could be

reflected in their respective seed performances. The germination test is found to be useful for identifying the genotypes capable of quickly establishing themselves under low soil moisture conditions which shows correlation with drought tolerance as suggested by Guoth et al. (2009).

The present investigation for screening water stress tolerance ability of foxtail millet accessions was found to be relevant in choosing the best accession for resisting drought. PEG-6000 is considered as the best macromolecular compound to simulate drought stress, and it is widely used in water stress physiology and genetic mechanism for plants (Christmann et al., 2005; Verslues and Bray, 2004; 2006). The characters germination percentage and root to shoot length ratio showed considerable variability under stress conditions in foxtail millet. There are many reports which are in agreement with the present findings indicating that drought stress induced by PEG severely reduced the growth and biomass of the plant. The laboratory screening using PEG-6000 was found to effective in selecting core set of tolerant and susceptible accessions in foxtail millet the major advantage being controlled environment where large population that can be handled in a lesser space within a short span of time, and the plant material being kept disease free (Patade et al. 2008; Patade and Suprasanna 2008).

Based on the various parameters like significant highest germination stress tolerance index, root length stress tolerance index and the water content were recorded to estimate the drought stress tolerance, and the core set of resistant and susceptible accessions were screened successfully. IC97087, IC97189, IC120159 and IC120239 were recorded as the most drought tolerant genotypes whereas IC97109, IC120234, IC120346 and Lepakshi were recorded as sensitive genotypes.

Table 4.5. Intra-cluster distance of different groups in D^2 analysis

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Group 1	6774.76	32121.30	13905.50	60612.85	78034.20	41954.41	**140864.00**	116132.10	17917.04
Group 2		9944.22	26193.27	12883.95	22528.60	17653.06	54476.59	39447.12	29881.04
Group 3			8215.09	49745.70	66378.83	20938.81	113552.10	88137.98	29948.71
Group 4				0.00	**6583.19**	26577.65	19071.84	19386.61	56654.49
Group 5					11950.17	35296.61	21709.42	20087.63	68750.08
Group 6						0.00	64002.69	32577.40	42747.63
Group 7							0.00	18199.86	138650.04
Group 8								0.00	94676.04
Group 9									0.00

Table 4.6 Contribution of various characters from physiological screening using PEG-6000 in foxtail millet under water stress for D^2 analysis

Source	Contribution
Promptness index stress tolerance index	3.23 %
Seed germination stress tolerance index	65.68 %
Shoot length stress tolerance index	20.47 %
Dry matter stress tolerance index	10.58 %

4.1.2 Physiological measurements for induced water stress experiment

To further evaluate the drought tolerant potentials of foxtail millet accessions, we analyzed relative water content, total chlorophyll content and leaf water potential in twenty-one day old foxtail millet seedlings subjected to water stress of 9 days. Plants showed significant variations in relative water content (RWC), chlorophyll content index (CCI) and leaf water potential (LWP) which are discussed below in detail.

4.1.2.1 Relative water content (RWC)

To understand the physiological changes in water stress condition in plants, changes in relative water content is a less error prone and useful means for determining the physiological water status of plants (Makbul, et al., 2011, González and González-vilar, 2001). The choice of RWC as the best representation of plant water status for assessing genetic differences in dehydration tolerance is supported by genetic association between RWC and plant biomass under dehydration (Blum 1996; Bhushan et al. 2007). Hence leaf RWC is an important parameter, which determines the ability of a plant to absorb water under water stress conditions and used as one of the indices to determine drought tolerance ability of the plants. Stressed plants have lower RWC than non-stressed plants. Likewise, in the present study, relative water content was found to be decreased significantly in all accessions under water stress however, the tolerant cultivars showed the capacity to maintain relatively high RWC (Figure 4.2).

The decline in RWC was reported by several investigators under stress conditions (Ramanjulu and Sudhakar, 1997; Madhusudan et al., 2002; Turkan et al., 2005; Farooq and Azam, 2006). The present study showed a decrease in relative water content with initiation of water stress. Shamsi (2010) also reported a decrease in relative water content with an increase in the intensity of drought stress on wheat cultivars. Mationn et al. (1989) represented a similar report as regards a drop in the amount of RWC in tolerant and sensitive cultivars of barley. RWC was ranged from 32.14 % to 94.09% under stressed conditions with the lowest value recorded in C120346 followed by IC120234 (32.54%), IC97109 (35%), IC120355 (35.5%) and IC120179 (38.09%) where RWC was found to be below 40%. If the RWC falls beyond 40%, plants do not survive even if they re-watered (Blum, 1996). A reduction

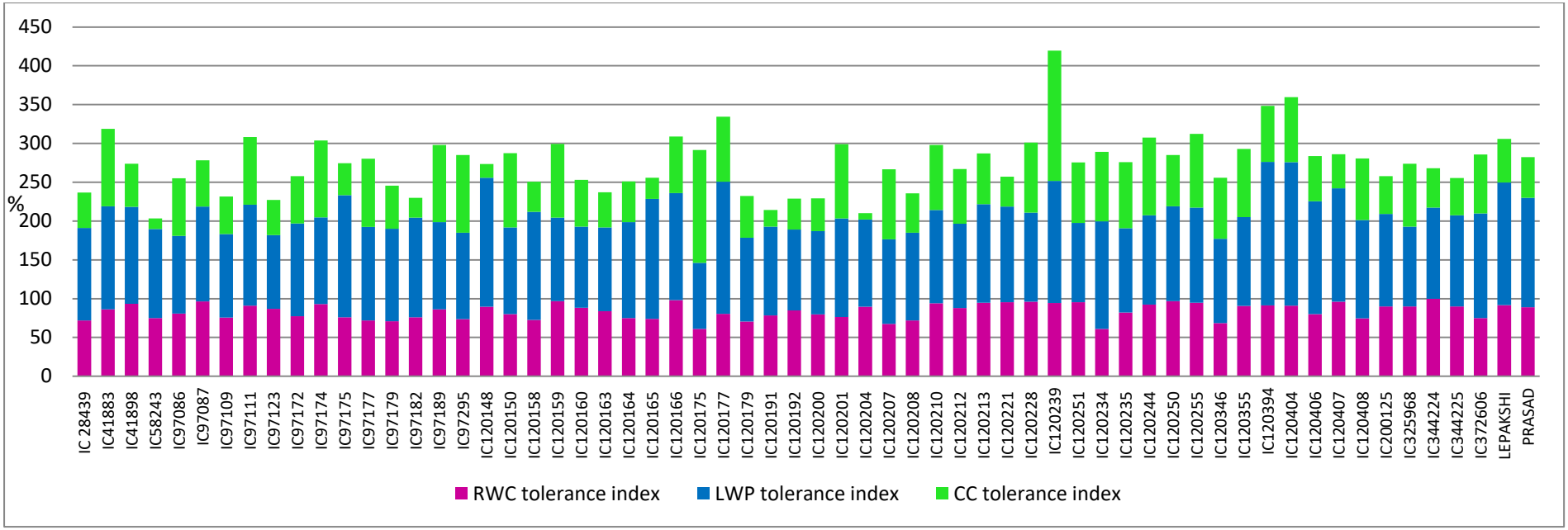


Figure 4.2 Physiological measurements for induced water stress experiments in response to dehydration stress. Data for relative water content tolerance index and leaf water potential tolerance index and chlorophyll content tolerance index is presented here.

LWP= Leaf water potential, RWC= Relative water content, CC= chlorophyll content (Percent values are represented here).

of this magnitude (39.5%) in RWC usually occurs in plants growing under severe drought conditions (Tripathy et al., 2000). Relative water content was significantly higher in IC120159 (94.09%) followed by IC97087 (92.05%), IC120239 (85.68%), PRASAD (81.94%) and IC97189 (80.49%) under water stressed condition. RWC tolerance index was calculated on the basis of RWC values of unstressed and stressed conditions which was found to highest in IC344224 (99.78%), IC120166 (98.30%), IC120159 (96.91%), IC120250 (96.85%) and IC97187 (96.44) while RWC tolerance index was found to be lower in accessions IC120175 (60.95%), IC120234 (61.15%), IC120207 (67.44), IC120346 (68.51%) and IC120179 (70.44) (Table 4.7). Percent decrease was also recorded which ranged from 0.2% to 39.05%. Being an elite drought tolerant crop, the sensitive accessions showed comparatively less reduction in RWC under water stress as compared to other crops. It is reported that high relative water content is a resistant mechanism to drought and that high relative water content is the result of more osmotic regulation or less elasticity of tissue cell wall (Shamsi, 2010). Overall decrease in RWC under drought stress was highly significant in all the accessions analysed here which in accordance with Allahmoradi et al. (2011) in Mungbean, Mohammadkhani and Heidari (2008) in maize, Moaveni (2011) and Farshadfar et al. (2011) in wheat. Similarly, Liu et al. (2006) reported a gradual decrease in RWC after application of PEG treatment as water stress.

In the present study, relative water content decreased significantly in leaves of all cultivars under water stress and was found to be significantly higher in tolerant accession than sensitive accessions. Hence, it is suggested that the relative water content could help the tolerant cultivar to perform physio-chemical processes more efficiently than sensitive cultivar. Erickson et al. (1991) reported that RWC of leaf indicate the internal water balance of plant tissue and changes in tissue water content change the tissue dry weight continually. The similar results were also obtained by Carter and Patterson (1985) in soybean. This suggested that the relative water content could help the tolerant cultivar to perform physio-chemical processes more efficiently than sensitive cultivars.

4.1.2.2 Leaf water potential (Ψ_L)

Leaf water potential also decreased significantly in leaves of all accessions under water stress conditions. The LWP ranged between -7.8 in tolerant accessions and -9.6 in susceptible accessions. Tolerant accessions showed relatively higher LWP than sensitive accessions. Many workers have reported that drought tolerant varieties have a smaller water deficit (relative saturation deficit) per unit decrease in water potential of leaf than drought sensitive plants (Levitt, 1972; Dedio, 1975; Ashraf et at., 1994a). LWP ranged from -28.11 to -4.19 with an average of -7.86 and -9.62 in unstressed led and stressed conditions respectively. Highest LWP was found in IC120244 (-4.19 MPa) followed by Prasad (-5.39 MPa), IC120159 (-5.57 MPa), IC97087 (-5.85 MPa), IC120160 (-5.96 MPa), IC97179 (-6.12 MPa), IC120251 (-6.38 MPa) and IC97189 (-6.4MPa). Lowest LWP was found to be present in IC120355 (-28.11 MPa) , IC120234(-23.61 MPa) , IC372606 (-18.52 MPa) , LEPAKSHI (-16.22 MPa), IC120207(-15.4 MPa) , IC120407(-14.54 MPa) and IC97109 (-14.17 MPa). LWP index was found to be highest in IC120394 (184.92%) followed by IC120404 (184.89) and IC120177 (170.18%), whereas, was found to be lowest in IC120175 (85.11%), IC97123 (94.93%) and IC97086 (100%) (Figure 4.2). Gonzalez et al. (2008) has also recorded significant decrease in Ψ_L and RWC in barley under drought stress. Significant differences in leaf water potential and RWC were recorded among the tolerant and susceptible accessions studied here and the results are consistent with Subrahmanyam et al. (2006); Tas and Tas (2007); Siddique et al. (2004); Zhu (2002) and Wahid and Close (2007), where reduction in RWC of the leaf and leaf water potential, had significant effect on photosynthesis under deficit moisture conditions.

4.1.2.3 Chlorophyll Content:

Chlorophyll index is a rapid method and forms one of the indices for estimating resistance to dehydration (Khidse et al., 1982). There are reports about decrease of chlorophyll content in drought stress conditions (Kuroda et. al., 1990). The present study shows that the chlorophyll content decreased with increasing water stress in majority of accessions over non stressed conditions, while some showed slight increase in the chlorophyll content. The

decrease in total chlorophyll content in the leaves of water stressed plants was reported by several workers earlier (Ramanjulu and Sudhakar, 2000; Gopalakrishna, 2001; Chandraobulreddy, 2005). In the present study, the chlorophyll index ranged from 8.02% to 168% under water stress. The accessions IC120239 showed highest CCI (168%) followed by IC97174 (151.6%), IC120175 (145.4%) and IC41883 (112.82%), while the lowest CCI was recorded in IC120204 (8.02%) followed by IC58243 (13.73%), IC120148 (17.92%) and IC120191 (21.42%) (Table 4.7). However, the rate of decrease in chlorophyll content was comparatively higher in susceptible accessions i.e. IC58243, IC120204, IC97123, IC120221 and IC200125. The tolerant accessions showed lesser decrease in chlorophyll content (IC120239, IC97174, IC120175, IC120159 and IC41883). This is in support of the report by Shamsi (2010) where resistant cultivar to drought and thermal stress conditions had high chlorophyll content (Shamsi, 2010). The chlorophyll content index measured in unstressed and water stressed leaves of foxtail millet accessions and results are depicted in Figure 4.2. Ramanjulu and Sudhakar (2000) also reported that lesser decrease in total chlorophyll content in tolerant mulberry cultivar than susceptible cultivar under salt stress. This decrease is because of reduced photosynthates production under water deficit conditions (Tezara et al., 1999; Anjum et al., 2003; Wahid and Rasul, 2005) as the chlorophyll content is positively associated with photosynthetic rate which increases biomass production and grain yield. In general photosynthetic apparatus is very sensitive and liable to drought stress injury. Drought stress inhibits enzymes of chlorophyll biosynthesis, particularly 5-aminolevilinic acid synthetase (Bharadwaj and Singhal, 1981). Similar findings were resulted by Ashraf and Yasmin (1995) in grasses and by Abrechit and Carberry (1993) in maize. A reduction in chlorophyll content was reported in drought stressed cotton (Massacci et. al., 2008), in *Catharanthus roseus* (Jaleel et al., 2008), in sunflower (Kiani et al., 2008) and in *Vaccinium myrtillus* (Tahkokorpi et al., 2007).

Table 4.7 Physiological parameters measured for the period of induced water stress.

SN	Accessions	Unstressed RWC	Stressed RWC	RWC index	LWP unstressed	LWP stressed	LWP index	CC unstressed	CC stressed	CC index
1.	IC 28439	86.17	62.02	71.97	-6.63	-7.89	119.00	17.90	8.20	45.81
2.	IC41883	68.53	59.07	86.19	-5.47	-7.26	132.72	7.80	7.80	100.00
3.	IC41898	71.00	66.36	93.45	-7.40	-9.25	125.00	6.50	3.60	55.38
4.	IC58243	76.16	57.01	74.85	-8.08	-9.28	114.85	18.20	2.50	13.73
5.	IC97086	72.19	58.33	80.80	-9.48	-9.48	100.00	3.90	2.90	74.35
6.	IC97087	95.45	92.05	96.44	-4.78	-5.85	122.38	5.90	3.50	59.32
7.	IC97109	46.29	35.00	75.59	-13.15	-14.17	107.75	11.80	5.70	48.30
8.	IC97111	56.02	51.04	91.11	-6.61	-8.59	129.95	9.40	8.20	87.23
9.	IC97123	62.34	54.16	86.88	-7.70	-7.31	94.93	20.90	9.50	45.45
10.	IC97172	52.80	40.83	77.33	-7.15	-8.54	119.44	11.30	6.90	61.06
11.	IC97174	52.20	48.55	93.00	-6.01	-6.72	111.81	9.20	9.10	98.91
12.	IC97175	55.30	42.01	75.96	-6.27	-9.87	157.41	16.30	6.70	41.10
13.	IC97177	83.33	59.84	71.81	-10.73	-12.93	120.50	6.70	5.90	88.05
14.	IC97179	82.90	58.76	70.87	-5.14	-6.12	119.06	7.00	3.90	55.70
15.	IC97182	63.15	48.01	76.01	-9.38	-12.05	128.46	10.20	2.60	25.49
16.	IC97189	93.28	80.49	86.28	-5.69	-6.40	112.47	12.50	12.40	99.20
17.	IC97295	81.12	59.68	73.58	-5.80	-6.46	111.37	11.80	11.80	100.00
18.	IC120148	55.82	50.04	89.63	-5.39	-8.95	166.04	10.60	1.90	17.92
19.	IC120150	77.40	61.94	80.02	-7.47	-8.34	111.64	7.40	7.10	95.94
20.	IC120158	59.00	42.76	72.48	-5.18	-7.22	139.38	3.10	1.20	38.70
21.	IC120159	97.08	94.09	96.91	-5.18	-5.57	107.52	14.10	13.40	95.03

SN	Accessions	Unstressed RWC	Stressed RWC	RWC index	LWP unstressed	LWP stressed	LWP index	CC unstressed	CC stressed	CC index
22.	IC120160	72.42	63.90	88.24	-5.70	-5.96	104.56	20.90	12.60	60.28
23.	IC120163	67.64	56.77	83.92	-9.42	-10.15	107.74	8.60	3.90	45.34
24.	IC120164	84.37	63.15	74.85	-7.13	-8.82	123.70	17.20	9.00	52.32
25.	IC120165	84.25	62.23	73.86	-4.16	-6.44	154.80	5.50	1.50	27.27
26.	IC120166	64.64	63.54	98.30	-4.73	-6.51	137.63	5.90	4.30	72.88
27.	IC120175	89.93	54.81	60.95	-9.47	-8.06	85.11	4.40	6.40	145.40
28.	IC120177	86.20	69.47	80.58	-6.64	-11.30	170.18	11.00	9.20	83.63
29.	IC120179	54.08	38.09	70.44	-6.42	-6.94	108.09	15.80	8.50	53.79
30.	IC120191	80.72	63.24	78.34	-8.07	-9.25	114.62	12.60	2.70	21.42
31.	IC120192	77.45	65.79	84.94	-8.05	-8.37	103.97	10.00	4.00	40.00
32.	IC120200	77.20	61.69	79.90	-8.15	-8.73	107.11	14.90	6.30	42.28
33.	IC120201	85.02	65.00	76.44	-5.33	-6.77	127.01	11.30	10.80	95.57
34.	IC120204	58.58	52.56	89.72	-7.24	-8.14	112.43	13.70	1.10	8.02
35.	IC120207	90.67	61.15	67.44	-14.14	-15.40	108.92	12.50	11.30	90.40
36.	IC120208	73.23	52.79	72.08	-6.75	-7.63	113.03	10.90	5.50	50.45
37.	IC120210	72.54	68.31	94.16	-8.22	-9.88	120.19	9.70	8.10	83.50
38.	IC120212	81.77	71.98	88.02	-8.33	-9.05	108.64	12.80	9.00	70.31
39.	IC120213	64.88	61.45	94.70	-9.73	-12.37	127.13	11.50	7.50	65.21
40.	IC120221	70.14	67.04	95.56	-6.88	-8.46	122.96	17.30	6.70	38.72
41.	IC120228	71.09	68.45	96.27	-10.25	-11.74	114.53	12.40	11.20	90.32
42.	IC120239	90.78	85.68	94.37	-3.67	-5.77	157.22	7.50	12.60	168.00
43.	IC120251	71.78	68.42	95.31	-6.23	-6.38	102.40	21.70	16.90	77.88
44.	IC120234	53.21	32.54	61.15	-17.05	-23.61	138.47	9.60	8.60	89.58

SN	Accessions	Unstressed RWC	Stressed RWC	RWC index	LWP unstressed	LWP stressed	LWP index	CC unstressed	CC stressed	CC index
45.	IC120235	88.14	72.52	82.27	-9.05	-9.81	108.39	11.60	9.90	85.34
46.	IC120244	68.00	62.80	92.35	-3.64	-4.19	115.10	8.10	8.10	100.00
47.	IC120250	66.66	64.57	96.85	-6.45	-7.87	122.01	11.20	7.40	66.07
48.	IC120255	71.33	67.49	94.61	-7.17	-8.80	122.73	12.20	11.60	95.08
49.	IC120346	46.91	32.14	68.51	-9.96	-10.82	108.63	6.60	5.20	78.78
50.	IC120355	39.21	35.50	90.53	-24.57	-28.11	114.40	22.50	19.80	88.00
51.	IC120394	69.86	63.82	91.35	-5.44	-10.06	184.92	18.30	13.20	72.13
52.	IC120404	61.93	56.42	91.10	-6.09	-11.26	184.89	11.50	9.60	83.47
53.	IC120406	69.04	55.22	79.97	-8.14	-11.84	145.45	11.50	6.70	58.26
54.	IC120407	59.37	56.98	95.96	-9.94	-14.54	146.27	11.90	5.20	43.69
55.	IC120408	67.67	50.58	74.74	-10.20	-12.90	126.47	23.30	18.50	79.39
56.	IC200125	71.52	64.45	90.11	-7.17	-8.54	119.10	19.80	9.60	48.48
57.	IC325968	69.19	62.28	90.01	-6.95	-7.14	102.73	16.50	13.40	81.21
58.	IC344224	61.08	60.95	99.78	-8.37	-9.85	117.68	14.00	7.10	50.71
59.	IC344225	61.64	55.36	89.81	-5.95	-7.00	117.64	16.30	7.80	47.85
60.	IC372606	73.16	54.78	74.87	-13.73	-18.52	134.88	25.00	19.00	76.00
61.	LEPAKSHI	47.14	43.28	91.81	-10.28	-16.22	157.78	15.80	8.90	56.32
62.	PRASAD	92.22	81.94	88.84	-3.82	-5.39	141.09	20.20	10.60	52.47
Average		**70.87**	**59.37**	**83.93**	**-7.86**	**-9.62**	**124.00**	**12.52**	**8.13**	**67.45**
SE(m)		0.17	0.79	-	0.01	0.02	-	0.09	0.50	-
CD (5%)		0.48	2.22	-	0.05	0.06	-	0.26	1.45	-
CV		0.42	2.31	-	0.04	0.04	-	1.31	11.23	-

Data represents the means of three independent experiments (n = 3), “P < 0.05.

As discussed earlier, foxtail millet accessions are mostly tolerant to abiotic stresses, and further exploiting these traits can enhance the utility of foxtail millet for dry and neglected lands and would also be further useful in development of stress tolerant cultivars (Li and Wu, 1996; Dekker, 2003). The present study showed that the foxtail millet responds to water stress in the form of changes in various physiological processes. Water deficit stress has exerted negative effect on all the physiological parameters considered. Based on the various parameters like significant highest germination stress tolerance index, root length stress tolerance index, relative water content and leaf water potential, the core set of resistant and susceptible genotypes were screened successfully and were selected for further analysis which are presented in Table 4.8

Table 4.8 Core set of accessions screened on the basis of physiological data

Tolerant accessions				Susceptible accessions			
PEG screening	**RWC**	**Ψ_L**	**CC**	**PEG screening**	**RWC**	**Ψ_L**	**CC**
IC97087	IC120251	IC120165	IC97189	IC97109	IC120346	IC120355	IC120204
IC97189	IC120228	IC97189	IC120160	IC120234	IC120234	IC120234	IC120158
IC120159	IC120177	IC120251	IC120239	IC120346	IC97109	IC372606	IC120165
IC120239	IC120212	IC97179	IC120394	IC120355	IC120355	LEPAKSHI	IC120148
PRASAD	IC120235	IC120160	IC325968	LEPAKSHI	IC120179	IC120207	IC58243
IC41883	IC97189	IC97087	IC120159	IC120201	IC97172	IC120407	IC97182
IC41898	PRASAD	IC120239	IC120251	IC120204	IC97175	IC97109	IC120191
IC58243	IC120239	IC120159	IC120408	IC120208	IC120158	IC97177	IC97086
IC97172	IC97087	PRASAD	IC372606	IC120235	LEPAKSHI	IC120408	IC97087
IC97182	IC120159	IC120244	IC120355	IC120244	IC97182	IC120213	IC41898

RWC= relative water content
Ψ_L= leaf water potential
CC= cholorophyl content

In the second phase of the study, the selected highly tolerant (IC97087, IC97189, IC120159, IC120239, PRASAD) and highly susceptible accessions (IC97109, IC120234, IC120346, IC120355, Lepakshi) from the physiological screening were screened for their water stress tolerance on the basis of some primary metabolites like proline, total carbohydrates etc. and antioxidant

enzymes like superoxide dismutase, peroxidase, catalase and glutathione reductase, as acclimation of plants to drought is considered to promote antioxidants defence systems to face the increased levels of reactive oxygen species (ROS).

4.2 Changes in drought responsive metabolites in tolerant and susceptible foxtail millet accessions under water stress.

One of the widely described plant responses to water deficit is osmotic adjustment, which requires accumulation of compatible solutes, such as amino acids, carbohydrates, polyols, tertiary sulfonium and quaternary ammonium compounds which play an important role in maintaining cell turgor, as well as stabilizing proteins and cell membranes.

4.2.1 Accumulation of primary metabolites

4.2.1.1 Amino acids

It has been documented that many amino acids accumulate in plants exposed to various abiotic stresses. Proline is one of the most widely distributed osmolyte, the level of which is elevated in different environmental stresses including drought, salinity and cold stress (Verbruggen and Hermans 2008; Szabados and Savoure 2010). This stress-responsive amino acid is predominantly synthesized from glutamate through pyrroline-5-carboxylate (P5C) by two reductions catalyzed by pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductases (P5CR) (Hu et al. 1992).

In the present study, free proline content was estimated in unstressed and water stressed foxtail millet accessions. Proline content was found to be comparatively higher in IC97189 (103.89 µmoles $(gFW)^{-1}$) with an percent increase of 41.67% followed by IC97087 (88.31 µmoles $(gFW)^{-1}$) with an percent increase of only 15.69% in water stressed condition which were found to be tolerant accessions in primary screening leaves and data is presented in Figure 4.3. Lowest proline accumulation was seen in IC120355 (34.63 µmoles $(gFW)^{-1}$) and IC120346 (34.63 µmoles $(gFW)^{-1}$). The mean proline content was found to be 68.3 µmoles/gm of tissue in stressed condition and 40.5 µmoles $(gFW)^{-1}$ in unstressed condition. Accession IC97087 showed highest amount of proline accumulation even in unstressed conditions, whereas, accession Prasad showed highest percent (70.45%) increase in proline accumulation in stressed conditions. Minimum percent increase in

accumulation of was seen in IC97109 i.e. 13.33% which is a susceptible accession (Table 4.9). Change in proline content has been correlated with its capacity to tolerate and adapt to stress conditions (Balibrea et al., 1997). It has been earlier reported that proline accumulates in larger amounts than other amino acids under salt or water stress (Niknam et al., 2006). Accumulation of proline under stress protects the cell by balancing the osmotic strength of cytosol with that of vacuole and external environment (Aspinall and Paleg, 1981). In addition its role as cytosolic osmotica, it is known to interacts with cellular macro-molecules such as enzymes and stabilizes the structure and function of such macromolecules (Jain et al, 2001). In the present investigation, the free proline content was found to be significantly increased in all accessions with water stress over unstressed plants. However, a difference in the accumulation of free proline content was observed among the accessions. Higher proline accumulation recorded in drought tolerant accessions, when compared to drought sensitive accessions indicates that proline metabolizes slowly as compared to the other antioxidative enzymes and thereby supplies the nitrogen to the cellular metabolic pathways post stress (Bartels and Sunkar, 2005). A direct consequence of higher proline concentration in foxtail millet was relatively higher water retaining capacity as reflected by RWC. Zaifnejad et al., (1997) also observed a higher proline content in a drought tolerant Sorghum under PEG-induced water deficit. Barron and De-Mejia (1998) reported a higher proline and its enzyme activities under water stress. It seems that water stress tolerance of foxtail millet could be due to biochemical processes related to proline metabolic enzymes.

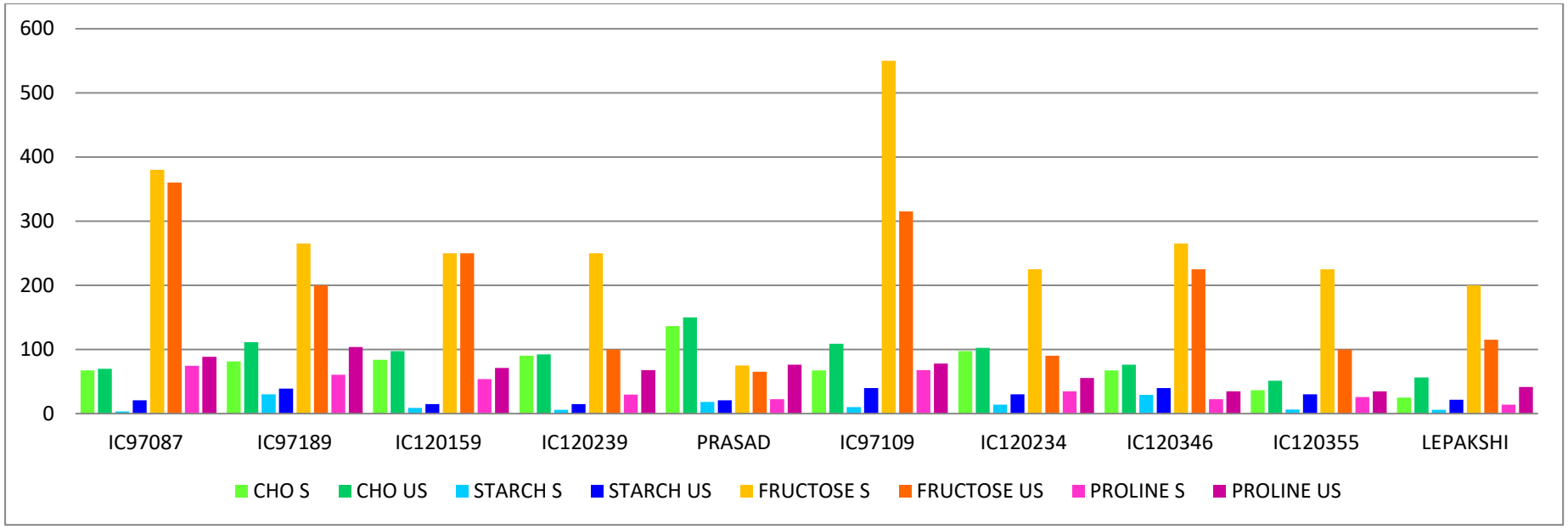


Figure 4.3 Changes in drought responsive metabolites in screened stress tolerant and susceptible foxtail millet accessions exposed to dehydration stress by water with-holding

CHO= carbohydrates, expressed as mg (g FW) $^{-1}$; Proline expressed as µmoles (gFW)$^{-1}$; US=unstressed; S=stressed

4.2.1.2 Carbohydrates

It is widely reported that abiotic stresses lead to accumulation of non-structural carbohydrates like sucrose, hexoses and polyhydric alcohols among many plant species. Soluble carbohydrates play an important role in plant metabolism as a source of carbon and energy within a cell. Their level is affected by different stresses, as the carbohydrate content is related to photosynthesis.

In the present study, total carbohydrates were found to be increased in all the accessions under water stress condition. Highest CHO value was recorded in Prasad (150 mg(gFW)$^{-1}$) with an increase of 9.1% followed by IC97189 with 11.25 mg(gFW)$^{-1}$ with an increase of 26.96% over unstressed condition. Lowest CHO content was found in IC120355 (51. mg(gFW)$^{-1}$) followed by Lepakshi (56.25 mg(gFW)$^{-1}$). CHO greatly increase in susceptible accession under water stress with a percent increase upto 55.55%, whereas tolerant accession showed a negligible increase in CHO content i.e. upto 26.96%. Strong correlation between the carbohydrate accumulation and tolerance to osmotic stresses, such as water deficit or salinity stress has been reported by Bartels and Sunkar (2005).

Similarly, starch increased in all the accessions studied, with highest starch content recorded in IC120346 i.e 39.9 mg(gFW)$^{-1}$ of tissue with 26.81% increase over unstressed condition. Lowest starch content mg(gFW)$^{-1}$ was recorded in IC120159 with percent increase of 39.59% over unstressed condition. Highest percent increase in starch contect was found in IC97087 with an increase of 83.57%. Starch content was found to be lower in unstressed condition in susceptible accessions than in tolerant ones. Drought stress significantly increase starch content. In contradiction to earlier reports, our study found good performance of drought susceptible cultivar, Lepakshi under water stress (Lata et al., 2011[a]). The increase in starch and carbohydrates might be due to their role as protectant during stress, protecting cell membranes (Janska´ et al., 2010) and their involvement in cell signaling (Hanson and Smeekens 2009). They also play a role in adaptive mechanisms to stress (Ramel et al. 2009).

Fructose showed a decrease in its content in stressed conditions. Prasad (65 mg(gFW)$^{-1}$) showed lowest fructose content with a decrease of 13.33 % over unstressed condition while IC97087 (360 mg(gFW)$^{-1}$) showed highest fructose content with only 5.2 % decrease over unstressed condition followed by IC97109 having 315 mg(gFW)$^{-1}$fructose. Accession IC120159 showed no change in fructose content in stressed and unstressed conditions. Similar observation was reported by Sicher et al. (2012) in water-stressed barley roots (Sicher et al., 2012). Soluble sugars function as osmoprotectants during water deficit, reducing the detrimental effects of osmotic stress, helping in maintaining turgor, stabilizing cell membranes and protecting plants from degradation (Basu et al., 2007). The increase in sugar content is mostly the effect of starch hydrolysis, which requires enzymes with a hydrolytic activity (Kaplan and Guy, 2004).

Table 4.9 Changes in drought responsive metabolites in tolerant and susceptible foxtail millet accessions in response to water stress

SN	Metabolite	CHO mg(gFW)$^{-1}$			Starch mg(gFW)$^{-1}$			Fructose mg(gFW)$^{-1}$			Proline µmoles (gFW)$^{-1}$		
	Treatment	US	S	% increase	US	S	% increase	US	S	% decrease	US	S	% increase
1.	IC97087	67.50	70.00	3.57	0.34	2.07	83.57	380.00	360.00	5.26	74.45	88.31	15.69
2.	IC97189	81.25	111.25	26.96	2.99	3.88	22.93	265.00	200.00	24.52	60.60	103.89	41.67
3.	IC120159	83.75	97.50	14.10	0.90	1.49	39.59	250.00	250.00	0.00	53.67	70.99	24.39
4.	IC120239	90.00	92.50	2.70	0.60	1.49	59.73	250.00	100.00	60.00	29.43	67.53	56.42
5.	PRASAD	136.25	150.00	9.16	1.80	2.09	13.87	75.00	65.00	13.33	22.51	76.19	70.46
6.	IC97109	67.50	108.75	37.9	1.00	3.99	74.93	550.00	315.00	42.72	67.53	77.92	13.33
7.	IC120234	97.50	102.50	4.87	1.40	2.99	53.17	225.00	90.00	60.00	34.63	55.41	37.50
8.	IC120346	67.50	76.25	11.47	2.92	3.99	26.81	265.00	225.00	15.09	22.51	34.63	35.00
9.	IC120355	36.25	51.25	29.27	0.65	2.99	78.26	225.00	100.00	55.55	25.97	34.63	25.01
10.	LEPAKSHI	25.00	56.25	55.55	0.60	2.16	72.22	200.00	115.00	42.50	13.85	41.50	66.62
AVERAGE		**75.20**	**91.60**	**19.55**	**1.30**	**2.40**	**47.48**	**268.60**	**182.00**	**31.87**	**40.50**	**68.30**	**38.60**
SE(m)		0.38	0.44	-	0.02	0.02	-	0.51	1.06	-	0.23	0.32	-
CD @ 5%		1.06	1.23	-	0.05	0.07	-	1.44	2.96	-	0.64	0.91	-
CV		0.87	0.80	-	2.80	2.02	-	0.33	1.00	-	0.99	0.82	-

CHO= carbohydrates; FW= fresh weight

Data represents the means of three independent experiments (n=3) at P<0.05.

CHO= carbohydrates, expressed as mg (g FW) $^{-1}$; Proline expressed as µmoles (gFW)$^{-1}$; US=unstressed; S=stressed

4.2.2 Accumulation of antioxidant enzymes

In the present study, antioxidant enzyme activities changed significantly in response to the water stress. These enzymes operate in different subcellular compartments and respond in concert when cells are exposed to oxidative stress. The effect of drought stress on the activities of antioxidant enzymes participating in the scavenging of ROS is presented in Table 4.10.

The results revealed an increase in SOD, POD, CAT and GR activities under drought treatment. Highest SOD activity was seen in IC97189 (0.892 Units min^{-1}) in water stressed condition with a 25.5% increase over unstressed condition. Accession IC97109 showed the least SOD activity under water stressed condition i.e. 0.522 Units min^{-1}. Highest percent increase in activity of SOD was found in Prasad with a 79.04% increase. Increased activity of SOD is often correlated with increased tolerance of the plant against environmental stresses. Overproduction of SOD has been reported to result in enhanced oxidative stress tolerance in plants (Gupta et al., 1993). It is also suggested that SOD can be used as an indirect selection criterion for screening drought-resistant plant materials (Nayyar and Gupta, 2006).

A significant and time-dependent increase in GR activity has been found in all the accessions exposed to dehydration stress as compared with their respective controls except in IC120139. Highest GR activity was seen in IC120159 (0.979 Units min^{-1}) with an increase of 49.00% increase over unstressed condition followed by IC97189 (0.248 Units min^{-1}) with an increase of 26.6% over unstressed plants. Whereas, Lepakshi (0.021 Units min^{-1}) was recorded with lowest activity of GR with only 9.61 % increase over unstressed condition. Decrease in the activity of GR was seen in IC120139 with 12.67% decrease over unstressed condition. Adaptation to drought has been reported to involve different protective mechanisms including the capacity to maintain high levels of antioxidants (ascorbate and GSH) and to regenerate them through the induction of GR (Loggini et al., 1999). Activation of ascorbate–glutathione cycle has earlier been suggested as essential in stressed plants to combat oxidative damage (Alscher et al., 1997). Similar observations were reported in foxtail millet, wheat, rice, sorghum, and pigeon pea (Lata et al.,

2011[a], Loggini et al., 1999; Jogeshwar et al., 2006; Guo et al., 2006; Kumutha et al., 2009) under various abiotic stresses.

Highest POD activity was seen in Prasad (2.207 Units min^{-1}) with an increase of 44.88% over unstressed conditions followed by IC97189 (2.036 Units min^{-1}). Accession IC120159 showed highest percent increase i.e. 64.21% in the activity POD in stressed condition. Lowest activity was seen in IC97109 (0.868 Units min^{-1}). Some previous studies, as parallel with our results, reported the increased POD activity under drought stress conditions in various plants, like sunflower (Gunes et al., 2008), poplar (Xiao et al., 2008), liquorice (Pan et al. 2006), brassica species (Das and Uprety, 2006), wheat (Csiszar et al., 2005).

Increase in CAT activity under water stress was seen in majority of accessions studied. IC120159 showed the highest CAT activity i.e. 1.917 Units min^{-1} with an increase of 47.05% over unstressed condition. Lowest CAT activity was seen in IC97087 (0.448 Units min^{-1}) with only 13.17% increase followed by IC97109 (0.796 Units min^{-1}). The results are in support of studies on antioxidative responses to dehydration-induced oxidative stress in core set of foxtail millet cultivars by Lata et al., (2011[a]). Increase in the activity of catalase (CAT) was also reported by Zarei et al., (2012) in tobacco under drought stress. CAT activity was found to be decreased in Prasad under stressed conditions. The decline in CAT activity is regarded as a general response to many stresses (Herbinger et al., 2002; Bakalova et al., 2004; Jung 2004; Guo et al., 2006; Pan et al., 2006; Gunes et al., 2008; Liu et al., 2008). The reduction of CAT activity is supposedly due to the inhibition of enzyme synthesis or change in the assembly of enzyme subunits under stress conditions. It may also be associated with degradation caused by induced peroxisomal proteases or may be due to the photo-inactivation of the enzyme. Hence, the noticed differences in the efficiency of maintaining higher catalase activity could reflect the better adaptability of genotypes to drought-induced oxidative stress. Figure 4.4 depicts the changes in antioxidative enzyme activity in foxtail millet accessions under stressed conditions.

In the present water stress tolerance study, significantly higher SOD, POD, GR and CAT was activity found in the tolerant accessions suggesting

that the higher antioxidant enzymes activity have a role in imparting tolerance against water stress (Mittova et al., 2003; Sharma and Dubey, 2005).

The balanced regulation in the activities of these enzymes can be induced by ROS directly or indirectly (Mittler, 2002). In conformity with the results concluded by Chen and Muruta, (2002) we found that the joint activity and inter correlation between these enzymes would have an important role in preventing the formation of ROS, and therefore the appearance of excessive damage by oxidative stress, achieving better tolerance. Increased SOD, POD and CAT activities are closely related to stress tolerance of many plants as reported by previous researches (Rahnama and Ebrahimzadeh, 2005; Azevedo Neto et al., 2006; Koca et al., 2007).

There are many reports in the literature that underline the intimate relationship between enhanced or constitutive antioxidant enzyme activities and increased resistance to environmental stresses in several plant species, such as rice (Guo et al., 2006), foxtail millet (Sreenivasulu et al., 2000), tomato (Mittova et al., 2003), sugar beet (Bor et al., 2003), oilseed rape (Abedi and Pakniyat, 2010), wheat (Zaefyzadeh et al., 2009; Shahbazi et al., 2010; Ahmadizadeh et al., 2011) and barley (Acar et al., 2001).

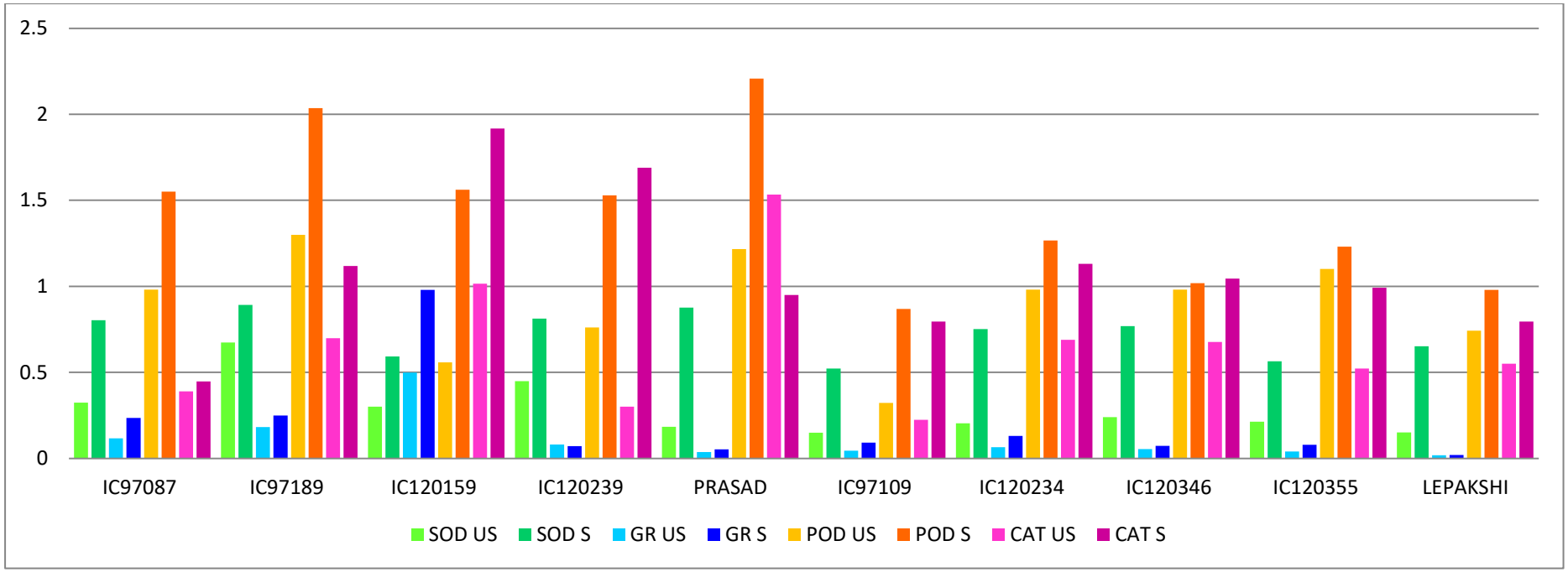


Figure 4.4 Changes in antioxidative enzyme activity in stress tolerant and susceptible foxtail millet accessions exposed to dehydration stress by water with-holding (a) SOD, one unit of enzyme activity defined as inhibiting the rate of reduction of NBT by 50% (b) GR, one unit defined by reduction in 1.0 µmole of oxidized glutathione min^{-1};(c) CAT, one unit of enzyme activity defined as 1 µmol H_2O_2 oxidized min^{-1}; (d) POD, one unit of enzyme activity defined by the formation 1.0 milligram of purpurogall in from pyrogallol

SOD= superoxide dismutase; GR= glutathione reductase; CAT=catalase; POD= peroxidise; US-unstressed; S-stressed

Table 4.10 Changes in antioxidative enzyme activity in stress tolerant and susceptible foxtail millet accessions exposed to dehydration stress

SN	Accessions	SOD			GR			CAT			POD		
		US	S	% increase	US	S	% increase	US	S	% increase	US	S	% increase
1	IC97087	0.324	0.803	59.670	0.116	0.235	50.649	0.389	0.448	13.170	0.981	1.551	36.747
2	IC97189	0.673	0.892	24.500	0.182	0.248	26.667	0.699	1.119	37.501	1.300	2.036	36.140
3	IC120159	0.300	0.593	49.410	0.499	0.979	49.000	1.015	1.917	47.057	0.559	1.561	64.212
4	IC120239	0.449	0.813	44.769	0.080	0.072	-12.245	0.301	1.690	82.186	0.761	1.529	50.211
5	PRASAD	0.184	0.877	79.042	0.037	0.052	28.846	1.533	0.950	-61.301	1.216	2.207	44.886
6	IC97109	0.150	0.522	71.254	0.045	0.091	50.909	0.224	0.796	71.890	0.322	0.868	62.869
7	IC120234	0.205	0.752	72.788	0.064	0.131	50.820	0.690	1.131	38.997	0.981	1.266	22.492
8	IC120346	0.240	0.769	68.771	0.054	0.073	25.926	0.676	1.045	35.273	0.981	1.019	3.729
9	IC120355	0.214	0.565	62.201	0.040	0.079	50.000	0.523	0.991	47.271	1.101	1.230	10.488
10	LEPAKSHI	0.151	0.652	76.828	0.019	0.021	9.617	0.551	0.796	30.850	0.742	0.980	24.284
AVERAGE		**0.289**	**0.724**	**60.923**	**0.114**	**0.198**	**33.019**	**0.660**	**1.088**	**34.290**	**0.895**	**1.425**	**35.606**
SE(m)		0.007	0.006	-	0.006	0.005	-	0.010	0.016	-	0.013	0.019	-
CD @ 5%		0.020	0.016	-	0.016	0.013	-	0.027	0.044	-	0.036	0.052	-
CV		4.356	1.385	-	9.131	4.168	-	2.563	2.516	-	2.479	2.281	-

Expressed as Enzyme unit per mg of protein

Data represent the means of three independent experiments (n=3); P<0.05.

SOD= superoxide dismutase; GR= glutathione reductase; CAT=catalase; POD= peroxidise; US-unstressed; S-stressed

4.3 Gene expression profiling using cDNA-SRAP and drought specific marker genes

Even though the drought-tolerance capacity of foxtail millet is ascribed to its cellular and morphological characteristics (Li, 1997), there have been very less study on differentially expressed genes that impart drought tolerance to foxtail millet.

Collectively based on the physiological and biochemical screening of all the foxtail millet accessions, two contrasting accessions (most tolerant: IC97189 and most susceptible: IC97109) were selected for further exploring the reason behind its drought tolerance (Plate 4.4). Comparative differential gene expression studies were carried out by using two approaches:

In the first approach, differential gene expression pattern was studied by using random primers like cDNA SRAP. In the second approach, the drought stress specific genes/TFs (identified earlier in various crops) were analyzed. Strikingly, the number of genes that were significantly upregulated under drought stress were much higher in tolerant than in susceptible accession. The details of differential expression studies are discussed below under appropriate headings-

The initial step in expression studies is isolation of good quality RNA. In this regard, RNA was extracted from 21 day old seedling from the respective plant type using trizol method and checked for quality and quantity on 1.5% denaturing agarose gel. Agarose gel electrophoresis and alpha imager analysis revealed that RNA extracted in this study can meet the quality requirement for cDNA-SRAP analysis (plate 4.5). Amplification products presented in all cDNA-SRAP reactions indicated that the reaction conditions adopted here were suitable for the analysis of cDNA-SRAP differential display in foxtail millet. There were neither primer-dimers nor the occurrence of non-specific amplifications. Accurate normalization of template is an important step in gene expression studies. Hence, to confirm the normalisation of cDNA, appropriate internal control gene like α tubulin and EF1α were used which showed constant expression in unstressed and stressed condition. EF-1 α was used in the present study which was suggested as reliable internal control gene in foxtail millet gene expression studies (Kumar et al., 2013). Semi-quantitative RT-PCR has emerged as a versatile technique in

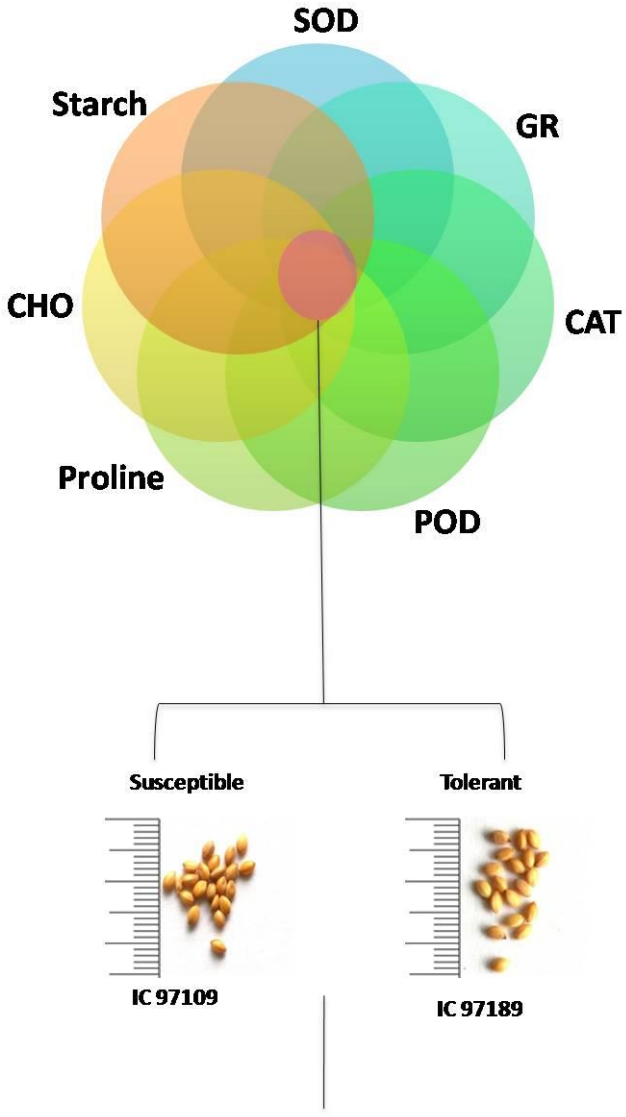


Plate 4.4 Selection of tolerant and susceptible accessions for gene expression studies on the basis of biochemical screening

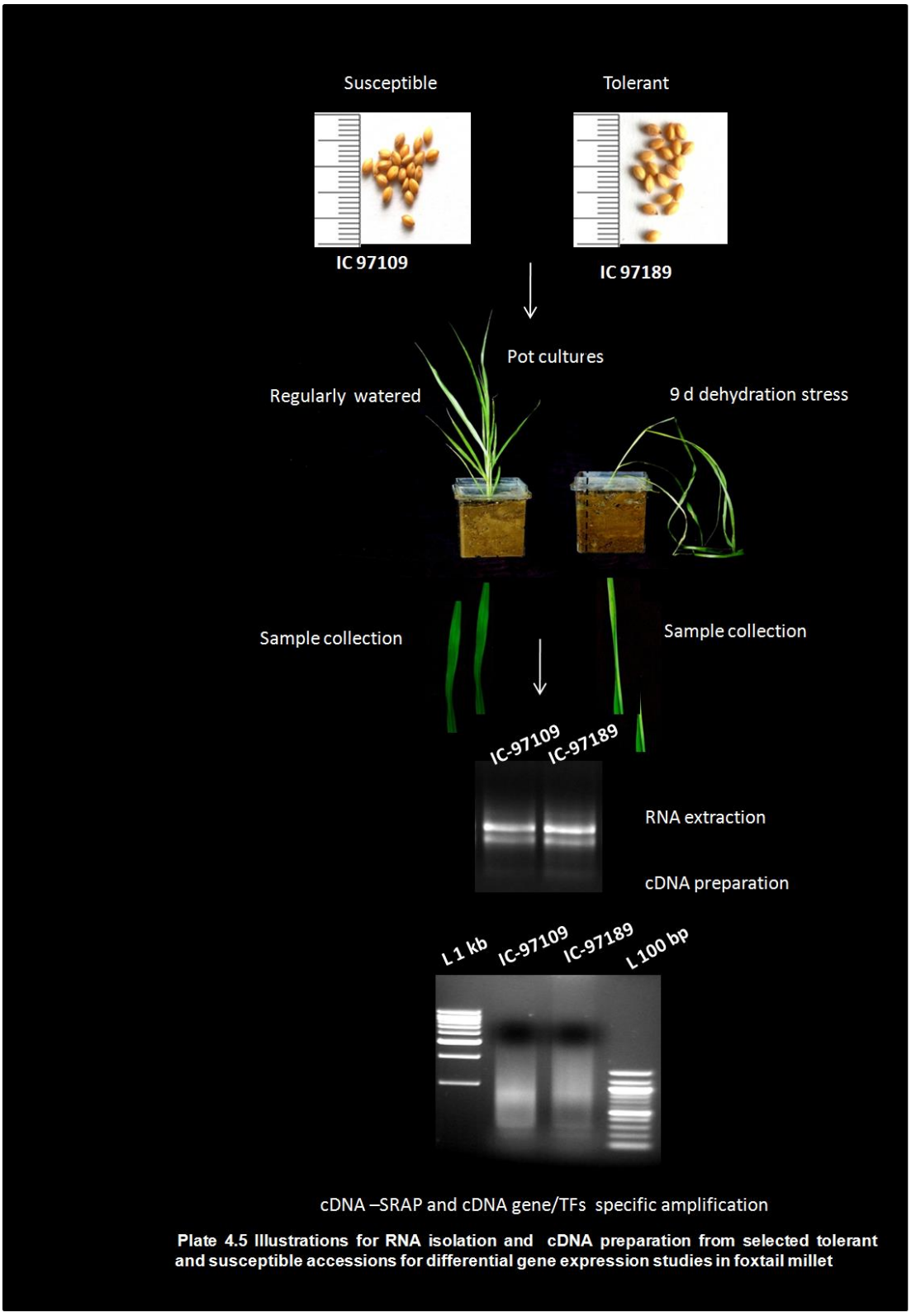


Plate 4.5 Illustrations for RNA isolation and cDNA preparation from selected tolerant and susceptible accessions for differential gene expression studies in foxtail millet

transcriptomics, as it can generate rapid measurement of mRNA levels in minimal tissue samples. Hence, the method was adopted in the present investigation.

Out of the 30 primer combinations of SRAP, only 12 primer combinations showed good amplification of which 4 combinations were monomorphic and 8 combinations were polymorphic. Semi-quantitative evaluation of the relative mRNA accumulation showed upregulation/downregulation in the various primer combinations studied. Nine of the twelve primer combination showed upregulated expression after treatment, two combinations F1-R5 and F4-R5 showed neither upregulated nor downregulated expression and combination F2-R1 showed downregulation in expression of the gene amplified in stressed condition. The number of amplicons produced by an individual primer combination was ranged from one (F4-R1) to eleven (F6-R4) with an average of 5.66 amplicons per primer combination i.e. Combination F6-R4 showed amplification of maximum genes whereas combination F4-R1 showed amplification of single gene (plate 4.6). Expression pattern of possibly water stress-related genes through cDNA-SRAP is presented in the form of heatmap (Plate 4.7). The amplicons obtained in each primer combination were designated as 1-68 in continuous manner. Primer combination F2-R5 and F1-R1 showed significant high expression in tolerant accession. Amplicon number 49, 50, 51, 54, 55, 56, 60, 61, 62, 63, 65, 66, 67 showed consistent expressions in both contrasting accessions in unstressed and stressed conditions. Details of SRAP gene expression profiling in foxtail millet under water stress are presented in Table 4.11.

The high expression of these genes under stress condition confirms their role in drought tolerance. The primer combination F2-R5 showed the expression of gene only in stress condition in accession IC97189. Further validation of these genes could help in identifying the potential candidate genes involved in drought tolerance. Differentially expressed new putative transcripts and/or genes can be a useful tool for understanding their function in response to stress. The cDNA-SRAP technique has been proved to be suitable for analysis of differential gene expression in several kinds of plants

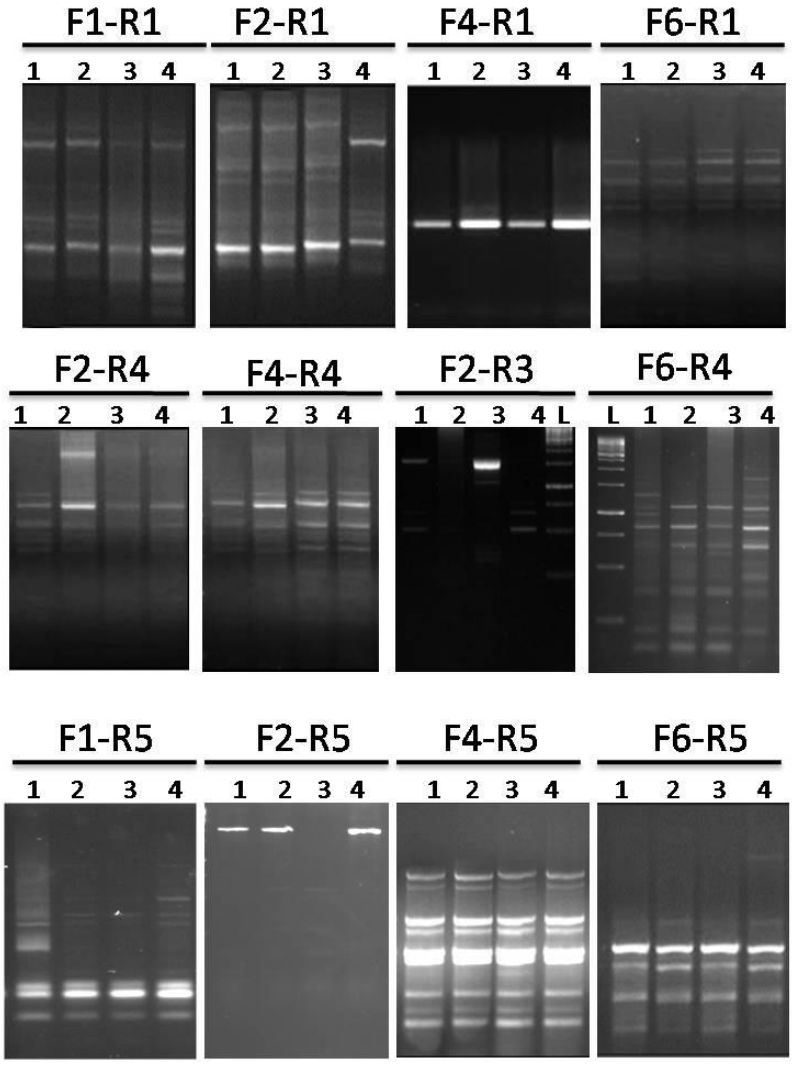


Plate 4.6 Sequence Related Amplification Polymorphism (SRAP) marker profiling in two contrasting foxtail millet accessions, IC-97109, IC-97189 under dehydration stress

Samples were run on 1.5% agarose gel; ; lane L: ladder, Lane 1: IC-97109 control, lane 2: IC-97109 stress, lane 3: IC-97189 control, lane 4: IC-97189 stress.; primer combination is indicated at top.

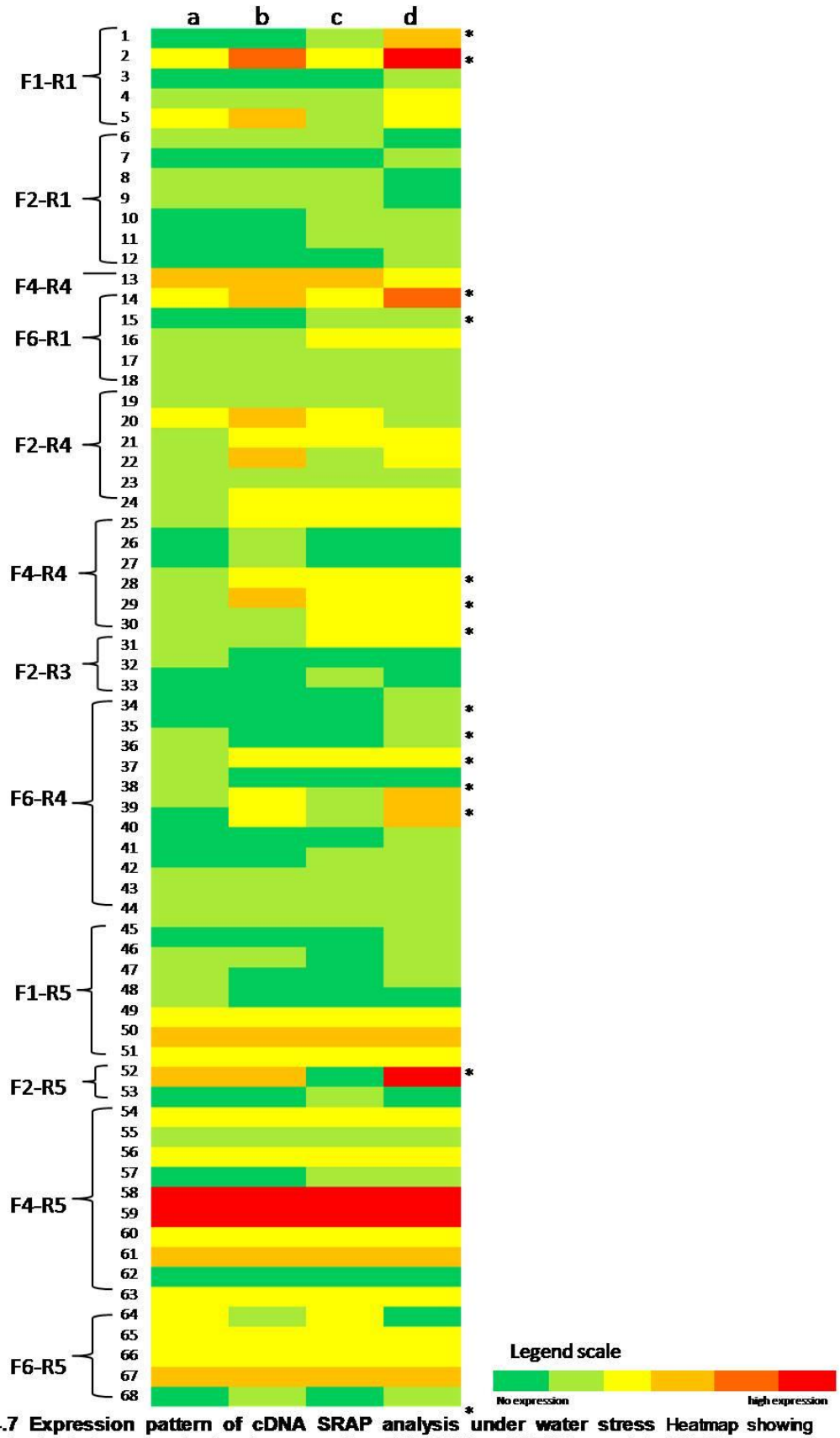


Plate 4.7 Expression pattern of cDNA SRAP analysis under water stress Heatmap showing transcription level of genes under drought stress using cDNA-SRAP; a: IC97109 control, b: IC97109 stress; c: IC97189 control; d: IC97189 stress; Green and red indicate low and high levels of transcript abundances based on IDV values, respectively; Asterisks represent significant changes in transcription level after drought stress.

discussed earlier (Li et al.,2003; Lu and Wu, 2006;Deng et al.,2007; Ma et al.,2008).

Table 4.11 SRAP gene expression profiling in foxtail millet under water stress

SN	Primer combination	Total no. of bands	Amplicon size (bp)	IC97109		IC97189	
				unstressed	stress	unstressed	stress
1	F1R1	5	210	-	-	+	++
			250	+	++	+	+++
			270	-	-	-	+
			279	+	+	+	++
			760	++	+++	+	++
2	F2-R1	7	250	+	+	+	-
			280	-	-	-	+
			295	+	+	+	-
			578	+	+	+	-
			590	-	-	+	+
			750	-	-	-	+
			800	+++	+++	+++	++
3	F4-R1	1	300	++	+++	++	++++
4	F6-R1	5	470	-	-	+	+
			460	+	+	++	++
			410	+	+	+	+
			380	+	+	+	+
			360	+	+	+	+
5	F2-R4	6	600	-	+++	-	+
			380	+	-	-	-
			367	+	+++	+	++
			350	+	+	+	+
			333	+	-	-	-
			321	+	-	-	-
6	F4-R4	6	382	-	+	-	-
			376	-	+	-	-
			370	+	++	++	++
			340	+	+++	++	++
			200	+	+	++	++
			140	+	+	++	++
7	F2-R3	3	1200	+	-	-	-
			1150	-	-	+	-
			250	-	-	-	+
8	F6-R4	11	1300	-	-	-	+
			1250	+	-	-	+
			1200	-	++	++	++
			1105	+	-	-	-
			1000	+	++	+	+++
			800	-	++	+	+++
			690	-	-	-	+
			520	-	-	+	+
			480	+	+	+	+
			200	+	+	+	+
			160	+	+	+	+

9	F1-R5	7	1000	-	-	-	+
			610	+	+	-	+
			500	+	-	-	+
			258	+	-	-	-
			240	++	++	++	++
			200	+++	+++	+++	+++
			140	++	++	++	++
10	F2-R5	2	1250	+++	+++	-	++++
			700	-	-	+	-
11	F4-R5	10	140	++	++	++	++
			152	+	+	+	+
			200	++	++	++	++
			490	-	-	+	+
			500	++++	++++	++++	++++
			510	++++	++++	++++	++++
			870	++	++	++	++
			1000	+++	+++	+++	+++
			1200	+	+	+	+
			1250	++	++	++	++
12	F6-R5	5	115	++	+	++	-
			250	++	++	++	++
			480	++	++	++	++
			500	+++	+++	+++	+++
			610	-	+	-	+

- : no expression
* : low expression
**** : high expression

After the cDNA-SRAP gene expression analysis, semi-quantitative evaluation of the relative mRNA accumulation of four genes namely, Aquaporin, DREB2, DREB1 and C_2H_2 induced by dehydration stress was performed. Relative function of the drought responsive genes/TFs is presented in Table 4.12. Tolerant accessions showed higher expression levels of drought responsive genes/TFs even in unstressed conditions as compared to susceptible accessions. Aquaporin belongs to major intrinsic protein super family which functions as a membrane channel. The designed primer amplified a segment of 145 bp in the selected contrasting accessions in unstressed and stressed conditions. qRT-PCR analysis showed an upregulation of aquaporin in stressed condition in both the accessions (Plate 4.8). Based on the IDV values, the relative upregulation was found to be 27.61 % due to dehydration stress in IC97189 (Table 4.13). Peng et al. (2007) also reported that aquaporin enhances drought and salt tolerance ability in transgenic Arabidopsis plants (Peng et al., 2007).

Dehydration Responsive Elements have been reported to be involved in various types of abiotic stress responses via ABA-dependent and ABA-independent pathways (Shinozaki and Yamaguchi-Shinozaki, 1997). The DREB transcription factor family is one of the largest and is broadly divided into DREB1 and DREB2 sub families and each sub family contain several paralogs. Among the eight DREB2-type proteins that are reported in Arabidopsis thaliana, DREB2A and DREB2B are thought to be major transcription factors that function under drought and high-salinity stress conditions (Sakuma et al., 2002; Sakuma et al., 2006). In the present study, the designed DREB2 primer amplified a fragment of 215 bp, whereas, DREB1 amplified a fragment of 500 bp in the selected contrasting accessions in unstressed and stressed conditions. qRT-PCR analysis showed an upregulation of transcript in stressed condition in both the accessions (Plate 4.8). Based on the IDV values, qRT-PCR analysis of DREB2 showed the relative upregulation of about 7.86 % due to dehydration stress in IC97189. Whereas, DREB1 showed the relative upregulation of about 11.80%. Both DREB1 and DREB2 showed higher expression in both unstressed and stressed condition in tolerant accession, IC97189. Several DREB1/DREB2 homologous genes have been isolated from many plants including wheat, rice, barley, rye, sorghum, and oat (Nakashima et al., 2009). The up-regulation of these transcripts in the tolerant cultivar clearly suggests their role in providing dehydration stress tolerance to the selected accession.

Table 4.12 Function and upregulation pattern of the drought responsive genes/TFs

SN	Gene	Function	Increase (%)
1.	Aquaporin	Function as membrane channels that selectively transport water, small neutral molecules, and ions out of and between cells	27.61
2.	DREB1	DNA-binding domain found in transcription regulators in plants such as APETALA2 and EREBP	11.80
3.	DREB2	DNA-binding domain found in transcription regulators in plants such as APETALA2 and EREBP	7.86
4.	C_2H_2	Sequence-specific DNA binding transcription factor activity	18.67

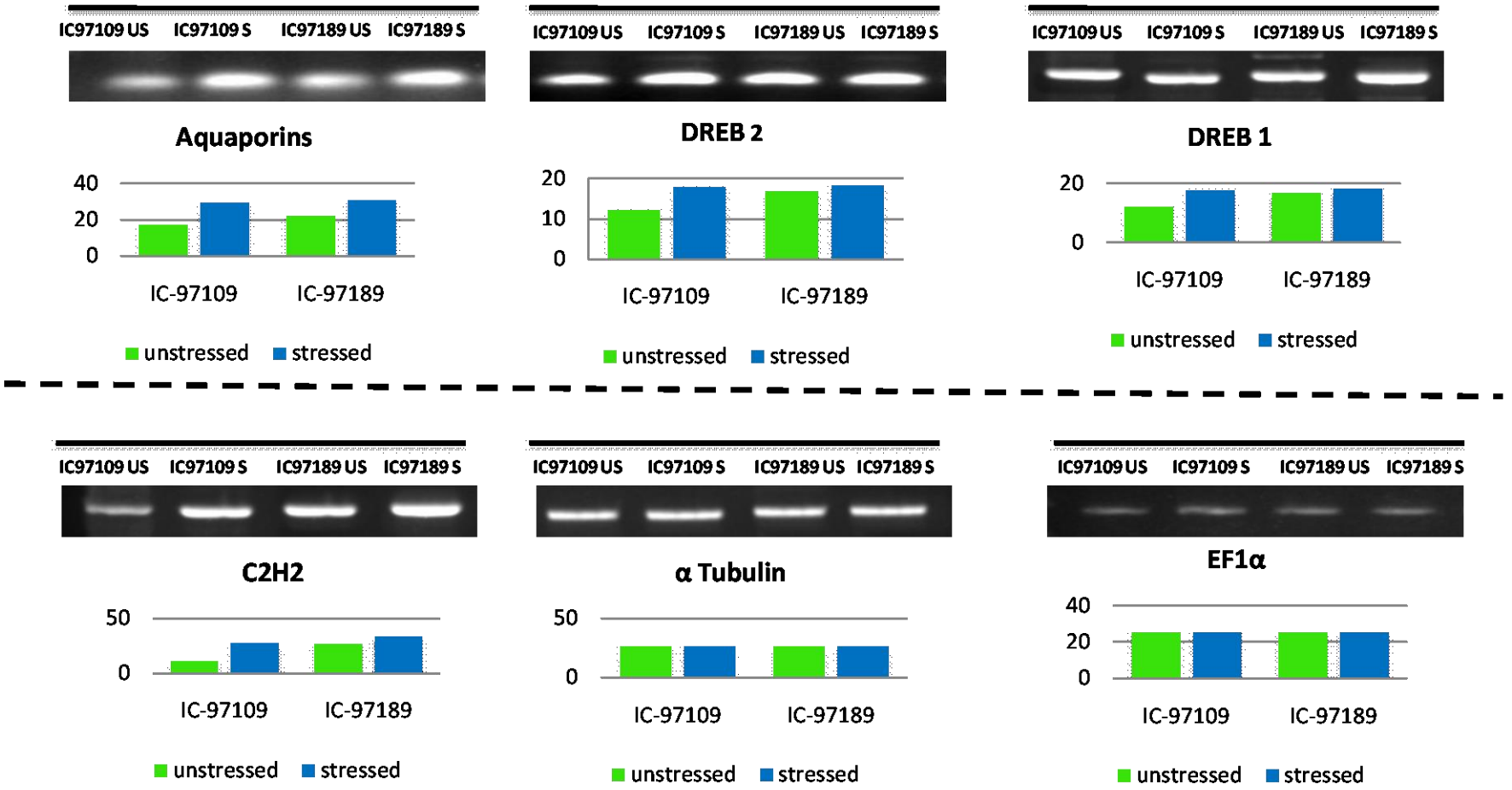


Plate 4.8 Semi-quantitative RT-PCR profile of drought related candidate genes

Samples from two contrasting foxtail millet accessions, IC-97109, IC-97189 in response to water stress were run on 1.5% agarose gel; Graphs show differential expression of Aquaporin, DREB2, DREB1 and C_2H_2; α tubulin and EF1 α used as internal controls; US: unstressed; S: stressed.

The present study also showed an upregulation of C_2H_2 type of zinc finger transcription factors of about 18.67 % in tolerant accession, IC97189 under water stressed condition. Expression was also found to be higher in unstressed condition in both tolerant and susceptible accession. C_2H_2 type of zinc finger transcription factors (TFs) play crucial roles in plant stress response and hormone signal transduction. Several members of the C_2H_2-type ZF family have been reported to play diverse roles in the plant stress response and the hormone signal transduction. Transcription profiling studies have shown that the transcript level of many C_2H_2-type ZF proteins is elevated under different abiotic stress conditions such as cold, salt, drought, osmotic stress and oxidative stress (Kiełbowicz-Matuk, 2012). A number of stress-responsive C_2H_2-type zinc finger TFs were also reported in response to drought stress (Tian et al. 2010; Sun et al. 2010; Kodaira et al. 2011).

Upregulated expression due to stress causing notable changes in gene expression particularly those involved in metabolism, proteolysis, and stress signaling has been suggested by many scientist (Zhang et al.,2007; Lata et al., 2010). Hence induction of these transcripts suggests that these genes might impart drought avoidance capacity to the tolerant accession in comparison to the sensitive one, as the expression analysis of up-regulated transcripts between drought tolerant and susceptible cultivars upon dehydration stress suggests their function in dehydration adaptation and tolerance in foxtail millet (Lata et al., 2010). The differential gene expression of drought responsible transcriptional factors like Aquaporins and C_2H_2 obtained could fetch out the molecular mechanism underneath drought tolerance in *Setaria italica.*

The main objective of this study was to identify putative genes that were consistently expressed in tolerant or susceptible accessions in response to water stress condition. Here, aquaporin showed considerable upregulation, hence, considering their importance, genome-wide identification was carried out for detailed study of their role in drought tolerance.

Table 4.13 IDV values of differentially expressed transcripts of water stress responsive genes.

Accessions	Aquaporin		DREB2		DREB1		C2H2		α TUBULIN		EF1 α	
	US	S	US	S	US	S	US	S	US	S	US	S
IC-97109	17.32	29.29	12.15	17.91	23.72	25.07	11.60	27.50	26.10	26.10	25.19	25.19
IC-97189	22.41	30.96	16.86	18.30	23.99	27.20	27.30	33.57	26.10	26.10	25.19	25.19

US-unstressed; S-stressed

Table 4.14 IDV values of differentially expressed PIPs in response to water stress.

Accession	PIP1;1		PIP1;2		PIP1;6		PIP2;5		PIP2;6a		PIP2;7		PIP2;5 LIKE	
	US	S	US	S	US	S	US	S	US	S	US	S	US	S
IC-97109	9.06	22.31	1.40	11.48	6.56	27.22	6.56	27.22	40.95	25.54	6.26	12.69	9.16	9.89
IC-97189	33.66	34.95	21.66	65.44	6.28	59.93	6.28	59.93	21.27	12.21	13.22	67.82	32.12	48.81

US-unstressed; S-stressed

4.4 Genome wide investigation of aquaporins gene family in foxtail millet and differential gene expression studies

Aquaporin belongs to a highly conserved group of membrane proteins (with molecular masses of between 26 and 30 kDa.) called major intrinsic proteins that facilitate water transport across biological membranes. Aquaporins contain six membrane spanning helices and 5 loops (loops A to E) with N- and C-termini residing in the cytosol (Jung et al., 1994). Loops A and C are extracellular and loop D is intracellular. Loops B and E fold back into the membrane from the inner (B) and the extracellular (E) sides forming two short half helices penetrating the membrane as a seventh helix. They contain the highly conserved duplicated asparagine-proline-alanine (NPA) signature motif (Figure 4.5).

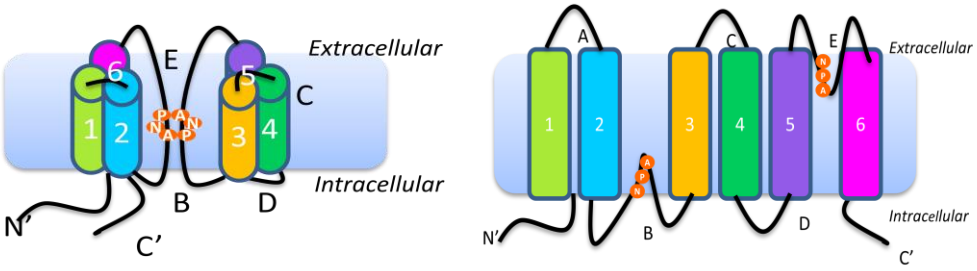


Figure 4.5 Hourglass model of AQP showing its membrane topology: Schematic representation of an AQP. Six transmembrane alpha helices (1 to 6) are connected by five loops (A to E). With folding of loop B and E the two helical domains containing the conserved NPA motifs overlap within the lipid bilayer to form a single aqueous pathway.

On the basis of sequence homology, aquaporins in most plant species can be divided into four subgroups. The plasma membrane intrinsic proteins (PIP) (with two phylogenic subgroups, PIP1 and PIP2) and the tonoplast intrinsic proteins (TIP) are the most abundant aquaporins in the plasma membrane and vacuolar membrane (tonoplast), respectively (Johanson et al., 2001; Quigley et al., 2001). The third subfamily comprises the nodulin- 26–like intrinsic membrane proteins (NIPs), which were named after soybean (*Glycine max*) nodulin-26 (*Gm*NOD26), an abundant aquaporin expressed in the peribacteroid membrane of N_2-fixing symbiotic root nodules. NIPs are also present in non-legume plant species. A fourth class comprises small basic intrinsic proteins (SIPs) (Ishikawa et al., 2005; Johanson et al., 2001; Quigley et al., 2001). Although these four classes are conserved among all plant

species, the aquaporin gene family shows signs of rapid and recent evolution and orthologs cannot necessarily be distinguished between species (Sakurai et al., 2005).

4.4.1 *In silico* investigations of aquaporin genes in foxtail millet

Aquaporin gene identification studies in plants have primarily relied on *in silico* methods. After critical searching the foxtail millet genome, 43 members were defined as aquaporin genes (Table 4.15). The length of aquaporin ORF in foxtail millet ranged from 195-1134 bp. BLAST analysis against the Pfam and SMART database indicated that all of them belonged to the aquaporin gene family. The predicted aquaporin protein contains the highly conserved duplicated asparagine-proline-alanine (NPA) signature motif specific to aquaporin family members. The 43 aquaporin genes were distributed on nine foxtail millet chromosomes with chromosomes 1, 5, and 9 having 7 genes each, chromosomes 6 and 7 having 5 gene each, chromosomes 2 and 4 having 4 genes each while chromosomes 3 and 8 having 3 and 1 gene respectively.

Aquaporins have been identified in rice (Sakurai et al., 2005), *Arabidopsis* (Johanson et al., 2001) *Vitis vinifera* L. (Fouquet et al., 2008), *Physcomitrella patens* (Danielson et al., 2008), *Populus trichocarpa* (Gupta et al., 2009), *Zea mays* L. (Chaumont et al., 2001), *Triticum aestivum* L. (Forrest and Bhave, 2007), *Nicotiana tabacum* L. (Siefritz et al., 2001), and *Pisum sativum* L. (Schuurmans et al., 2003). In addition, many research groups have investigated the effects of salt, drought, or cold stress on the expression of aquaporin genes in various plant species (Liu et al., 1994; Yamada et al., 1995, 1997; Mariaux et al., 1998; Uno et al., 1998; Gao et al., 1999; Li et al., 2000; Kawasaki et al., 2001; Smart et al., 2001; Hoth et al., 2002; Kreps et al., 2002; Suga et al., 2001).

Table 4.15 *In silico* investigations of aquaporin genes in foxtail millet and their sequence characteristics

Sr. No.	Gene	Transcript name	Chromosome	CDS (bp)	Phosphorylation			No. of introns
					Ser	Thr	Tyr	
1.	PIP2;5 like	Si036923m	9	855	7	2	2	0
2.	PIP1;1	Si010758m	7	867	7	1	1	3
3.	SIP1;1	Si027934m	8	600	1	1	0	1
4.	TIP1;1	Si037174m	9	750	5	1	3	1
5.	NIP1;1	Si018035m	1	837	6	1	0	3
6.	PIP2;1	Si030703m	2	870	5	1	1	2
7.	PIP2;1	Si039883m	9	885	10	2	2	3
8.	SIP2;1	Si037152m	9	759	2	1	0	2
9.	TIP2;1 x1	Si004690m	5	732	3	0	0	1
10	TIP2;1	Si018184m	1	750	6	0	1	1
11	NIP2;1	Si017716m	1	1026	13	2	0	5
12	TIP3;1	Si037080m	9	789	4	3	1	1
13	TIP3;1	Si037121m	9	774	4	2	1	1
14	NIP3;1	Si036817m	9	900	4	4	2	3
15	NIP4;1	Si002921m	5	633	2	0	2	3
16	TIP5;1	Si011789m	7	780	5	1	1	2
17	TIP5;1	Si019510m	1	780	10	3	1	2
18	PIP1;2	Si017731m	1	1014	14	1	1	3
19	SIP1;2	Si023083m	3	732	1	2	1	2
20	TIP1;2	Si004276m	5	807	12	2	1	1
21	TIP2;2	Si007145m	4	759	5	0	0	2
22	NIP2;2	Si007007m	4	894	11	4	0	4
23	TIP3;2	Si012227m	7	729	1	0	0	2
24	NIP3;2	Si015217m	6	834	4	1	0	3
25	NIP3;2	Si015338m	6	876	7	3	1	0
26	NIP3;2	Si014171m	6	891	8	3	2	3
27	NIP3;2	Si015485m	6	861	8	1	1	3
28	NIP3;2	Si015350m	6	663	7	3	1	4
29	TIP4;2 x1	Si022367m	3	1134	8	4	1	3
30	NIP1;3	Si005574m	5	195	2	1	0	0
31	NIP1;3	Si022896m	3	846	5	3	0	3
32	TIP2;3	Si010925m	7	747	6	1	0	1
33	TIP4;3	Si003768m	5	741	2	2	0	1
34	TIP4;3 x1	Si002696m	5	741	2	2	0	2
35	NIP1;4	Si008400m	4	861	7	0	3	3
36	PIP2;4	Si017990m	1	867	8	0	1	2
37	TIP4;4	Si002674m	5	753	1	4	1	1
38	PIP1;5	Si017991m	1	867	10	2	3	1
39	PIP2;5	Si010750m	7	873	8	1	3	2
40	PIP1;6	Si007002m	4	900	8	1	2	1
41	PIP2;6	Si030713m	2	861	10	0	1	2
42	PIP2;6	Si030712m	2	861	9	1	2	2
43	PIP2;7	Si030718m	2	858	8	2	2	3

Ser=serine; tht=theronine; Tyr=tyrosine.

4.4.2 Phylogenetic analysis, gene structure and conserved motifs of aquaporin genes in foxtail millet

To examine the phylogenetic relationships among the identified aquaporins in foxtail millet an unrooted phylogenetic tree was constructed from alignments of the full length aquaporin sequences (Plate 4.9). The 43 aquaporin genes were classified into four different subfamilies based on their sequence similarity comprising of 12 plasma membrane intrinsic proteins (PIPs), 17 tonoplast intrinsic proteins, 11 NOD26-like MIPs or NOD26-like intrinsic proteins, and 3 small basic intrinsic proteins. The PIP subfamily is further divided into two subgroups named PIP1 and PIP2 that have specific arrays of amino acids at the N and C-termini (Schaffner, 1998). According to the nomenclature by Johanson et al. (2001), the PIP1 subgroup representing five members of aquaporins is named PIP1;1 to PIP1;5, and PIP2 subgroup consisting of eight members of aquaporins is named PIP2;1 to PIP2;8 in *Arabidopsis.* In the present investigation, PIP1;1, PIP1;2, PIP1;5, PIP1;6 of PIP1 subgroup are found to be present in *Setaria italica* L. The PIP2 subgroup in *Setaria* compromises of six homologues comprising of PIP2;1, PIP 2;2, PIP 2;4, PIP2;5, PIP2;6, and PIP2;7.

To gain further insights into the structural diversity of aquaporin genes, we analyzed the exon/intron organization in the full-length cDNAs with their corresponding genomic DNA sequences in foxtail millet (Plate 4.9). Most closely related aquaporin members within the same subgroups shared similar gene structures in terms of either intron numbers or exon lengths. The coding sequences of all the aquaporins genes were disrupted by introns, with numbers varying from one to five except for gene PIP 2-5 like(Si036923m) and NIP 3-2 (Si015338m) which showed no intron. The identified genes showed more than one gene model, which could be attributed to alternative splicing. Gene structural diversity is a possible mechanism for the evolution of multigene families (Hu et al., 2010).Putative protein motifs were predicted using MEME to further confirm the presence of duplicated NPA motifs (Figure 4.6).

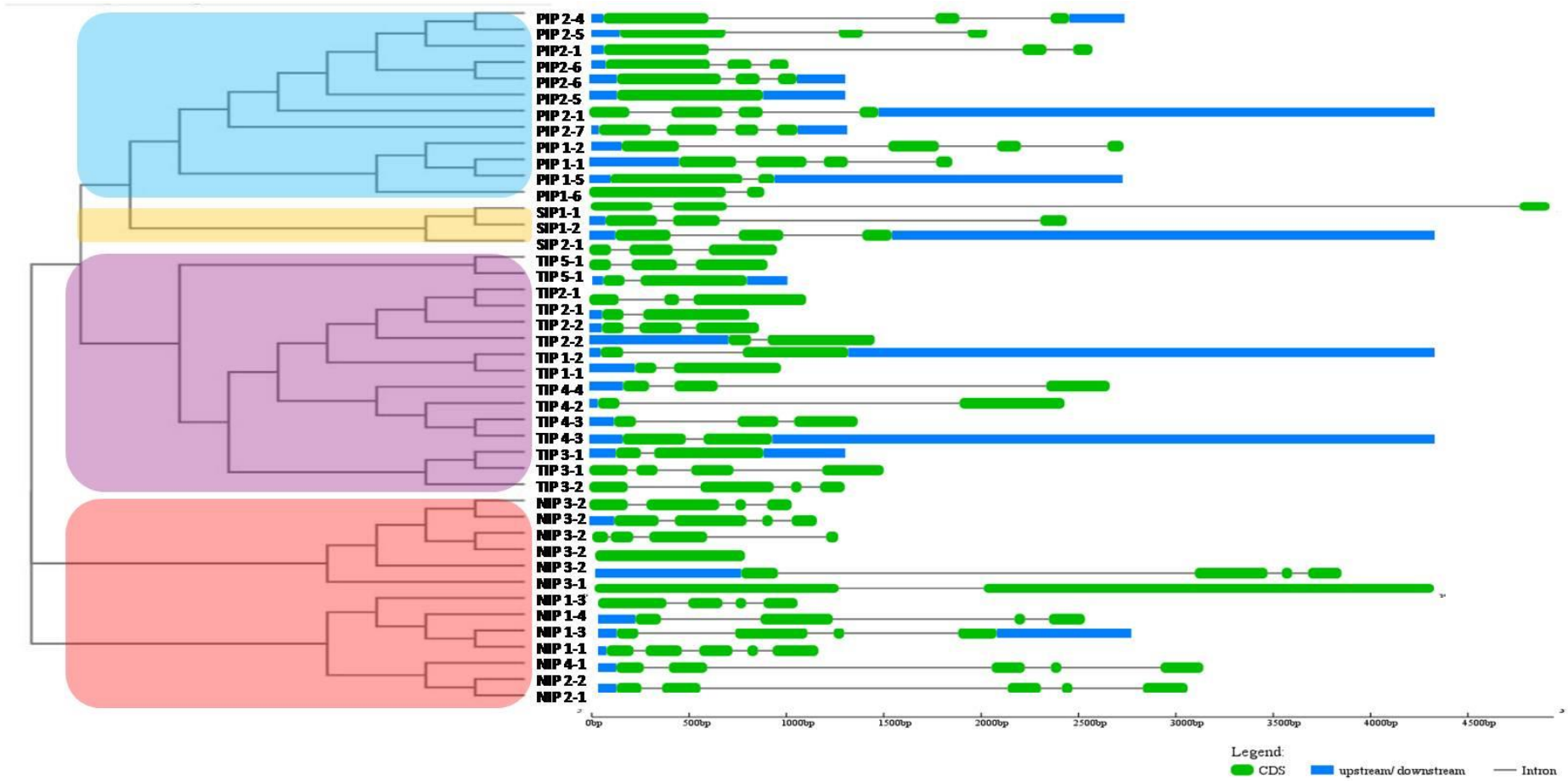


Plate 4.9 Phylogenetic tree and gene structural organization of identified 43 Aquaporins in *Setaria italica* L.

The tree shows presence of 4 classes viz. PIP, SIP. TIP and NIP, colored in various shades . The intron-exon positions of respective genes are shown in the right, represented by colored boxes and black lines, respectively. Exon/intron structures were obtained from the Gene Structure Display Server.

Motif 1

Motif 2

Figure 4.6 Graphical representation of two conserved amino acid motifs in aquaporins displayed using MEME logo tool

The overall height of the stack indicates the conserved sequence at that position, while the height of symbols within the stack indicates the relative frequency of each amino acid at that position.

Several post-translational modifications have been found to regulate aquaporin activity. Phosphorylation of aquaporin seems to be an important mechanism for aquaporin regulation involved in a broad range of processes and that can itself be regulated by external parameters. Phosphorylation of soybean nodulin 26 at serine residue 262 is stimulated in response to water deficit, resulting in enhanced transport activity (Guenther et al., 2003). Furthermore, phosphorylation of a plasma membrane aquaporin in tulip has been shown to accompany opening of petals (Azad et al., 2004). Hence the phosphorylation sites were predicted using the NETphos online tool. The phosphorylation sites varied from 1 to 16 with Maximum phosphorylation sites predicted in PIP1;2 (Si017731m) while only 1 phosphorylation site was predicted in TIP3;1(Si012227m). Phosphorylation is thought to be important post-translational modifications in aquaporins for their activity (Table 4.15).

4.5 Expression profile of foxtail millet aquaporins genes in response to water stress

Investigating the function of specific isoform(s) is a crucial objective for implementing the molecular understanding of plant water relations. In particular, knowing expression patterns of the PIPs that are considered to be involved in water exchange between the environment and plant cells is an indispensable step toward understanding the functions of PIPs and their

importance in plant responses to environmental stimuli. Hence the PIP sub family of aquaporin was selected for further detailed investigation. Several reports have shown the differential responsiveness of PIP genes to water stresses (Kreps et al., 2002; Seki et al., 2002[a]; Seki et al., 2002[b]; Affenzeller, 2003; Maathuis et al., 2003; Jang et al., 2004; Kawaguchi et al., 2004; Alexandersson et al., 2005; Boursiac et al., 2005). Out of the 12 PIPs of aquaporins found in foxtail millet, only 7 PIPs showed noticable amplification, whereas the other PIP aquaporins were not amplified. Semi-quantitative evaluation of PIP aquaporins induced by dehydration stress was performed which showed differential expression in all the PIPs with tolerant accessions showing higher expression levels. Water stress treatment most significantly altered the expression of PIPs. To maintain the normalisation of cDNA, reference gene primer like tubulin and EF1α were used which showed constant expression in unstressed and stressed conditions as discussed in section 4.3.

In the gene expression profiling studies, the primer designed for PIP1;1, amplified a segment of 351bp in the selected contrasting accessions in unstressed and stressed conditions. Based on the IDV values, qRT-PCR analysis showed an upregulation of aquaporin in stressed condition in the accessions IC97109 (59.37%), whereas, only 3% upregulation of aquaporin was obsereved in IC97189, but the expression in unstressed condition was higher in tolerant accession (Plate 4.10) (Table 4.14). The expression analysis of PIP1;2 amplified a segment of 557bp with very less expression in susceptible accession IC97109. Its relative expression in stressed condition in tolerant accession was found to be high and significant (with 66.89% increase). Weig et al. (1997) also demonstrated that among the PIPs, PIP1;1 and PIP1;2 were most abundantly expressed under drought stress in *Arabidopsis*.

PIP1-6 showed good amplification and upregulated expression pattern under stressed condition. IC97109 showed 75.90% upregulation whereas IC97109 showed 89.51% upregulation. Similarly expression analysis of PIP2;5 showed an 89.51% upregulation under water stressed condition in IC97189. PIP2;6a showed more upregulation (42.57%) in stressed condition

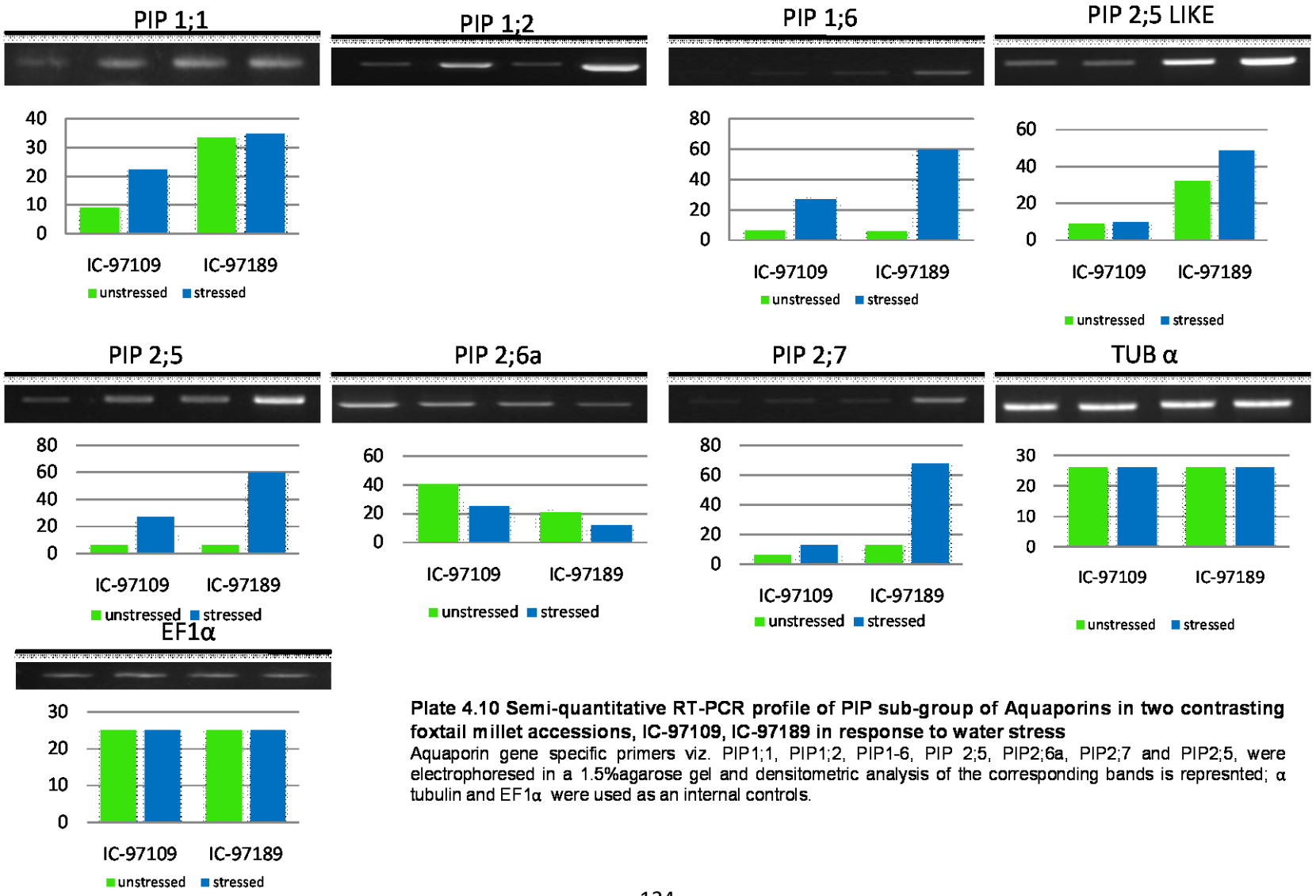


Plate 4.10 Semi-quantitative RT-PCR profile of PIP sub-group of Aquaporins in two contrasting foxtail millet accessions, IC-97109, IC-97189 in response to water stress

Aquaporin gene specific primers viz. PIP1;1, PIP1;2, PIP1-6, PIP 2;5, PIP2;6a, PIP2;7 and PIP2;5, were electrophoresed in a 1.5%agarose gel and densitometric analysis of the corresponding bands is represnted; α tubulin and EF1α were used as an internal controls.

in the accessions IC97109, however the upregulation in IC97189 was relatively less (37.62%), but the expression in unstressed condition was higher in tolerant accession.

Amongst all the PIPs, PIP2;7 showed highest upregulation under water stressed condition with a percent increase of 80.48%. Up-regulation of the PIP subfamily in the tolerant cultivar clearly suggests its role in providing dehydration stress tolerance to the genotype. In the present study, PIP1;2, 2;7 and PIP2;5 like aquaporin showed higher expression in stressed condition. Similar results have been observed by Jang et al. (2004); Weig et al. (1997);Quigley et al. (2001); Javot et al. (2003) in *Arabidopsis.*

In the present study, only PIP2;6 was found to be downregulated under water stress. These results are similar to that of Jang et al.(2004) where PIP1;1, PIP1;2, and PIP2;3 were up-regulated, and PIP1;5 and PIP2;6 were down-regulated by salt treatment in *Arabidopsis* (Jang et al., 2004). The initial down-regulation and the subsequent up-regulation of aquaporin gene expression have also been observed in rice cultivars (Kawasaki et al., 2001) and Arabidopsis (Maathuis et al., 2003). This different down- or upregulation of aquaporin gene expression during stress may play roles in limiting initial water loss during the early stage of stress and assisting the subsequent uptake of water to maintain water homeostasis. The up- or down-regulation of a variety of PIP genes in plants subjected to drought stress imply that lower or higher expression of these aquaporin genes is beneficial to keep a suitable status of water under stressed conditions.

The complex expression pattern of PIP genes suggests that maintenance of a proper water status drought stress requires both increased water transport via aquaporins in some cells and reduced water transport via aquaporins in other cells and tissues. However, it is too early to confirm the relationship between its expression and the beneficial or deleterious effect of each aquaporin on plants simply by comparing the expression patterns of PIP genes under stressed conditions. Although the expression levels of specific aquaporin genes are important to control water transport in plants under environmental stimuli, the molecular and cellular mechanisms that link stress stimuli to the activity of aquaporins in cell membranes remain poorly understood.

In the present study, we focused on the aquaporins that are responsible for the transcellular movement of water across the cell membrane, and deciphered that the expression of the PIP genes responded significantly and differently to environmental stress conditions. However, regulation of the expression or activity of water channel by itself is unlikely to be able to explain the large variations in hydraulic conductivity at the level of tissues and organs in plants exposed to various environmental conditions. More complete analyses for dissecting the contribution of the transport paths to overall water movement are required to better understand the function of aquaporins in stress-related physiological processes. Here, as the PIP2;7 aquaporin showed highest upregulation under water stress, an attempt was made fully characterise the gene.

4.6 Molecular Cloning and Sequence Characterization of PIP 2;7 aquaporin gene

The availability of complete genomic sequences for foxtail millet (*Setaria italica* L.) has helped us to clone and characterise full sequence of PIP2;7 gene. Amplification with PIP2;7 gene CDS primers was done to obtain full length gene. Here, we tried to characterise the CDS sequence and we were able to isolate a 770bp long DNA stretch out of the 857 base pair CDS. Gene structure prediction using the genomic and CDS sequences confirmed the presence of two introns (Figure 4.7). Database analysis with BLAST revealed that the deduced sequence has high homology to a UNPRIDICTED PIP2;7 gene in *Setaria italica* L. with an identity of 99% and query coverage of 95%. Details of BLAST search are presented in Table 4.16.

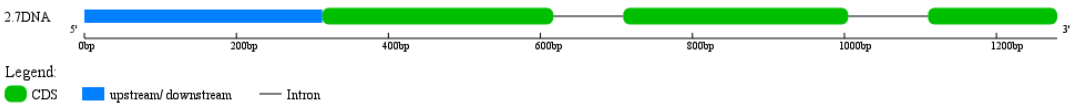


Figure 4.7 Exon-intron structures of sequenced 2;7 aquaporin in foxtail millet Coding exons, represented by green, lines connecting two exons represent introns.

>PIP 2;7 Genomic sequence

GACCTGTACCTCTACGTGCCAACATTCTTGTGGCTTTCCTCCCGCCCATATATGTGTATCGC
AACTTGGACCCTATCGAACTTCACCATCTTTATTTTGAAATTCTAGCGAAGTACGGAAAAAG
ATGGATAAATTCATGGCAACCGGTGGGTCGCAAGGAAGGAATATAATCTTTTGAAAGATCTT
CTTCGGCCGTGTTCACAGCTCTTCCTCCGGTCCCTGGCCTCCCTATTTAAACCACGGCCATT
GCCTTATCCTCCGCGCGTCCGAAGAAGTAACCCTAGTTAACAGTCGAAGCTACGGAGACCGA
GCCATGCCGATCGAGGACGTGAGCATCGAGACGACCGAGGCGGCGGGCCCCCAAAAGGTGCC
GTACTGGGACCCGCCGCCGGCGCCGCTCCTGGAAACGAGCGAGCTGATGAAGTGGTCGCTGT
ACCGCGCGCTCATCGCCGAGTTCGTGGCCACCCTCATCTTCCTCTACGTGAGCATCACCACC
GTCATCGGGTACAAGGACCAGTCCAAGGCCCTGGCGTGCAACGGCGTGGATTCCTCGGCGTC
GCCTGGTCCTTTTGGCGCCACCATCTTCATCCTCGTGTACTGCATCGGCGGCATCTCAGGTA
CTGTAGCATGCAATGTGCGTGCATGGACGCGCTCGCTAGCTGCTGTACGAATCGAAATGAAT
TGACGCGAGCATGGGACATGGGACCAGGTGGGCACATCAACCCGGCCGTGACGTTCGGGCTG
TTCGTGGGGCGGAAGCTGTCGCTGTTGCGCACCGTGCTGTACATCGTGGCGCAGTGCCTGGG
CGCCATCTGCGGCGTGGCCATCGTGAAGGGCATCACGGGGGATCAGTACAGCCTCCTCGGCG
GCGGCGCAAACTCAGTGGCCGACGGCTTTTCCGTCGTGGCCGGCCTCGGCGCCGAGATCATG
GGCACGTTCGTCCTCGTTTACACCGTCTTCTCCGCCACCGACCCCAAGCGCACCGCGCGAGA
CTCATTCATCCCGGTACGAGCGCATCTCCTCTCCATCTGTACATTTAATCTTGTTACAAAAG
TGCAAGTTAATAGTGTAATCAATTAATGTGCTGACCTGGAACGAGTGGCCGTGCAGGTGCTG
GTGCCGCTGCCGATTGGGTTCGCGGTGTTCGTGGTGCACCTGGCGACCATCCCCATCACCGG
CACGGGCATCAACCCGGCCAGGAGCCTTGGGGCCGCCGTAATCTTCGCTGAGGCCCGGAAAA
ACCAAGGAAGATCAATCCGTGCGACAAGTCCCGCTAGAGG

>PIP 2;7 CDS sequence

ATGCCGATCGAGGACGTGAGCATCGAGACGACCGAGGCGGCGGGCCCCCAAAAGGTGCCGTA
CTGGGACCCGCCGCCGGCGCCGCTCCTGGAAACGAGCGAGCTGATGAAGTGGTCGCTGTACC
GCGCGCTCATCGCCGAGTTCGTGGCCACCCTCATCTTCCTCTACGTGAGCATCACCACCGTC
ATCGGGTACAAGGACCAGTCCAAGGCCCTGGCGTGCAACGGCGTGGATTCCTCGGCGTCGCC
TGGTCCTTTTGGCGCCACCATCTTCATCCTCGTGTACTGCATCGGCGGCATCTCAGGTGGGC
ACATCAACCCGGCCGTGACGTTCGGGCTGTTCGTGGGGCGGAAGCTGTCGCTGTTGCGCACC
GTGCTGTACATCGTGGCGCAGTGCCTGGGCGCCATCTGCGGCGTGGCCATCGTGAAGGGCAT
CACGGGGGATCAGTACAGCCTCCTCGGCGGCGGCGCAAACTCAGTGGCCGACGGCTTTTCCG
TCGTGGCCGGCCTCGGCGCCGAGATCATGGGCACGTTCGTCCTCGTTTACACCGTCTTCTCC
GCCACCGACCCCAAGCGCACCGCGCGAGACTCATTCATCCCGGTGCTGGTGCCGCTGCCGAT
TGGGTTCGCGGTGTTCGTGGTGCACCTGGCGACCATCCCCATCACCGGCACGGGCATCAACC
CGGCCAGGAGCCTTGGGGCCGCCGTAATCTTCGCTGAGGCCCGGAAAAACCAAGGAAGATCA
ATCCGTGCGACAAGTCCCGCTAGAGG

Table 4.16 Details of BLAST homology search against the characterised CDS

Description	Query cover	E value	Ident	Accession
PREDICTED: *Setaria italica* probable aquaporin PIP2;7 (LOC101753181), mRNA	95%	0.0	99%	XM_004957448.2
PREDICTED: *Setaria italica* aquaporin PIP2;5-like (LOC105913608), mRNA	83%	4e-120	79%	XM_012842720.2
PREDICTED: *Setaria italica* aquaporin PIP2-4 (LOC101753029), mRNA	84%	5e-119	79%	XM_004953115.2
PREDICTED: *Setaria italica* aquaporin PIP2;1 (LOC101755066), mRNA	84%	4e-110	78%	XM_004956056.3
PREDICTED: *Setaria italica* aquaporin PIP2;5 (LOC101761554), mRNA	84%	1e-100	77%	XM_004976197.2
PREDICTED: *Setaria italica* aquaporin PIP2;6 (LOC101756292), mRNA	83%	2e-97	77%	XM_004956059.2
PREDICTED: *Setaria italica* aquaporin PIP2;1-like (LOC105914220), mRNA	11%	5e-29	93%	XM_012845268.1

4.6.1 Pair wise alignment

ClustalW sequence alignment with the CDS from phytazome showed 87.4% similarity with highly conserved regions (Plate 4.11). Sequence alignment was also done using Mview to align the sequences based on identity and property of amino acids which showed 72.5% similarity (Plate 4.12).

Following the alignment, virtual frame for amino acid was determined using ExPASy translate tool (http://web.expasy.org/translate/). The correct ORF of 256AA showing NPA region was selected as it is an important characteristic of plant aquaporins. Protein BLAST with selected frame as query shows significant sequence similarity with PIP2;7 unpredicted protein in *Setaria italica* L.

Virtual amino acid sequence (256 AA)

MPIEDVSIET TEAAGPQKVP YWDPPPAPLL ETSELMKWSL YRALIAEFVA TLIFLYVSIT
TVIGYKDQSK ALACNGVDSS ASPGPFGATI FILVYCIGGI SGGHINPAVT FGLFVGRKLS
LLRTVLYIVA QCLGAICGVA IVKGITGDQY SLLGGGANSV ADGFSVVAGL GAEIMGTFVL
VYTVFSATDP KRTARDSFIP VLVPLPIGFA VFVVHLATIP ITGTGINPAR SLGAAVIFAE
ARKNQGRSIR ATSPAR

4.6.2 Structure prediction

The 3D structure of protein is very important in understanding the protein interactions, functions, and localisations (Parasuram et al., 2010). Structure of PIP 2;7 aquaporin was predicted using Phyre2 online tool for protein structure prediction. Homology modelling is the most common structure prediction method. Moreover, finding a best matching template using similarity searching programs like PSI BLAST against a PDB database has been considered the basic step in homology modelling. Templates were selected based on their sequence similarity with query sequence. The structures were sealed in PDB file format for further analysis. Theoretical model of the tertiary structure shows the presence of amphipathic channel and Asn-Pro-Ala signature motifs that it has a highly conserved feature in aquaporins. The secondary structure revealed the presence of 62% alpha helices, 0% beta turns, and 49% TM helices.

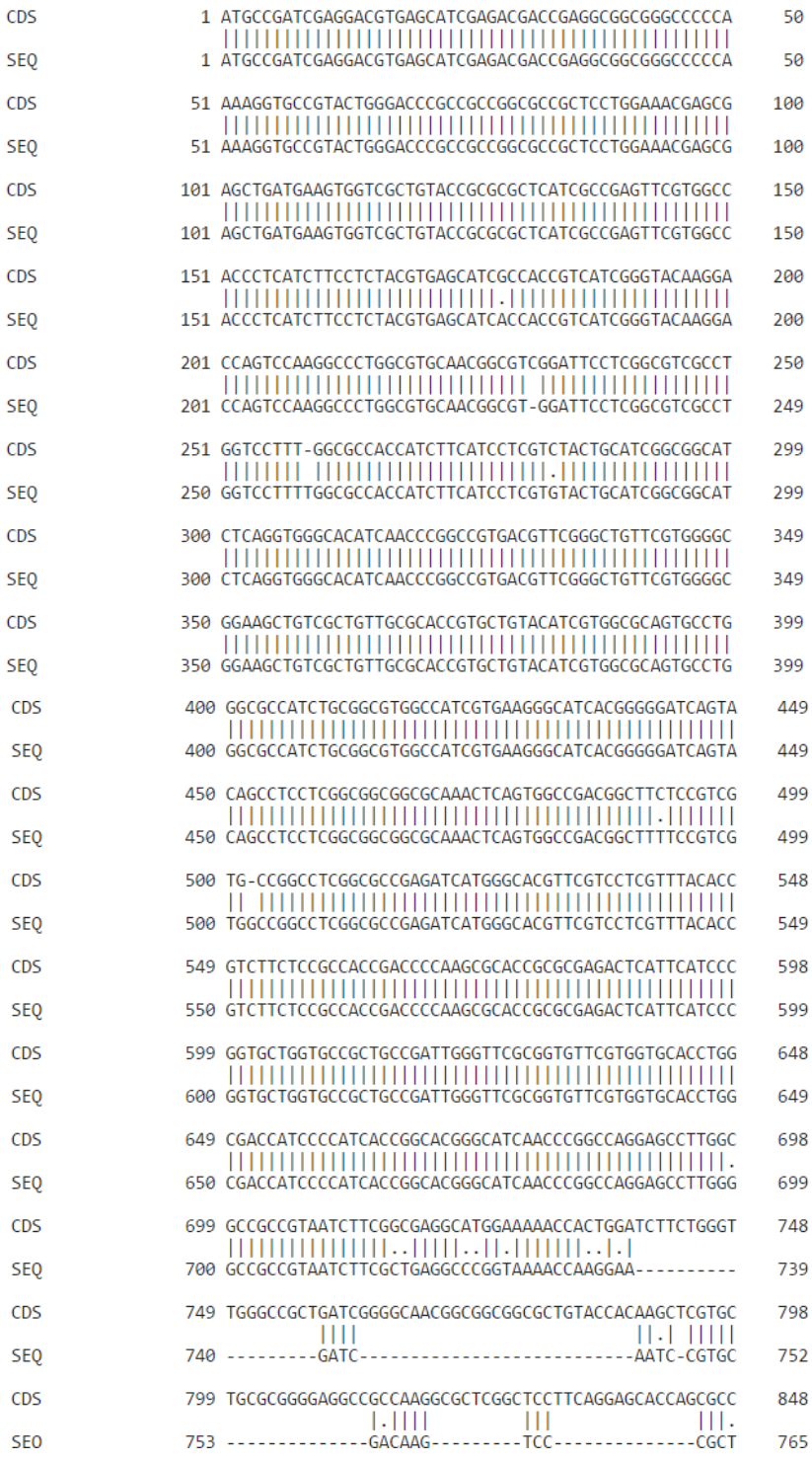


Plate 4.11 Sequence alignment of the sequenced PIP 2;7 aquaporin (Si030718m) using ClustalW,

Identity: (87.4%) ; Similarity: (87.4%) ; Gaps: (10.6%); Score: 3203

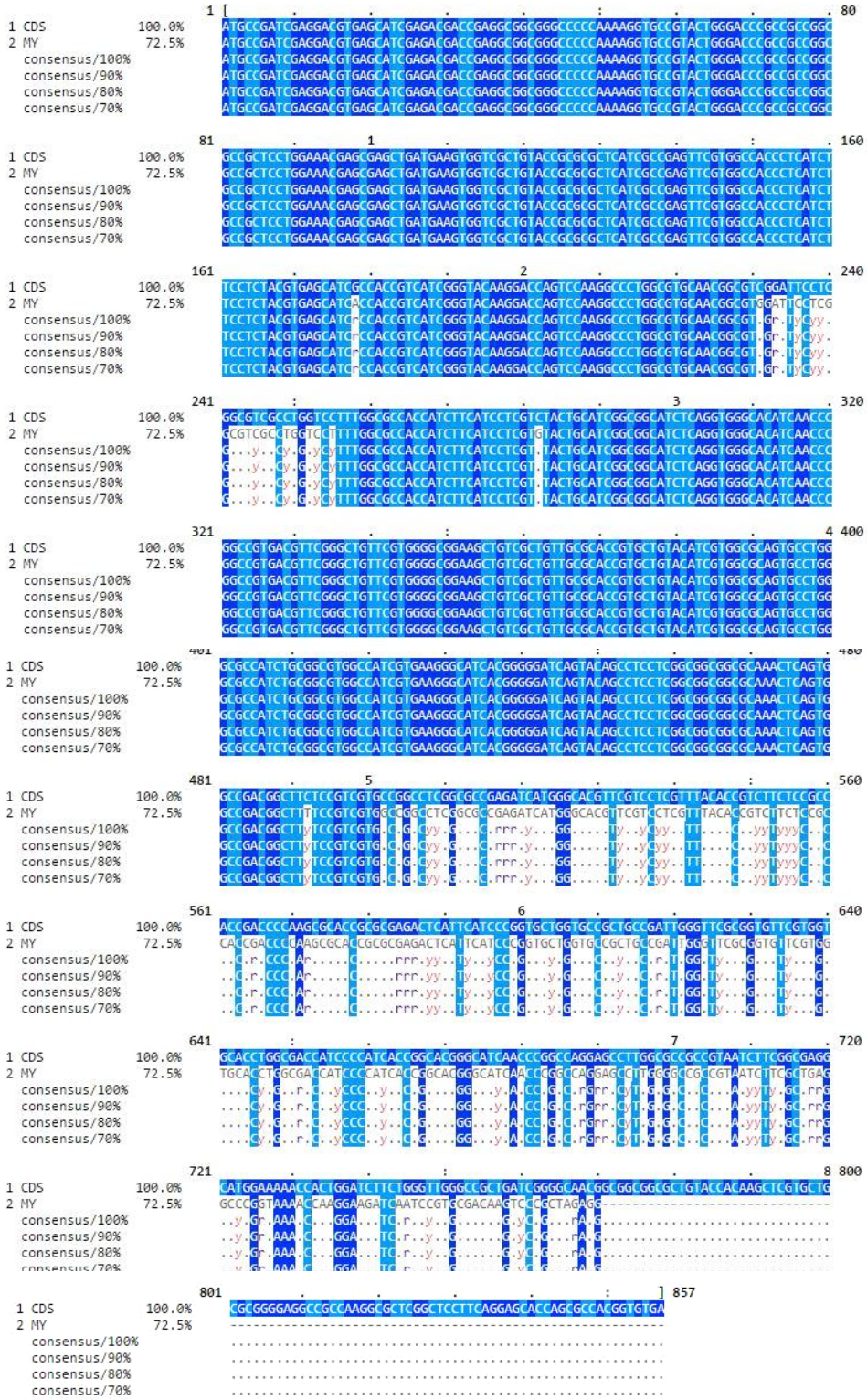


Plate 4.12 Sequence alignment of the sequenced PIP 2;7 aquaporin (Si030718m) from foxtail millet with CDS retrieved from Phytazome

Sequences were aligned using Mview and are colored by identity and property; the light or dark shaded backgrounds indicate partial or entirely conserved amino acid residues, respectively.

4.6.3 Structure validation

After protein structure prediction, the quality and reliability of structure was assessed by several structure assessment methods, including Z-score and Ramachandran plot. The Z-score is indicative of overall model quality and is used to check whether the input structure is within the range of scores typically found in native proteins of similar size. PROSA online tool was used to find the Z-score of the predicted structure. The Z-score of the protein was -2.16. Plot of residue scores shows local model quality by plotting energies as a function of amino acid sequence position *i*. In general, positive values correspond to problematic or erroneous parts of the input structure. RAMPAGE online server was used for preparation of Ramachandran plot. The Ramachandran plots of the initial and final models were depicted and compared after refinement. The result of the Ramachandran plot showed 92.5% of residues in the favoured region (Plate 4.13). Details of favoured and allowed regions are given in Table 4.17. The Z-scores and Ramachandran plot confirmed the quality of the predicted protein for PIP 2;7 aquaporin.

Table 4.17 Geometric characteristics of amino acid residue in favoured, allowed and outliner region of Ramachandran plot for PIP2;7 aquaporin

Allowed region			**Outlier region**		
Amino acid Residue	**Φ**	**Ψ**	**Amino acid Residue**	**Φ**	**Ψ**
[34 :GLU]	-105.4	-67.28	[19 :VAL]	-67.26	-10.90
[40 :LEU]	-60.21	169.68	[25 :PRO]	-122.75	125.73
[71 :ALA]	-86.43	-66.96	[26 :PRO]	-121.05	90.69
[106 :ASN]	-171.60	114.19	[76 :GLY]	-45.95	0.18
[157 :ALA]	57.01	10.29	[148 :ASP]]	84.16,	174.22
[159 :SER]	-51.18	-17.18	[199 :ILE]	167.71	1.52
[193 :THR]	-61.22	172.56	[224 :THR]	66.12	91.76
[196 :ASP]	93.88,	-7.64	**Outliner region**	**7 (3.1%)**	
[225 :GLY]	-85.60	-73.19	**Expected**	**-**	
[227 :ASN]	-178.57	100.48			
Allowed region	**10 (4.4%)**				
Expected	**~2.0 %**				
Residues in favoured region	**208 (92.4%)**				
Expected	**~98.0%**				
Total residues	**225**				

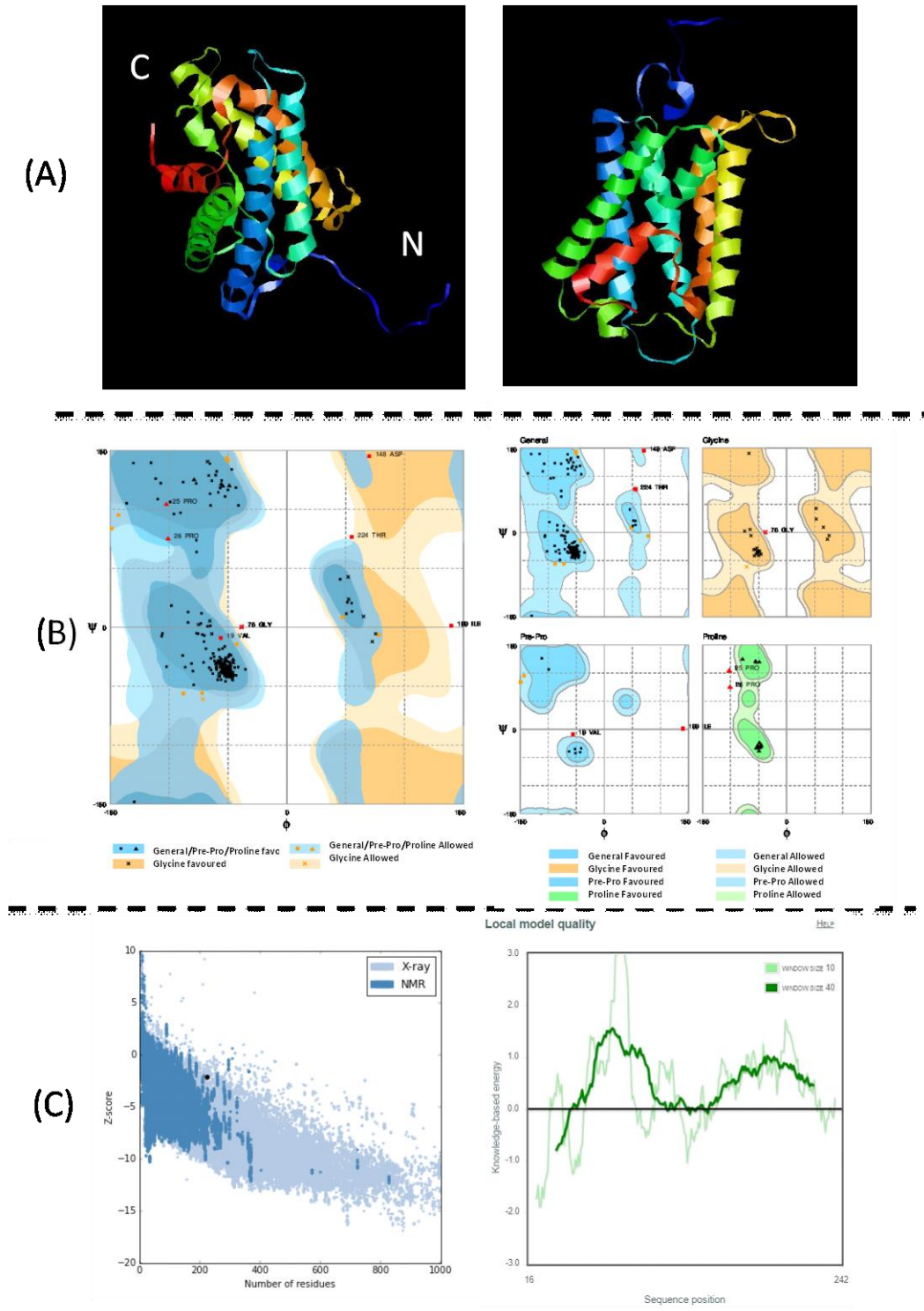


Plate 4.13 Structure prediction an validation of PIP2;7 aquaporin from tolerant accession, IC97189

(A) Three dimensional structure prediction of PIP 2;7 aquaporin in Foxtail millet with N and C terminals labeled , side and top view; (B) Structural validation using Ramchandran plot; (C) Quality validation using Z-score

4.7 Analysis of the promoter regions of PIP2;7 aquaporin gene

To fetch the reason behind the high expression of PIP2;7, we analysed the *cis*-regulatory elements in the promoter region which is important for delineating their function and regulation. Hence, an upstream 1000 bp towards 5' direction from the transcription start site (TSS) was analysed. *Cis*-elements play essential roles in the regulation of gene expression by controlling the efficiency of promoters. Thus research on *cis*-elements could lay a foundation for further functional analysis of these genes. *In-silico* analysis has been used with success in the identification of putative regulatory elements in plant promoters (Pujade-Renaud et al., 2005; Kaur et al, 2008).

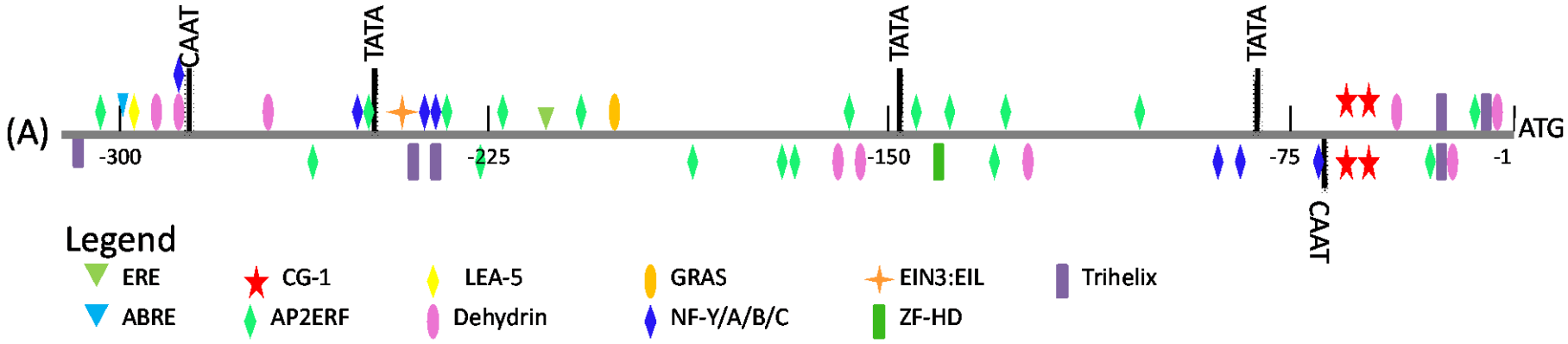
In this regard, the primer for PIP2;7 was designed using 1 kb upstream region of respective gene (section 3.2.5.1). Primers were designed such that they will amplify a segment of respective gene so as to amplify only UAS (upstream acting region) of the particular gene and no other gene. Genomic DNA was extracted from 21 day old seedling of tolerant accession IC97189 using CTAB genomic extraction method. The quality and quantity of extracted DNA was checked on 0.8% agarose gel. The individual genomic DNA templates were diluted to a final concentration of 50 ng/µl. Optimum annealing temperature (Ta°C) for individual primer pair was worked out. Amplification of products indicated that the reaction conditions adopted here were suitable for the analysis of promoter regions in foxtail millet. There were neither primer-dimers nor the occurrence of non-specific amplifications.

Sequence characterisation revealed a stretch of 312 bp DNA upstream of the transcription start site (ATG). Scanning the upstream sequences using PlantPAN 2.0 identified more than 200 *cis*-regulatory elements present in the promoter region of PIP2;7 aquaporin gene in foxtail millet which revealed the presence of a number of abiotic stress responsive acting elements such as, ABRE binding site, MYB binding site, AP2/ERF binding site, LEA-5 binding site, NF-YB/A/C binding site, Trihelix binding site, EIN3:EIL binding site etc. Summary of putative *cis* acting regulatory elements is presented in Table 4.18). The AP2 family, that binds to the GCAC(A/G)N(A/T) TCCC(A/G)ANG(C/T) element (Nole-Wilson et al., 2000; Gong et al., 2008) and the ERF subfamily that binds to an AGCCGCC sequence, i.e. the GCC box (Ohme-Takagi et al., 1995) were found to be abundantly present at -302,

-264, -263, -237, -224, -209, -190, -186, -147, -142, -133, -130, -81, -30, -16 upstream of the transcription start site. The AP2/ERF (APETALA2/ethylene response factor) transcription factors perform a very important role in the plant's stress defense mechanism (Okamuro et al., 1997; Sakuma et al., 2002; Wessler, 2005; Nakano et al., 2006). Similarly presence of gTGTATcgc sequence found at -258 upstream confirms the binding of EIN3; EIL transcription factor. EIN3 (ethylene insensitive 3; EIN3/EINL induce the transcription of target genes, mainly the AP2/ERF transcription factor superfamily (Ju et al., 2012). Map of the regulatory sequences of PIP2;7 aquaporin is presented as Plate 4.14.

The GRAS binding domain was found to be present at -200 bp upstream of ATG and the sequence was found to be highly conserved. GRAS proteins are plant specific proteins and the name is derived from the three initially identified members, GIBBERELLIN-ACID INSENSITIVE (GAI), REPRESSOR of GA1 (RGA) and SCARECROW (SCR) (Pysh et al., 1999). GRAS family proteins are divided into several sub-families such as DELLA, SHR, SCR, PAT, LISCL, and SCL3 (Tian et al., 2004). GRAS play important roles in drought resistance through regulating stomata closure, reactive oxygen species (ROS) scavenging, or other physiological processes. Transcription factor binding site for Nuclear Factor Y (NF-Y), also called hemi-activated protein (HAP) or CCAAT binding factor (CBF) was found to be present throughout the upstream region suggesting its role in drought sensing. It is a hetero trimeric transcription factor comprised of three distinct subunits: NF-YA (HAP2 or CBF-B), NF-YB (HAP3 or CBF-A), and NF-YC (HAP5 or CBF-C) (Romier et al., 2003). NF-YB and NF-YC initially form a dimer in the cytoplasm and then translocate to the nucleus where they interact with NF-YA and bind CCAAT sites, one of the most common elements in eukaryotic promoters (FitzGerald et al., 2004; Testa et al., 2005). The NF-Y complex coordinates oxidative stress response in eukaryotes (Hackenberg et al., 2012; Ikbal et al., 2014).

Trihelix binding domains were present at -306,-256,-251,-172,-37,-28,-27, -15 on the DNA stretch. Members of Trihelix family, also known as GT factors (DNA binding proteins with specificity for GT-elements) are characterized by binding specificity for GT-elements present in the promoter



(B)

Position	Strand	Similar Score	Hit Sequence
53	+	1	CCGCGc
53	-	1	cCGCGC
51	+	1	GCGCGt
51	-	1	gCGCGT

Plate 4.14 Map of the regulatory sequences of PIP2;7 aquaporin

(A) The consensus sequences corresponding to the various putative *cis*-elements are described in Table 4.18. Positions are with respect to the first base of the translation start site (ATG).**(B)** Motif logo for binding site for CG-1 (DNA binding domain).

region of many plant genes (Hiratsuka et al., 1994; Nagano et al., 2001) and are among the first transcription factors identified in plants (McCarty and Chory, 2000). They share one or two trihelix (helix – loop – helix – loop – helix) structures, each consisting of three putative-helices, which are responsible for binding to DNA (Zhou, 1999). Nevertheless, further studies in rice and Arabidopsis showed that some GT factors are not light-responsive at the transcriptional level (Dehesh et al., 1990; Kuhn et al., 1993). The involvement of this response to environmental cues (O'Grady et al., 2001; Park et al., 2004; Wang et al., 2004; Xie et al., 2009; Fang et al., 2010) has also been reported.

Another important TF binding site was found to be present at -145 bp upstream of the TSS. This class of Zn finger TFs that has been implicated in abiotic stress signalling is the Zn-finger homeodomain proteins (ZF-HD), which are characterized by the presence of Zn-finger-like motifs upstream of a homeodomain (Windhovel et al., 2001). The gene expression of ZF-HD and C_2H_2-type TFs has been already described as regulated by abiotic stress conditions in Arabidopsis (Sakamoto et al., 2004; Tan and Irish, 2006)

Among the other significant elements, there were a number of AAAG motifs that are determinants for Dof domain proteins binding which are plant-specific transcription regulators with highly conserved single DNA-binding zinc finger domain (Yanagisawa and Schmidt, 1999). Dof domain proteins are associated with diverse plant-specific physiological roles as suggested by the gene expression studies. They include stress responses (Zhang et al., 1995; Kang et al., 2003), light responses (Yanagisawa and Sheen, 1998), phytochrome signaling and responses to plant hormones including auxin (DePaolis et al, 1996; Kisu et al., 1998).

Promoter anlaysis also revealed the presence of the binding site for a sequence-specific DNA-binding domain (designated CG-1) found in Calmodulin-Binding Transcription Activators (CAMTAs) in the upstream region of PIP2;7 aquaporin. This domain could bind DNA directly and activate transcription, or interact with other transcription factors, not through DNA binding, thus acting as a co-activator of transcription (Figure 4.8).The DNA *cis* element that binds to CAMTA was identified as CGCG and CGTG binding motif in Arabidopsis, AtCAMTA3 (Yang et al., 2002) and Rice, Os-CBT (Choi

et al., 2005). The consensus sequence of CGCG core motif is (A/C)CGCG(C/G/T), giving the name to the DNA binding domain of the protein as CG-1, a novel cis-element which was first isolated from the parsley cDNA library (OdCe ,1994). The consensus sequence of CGTG core motif is (A/C)CGTGT and includes classical abscisic acid responsive element (ABRE) motif (ACGTGT), which is recognised by bZIP proteins (Hobo et al., 1999).

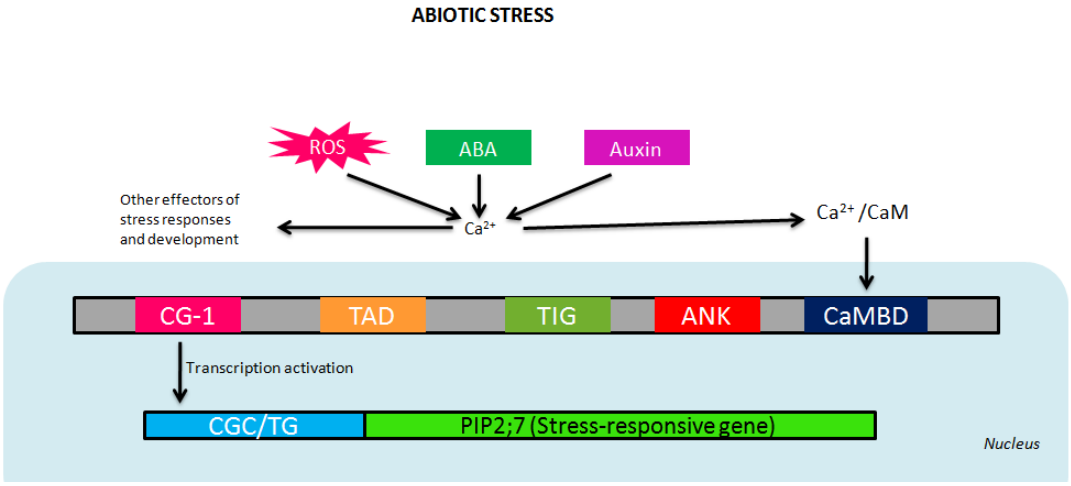


Figure 4.8 Plant CAMTAs integrate stress and growth signals

Plants respond and adapt to environmental stresses by multiple signaling pathways. Ca^{2+} concentrations are transiently elevated, via increased Ca^{2+} influx in response to environmental stimuli (drought) .Ca^{2+} transients are transduced by various types of Ca^{2+}-binding proteins including CaM, which affect numerous downstream targets and cellular processes. Auxin is a multifunctional plant hormone that plays a central role in growth and development, whose signal transduction is also mediated by Ca^{2+}/CaM (TAD: transcription activation domain; ANK: ankyrin repeats; CaMBD: calmodulin binding domain).

The presence of binding site for CG-1 domain is thought to be an important factor in tolerance to abiotic stress. These genes are regulated by cytosolic and nuclear Ca^{2+} signals (van Der Luit et al., 1999; Ikura et al., 2002). Thus, nuclear and cytoplasmic Ca^{2+} signals control transcription by distinct mechanisms which include numerous and include various signal transducers, such as the superfamily of EF-hand Ca^{2+}-binding proteins (e.g. calmodulin, CaM) (Ikura etal., 2002). These regulate the activity of a number of transcriptional regulators such as the cAMP transcriptional activator CREB and its versatile co-activator CREB-binding protein CBP300 (Chawla et al., 1998). In addition, certain TFs of the bHLH family directly bind to CaM, thus inhibiting DNA-binding by masking the DNA-binding domain (Corneliussen et al., 1994; Onions, et al., 1997; 2000). In plants, the occurrence of other types

of CaM-binding TFs including WRKY (Journot-Catalino, et al., 2006) and Myb (Yoo et al., 2005).

The results establish a role for CAMTA in drought acclimation. The research provides a possible point of integrating various molecular and biological pathways with water stress regulated gene expression. The interaction with several stress responsive genes, maintenance of osmoticum, regulating membrane biogenesis, generating ABA response, guarding photosynthesis and interaction with AP2-EREBP were some of the key regulatory components in response to drought stress. These findings provide insight for further investigation of CG-1 domain in plant aquaporins in opening new perspectives for improving drought tolerance which could eventually lead to better crop production.

Altogether, based on the differential response of contrasting foxtail millet accessions to water stress, a working hypothesis for the mechanism of drought tolerance shown by the foxtail millet is depicted in Plate 4.15. Data from transcriptional profiling expressed under drought helps functional characterization the stress responsive genes. Cells constantly respond to adverse changes by certain mechanisms. A crucial part to establish a tolerant state involves transcriptional control of the expression of stress-responsive genes and regulation of their temporal and spatial expression patterns (Rushton and Somssich 1998).

From the present study, we have identified a aquaporin PIP2;7 from tolerant foxtail millet accession, IC97189. To the best of our knowledge, there have been no reports on the cloning of the aquaporin gene from *S. italica* species. *Cis*-regulatory sequences function not only as molecular switches for gene expression, but also as terminal points of signal transduction. Promoter anlaysis of PIP 2;7 aquaporin revealed the presence of the binding site for a sequence-specific DNA-binding domain (designated CG-1) found in Calmodulin-Binding Transcription Activators (CAMTAs) in the upstream region that could activate transcription, or interact with other transcription factors.

Transcription factors (TFs) are proteins that bind specific sequences of DNA (*cis*-regulatory sequences) in the promoter regions of various genes and thus are capable of activating and/or repressing transcription of many secondary responsive genes.

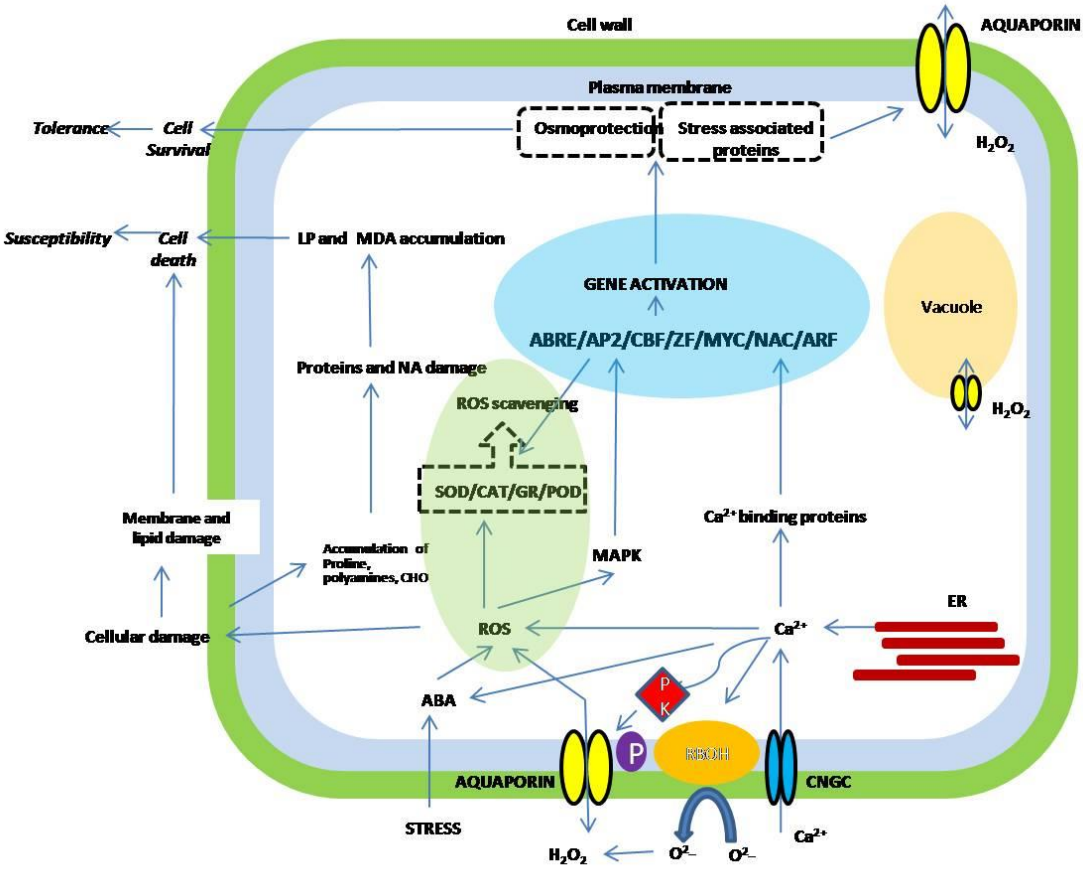


Plate 4.15 A model of the drought-tolerance mechanisms operating in foxtail millet
Major generation sites of ROS and transient calcium increase from different intracellular stores and the influx of extracellular calcium in to the cell induced by the opening of CNGC in the plasmamembrane in response to stress. Stress induces activation of calcium channels in ER membranes, leading to the release of calcium in to the cytosol. Chloroplast is a major producer of ROS during photosynthesis under stress and contains a large array of ROS-scavenging mechanisms. ROS production also occurs in mitochondria. Under stress, the maintenance of ROS homeostasis is involved in redox enzymes and metabolites. RBOH in the plasma membrane becomes activated by stress via increased membrane fluidity and/or via a consequent increase in cytosolic levels of Ca^{2+} controlled by a Ca^{2+} permeable channel (CNGC). Ca^{2+} influx activates RBOH by promoting its phosphorylation, leading to the increase of ROS. Reactive oxygen species (ROS), high solute concentrations, and cell hydrostatic pressure act on aquaporin activity. When the plant cell senses a lowered apoplastic water potential, the aquaporins become dephosphorylated, thereby lowering the water permeability of the plasma membrane and minimizing water loss. The aquaporins of the vacuolar membrane remain open and allow water to flow into the cytosol to compensate for water lost to the apoplast. Abbreviations: CNGC, cyclic nucleotide gated channels; RBOH, respiratory burst oxidase homolog; PK, protein kinase; P, phosphate group. *(based on well known concepts reported in model species such as Arabidopsis.)*

Table 4.18 Summary of putative *cis* acting regulatory elements in PIP2;7 promoter region

SN	TF	Position and strand	Hit Sequence
1.	Alpha-amylase	-281(-); -109 (-); -221(-); -26(+); -187(-)	tggctTTCCT; agctcTTCCT; tTTATT; TAACAgt; atGGATA
2.	AP2 ;ERF	-302(+); -264(+); -263(-); -237(+); -224(+); -209(+); -190(-); -186(-); -182(-); -147(+); -142(+); -133(-); -130(+); -81(+); -30(-); -16(+);	CTCTA; ATATA; TATAT; ATCGA; ATCTT; TTCTA; AAGAT; TGGAT; TAAAT; ATATA; ATCTT; AAGAT; ATCTT; ATTTA; TAGTT; AGCTA
3.	AP2 ;RAV ;B3	-292(+)	CAACA
4.	AT-Hook	-149(+); -149(-); -149(+); -148(+); -221(-);-187(+); -197(+); -221(-); -221(-); -221(-); -149(+)	gAATATaatc; gaatATAATc; gAATATaatc; aATATAatct; TTTATtttg; atggATAAA; acggAAAAA; TTTATttt; ttTATTTt; tTTATTttga; gaatATAAT
5.	B3	-297(+); -289(+); -177(-); -176(+); -158(-)	CGTGC; CATTC; TCATG; CATGG; GCAAG
6.	bHLH	-301(-); -300(-); -51(+); -51(-); -251(+);-299(+); -299(-); -300(+); -262(+)	tctACGTGcc; ctaCGTGC; GCGCGtcc; gcgCGTCC; gcaACTTGg; TACGTg; tACGTG; CTACGtgc; ATATGtgt
7.	bZIP	-301(+);-262(-);-251(+);-300(+);-299(-); -298(+); -231(-); -48(-); -288(-); -111(+)	tctACGTGccaac; ataTGTGT; GCAACttgga; cTACGTgcca; TACGT; ACGTG; CTTCA; CGTCC; aTTCTT; ACAGCt
8.	bZIP ;(Others)	-301(-)	tctACGTG
9.	CG-1 ;CAMTA	-51(+); -54(+); -53(+); -53(-); -51(+); -51(-)	gCGCGTccg; tccgCGCGT; CCGCGc; cCGCGC; GCGCGt; gCGCGT
10.	Dehydrin	-293(+); -270(+); -165(-); -161(-); -124(-);-47(+); -45(+); -21(-); -5(+)	CCAAC; CCGCC; GTGGG; GTCGC; TTCGG; GTCCG; CCGAA; GTCGA; CCGAG
11.	Dof	-225(-); -194(+); -279(-); -279(-); -225(-); -281(-); -193(+); -279(-); -223(-); -191(+);-156(+); -152(+); -141(-); -134(+); -63(-)	catCTTTAttt; gaAAAAG; GCTTT; GCTTT; catCTTTAttt; tgGCTTTcctc; aaAAAGAtgg; GCTTT; TCTTT; AAAGA; AAGGA; AAGGA ;TCTTT; AAAGA; GCCTT
12.	E2F	-54(+); -54(-)	tcCGCGCgtc; tccGCGCGtc
13.	EIN3 ;EIL	-258(+)	gTGTATcgc
14.	GATA	-134(-); -132(+); -136(-); -132(+); -134(+); -134(-); -133(+); -133(-); -134(+); -134(-);-135(-); -133(+); -134(-); -133(+); -134(+);-134(-); -	aaAGATCt; aGATCTtc; tgaaAGATCt; aGATCTtctt; aaAGATCttc; aaaGATCTtc; aAGATCtt; aaGATCTt; aaAGATCttc; aaaGATCTtc; gaaaGATCTt; aAGATCttct; aaAGATCtt; aaGATCTtc; aaAGATCttc;

		135(-); -134(+); -134(+); -134(-);-255(-); -238(-); -225(-);-189(+); -185(+);-143(-); -132(+); -131(-); -59(-);-256(-); -239(-); -184(+); -60(-)	aaaGATCTtc; gaaAGATCttc; aaaGATCTtct; aaaGATCTtc; aaAGATCttc; TATCG; TATCG; CATCT; AGATG; GGATA; AATCT; AGATC; GATCT; TATCC; GTATC; CTATC; GATAA; TTATC
15.	GRAS	-200(+)	aGTACGgaa
16.	Homeodomain ;HB-PHD	-79(+)	tTAAACcacg
17.	Homeodomain ;HD-ZIP	-182(+)	TAAATtca
18.	LEA type 1	-251(+);-172(+)	GCAACttgga; GCAACcggtg
19.	LEA_5	-299(+)	TACGTgcc
20.	MADF	-30(-); -28(+); -29(+); -29(-); -37(-)	tagTTAAC; GTTAAcag; aGTTAAca; agTTAACa; gTAACCctag
21.	MADS box	-88(-);-88(-)	cctcccTATTTaaaccacggcc
22.	MYB ;ARR-B	-134(+);-134(-)	aaAGATCttc; aaaGATCTtc
23.	Myb/SANT	-188(+); -61(-); -187(-); -62(+); -186(+); -61(-); -61(-); -187(-); -62(+); -188(+); -61(-); -170(+); -170(-); -63(+); -186(-); -62(+);-187(-); -62(+); -35(-); -62(+); -36(-); -189(+); -63(-); -35(-); -153(-); -36(-); -186(-); -63(+); -35(-)	gatGGATAaa; ctTATCCtcc; atGGATAaat; cctTATCCtc; tGGATAaa; ctTATCCt; cTTATCctcc; atGGATAaat; cctTATCCtc; gatGGATAaa; ctTATCCtcc; aACCGGtg; aaCCGGTg; gccTTATCct; tgGATAAat; ccTTATCct; atgGATAAat; ccTTATCctc; aaCCCTAgtt; cctTATCC; taACCCTagt; agatGGATAaatt; gcctTATCCtccg; aaCCCTAgtt; gaagGAATAtaa; taACCCTag; tgGATAAatt;
24.	Myb/SANT ;G2-like ;MYB	-150(+)	ggAATATaat
25.	Myb/SANT ;MYB ;ARR	-134(+);-134(-);-143(-)	aaAGATCttc; aaaGATCTtc; AATCT
26.	Myb/SANT ;MYB ;G2-like	-150(-)	gGAATAtaat
27.	Myb/SANT ;MYB-related	-35(-)	aaCCCTAg
28.	NF-YB ;NF-YA	-293(+); -267(+); -247(-); -240(+); -84(+);-	CCAAC; CCCAT; CTTGG; CCTAT; CCTAT; CCATT; ATTGC

	;NF-YC	68(+); -66(-)	
29.	PsaH	-78(+)	TAAACcacggcc
30.	SBP	-201(+); -201(+);	aaGTACGga; aaGTACGgaa
31.	SBP		
32.	SBP	-202(+); -203(+); -200(+); -202(-); -201(+);-200(+); -200(+); -200(+); -201(+); -201(+);-203(+); -205(+); -206(+); -201(+); -307(-);-306(+); -200(-); -199(+); -299(+); -201(-)	gaaGTACGg; cgaaGTACGgaa; aGTACGgaa; gaaGTACGga; aaGTACGga; aGTACGga; aGTACGgaa; aGTACGg; aaGTACGga; aaGTACGga; cgaaGTACGg; agcgaaGTACGgaaaa; tagcgaaGTACGgaaaaag; aaGTACGgaa; TGTAC; GTACC; AGTAC; GTACG; taCGTGCca; aaGTACGga
33.	Storekeeper	-123(+); -123(-); -164(-)	tCGGCCgt; tcGGCCGt; tgGGTCGca
34.	TBP	-184(+)	gATAAAtt;
35.	TCP	-306(+); -306(-); -296(+); -296(-); -269(+);-269(-); -244(+); -244(-); -243(+); -243(-);-163(+); -163(-); -162(+); -162(-);122(+); -122(-); -121(+); -121(-); -97(+); -97(-); -96(+); -96(-); -91(+); -91(-); -90(+); -90(-); -71(+);-71(-);-70(+);-70(-);-48(+);-48(-);-8(+); -8(-); -3(+); -3(-); -247(-); -162(+); -97(+)	GTACC; GTACC; GTGCC; GTGCC; CGCCC; CGCCC; GGACC; GGACC; GACCC; GACCC; GGGTC; GGGTC; GGTCG; GGTCG; CGGCC; CGGCC; GGCCG; GGCCG; GGTCC; GGTCC; GTCCC; GTCCC; TGGCC; TGGCC; GGCCT; GGCCT; CGGCC; CGGCC; GGCCA; GGCCA; CGTCC; CGTCC; AGACC; AGACC; GAGCC; GAGCC; cttGGACC; GGTCGcaa; GGTCCctg
36.	TCR	-82(+); -82(-); -217(+); -139(+);	taTTTAAacc; tatTTAAAcc; tTTTGAaatt; tTTTGAaaga
37.	TCR ;CPP	-81(+); -81(-)	aTTTAAac; atTTAAAc
38.	Trihelix	-306(-);-256(-);-251(-);-172(-);-37(+);-37(-);-28(+);-27(-);-15(+)	GTACC; GTATC; GCAAC; GCAAC; GTAAC; GTAAC; GTTAA; TTAAC; GCTAC
39.	Tryp_alpha_amyl	-260(-)	atGTGTA
40.	ZF-HD	-145(-)	ATAAT
41.	(Others)	-268(-)	gcccatatatgtGTATC

The presence of binding site for CG-1 domain is thought to be an important factor in tolerance to abiotic stress. The results establish a role for CAMTA in drought acclimation and further investigation of CG-1 domain could help in exploring new perspectives in engineering drought stress.

Accomplishments of these goals permit better understanding of molecular and physiological mechanisms utilized by plants during adverse conditions and define the biomarkers of tolerance against stresses. The research provides a possible point of integrating various molecular and biological pathways with water stress regulated gene expression.

CHAPTER V

SUMMARY AND CONCLUSIONS

The present investigation entitled “Deciphering the differentially expressed genes in Foxtail millet (*Setaria italica* L.) in response to water stress” was carried out at the Biotechnology Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during 2013-2016.

Plant growth, productivity, and distribution are greatly affected by environmental stresses and plants undergo a variety of changes at the molecular level (gene expression) leading to physiological adaptation. Identification of stress-responsive genes and their expression kinetics in the tolerant crop is an interesting area of research. It was therefore important to explore stress tolerance mechanism in a relatively abiotic stress tolerant crop. In this study, foxtail millet *(Setaria italica),* was used to study gene expression analysis.

The investigation was carried out with following objectives,

1. Screening and evaluation of foxtail millet accessions for dehydration tolerance under controlled conditions
2. Biochemical screening for presence of water stress responsive metabolites
3. To identify differentially expressed genes in foxtail millet during water stress by cDNA-SRAP and gene specific amplification
4. To study the gene expression pattern in the screened water stress tolerant and susceptible foxtail millet accessions

The objectives were accomplished at three phases, physiological screening and evaluation of foxtail millet accession for water stress tolerance, screening for drought responsive metabolites and differential expression studies for drought responsive genes. The experimental material comprised of sixty two accessions of foxtail millet procured from NBPGR, Dr. PDKV, Akola. In the initial phase, 62 accessions of foxtail millet were screened for water stress tolerance using PEG-6000 and induced water stress experiments. The results showed that all accessions of foxtail millet have different responses to water stress, indicating that it is feasible to choose the best accession for

resisting drought as some accessions have genetic potential to maintain the higher growth under stress conditions. Based on the various parameters like significant highest germination stress tolerance index, shoot and root length stress tolerance index, RWC and LWP, the core set of resistant and susceptible accessions were screened successfully. IC97087, IC97189, IC120159 and IC120239 were recorded as the most drought tolerant accessions whereas IC97109, IC120234, IC120346 and Lepakshi were recorded as susceptible accessions.

The selected tolerant and susceptible accessions from the physiological screening were then screened for their water stress tolerance on the basis of some primary metabolites like proline, total carbohydrates and antioxidant enzymes like superoxide dismutase, peroxidase, catalase and glutathione reductase. Observation of the influence of environmental stimuli on plants and their responses registered on protein and metabolite level provides lots of valuable information about mechanisms underlying plant acclimation. Osmolytes viz. proline and carbohydrates increased with increasing drought stress. The proline content in unstressed tolerant plant is found to be much higher as compared to that in unstressed susceptible plants suggesting its important role in drought tolerance of plants. Photosynthetic pigment decreased with increasing drought stress. Activities of antioxidant enzymes increased with drought stress in most of the accessions. The activity of SOD was found to be highest in IC120239 which was found to be a tolerant accession in prior physiological screening. The activity of GR was high in all tolerant accession than in susceptible accessions. Catalase and POD activity was found to be highest in Prasad. Generally in tolerant accessions the reduction of these enzymes is decreased, when compared with susceptible accessions. Enzymatic and non-enzymatic ROS scavengers are reported to be upregulated, especially in tolerant genotypes. Primary metabolites involved in osmotic adjustment, especially in conditions of limited water availability, are generally found to have elevated concentration level. However, in some sensitive accessions this parameter was higher than in tolerant species.

Applying different physiological and biochemical tests to appreciate drought tolerance in plant leads to faster selection methods. Therefore, these

characters can be used as an indirect selection criterion for screening drought tolerance plant materials which will lead to new cultivars with high yield potential and high yield stability that in turn will result in superior performance in dry environments. Hence, this research can provide documentation for breeding/selection of higher drought resistant foxtail millet in arid regions and acquisition of good information for future molecular research. The physio-biochemically screened accessions can be further prove to be useful in studies involving transcriptome changes to fetch out the molecular mechanism underneath of its drought adaptation.

Contrasting accessions IC97189 (tolerant) and IC97109 (susceptible), were selected for further gene expression studies as the transcription regulation of abiotic stress-related genes is a potential area of interest for improving stress tolerance in plants. cDNA SRAP analysis showed a relatively higher number of genes expressed in IC97189. The differential gene expression of drought responsible transcriptional factors like DREB1, DREB2, Aquaporins and C_2H_2 was studied in selected drought tolerant and susceptible plants each one showing considerable difference gene expression. Aquaporin protein showed high expression ratio in a foxtail millet which made us curious to know the possible role of this gene in stress tolerance mechanisms. Aquaporins act as multifunctional channels but the lack of characterization of transport selectivity renders a complete comprehensive analysis difficult. As a next objective, genome–wide investigation of plant aquaporin protein from foxtail millet was carried out. The study revealed that, Aquaporins comprise of MIP superfamily with conserved motif. Primers for PIP subclass of aquaporins were developed targeting the MIP region. The profile showed differences in gene expression pattern.

Further an attempt was made to characterise full length sequence of PIP 2;7 to isolate a 770bp long DNA stretch out of the 857 base pair CDS. Gene structure prediction using the genomic and CDS sequences confirmed the presence of two introns. Database analysis with BLAST revealed that the deduced sequence has high homology to a UNPRIDICTED PIP2;7 gene in *Setaria italica* L. with an identity of 99%. Theoretical model of the tertiary structure shows that it has a highly conserved containing the NPA motif which was further validated by Ramachandran plot.

The gene was further analysed for *cis* regulatory elements present upstream the gene. Sequence characterization revealed a stretch of 312 bp DNA upstream of the transcription start site (ATG). Scanning the upstream sequences using PlantPAN 2.0 identified more than 250 *cis*-regulatory elements present in the promoter region of PIP2;7 aquaporin gene in foxtail millet which revealed the presence of a number of abiotic stress responsive acting elements such as, ABRE binding site, MYB binding site, AP2/ERF binding site, LEA-5 binding site, NF-YB/A/C binding site, Trihelix binding site, EIN3:EIL binding site etc.

Promoter analysis also revealed the presence of a sequence-specific DNA-binding domain (designated CG-1) for binding to the calmodulin-binding transcription activators (CAMTAs) in the upstream region of PIP2;7 aquaporin. This domain could bind DNA directly and activate transcription, or interact with other transcription factors, not through DNA binding, thus acting as a co-activator of transcription.

The research provides a possible point of integrating various molecular and biological pathways with water stress regulated gene expression. These findings provide insight for further investigation of CG-1 domain in plant aquaporins in opening new perspectives for improving drought tolerance which could eventually lead to better crop production. The identified aquaporin could be explored in development of tolerant lines by MAS using PIP 2;7 as a functional marker and also in transgenic development in other related crops.

The study can prove helpful to the farmers in selecting foxtail millet cultivars for unaffected yields in diverse agronomic conditions. Also can be further proving useful in studies involving transcriptome changes to fetch out the molecular mechanism underneath of its drought adaptation. Accomplishments of these goals permit better understanding of molecular and physiological mechanisms utilized by plants during adverse growth conditions and define the biomarkers of tolerance against stresses. These assignments should be a valuable starting point for further research to dissect the contribution of each aquaporin to plant water transport.

CHAPTER VI
IMPLICATIONS

The research provides a possible point of integrating various molecular and biological pathways with water stress regulated gene expression. The physiological and biochemical parameters studied here could be used as faster selection methods to appreciate drought tolerance in plants leading to development of new cultivars with superior performance in dry environments.

The study can prove helpful to the farmers in selecting foxtail millet cultivars for unaffected yields in diverse agronomic conditions. The research can provide documentation for breeding/selection of higher drought tolerant foxtail millet in arid regions. The upregulated enzymatic and non-enzymatic osmolytes identified here could be used as the biomarkers of tolerance against stresses. The physio-biochemically screened accessions can be further prove to be useful in studies involving transcriptome changes to fetch out the molecular mechanism underneath of its drought adaptation and acquisition of good information for future molecular research..

The identified aquaporin could be explored in development of tolerant lines by MAS using PIP 2;7 as a functional marker and also in transgenic development in other related crops.These assignments should be a valuable starting point for further research to dissect the contribution of each aquaporin to plant water transport.

CHAPTER VI

LITERATURE CITED

Abbate PE, Dardanellib JL, Cantareroc MG, Maturanoc M, Melchiorid RJM, Sueroa EE (2004) Climatic and water availability effects on water-use efficiency in wheat. Crop Sci 44:474–483

Abe H, Urao T, Ito T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. The Plant Cell 15: 63–78

Abedi T, and Pakniyat H (2010) Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.). Czech J Genet Plant Breed 46(1): 27-34

Abrechit DG, and Carberry PS (1993) The influence of water deficit prior to tassel initiation in maize growth, development and yield. Field Crop Res 31: 55-69

Acar O, Turkan I, and Zdemir FO (2001) Superoxide dismutase and peroxidase activities in drought sensitive and resistant barley (*Hordeum vulgare* L.) varieties. Acta Physiologiae Plantarum 3: 351-356

Acquaah (2007) Principles of plant genetics and breeding. Blackwell, Oxford, UK

Affenzeller MJ (2003) Arabidopsis thaliana unter Wasserstress: Transkriptionsprofile der MIP-Familie und von Genen aus dem Stress- und Sekundarstoffwechsel. Dissertation. Ludwig-Maximilians-Universitat, Munchen

Agarwal PK, Agarwal P, Reddy MK, Sopory SK (2006) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep 25:1263–1274

Agarwal P, Agarwal PK, Nair S, Sopory SK, Reddy MK (2007) Stress inducible DREB2A transcription factor from Pennisetum glaucum is a phosphoprotein and its phosphorylation negatively regulates its DNA binding activity. Molecular Genetics and Genomics 277: 189–198

Aharon R, Shahak Y, Wininger S, Bendov R, Kapulnik Y, Galili G (2003) Overexpression of a plasma membrane aquaporins in transgenic tobacco improves plant vigour under favourable growth conditions but not under drought or salt stress. Plant Cell 15:439–447

Ahmadi A, and Baker DA (2001) The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathway in wheat. Plant Growth Regul 35:81–91

Ahmadizadeh M, et al. (2011) Genetic diversity of durum wheat landraces using multivariate analysis under normal irrigation and drought stress conditions. African J Agri Res 6(10): 2294-2302

Alaei M, Zaefizadeh M, Khayatnezhad M, Alaei Z, and Alaei Y (2010) Evaluation of germination properties of different durum wheat genotypes under osmotic stress. Middle East J Sci Res 6: 642-646

Alexandersson E, Fraysse L, Sjovall-Larsen S, Gustavsson S, Fellert M, Karlsson M, Johanson U, Kjellbom P (2005) Whole gene family expression and drought stress regulation of aquaporins. Plant Mol Biol 59: 469-484

Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ 24:1337–1344

Al-Karaki G, Al-Ajimi A, Othman Y (2007) Seed germination and early root growth of three barley cultivars as affected by temperature and water stress. Am Eurasian J Agric Environ Sci 2:112–117

Allahmoradi P, Ghobadi M, Taherabadi S and Aherabadi S (2011) Physiological aspects of mungbean (*Vigna radiata* L.) in response to drought stress. Intl Conf Food Engg Biotechnol, IPCBEE 9, IACSIT Press, Singapore.

Alscher RG, Donahue JL, Cramer CL (1997) Reactive oxygen species and antioxidants: relationships in green cells. Physiol Plant 100:224–233

Amara I, Zaidi I, Masmoudi K, Ludevid MD, Pagès M, Goday A, Faiçal B, (2014) Insights into Late Embryogenesis Abundant (LEA) Proteins in Plants: From Structure to the Functions, American J Plant Sci 5:3440-3455

Anjum F, Yaseen M, Rasul E, Wahid A, Anjum S (2003) Water stress in barley (Hordeum vulgare L.). I. Effect on chemical composition and chlorophyll contents, Pakistan J Agr Sci 40:45–49.

Ashraf MY, Khan AH, Azmi AR (1992) Cell membrane stability and its relation with some physiological processes in wheat. Acta Agronomica Hungarica 41: 183-191

Ashraf MY, Azmi AR, Khan AH and Naqvi SSM (1994) Water relations in different wheat (*Triticum aestivum* L.) genotypes under soil water deficits. Acta Physiologiae Plantarum 16: 231-240

Ashraf M, and Yasmin N (1995) Responses of four arid zone grass species from varying habitat to drought stress. Biol Plant 37(4): 567-575

Ashraf MY, Ali SA and Bhatti AS (1998) Nutritional imbalance in wheat genotypes grown at soil water stress. Acta Physiol Plant 20(3): 307-310

Ashraf M (2002) Salt tolerance of cotton: some new advances. Crit Rev Plant Sci 21: 1-30

Ashwell G (1957) Methods in Enzymol. *In* Colowick SJ and Kaplan NO eds, Academic Press, New York, pp. 75

Aspinall D and Paleg G (1981) Proline accumulation: physiological aspects. In: The physiology and biochemistry of drought resistance in plants. (Eds.). L, Paleg, D. Aspinall, Academic Press, Sidney, pp 215-228

Atkinson CJ, Policarpo M, Webster AD, Kingswell G (2000) Drought tolerance of clonal Malus determined from measurements of stomatal conductance and leaf water potential. *Tree Physiol* 20(8): 557-563

Azad AK, Sawa Y, Ishikawa T, Shibata H (2004) Phosphorylation of plasma membrane aquaporin regulates temperature-dependent opening of tulip petals. Plant Cell Physiol 45: 608-617

Azevedo Neto AD, Prico JT, Eneas-Filho J, Braga de Abreu CE, Gomes-Filho E (2006) Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environ Exp Bot 56: 235–241

Bakalova S, Nikolova A, Wedera D (2004) Isoenzyme profiles of peroxidase catalase and superoxide dismutase as affected by dehydration stress and ABA during germination of wheat seeds. J Plant Physiol 30: 64–77

Baker J, Steele C, Dure L (1998) Sequence and characterisation of 9 LEA proteins and the genes from cotton. Plant Mol Biol 11:277–291

Balibrea ME, Rus-Alvarez AM, Bolarin MC, Perez-Alfocea F (1997) Fast changes in soluble carbohydrate and proline contents in tomato seedlings in response to ionic and non-ionic iso-osmotic stresses. J Plant Physiol 151:221-22

Baloch MJ, Dunwell J, Khakwani AA, Dennet M, Jatoi WA, Channa SA (2012) assessment of wheat cultivars for drought tolerance via osmotic stress imposedat early seedling growth stages. J Agric Res 50:299-310

Barron MC and De-Mejia EG (1998) Comparative study of enzymes related to proline metabolism in tepary bean *(Phaseolus acutifolius*) and common bean (Phaseolus vulgaris) under drought and irrigated conditions, and various urea concentrations. Plant Food and Huma Nutri 52**:** 119-132

Barrs HD (1968) Determination of water deficits in plant tissue. In Kozlowski TT (Eds) Water deficits and plant growth. New York, Academic Press,1, pp 235-368

Bartels D, Salamini F (2001) Desiccation tolerance in the resurrection plant Craterostigma plantagineum. A contribution to the study of drought tolerance at the molecular level. Plant Physiol 127:1346–1353

Bartels D and Sunkar R (2005) Drought and salt tolerance in plants. Crit Rev in Plant Sci 24: 23-58

Bartels D, Phillips J, Chandler J (2007) Desiccation tolerance: gene expression, pathways, and regulation of gene expression. In: Jenks MA, Wood AJ (eds) Plant Desiccation Tolerance. Blackwell, Ames, IA, pp 115–137

Barton L, Newsome SD, Chen FH, Wang H, Guilderson TP, Bettinger RL (2009) Agricultural origins and the isotopic identity of domestication in northern China. Proc Natl Acad Sci USA 106: 5523–5528

Basu PS, Ali M, Chaturvedi SK (2007) Osmotic adjustment increases water uptake, remobilization of assimilates and maintains photosynthesis in chickpea under drought. Indian J Exp Biol 45:261–267

Bates LS, Waldren RP and Teare ID (1973) Rapid determination of free proline for water studies. Plant and Soil 39:205-208

Batistic O, and Kudla J (2004) Integration and channeling of calcium signaling through the CBL calcium sensor/CIPK protein kinase network. Planta 219: 915-924

Beard JB, Sifers SI (1997) Genetic diversity in dehydration avoidance and drought resisitance within the Cynodon and Zoysia species. Int Turfgrass Soc Res J 8:603–610

Beauvois P (1812) Essai d'une nouvelle agrostographie; ou nouveaux genres des Graminiees.Paris.

Beck EH, Fettig S, Knake C, Hertig, Bhattarai T (2007) Specific and unspecific responses of plants to cold and drought stress. Journal of Bioscience 32(3):501-510

Benabdelmouna A, Darmency MA and Darmency H (2001) Phylogenetic and genomic relationships in Setaria italica and its close relatives based on the molecular diversity and chromosomal organization of 5S and 18S-5.8S-25S rDNA genes. Theor Appl Genet 103: 668-677

Bennetzen JL, Schmutz J, Wang H et al. (2012) Reference genome sequence of the model plant *Setaria*. Nat Biotechnol 30:555–561

Bharadwaj R, and Singhal GS (1981) Effect of water stress on photochemical activity of chloroplasts during greening of etiolated barley seedlings. Plant and Cell Physiol 79: 266-269

Bhushan D, Pandey A, Choudhary MK, Datta A, Chakraborty S, Chakraborty N (2007) Comparative proteomics analysis of differentially expressed proteins in chickpea extracellular matrix during dehydration stress. Mol and Cellul Proteom 6:1868–1884

Biber PD (2007) Evaluating a chlorophyll content meter on three coastal wetland plant species. J of Agri Food and Env Sci 1:1-10

Biehler K, and Fock H (1996) Evidence for the contribution of the Mehler peroxidase reaction in dissipating excess electrons in drought stressed wheat. Plant Physiol 112:265–272

Blum A, Sinmena B, Ziv O (1980) An evaluation of seed and seedling drought tolerance screening tests in wheat. Euphytica 29: 727-736

Blum A (1996) Developing drought and low N-tolerant maize. In: Edmeades GO, Banziger M, Mickelson HR, Pena-Valdivia CB, eds. Proc of a Sympo at the International Maize and Wheat Improvement Cente. El-Batan, Mexico, CIMMYT, 131–135

Bohnert HJ, Jensen RG (1996) Strategies for engineering water-stress tolerance in plants. Trends Biotechnol 14:89–97

Bonthala VS, Muthamilarasan M, Roy R, Prasad M (2014) FmTFDb: a foxtail millet transcription factors database for expediting functional genomics in millets. Mol Biol Rep 41 (10): 6343–6348

Bor MF, Ozdemir and Turkan I (2003) The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L and wild beet *Beta maritima* L. Plant Sci 164: 77-84

Boston RS, Viitanen PV, Vierling E (1996) Molecular chaperones and protein folding in plants. Plant Mol Biol 32:191–222

Bota J, Flexas J, Medrano H, (2004) Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? New Phytol 162:671–681

Boursiac Y, Chen S, Luu DT, Sorieul M, van den Dries, Maurel C (2005) Early effects of salinity on water transport in Arabidopsis roots. Molecular and cellular features of aquaporin expression. Plant Physiol 139: 790-805

Bouslama M, and Schapaugh WT (1984) Stress tolerance in soybean. Part 1: evaluation of three screening techniques for heat and drought tolerance. Crop Sci J 24:933-937

Bowler C, Van Montagu M, Inze D (1992) Superoxide dismutase and stress tolerance. Annu Rev Plant Physiol Plant Mol Biol 43:83–116

Bray EA (1993) Molecular responses to water deficit. Plant Physiol 103:1035–1040

Bray EA (1997) Plant responses to water deficit. Trends Plant Sci 2: 48-54.

Bray EA (2002) Abscisic acid regulation of gene exressions during water deficit stress in the era of the Arabidopsis genome. Plant cell Environ 25:153-161

Bruckner PL, and Frohberg RC (1987) Stress tolerance and adaptation in spring wheat. Crop Sci 27: 31-36

Brutnell TP, Wang L, Swartwood K, Goldschmidt A, Jackson D, Zhu XG, Kellogg E, Van Eck J (2010) Setaria viridis: a model for C4 photosynthesis. Plant Cell 22: 2537–2544

Bulbotko GV (1973) The effect of the physical properties of soils on the development of the root system of apple trees. Soviet Soil Sci USSR 5: 219-224

Carter Jr.TE, Patterson RP (1985) Use of relative water content as a selection tool for drought tolerance in soybean. Fide Agron abstr 77th Annu Meeting, pp 77

Cattivelli L, Rizza F, Badeck FW, Mazzucotelli E, Mastrangelo AM, Francia E, Mare C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crops Res 105:1–14

Ceccardi TL, Meyer NC, Close TJ (1994) Purification of a maize dehydrin. Protein Expr Purif 5:266–269

Chandler PM, and Robertson M (1994) Gene expression regulated by abscisic acid and its relation to stress tolerance. Annu Rev Plant Physiol Plant Mol Biol 45:113–141

Chandraobulreddy P (2005) Molecular cloning and characterization of differentially expressed genes from horsegram (*Macrotyloma uniflorum* (Lam.) Verde.) and groundnut (*Arachis hypogea* L.) under drought stress. Ph.D., thesis, Sri Krishnadeveraya University, Anantapur

Chapman KD (1998) Phospholipase activity during plant growth and development and in response to environmental stress. Trends Plant Sci 3:419–426

Chaumont F, Barrieu F, Wojcik E, Chrispeels MJ, Jung R (2001) Aquaporins constitute a large and highly divergent protein family in maize. Plant Physiol 125(3):1206-1215

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Annals of Botany 103: 551–560

Chawla S, Hardingham GE, Quinn DR, and Bading H (1998) CBP: a signal-regulated transcriptional coactivator controlled by nuclear calcium and CaM kinase IV. Science 281:1505– 1509

Chehab EW, Patharkar OR, Hegeman AD, Taybi T, Cushman JC (2004) Autophosphoryiation and subcellular localization dynamics of a salt- and water deficit induced calcium-dependent protein kinase from ice plant. Plant Physiol135: 1430-1446

Chen TH, and Murata N (2002) Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. Curr Opin Plant Biol 5:250-257

Chen CW, Yang YW, Lur HS, Tsai YG, Chang MC (2006) A novel function of abscisic acid in the regulation of rice (*Oryza sativa* L.) root growth and development. Plant Cell Physiol 47:1–13

Cho EK, Hong CB (2006) Over-expression of tobacco NtHSP70–1 contributes to drought-stress tolerance in plants. Plant Cell Rep 25:349–358

Choi H, Hong J, Ha J, Kang J, and Kim SY (2000) ABFs, a family of ABA responsive element binding factors. J Bio Chem 275:1723–1730

Choi H, Park HJ, Park JH, Kim S, Im MY, Seo HH, Kim YW, Hwang I, Kim SY (2005) Arabidopsis calcium-dependent protein kinase AtCPK32 interacts with ABF4, a transcriptional regulator of abscisic acid-responsive gene expression, and modulates its activity. Plant Physiol 139(4):1750–1761

Christmann A, Hoffmann T, Teplova I (2005) Generation of active pools of abscisic acid revealed by in vivo imaging of water-stressed Arabidopsis. Plant Physiol 1: 209-219

Clarke JM, and McCaig TN (1982) Evaluation of techniques for screening for drought resistance in wheat. Crop Sci 22: 503-506

Close KR, Gallagher-Ludeman LA (1989) Structure-activity relationships of auxin-like plant growth regulators and genetic influences on the culture induction response in maize (Zea mays L.). Plant Sci 61:245–252

Close TJ (1996) Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. Physiol Plant 97:795–803

Coca MA, Almoguera C, Jordano J (1994) Expression of sunflower low molecular weight heat shock proteins during embryogenesis and persistence after germination: localization and possible functional implications, Plant Mol Biol 25:479–492

Cohen P (1989) Structure and regulation of protein phosphatases. Annu Rev Biochem 58: 453-508

Corneliussen B, Holm M, Waltersson Y, Onions J, Hallberg B, Thornell A and Grundstrom T (1994) Calcium/calmodulin inhibition of basic-helix–loop–helix transcription factor domains. Nature 368:760–764

Cornic G, and Massacci A (1996) Leaf photosynthesis under drought stress. *In* Baker NR, Eds, Photosynthesis and the Environment, Kluwer Academic Publishers, Springer Netherlands, pp 347-366

Csiszar J, Feher-Juhasz E, Kotai E, IvankovitsKiss O, Horvath GV, Mai A, Galle A, Tari I, Pauk J, Dudits D, Erdei L (2005) Effect of osmotic stress on antioxidant enzyme activities in transgenic wheat calli bearing MsALR gene. Acta Biologica Szegediensis 49: 49–50

Cui XH, Hao FS, Chen H, Chen J, Wang XC (2008) Expression of the Vicia faba VfPIP1 gene in Arabidopsis thaliana plants improves their drought resistance. J Plant Res 121:207–214

Dai HP, Shan CJ, Wei AZ, Yang T, Sa WQ, and Feng BL (2012) Leaf senescence and photosynthesis in foxtail millet [*Setaria italica* (L.) P. Beauv] varieties exposed to drought conditions Aus J Crop Sci 6(2):232-237

Danielson JA, and Johanson U (2008) Unexpected complexity of the aquaporin gene family in the moss Physcomitrella patens. BMC Plant Biol 8:45

Das R, and Uprety DC (2006) Interactive effect of moisture stress and elevated CO_2 on the oxidative stress in Brassica species. J Food Agri and Enviro 4: 298–305

Davoody N, Seghatoleslami MJ, Mousavi SGH, Nasrabad AA (2013) Foxtail Millet Responses to Bulk and Nano Zinc Oxcide Particles in Water Stress Conditions. Ann Rev and Res Bio 3(4): 959-973

de Wet JMJ, Oestry-Stidd LL and Cubero JI (1979) Origins and evolution of foxtail millet (*Setaria italica*). J Agr Bot 26:53–54

Dedio (1975) Water relations in wheat leaves as screening test for drought resistance. Canadian J Plant Sci 55: 369-378

Dehesh K, Bruce WB, and Quail PH (1990) A trans-acting factor that binds to a GT-motif in a phytochrome gene promoter. Science 250:1397-1399

Dekker J (2003) The foxtail (*Setaria*) species-group. Weed Sci 51:641–656

Denekamp M, and Smeekens SC (2003) Integration of wounding and osmotic stress signals determines the expression of the AtMYB102 transcription factor gene. Plant Physiol 132:1415–1423

Deng XH, Zhang SN, and Hou XL (2007) Differential expression analysis of bud of pol CMS and its maintainter line of *Brassica rapa*.ssp.chinensis through SRAP. Acta Horticulturae Sinica (34):655–658

DePaolis A, Sabatini S, DePascalis L, Constantino P, Capone I (1996) A rolB regulatory factor belongs to a new class of single zinc finger plant proteins. Plant J 10: 215-223

Devos KM, Wang ZM, Beales J, Sasaki T and Gale MD (1998) Comparative genetic maps of foxtail millet (*Setaria italica*) and rice (*Oryza sativa*). Theor Appl Genet 96:63–68

Dhanda SS, and Sethi GS (2002) Tolerance to drought stress among selected Indian wheat cultivars. J Agric Sci 139: 319-326

Dhanda SS, Sethi GS and Behl RK (2004) Indices of drought tolerance in wheat genotypes at early stages of plant growth. J Agron Crop Sci 190:1-6

Dhindsa RS, Dhindsa PP, Thorpe TA (1980) Leaf senescence correlated with increased levels of membrane permeability and lipid-peroxidation and decreased of superoxide dismutase and catalase. J Exp Bot 32:93–101

Diamant S, Eliahu N, Rosenthal D, Goloubinoff P (2001) Chemical chaperones regulate molecular chaperones in vitro and in cells under combined salt and heat stresses. J Biol Chem 276:39586–39591

Diatchenko L, Lau YFC, Campbell AP, Chenchik A, Moqadam F, Huang B, Lukyanov S, Lukyanov K, Nadya Gurskaya N, Eugene DS, and Siebert PD (1996) Suppression subtractive hybridization: A method for generating differentially regulated or tissue specific cDNA probes and libraries. Proc Natl Acad Sci USA 93: 6025-6030

Ding Z, Li S, An X, Liu X, Qin H, Wang D (2009) Transgenic expression of MYB15 confers enhanced sensitivity to abscisic acid and improved drought tolerance in *Arabidopsis thaliana*. J Genet Genomics 36:17–29

Doust AN, Kellogg EA, Devos KM, and Bennetzen JL (2009) Foxtail millet: a sequence-driven grass model system. Plant Physiol. 149:137–141

Doyle JJ, and Doyle JL (1987) A rapid isolation procedure for small quantities of fresh leaf tissue.Phytochem Bulletin 19: 11–15

Dubois M, Gilles KA, Hamilton J, Rebers PA and Smith F (1956) colorimetric method for determination of sugars and related substances. Anal Chem 28:350

Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, Seki M, Shinozaki K, Yamaguchi- Shinozaki K (2003) OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J 33:751–763

Dure III L (1993) A repeating 11-mer amino acid motif and plant desiccation. Plant J 3:363–369

En H, Pang ZH, and Xiong BH (2008) Comparative analysis of composition and nutritive value of millet bran feed. China Feed18:39–41

Erickson PI, Ketring DL, Stone JF (1991) Response of internal tissue water balance of peanut to soil water. Agronomy Journal 83:248-253

Estrada-Campuzano G, Miralles DJ, Slafer GA (2008) Genotypic variability and response to water stress of pre- and post-anthesis phases in triticale. Eur J Agron 28:171–177

Eulgem T, Rushton PJ, Robatzek S, and Somssich IE (2000) The WRKY superfamily of plant transcription factors. Trends Plant Sci 5:199–206

Fang Y, Xie K, Hou X, Hu H and Xiong L (2010) Systematic analysis of GT factor family of rice reveals a novel subfamily involved in stress responses. Mol Genet Genomics 283:157- 169

Farooq S, and Azam F (2006) The use of cell membrane stability (CMS) technique to screen for salt tolerant wheat varieties. Journal of Plant Physiology 163**:** 629-637

Farooq M, Aziz T, Basra SMA, Cheema MA, Rehamn H (2008) Chilling tolerance in hybrid maize induced by seed priming with salicylic acid. J Agron Crop Sci 194:161–168

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development, Springer Verlag (Germany) 29(1):185-212

Farshadfar E, Rasoli V, Teixeira da Silva JA, Farshadfar M (2011) Inheritance of drought tolerance indicators in bread wheat (*Triticum aestivum* L.) using a diallel technique. Aust J Crop Sci 5:870-878

Fazeli F, Ghorbanli M, Niknam V (2007) Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. Biol Plant 51:98–103

Fernandez GCJ (1992) Effective selection criteria for assessing plant stress tolerance. *In* Proceedings of the International Symposium on Adaptation of Vegetables and other Food Crops in Temperature and Water Stress, Taiwan, pp 257-270

Ferriol M, Pic B, and Nuez F (2003) Genetic diversity of a germplasm collection of Cucurbita pepo using SRAP and AFLP markers.Theo and App Genet 107(2):271–282

Finkelstein RR and Gibson SI (2002) ABA and sugar interactions regulating development: cross-talk or voices in a crowd? Current Opinion in Plant Biology 5:26–32

FitzGerald PC, Shlyakhtenko A, Mir AA, and Vinson C (2004) Clustering of DNA sequences in human promoters. Genome Res 41:1562–1574

Forrest KL, and Bhave M (2007) Major intrinsic proteins (MIPs) in plants: a complex gene family with major impacts on plant phenotype. Funct Integr Genomics 7(4):263–289

Fouquet R, Leon C, Ollat N, Barrieu F (2008) Identification of grapevine aquaporins and expression analysis in developing berries. Plant Cell Rep 27(9):1541-1550

Foyer CH, and Fletcher JM (2001) Plant antioxidants: colour me healthy, Biologist 48:115–120

Fu J, and Huang B (2001) Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress, Environ. Exp Bot 45:105–114

Fujita Y, Fujita M, Satoh R, Maruyama K, Parvez MM, Seki M, Hiratsu K, Masaru OT, Kazuo S, Kazuko YS (2005) AREB1 is a transcription activator of novel ABRE-dependent ABA signalling that enhances drought stress tolerance in Arabidopsis. Plant Cell 17:3470–3488

Fukunaga K and Kato M (2002) Mitochondrial DNA variation in foxtail millet, *Setaria italica* (L.)P. Beauv. Euphytica 129:7–13

Fukunaga K, Wang ZM, Kato K, and Kawase M (2002) Geographical variation of nuclear genome RFLPs and genetic differentiation in foxtail millet, *Setaria italica* (L.) P. Beauv. Genet Resour Crop Evol 49:95–101

Gao YP, Young L, Bonham-Smith P, Gusta LV (1999) Characterization and expression of plasma and tonoplast membrane aquaporins in primed seed of *Brassica napus* during germination under stress conditions. Plant Mol Biol 40:635–44

Garay-Arroyo A, Colmenero-Flores JM, Garciarrubio A, Covarrubias AA (2000) Highly hydrophilic proteins in prokaryotes and eukaryotes are common during conditions of water deficit. J Biol Chem 275:5668–5674

Garg G (2010) Response in germination and seedling growth in Phaseolus mungo under salt and drought stress. J Environ Biol 31: 261-264

Gholamin R, Zaefizadeh M, Khayatnezhad M (2010) Study of drought tolerance of durum wheat genotypes under drought and irrigated conditions. World App Sci j 10(9): 1020-2023

Gill RK, Sharma AD, Singh P and. Bhullar SS (2002) Osmotic stress-induced changes in germination, growth and soluble sugar content of *Sorghum bicolor* (L.) Moench seeds. Bulg J Plant Physiol 28:12-25

Goday A, Jensen AB, Culianez-Macia FA, Mar Alba M, Figueras M, Serratosa J, Torrent M,Pages M (1994) The maize abscisic acid-responsive protein Rab17 is located in the nucleus and interacts with nuclear localization signals. Plant Cell 6:351–360

Goff SA, Cone KC, Chandler VL (1992) Functional analysis of the transcriptional activator encoded by the maize B gene: evidence for a direct functional interaction between two classes of regulatory proteins. Genes Dev 6: 864-875

Goff SA, Ricke D, Lan TH, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, et al. (2002) A draft sequence of the rice genome (Oryza sativa L. ssp. japonica). Science 296: 92-100

Gong H, Zhu X, Chen K, Wang S, Zhang C (2005) Silicon alleviates oxidative damage of wheat plants in pots under drought, Plant Sci 169:313–321

Gong W, He K, Covington M, Dinesh-Kumar SP, Snyder M, Harmer SL, Zhu YX, Deng XW (2008)The development of protein microarrays and their applications

in DNA-protein and protein-protein interaction analyses of Arabidopsis transcription factors. Mol Plant 1(1):27–41

Gonzalez L and Gonzalez-vilar M (2001) Determination of relative water content. In: Reigosa MJ (ed.) Handbook of Plant Ecophysiology Techniques, Dordrecht, Kluwer Academic Publishers, pp 207-212

Gopalakrishna R (2001) Cloning and characterization of moisture stress responsive genes from stress tolerant crop groundnut *(Arachis hypogaea* L.). Ph.D thesis, University of Agricultural Sciences, Bangalore.

Gorantla M, Babu PR, Lachagari VBR, Reddy AMM, Wusirika R, Bennetzen JL, Reddy AR (2006) Identification of stress responsive genes in an indica rice (Oryza sativa L.) using ESTs generated from drought-stressed seedlings. J Exp Bot 58:253– 265

Grandori C, Cowley SM, James LP, Eisenman RN (2000) The Myc/Max/ Mad network and the transcriptional control of cell behavior. Annu Rev Cell Dev Biol 16: 653–699

Grzesiak S, Filek W, Skrudlik G, and Niziol B (1996) Screening for drought tolerance: Evaluation of seed germination and seedling growth for drought resistance in legume plants. J Agron Crop Sci 177(4): 245-252

Gu SL, Liu J, Ren HR, et al. (1987) The relationship between foxtail millet and its environment. *In*: Shanxi Academy of Agricultural Sciences, eds, Foxtail millet cultivation in China, (In Chinese). Bejing: China Agricultural Press,pp 64–65

Guenther JF, Chanmanivone 1, Galetovic MP, Wallace IS, Cobb JA, Roberts DM (2003) Phosphorylation of soybean nodulin 26 on serine 262 enhances water permeability and is regulated developmentally and by osmotic signals. Plant Cell 15: 981-991

Gunes A, Pilbeam D, Inal A, Coban S (2008) Influence of silicon on sunflower cultivars under drought stress, I: Growth, antioxidant mechanisms and lipid peroxidation. Commun Soil Sci & Plant Nutri 39:1885–1903

Guo Z, Ou W, Lu S, Zhong Q (2006) Differential responses of antioxidative system to chilling and drought in four rice cultivars differing in sensitivity. Plant Physiol Biochem 44:828–836

Guoth A, Tari I, Galle A, Csiszar J, Pecsvaradi A, Cseuz L, and Erdei L (2009) Comparison of the drought stress responses of tolerant and sensitive wheat cultivars during grain filling: changes in flag leaf photosynthetic activity, ABA levels, and grain yield. J Plant Growth Regul 28:167–176

Gupta AS, Heinen JL, Holaday AS, Burke JJ, and Allen RD (1993) Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc Nat Ac Sci USA 90(4):1629–1633

Gupta AB, and Sankararamakrishnan R (2009) Genome-wide analysis of major intrinsic proteins in the tree plant Populus trichocarpa: characterization of XIP subfamily of aquaporins from evolutionary perspective. BMC Plant Biol 9:134

Gupta S, Kumari K, Das J, Lata C, Puranik S, and Prasad M (2011) Development and utilization of novel intron length polymorphic markers in foxtail millet [*Setaria italica* (L.) P. Beauv.]. Genome 54: 586–602

Haake V, Cook D, Riechmann JL, Pineda O, Thomashow MF and Zhang JZ (2002) Transcription factor CBF4 is a regulator of drought adaptation in Arabidopsis. Plant Physiology 130: 639-648

Hackenberg D, Keetman U, and Grimm B (2012) Homologous NF-YC2 subunit from Arabidopsis, tobacco is activated by photo oxidative stresss and induces flowering. Int J Mol Sci 13:3458–347

Hanson J, and Smeekens S (2009) Sugar perception and signaling—an update. Curr Opin Plant Biol 12: 1–6

Hare PD, Cress WA, Van Staden J (1998) Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ 21:535–553

Harlan JR, and de Wet JMJ (1971) Towards a rational taxonomy of cultivated plants. Taxon 20:509-517

Harmon AC, Gribskov M, Harper JF (2000) CDPKs – a kinase for every Ca^{2+} signal? Trends Plant Sci 5:154–159

Harris D, Tripathi RS, Joshi A (2002) On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice. *In* Pandey S, Mortimer M, Wade L, Tuong TP, Lopes K, Hardy B (Eds.), Direct seeding: Research Strategies and Opportunities, International Research Institute, Manila, Philippines, pp 231–240

Hartl FU (1996) Molecular chaperones in cellular protein folding. Nature 381:571–580

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol 51: 463-499

Hector JH (1936) Introduction to the botany of field crops. *In* Millets,Cereals, Central New Agency Johannesburg, South Africa, 1, pp 307–319

Heidari H (2012) Alternate Furrow Irrigation Effect on Yield, Yield Components and Seed Germination of Foxtail Millet (Setaria italica) In Double Cropping System. Int Res J Appl and Basic Sci 3(1):64-69

Hendrick JP, and Hartl FU (1993) Molecular chaperone functions of heat-shock proteins. Annu Rev Biochem 62:349–384

Herbinger K, Tausz M, Wonisch A, Soja G, Sorger A, Grill D (2002) Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. Plant Physiol and Biochem 40: 691–696

Hiratsuka K, Wu X, Fukuzawa H, and Chua NH (1994) Molecular dissection of GT-1 from Arabidopsis. Plant Cell 6:1805- 1813

Hobo T, Kowyama Y, and Hattori T (1999) A bZIP factor, TRAB1, interacts with VP1 and mediates abscisic acid-induced transcription. Proc Natl Acad Sci 96(26):15348

Hodge JE, and Hofreiter BT (1962) *In* Methods in Carbohydrate Chemistry, (Eds. Whistler, R.L. and Be Miller, J.N.), Academic Press, New York.

Homayoun H, Sam Daliri M, Mehrabi P (2011) Study of PGA stress effect on wheat cultivars at germination stage. Middle-East J Sci Res 9 (1): 71-74

Hong JP, and Kim WT (2005) Isolation and functional characterization of the Ca-DREBLP1 gene encoding a dehydration-responsive element binding-factor-like protein 1 in hot pepper (Capsicum annuum L. cv Pukang). Planta 220:875–888

Hoth S, Morgante M, Sanchez JP, Hanafey MK, Tingey SV and Chua NH (2002) Genome-wide gene expression profiling in Arabidopsis thaliana reveals new targets of abscisic acid and largely impaired gene regulation in the abi1-1 mutant. J Cell Sci 115: 4891–4900

Houde M, Daniel C, Lachapelle M, Allard F, Laliberte S, Sarhan F (1995) Immunolocalization of freezing-tolerance-associated proteins in the cytoplasm and nucleoplasm of wheat crown tissues. Plant J 8:583–593

Hu CA, Delauney AJ, Verma DP (1992) A bifunctional enzyme (Δ1-pyrroline-5-carboxylate synthetase) catalyzes the first two steps in proline biosynthesis in plants.Proc. Natl. Acad. Sci. U.S.A. 89 9354–9358

Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, Xiong L (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc Nat Acad Sci USA 103: 12987-12992

Hu R, Qi G, Kong Y, Kong D, Gao Q, Zhou G (2010) Comprehensive analysis of NAC domain transcription factor gene family in Populus trichocarpa. BMC Plant Biol 10:145

Huang N, Zhang YY, Xiao XH, Huang L, Wu QB, Que YX, and Xu LP (2015) Identification of smut-responsive genes in sugarcane using cDNA-SRAP. Genet Mol Res 14 (2): 6808-6818

Hulse J, Laing EM, and Pearson OE (1980) Sorghum and millets: their chemical composition and nutritive value. Acad press, New York

Ibrahim MJ, Akhtar M, Younis M A, Riaz M, Anwarul-Haq, Tahir M (2007) Selection of cotton (*Gossypium hirsutum* L.) genotypes against NaCl stress. Soil and Environment 26(1): 59-6

Ikbal, FE, Hernández JA, Barba-Espín G, Koussa T, Aziz A, Faize M, et al. (2014) Enhanced salt-induced antioxidative responses involve a contribution of polyamine biosynthesis in grape vine plants. J Plant Physiol 171:779–788

Ikura M, Osawa M, and Ames JB (2002) The role of calciumbinding proteins in the control of transcription: structure to function. Bioessays 24:625–636

Ingram J, and Bartels D (1996) The molecular basis of dehydration tolerance in plants. Annu Rev Plant Phys Plant Mol Biol 47:377–403

Ishikawa F, Suga S, Uemura T, Sato MH, Maeshima M (2005) Novel type aquaporin SIPs are mainly localized to the ER membrane and show cell-specific expression in *Arabidopsis thaliana*. FEBS Lett 579: 5814-5820

Islam MS, Akhter MM, Sabagh AE, Liu LY, Nguyen NT, Ueda A, Masaoka Y and Saneoka H (2011) Comparative studies on growth and physiological responses to saline and alkaline stresses of Foxtail millet (*Setaria italica* L.) and Proso millet (*Panicum miliaceum* L.). AJCS 5(10):1269-1277

Jagtap V, Bhargava S, Sterb P, Feierabend J (1998) Comparative effect of water, heat and light stresses on photosynthetic reactions in *Sorghum bicolor* (L.) Moench. J Exp Bot 49: 1715-1721

Jain M, Mathur G, Koul S and Sarin NB (2001) Ameliorative effects of proline on salt stress induced lipid peroxidation in cell lines of groundnut *(Arachis hypogea* L.). Plant Cell Reprod 20**:** 463-468

Jakoby M, Weisshaar B, Dröge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, Parcy F (2002) bZIP transcription factors in Arabidopsis. Trends Plant Sci 7:106–111

Jaleel CA, Sankar B, Murali PV, Gomathinayagam M, Lakshmanan GMA and Panneerselvam R (2008) Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus*; impacts on ajmalicine accumulation. Colloids Surf. B: Biointerfaces 62: 105–111

Jang JY, Kim DG, Kim YO, Kim JS, Kang H (2004) An expression analysis of a gene family encoding plasma membrane aquaporins in response to abiotic stresses in Arabidopsis thaliana. Plant Mol Biol 54: 713-725

Jang JY, Lee SH, Rhee JY, Chung GC, Ahn SJ, Kang H (2007) Transgenic Arabidopsis and tobacco plants overexpressing an aquaporin respond differently to various abiotic stresses. Mol Biotechnol 40:280–292

Janska A, Marsik P, Zelenkova S, Ovesna J (2010) Cold stress and acclimation: what is important for metabolic adjustment? Plant Biol 12:395-405

Javot H, and Maurel C (2002) The role of aquaporins in root water uptake. Ann Bot 90:301–313

Javot H, Lauvergeat V, Santoni V, Martin-Laurent F, Guclu J, Vinh J, Heyes J., Franck K.I., Schaffner A.R., Bouchez D, Maurel C (2003) Role of a single aquaporin isoform in root water uptake. Plant Cell 15:509–522

Jayaraman A, Puranik S, Rai NK, Vidapu S, Sahu PP, Lata C and Prasad M (2008) cDNA-AFLP analysis reveals differential gene expression in response to salt stress in foxtail millet (*Setaria italica* L.). Mol Biotechnol 40: 241–251

Jia X, Zhang Z, Liu Y, Zhang C, Shi Y, et al. (2009) Development and genetic mapping of SSR markers in foxtail millet [*Setaria italica* (L.) P. Beauv.]. Theor Appl Genet 118: 821-829

Jiang Y, and Huang B (2002) Protein alterations in tall fescue in response to drought stress and abscisic acid. Crop Sci 42: 202-207

Jiang CJ, Aono M, Tamaoki M, Maeda S, Sugano S, Mori M, Takatsuji H (2008) SAZ, a new SUPERMAN-like protein, negatively regulates a subset of ABA-responsive genes in Arabidopsis. Molecular Genetics and Genomics 279:183–192

Jogeshwar G, Pallela R, Jakka NM, Reddy PS, Rao JV, Sreenivasulu N, Kishor PBK (2006) Antioxidative response in different sorghum species under short-term salinity stress. Acta Physiol Plant 28:465–475

Johansson I, Karlsson M, Shukla VK, Chrispeels MJ, Larsson C, Kjellbom P (1998) Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation, Plant Cell 10:451–459

Johanson U, Karlsson M, Johansson I, Gustavsson S, Sjovall S, Fraysse L, Weig AR, Kjellbom P (2001) The complete set of genes encoding major intrinsic proteins in Arabidopsis provides a framework for a new nomenclature for major intrinsic proteins in plants. Plant Physiol 126(4):1358-1369

Jonak C, Kiegerl S, Ligterink W, Barker PJ, Huskisson NS, Hirt H (1996) Stress signaling in plants: a mitogen-activated protein kinase pathvi'ay is activated by cold and drought. Proc Natl Acad Sci USA 93:11274-11279

Jonak C, Ligterink W, Hirt H (1999) MAP kinases in plant signal transduction. Cell Mol Life Sci 55:204-213

Journot-Catalino N, Somssich IE, Roby D and Kroj T (2006) The transcription factors WRKY11 and WRKY17 act as negative regulators of basal resistance in Arabidopsis thaliana. Plant Cell 18:3289–3302

Ju C, Yoon GM, Shemansky JM, Lin DY, Ying ZI, Chang J, et al. (2012) CTR1 phosphorylates the central regulator EIN2 to control ethylene hormone signaling from the ER membrane to the nucleus in Arabidopsis. Proc Natl Acad Sci 109(47):19486–91

Jung JS, Preston GM, Smith BL, Guggino WB, Agre P (1994) Molecular structure of the water channel through aquaporin CHIP. The hourglass model. J Biol Chem 269: 14648-14654

Jung S (2004) Variation in antioxidant metabolism of young and mature leaves of Arabidopsis thaliana subjected to drought. Plant Sci 166: 459–466

Jung C, Seo JS, Han SW, Koo YJ, Kim CH, Song SI, Nahm BH, Choi YD, Cheong J-J (2008) Overexpression of AtMYB44 enhances stomatal closure to confer abiotic stress tolerance in transgenic Arabidopsis . Plant Physiol 146:623–635

Jusuf M, and Pernes J (1985) Genetic variability of foxtail millet (Setaria italica (L.) P. Beauv) Electrophoretic study of five isoenzyme systems. Theor Appl Genet 71:385–391

Kahrizsangi AG and Zargani M (2013) The comparison of the yield and water consumption of common millet (*Panicum miliaceumm*) and foxtail millet (*Setaria italica*) under different soil tillage. Int J Agri and Crop Sci 6(15):1040-1047

Kamara AY, Menkir A, Badu–Apraku B, Ibikunle O (2003) The influence of drought stress on growth, yield and yield components of selected maize genotypes. J Agr Sci 141:43–50

Kang HG, Foley RC, Onate-Sanchez L, Lin C, Singh KB (2003) Target genes for 0BP3, a Dof transcription factor, include novel basic helix-loop-helix domain proteins inducible by salicylic acid. Plant J 35: 362-372.

Kaplan F, and Guy CL (2004) β-Amylase induction and the protective role of maltose during temperature shock. Plant Physiol 135:1674–1684

Karyudi and Fletcher RJ (2002) Osmoregulative capacity in birdseed millet under conditions of water stress. I. Variation in *Setaria italica* and *Panicum miliaceum*. Euphytica125 (3): 337-348

Kaur H, Shukla RK, Yadav G, Chattopadhya D, Majee M (2008) Two divergent genes encoding L-myo-inositol 1-phosphate synthase 1 (CaMIPSl) and 2 (CaMIPS2) are differentially expressed in chickpea. Plant Ceil Environ 31: 1701-1716

Kawaguchi R, Girke T, Bray EA, Bailey-Serres J (2004) Differential mRNA translation contributes to gene regulation under non-stress and dehydration stress conditions in Arabidopsis thaliana. Plant J 38: 823-839

Kawar, PG, Pagariya MC, Dixit GB, Theertha Prasad D (2010) Identification and Isolation of SCGS phytoplasma-specific fragments by riboprofiling and development of specific diagnostic tool. J Plant Biochem Biotechnol 19: 185-194

Kawasaki S, Borchert C, Deyholos M, Wang H, Brazille S, Kawai K, Galbraith D, Bohnert HJ **(**2001) Gene expression profiles during the initial phase of salt stress in rice. Plant Cell 13: 889–905

Kawase M, and Sakamoto S (1987) Geographical distribution of landrace groups classified by hybrid pollen sterility in foxtail millet, *Setaria italica* (L.) P. Beavu. Japan. J Breed 37:1-9

Kaya MD, Okçub G, Ataka M, Çıkılıc Y, Kolsarıcıa Ö (2006) Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). Eur J Agron 24:291–295

Khaled AM, Saleh M, Al-Doss AA, Elshafei AA, Salem AK, Al- Qurainy FH, Barakat MN (2014) Identification of TRAP and SRAP markers linked with yield components under drought stress in wheat (*Triticum aestivum* L.), POJ 7(4):253-259

Khan Y, Yadav A, Suresh BV, Muthamilarasan M, Yadav CB, Prasad M (2014) Comprehensive genome-wide identification and expression profiling of foxtail millet [*Setaria italica* (L.)] miRNAs in response to abiotic stress and development of miRNA database. Plant Cell Tiss Organ Cult 118:279–292

Khidse SR, Bhale NL, and Boriker ST (1982) Proline accumulation and chlorophyll stability index in *Sorghum.* Sorghum News Letters 25: 123- 128

Kiani SP, Maury P, Sarrafi A, and Grieu P (2008) QTL analysis of chlorophyll fluorescence parameters in sunflower (Helianthus *annuus* L.) under well-watered and waterstressed conditions. Plant Sci 175: 565–573

Kiełbowicz-Matuk A (2012) Involvement of plant C_2H_2-type zinc finger transcription factors in stress responses. Plant Science 185–186, 78–85

Kim S, Kang JY, Cho DI, Park JH, Kim SY (2004) ABF2, an ABRE-binding bZIP factor, is an essential component of glucose signaling and its overexpression affects multiple stress tolerance. Plant J 40:75–87

Kisu Y, Ono T, Shinoflimtani N, Suzuki M, Esaka M (1998) Characterization and expression of a new class of zinc finger protein that binds to silencer region o f ascorbate oxidase gene. Plant Cell Physiol 39: 1054-1064.

Koag MC, Fenton RD, Wilkens S, Close TJ (2003) The binding of maize DHN1 to lipid vesicles. Gain of structure and lipid specificity. Plant Physiology 131:309–316

Kobayashi F, Maeta E, Terashima A, Kawaura K, Ogihara Y, and Takumi S (2008) Development of abiotic stress tolerance via bZIP-type transcription factor LIP19 in common wheat. J Exp Bot 59: 891-905

Koca H, Bor M, Özdemir F, Türkan İ (2007) The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ Exp Bot 60: 344–351

Kodaira KS, Qin F, Tran LS, Maruyama K, Kidokoro S, Fujita Y, Shinozaki K, Yamaguchi-Shinozaki K. (2011) Arabidopsis Cys2/His2 zinc-finger proteins AZF1 and AZF2 negatively regulate abscisic acid-repressive and auxin-inducible genes under abiotic stress conditions. Plant Physiol 157: 742–756

Kohler C, Hennig L, Spillane C, Pien S, Gruissem W, Grossniklaus U (2003) The polycomb-group protein MEDEA regulates seed development by controlling expression of the MADS-box gene PHERES1. Genes Dev 17: 1540–1553

Kolukisaoglu U, Weinl S, Blazevic D, Batistic O, Kudia J (2004) Calcium sensors and their iiiteracting protein kinases: genomics of the Arabidopsis and rice CBL-CIPK signaling networks. Plant Physiol 134: 43-58

Koornneef M, Bentsink L, and Hilhorst H (2002) Seed dormancy and germination. Current Opinion in Plant Biology 5:33–36

Kosman E, and Leonard KJ (2007) Conceptual analysis of methods applied to assessment of diversity within and distance between populations with asexual or mixed mode of reproduction. New Phytologist 174(3):683–696

Kreps JA, Wu Y, Chang HS, Zhu T, Wang X, Harper JF (2002) Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress. Plant Physiol 130: 2129–2141

Kudla J, Xu Q, Harter K, Gruissem W, Luan S (1999) Genes for calcineurin B-like proteins in Arabidopsis are differentially regulated by stress signals. Proc Natl Acad Sci USA 96:4718–4723

Kuhn RM, Caspar T, Dehesh K and Quail PH (1993) DNA binding factor GT-2 from Arabidopsis. Plant Mol Biol 23:337- 348

Kumar K, Muthamilarasan M, Prasad M (2013) Reference genes for quantitative Real-time PCR analysis in the model plant foxtail millet (*Setaria italica* L.) subjected to abiotic stress conditions.Plant Cell Tiss Organ Cult 115:13–22

Kumari K, Muthamilarasan M, Misra G, Gupta S, Subramanian A, et al. (2013) Development of eSSR-Markers in Setaria italica and Their Applicability in Studying Genetic Diversity, Cross-Transferability and Comparative Mapping in Millet and Non-Millet Species. PLoS ONE 8(6): e67742

Kumutha D, Ezhilmathi K, Sairam RK, Srivastava GC, Deshmukh PS, Meena RC (2009) Waterlogging induced oxidative stress and antioxidant activity in pigeonpea genotypes. Biol Plant 53:75–84

Kuroda M, Qzawa T, Imagawa H (1990) Changes in chloroplast peroxidase activities in relation to chlorophyll loss in barley leaf segments. Physiologia plantarum, 80: 555-560

Kyparissis A, Petropoulou Y, Manetas Y (1995) Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., Labiates) under Mediterranean field

conditions: Avoidance of photoinhibitory damage through decreased chlorophyll contents. J Exp Bot 46:1825- 1831

Lata C, Sahu PP, Prasad M (2010) Comparative transcriptome analysis of differentially expressed genes in foxtail millet (*Setaria italica* L.) during dehydration stress. Biochem Biophy Res Commun 393:720–727

Lata C, and Prasad M, (2011) Role of DREBs in regulation of abiotic stress responses in plants. J Exp Bot 62:4731—4748

Lata C, Bhutty S, Bahadur RP, Majee M, and Prasad M (2011[a]) Association of an SNP in a novel DREB2-like gene SiDREB2 with stress tolerance in foxtail millet [*Setaria italica* (L.)]. J Exp Bot 62:3387—3401

Lata C, Jha S, Dixit V, Sreenivasulu N, and Prasad M (2011[b]) Differential antioxidative responses to dehydration-induced oxidative stress in core set of foxtail millet cultivars [*Setaria italica* (L.)]. Protoplasma 248:817—828

Lata C, Yadav A, Prasad M (2011[c]) Role of plant transcription factors in abiotic stress tolerance. *In* Shanker A, Venkateshwarulu B (eds) Abiotic stress response in plants. INTECH Open Access, Rijeka, pp 269–296

Lata C, Gupta S, and Prasad M (2013) Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses. Crit Rev Biotechnol 33:328–343

Lata C, Mishra AK, Muthamilarasan M, Bonthala VS, Khan Y, et al. (2014) Genome-Wide Investigation and Expression Profiling of AP2/ERF Transcription Factor Superfamily in Foxtail Millet (*Setaria italica* L.). PLoS ONE 9(11): e113092

Lawlor DW, and Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ 25: 275–294

Lawlor DW, and Tezara W (2009) Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. Annals of Botany 103: 561–579, 2009

Lee GJ, Roseman AM, Saibil HR, Vierling E (1997) A small heat shock protein stably binds heat denatured model substrates and can maintain a substrate in a folding-competent state. EMBO J 16:659–671

Levitt J (1972) Response of plants to stresses. Academic Press. New York and London. p.697

Li CH, Pao WK, and Li HW (1942) Interspecific crosses in Setaria. II. Cytological studies of interspecific hybrids involving 1, *S. faberii* and *S. italica,* and 2. A three way cross, F2 of *S. italica* × *S. viridis* and *S.faberii.* J Hered 33: 351-355

Li Y, Wu S, Cao YS, Zhang XZ (1995) A phenotypic diversity analysis of foxtail millet (*Setaria italica* (L.) P. Beauv.) landraces of Chinese origin. Genet Resour Crop Evol 43:377–384

Li Y, Wu SZ (1996) Traditional maintenance and multiplication of foxtail millet (*Setaria italica* (L) P. Beauv.) landraces in China. Euphytica 87:33–38

Li YM (1997[a]) Drought-resistant mechanism and genetic expression of foxtail millet. *In* Li YM (eds) Foxtail millet breeding. Beijing: China Agricultural Press, pp 433–434

Li YM (1997[b]) Breeding for foxtail millet drought tolerant cultivars (in Chinese). *In* Li Y, eds, Foxtail millet breeding. Beijing: Chinese Agr Press, pp 421–446

Li LL, Li SF, Tao Y, and Kitagawa Y (2000) Molecular cloning of a novel water channel from rice: its products expression in Xenopus oocytes and involvement in chilling tolerance. Plant Sci 154: 43–51

Li G, and Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor Appl Genet 103:455–461

Li G, Gao M, Yang B, and Quiros CF (2003) Gene for gene alignment between the Brassica and Arabidopsis genomes by direct transcriptome mapping. Theot and App Gent 107(1):168–180

Li B, Wei A, Song C, Li N, Zhang J (2008) Heterologous expression of the TsVP gene improves the drought resistance of maize. Plant Biotechnol J 6:146–159

Li P, and Brutnell TP (2011) Setaria viridis and Setaria italica, model genetic systems for the Panicoid grasses. J Exp Bot 62: 3031–3037

Li SY, An SJ, Liu ZL, Cheng RH and Wang ZJ (2014) Innovation of the new superior quality foxtail millet [*Setaria italica* (L.)P.Beauv] variety-Jigu32 with characteristics of stress resistance, stable and high yield and its physiological mechanism. Agricultural Sciences 5:304-316

Li ZY, Wang N, Dong L, Bai H, Quan JZ, Liu L, et al. (2015) Differential Gene Expression in Foxtail Millet during Incompatible Interaction with Uromyces setariae-italicae. PLoS ONE 10(4): e0123825

Lin Z, He D, Zhang X et al. (2005) Linkage map construction and mapping QTL for cotton fibre quality using SRAP, SSR and RAPD. Plant Breeding 124(2):180–187

Linnaeus C (1753) Species plantarum. Stockholm.

Lisitsyn N, and Wigler M (1993) Cloning the differences between two complete genomes. Science 259: 946-951

Liu Q, Umeda M, Uchimiya H (1994) Isolation and expression analysis of two rice genes encoding the major intrinsic protein. Plant Molecular Biology 26: 2003–2006

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Goda H, Shimada Y, Yoshida S, Shinozaki K, Yamaguchi-Shinozaki K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell 10:1391–1406

Liu L, White MJ, MacRae TH (1999) Transcription factors and their genes in higher plants Functional domains, evoludon and regulation. Eur J Biochem 262: 247-257

Liu JH, Nadda K, Honda C, Kitashiba H, Wen XP, Pang XM, Moriguchi T (2006) Polyamine biosynthesis of apple callus under salt stress: importance of arginine decaroxylase pathway in stress response. J Exp Bot 57:2589-2599

Liu J, Xie X, Du J, Sun J, Bai X (2008) Effects of simultaneous drought and heat stress on Kentucky bluegrass. J Horti Sci 115: 190–195

Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F (1999) Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiol 119:1091–1099

Lopez CG, Banowetz G, Peterson CJ and Kronstad WE (2001) Differential accumulation of a 24- kd dehydrin protein in wheat seedlings correlates with drought stress tolerance at grain filling. Hereditas 135: 175-181

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265

Lu YQ and Wu WR (2006) Identification of salt-responsive genes in English cordgrass (Spartina anglica) roots using SRAP technique. J Zhejiang Univ 32(5):511–514

Luan S, Kudia F, Rodn'guez-Concepcio'n M, Yalovsky S, Gruissem W (2002) Cahiiodulins and calcineurin B-like proteins: calcium sensors for specific signal response coupling in plants. Plant Cell 14: S389-S400

Luck H (1974) Methods in enzymatic analysis. Academic Press, New York, 885

Ma AF, Li JN, Chen L, Qian W, Fu FY, and Liu LZ (2008) Differential display of related genes to seed-coat color by cDNA-SRAP in *Brassica napus* L. Acta Agro Sinica 34:526–529

Maathuis FJ, Filatov V, Herzyk P, Krijger GC, Axelsen KB, Chen S, Green BJ, Li Y, Madagan KL, Sanchez-Fernandez R, Forde BG, Palmgren MG, Rea PA, Williams LE, Sanders D, Amtmann A (2003) Transcriptome analysis of root transporters reveals participation of multiple gene families in the response to cation stress. Plant J 35: 675-692

Madhusudan KV, Giridarakumar S, Ranganayakulu GS, Chandraobulreddy, P and Sudhakar C (2002) Effect of water stress on some physiological responses in two groundnut *(Arachis hypogea* L.) cultivars with contrasting drought tolerance. J Plant Biol 29:199-202

Makbul S, Saruhan-guler N, Durmus N and Guven S (2011) Changes in anatomical and physiological parameters of soybean under drought stress. Turk J Bot 35:369-377

Malm NR, and Rachie KO (1971) Setaria millets: A review of the world literature. S.B. 513. University of Nebraska, Lincoln pp 19–29

Manabendra DK and Baruah K (1998) Studies on physiological traits of rice (Oryza sativa L.) cultivars under moisture stress situations. Ind J Ecol 25: 192-196.

Manikavelu A, Nadarajan N, Ganesh SK, Gnanamalar RP, Babu RC (2006) Drought tolerance in rice: morphological and molecular genetic consideration, Plant Growth Regul 50:121–138

Manjula K, Sharma PS, Ramesh Tatikunta, Nageshwara Rao T (2003) Evaluation of castor (*Ricinus communis* L) genotypes for moisture stress. Indian J Plant Physiol 8(3): 319-322

March TJ, Able JA, Schultz CJ, Able AJ (2007) A novel late embryogenesis abundant protein and peroxidase associated with black point in barley grains. Proteomics 7:3800–3808

Mariaux JB, Bockel C, Salamini F, Bartels D **(**1998) Desiccation- and abscisic acid-responsive genes encoding major intrinsic proteins (MIPs) from the resurrection plant Craterostigma plantagineum. Plant Molecular Biology 38: 1089–1099

Masle J, Gilmore SR, Farquhar GD (2005) The ERECTA gene regulates plant transpiration efficiency in Arabidopsis. Nature 436:866–870

Massacci A, Nabiev SM, Petrosanti L, Nematov SK, Chernikova TN, Thor K, and Leipner J (2008) Response of the photosynthetic apparatus of cotton (*Gossypium hirsutum* L.) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging. Plant Physiol Biochem 46:189-195

Massonnet EC, Serge R, Erwin D, Regnard JL (2007). Stomatal Regulation of Photosynthesis in Apple Leaves: Evidence for Different Water-use Strategies between Two Cultivars Catherine. Ann Bot 100(6):1347-1356

Mationn MA, Brown JH and Ferguon H (1989) Leaf water potential, relative water content and diffusive resistance as screening techniques for drought resistance in barley. Agron J 81: 100-105

Maurel C, and Chrispeels MJ (2001) Aquaporins: a molecular entry into plant water relations. Plant Physiol 125:135–138

Mavis RD, and Stellwagen E (1968) Purification and subunit structure of glutathione reductase from bakers' yeast. J Biol Chem 243:809–814

McCarty DR, and Chory J (2000) Conservation and innovation in plant signaling pathways. Cell 103:201-209

McCourt P, and Creelman R (2008) The ABA receptors – we report you decide. Curr Opin Plant Biol 11:474–478

McNeil SD, Nuccio ML, Hanson AD (1999) Betaines and related osmoprotectants. Targets for metabolic engineering of stress resistance. Plant Physiol 120:945–949

Michel BE, and Kaufmann MR (1973) The Osmotic Potential of Polyethylene Glycol 6000. Plant Physiol 51(5): 914-916

Mishra A, Muthamilarasan M, Khan Y, Parida SK, Prasad M (2014) Genome-Wide Investigation and Expression Analyses of WD40 Protein Family in the Model Plant Foxtail Millet (*Setaria italica* L.),PLOS ONE 9(1):e86852

Mittler R (2002) Oxidative stress, antioxidants and stresstolerance. Trends Plant Sci 7:405-410

Mittova V, Tal M, Volokita M, and Guy M (2003) Up−regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt−induced oxidative stress in the wild salt−tolerant tomato species *Lycopersicon pennellii*. Plant Cell Environ 26:845–856

Miyazawa SI, Yoshimura S, Shinzaki Y, Maeshima M, Miyake C (2008) Deactivation of aquaporins decreases internal conductance to CO_2 diffusion in tobacco leaves grown under long-term drought. Funct Plant Biol 35:556–564

Moaveni P (2011) Effect of water deficit stress on some physiological traits of wheat (*Triticum aestivum*). Agric Sci Res J 1:164-68

Mohammad M El, Benbella M and Talouizete A (2002) Effect of sodium chloride on sunflower (*Helianthus annuus* L.) seed germination. Helia*,* 37: 51-58

Mohammadkhani N, and Heidari R (2008) Drought induced accumulation of soluble sugars and proline in two maize varieties. Wld Appl Sci J 3:448-453

Monakhova OF and Chernyadev II (2002) Protective role of kartolin-4 in wheat plants exposed to soil drought. Applied and Environmental Microbiology. 38:373–380

Monclus R, Dreyer E, Villar M, Delmotte FM, Delay D, Petit JM, Barbaroux C, Thiec DL, Bréchet C, Brignolas F (2006) Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoids* X *Populus nigra*. New Phytol 169:765–777

Morgan PW (1990) Effects of abiotic stresses on plant hormone systems. *In* Stress Responses in plants: adaptation and acclimation mechanisms, Wiley-Liss, Inc, pp113–146

Mukhopadhyay A, Vij S, Tyagi AK (2004) Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. Proc Natl Aca Sci USA 101: 6309–6314

Munné-Bosch S, and Penuelas J (2003) Photo and antioxidative protection, and a role for salicylic acid during drought and recovery in fieldgrown Phillyrea angustifolia plants. Planta 217:758–766

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25: 239-250

Murugan R, and Nirmalakumari A (2006) Genetic divergence in foxtail millet (*Setaria italica* (L.) Beauv.). Indian J Genet 66(4): 339-340

Muthamilarasan M, Khandelwal R, Yadav CB, Bonthala VS, Khan Y, et al. (2014[a]) Identification and Molecular Characterization of MYB Transcription Factor Superfamily in C4 Model Plant Foxtail Millet (*Setaria italica* L.). PLoS ONE 9(10): e109920

Muthamilarasan M, Bonthala VS and Awdhesh VS, Mishra K, Khandelwal R, Khan, Roy R and Prasad M (2014[b]) C_2H_2 type of zinc finger transcription factors in foxtail millet define response to abiotic stresses, Funct Integr Genomics, Springer-Verlag Berlin Heidelberg

Muthamilarasan M, and Prasad M (2015) Advances in Setaria genomics for genetic improvement of cereals and bioenergy grasses. Theor Appl Genet 128: 1–14

Muthamilarasan M, Bonthala VS, Khandelwal R, Jaishankar J, Shweta S, Nawaz K and Prasad M (2015) Global analysis of WRKY transcription factor superfamily in Setaria identifies potential candidates involved in abiotic stress signaling. Front Plant Sci 6:910

Muthamilarasan M, Mangu VR, Zandkarimi H, Prasad P and Baisakh N (2016) Structure, organization and evolution of ADP-ribosylation factors in rice and foxtail millet, and their expression in rice. Scientific Reports 6:24008

Nagano Y, Inaba T, Furuhashi H, and Sasaki Y (2001) Trihelix DNA-binding protein with specificities for two distinct ciselements: Both important for light down-regulated and dark-inducible gene expression in higher plants. J Biol Chem 276:22238-22243

Nagarajan S, and Rane J (2000) Relationship of seedling traits with drought tolerance in spring wheat cultivars. Indian J Plant Physiol 5(3): 264-270

Nageswara RRC, Talwar HS, Wright GC (2001) Rapid assessment of specific leaf area and leaf nitrogen in peanut (*Arachis hypogaea* L.) using chlorophyll meter. J Agron Crop Sci 189: 175-182

Nakano T, Suzuki K, Fujimura T, Shinshi H (2006) Genome-wide analysis of the ERF gene family in Arabidopsis and rice. Plant Phys 140: 411–432

Nakashima K, Kiyosue T, Yamaguchi-Shinozaki K, Shinozaki K (1997) A nuclear gene, erdl, encoding a chloroplast-targeted Clp protease regulatory subunit homolog is not only induced by water stress but also developmentally up-regulated during senescence in *Arahidopsis thaliana.* Plant J 12: 851-861

Nakashima K, Shinwar ZK, Sakuma Y, Seki M, Miura S, Shinozaki K, Yamaguchi-Shinozaki K (2000) Organization and expression of two Arabidopsis DREB2 genes encoding DRE-binding proteins involved in dehydration- and high salinity-responsive gene expression. Plant Mol Biol 42:657–665

Nakashinia K, Ito Y, Yamaguchi-Shinozaki K (2009) Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. Plant Physiol 149: 88-95

Nawaz M, Iqbal N, Idrees S, Ullah I (2014) DREB1A from Oryza sativa var. IR6: homology modelling and molecular docking. Turk J Bot 38: 1095-1102

Nayyar H, and Gupta D (2006) Differential sensitivity of C3 and C4 plants to water deficit stress: Association with oxidative stress and antioxidants. Environ Exp Bot 58:106-113

Nelson DE, Repetti PP, Adams TR, Creelman RA, Wu J, Warner DC, Anstrom DC, Bensen RJ, Castiglioni PP, Donnarummo MG, Hinchey BS, Kumimoto RW, Maszle DR, Canales RD, Krolikowski KA, Dotson SB, Gutterson N, Ratcliffe OJ, Heard JE (2007) Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on waterlimited acres. Proc Natl Acad Sci USA 104:16450–16455

Nicolas ME, Lambers H, Simpson RJ, Dalling MJ (1985) Effect of drought on metabolism and partitioning of carbon in two wheat varieties differing in drought tolerance. Ann Bot (London) 55:727-742

Niknam V, Razavi N, Ebrahimzadeh H, Sharifizadeh B (2006) Effect of NaCl on Biomass, Protein and Proline Contents, and Antioxidant Enzymes in Seedlings and Calli of Two Trigonella Species. Biol Plant 50: 591-596

Nirmalakumari A, and Vetriventhan M (2010) Characterization of foxtail millet germplasm collections for yield contributing traits. Elect J Plant Breed 1: 140–147

Niyogi KK (1999) Photoprotection revisited: genetic and molecular approaches, Annu Rev Plant Phys 50:333–359

Noctor G, and Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Biol 49:249–279

Nole-Wilson S, and Krizek BA (2000) DNA binding properties of the Arabidopsis floral development protein AINTEGUMENTA. Nucleic Acids Res 28(21):4076–4082

Nonami H (1998) Plant water relations and control of cell elongation at low water potentials. J Plant Res 111:373–382

O'Grady K, Goekjian VH, Naim CJ, Nagao RT and Key JL (2001) The transcript abundance of GmGT-2, a new member of the GT-2 family of transcription factors from soybean, is down-regulated by light in a phytochrome-dependent manner. Plant Mol Biol 47:367-378

Ober ES, Setter TL, Madison JT, Thompson JF, Shapiro PS (1991) Influence of water deficit on maize endosperm development: enzyme activities and RNA transcripts of starch and zein synthesis, abscisic acid, and cell division. Plant Physiol 97:154–164

OdCe S (1994) CG-1, a parsley light-induced DNA-binding protein. Plant Mol Biol 25(5):921

Ohme-Takagi M, Shinshi H (1995) Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. Plant Cell 7(2):173–182

Okamuro JK, Caster B, Villarroel R, Montagu MV, Jofuku KD (1997) The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in Arabidopsis. Proc Natl Acad Sci USA. 94: 7076– 7081

Onions J, Hermann S, and Grundstrom T (1997) Basic helixloop- helix protein sequences determining differential inhibition by calmodulin and S-100 proteins. J Biol Chem 272:23930–23937

Onions J, Hermann S, and Grundstrom T (2000) A novel type of calmodulin interaction in the inhibition of basic helix-loophelix transcription factors. Biochemistry 39:4366–4374

Pabo CO, Sauer RT (1992) Transcription factors: structural families and principles o f DNA recognition. Annu Rev Biochem 61: 1053-1095

Pan Y, Wu LJ, Yu ZL (2006) Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (*Glycyrrhiza uralensis* Fisch), Plant Growth Regul 49:157–165

Parasuram R, Lee JS, Yin P, Somarowthu S, Ondrechen MJ (2010). Functional classification of protein 3D structures from predicted local interaction sites. J Bioinform Comput Biol 8(1): 1–15

Park HC, Kim ML, Kang YH, Jeon JM, Yoo JH, Kim MC, Park CY, Jeong JC, Moon BC, Lee JH, et al. (2004) Pathogenand NaCl-induced expression of the SCaM-4 promoter is mediated in part by a GT-1 box that interacts with a GT-1-like transcription factor. Plant Physiol 135:2150-2161

Park JA, Cho SK, Kim JE, Chung HS, Hong JP, Hwang B, Hong CB, Kim WT (2003) Isolation of cDNAs differentially expressed in response to drought stress and characterization of the Ca- LEAL1 gene encoding a new family of atypical LEA-like protein homologue in hot pepper (*Capsicum annuum* L. cv. Pukang). Plant Sci 165:471–481

Pastori G, Foyer CH, Mullineaux P (2000) Low temperature-induced changes in the distribution of H_2O_2 and antioxidants between the bundle sheath and mesophyll cells of maize leaves.J Exp Bot 51:107–113

Patade VY, and Suprasanna P (2008) Radiation induced in vitro mutagenesis for sugarcane improvement. Sugar Tech 10(1):14–19

Patade VY, Suprasanna P, Bapat VA (2008) Gamma irradiation of embryogenic callus cultures and in vitro selection for salt tolerance in sugarcane (*Saccharum offcinarum* L.). Agril Sci China 7(9):101–105

Paul AK and Panneerselvam R (2013) Osmolyte accumulation, photosynthetic pigment and growth of [*Setaria italica* (L.) P. Beauv.] under drought stress, Asian Pacific Journal of Reproduction 2(3): 220-224

Pawar RM, Prajapati RM,. Sawant DM and Patil AH (2013) Genetic divergence in Indian bean (*Lablab purpureus* L. Sweet). Elec J Plant Breed 4(2): 1171- 1174

Pearson WR (1995) Comparison of methods for searching protein sequence databases. Prot Sci 4:1145-1160

Peng LX, Gu LK, Zheng CC, Li DQ, Shu HR (2006) Expression of MaMAPK gene in seedlings of Malus L. under water stress. Acta Biochim Biophys Sin 38:281–286

Peng Y, Lin W, Cai W, Arora R (2007) Overexpression of a Panax ginseng tonoplast aquaporin alters salt tolerance, drought tolerance and cold acclimation ability in transgenic Arabidopsis plants. Planta 226 (2007) 729–740

Perruc E, Charpenteau M, Ramirez BC, Jauneau A, Galaud JP, Ranjeva R, Ranty B (2004) A novel calmodulin-binding protein functions as a negative regulator of osmotic stress tolerance in Arabidopsis thaliana seedlings. Plant J 38:410–420

Peschke G, Seidler C and Yogal (1997) Effect of drought during the growing season on Agricultural (*Triticum aestivum* L.) and forest plant canopy (*Piceae abies* L.). *In* Proc 14th Int Cong Biomet 2(2): Solvenia

Pinheiro GL, Marques CS, Costa MDBL, Reis PAB, Alves MS, Carvalho CM, Fietto LG, Fontes EPB (2009) Complete inventory of soybean NAC transcription factors: sequence conservation and expression analysis uncover their distinct roles in stress response. Gene 444:10–23

Plaut Z (2003) Plant exposure to water stress during specific growth stages, Encyclopedia of Water Science, Taylor & Francis, pp 673– 675

Porcel R, Gomez M, Kaldenhoff R, Ruiz-Lozano JM (2005) Impairment of NtAQP1 gene expression in tobacco plants does not affect root colonisation pattern by arbuscular mycorrhizal fungi but decreases their symbiotic efficiency under drought. Mycorrhiza 15:417–423

Prasada Rao KE, de Wet JMJ, Brink DK and Mengesha MH (1986) Intraspecific variation and systematics of cultivated *Setaria italica*, foxtail millet (Poaceae). Econ Bot 41:108–116

Prashant SH, Namakkal SR, and Chandra TS (2005) Effect of the antioxidant properties of millet species on oxidative stress and glycemic status in alloxan-induced rats. Nutrition Research 25:1109–1120

Pujade-Renaud V, Sanier C, Canibillau L, Pappusamy A, Jones H, Ruengsri N (2005) Molecular characterization of new members o f the *Hevea hrasiliemis* hevein multigene family and analysis o f their promoter region in rice. Biochimica at Biophysica Acta 1727: 15 M61

Puranik S, Bahadur RP, Srivastava PS, and Prasad M (2011[a]) Molecular cloning and characterization of a membrane associated NAC family gene, SiNAC from foxtail millet [*Setaria italica* (L.) P. Beauv.]. Mol Biotechnol 49:138–150

Puranik S, Jha S, Srivastava PS, Sreenivasulu N, Prasad M (2011[b]) Comparative transcriptome analysis of contrasting foxtail millet cultivars in response to short-term salinity stress. J Plant Physiol 168: 280–287

Puranik S, Sahu PP, Srivastava PS, Prasad M (2012) NAC proteins: regulation and role in stress tolerance. Trends Plant Sci 17:369–381

Puranik S, Sahu PP, Mandal SN, B. VS, Parida SK, et al. (2013) Comprehensive Genome-Wide Survey, Genomic Constitution and Expression Profiling of the NAC Transcription Factor Family in Foxtail Millet (Setaria italica L.). PLoS ONE 8(5): e64594

Pysh LD, Wysocka-Diller JW, Camilleri C, Bouchez D, Benfey PN (1999) The GRAS gene family in Arabidopsis: sequence characterization and basic expression analysis of the SCARECROW-LIKE genes. Plant J 18(1):111–9

Qi X, Xie S, Liu Y, Yi F, and Yu J (2013) Genome-wide annotation of genes and noncoding RNAs of foxtail millet in response to simulated drought stress by deep sequencing. Plant Mol Biol 83:459–473

Que YX, Yang ZX, Xu LP, and Chen RK (2009) Isolation and identification of differentially expressed genes in sugarcane infected by Ustilago scitaminea. Acta Agronomica Sinica 35(3):452–458

Que YX, Lin JW, Song XX, Xu LP, and Chen RK (2011) Differential gene expression in sugarcane in response to challenge by fungal pathogen Ustilago scitaminea revealed by cDNA-AFLP. J Biomed and Biotech Article ID 160934

Que YX, Xu LP, Lin JW, Luo J, et al. (2012) cDNA-SRAP and its application in differential gene expression analysis: A case study in *Erianthus arundinaceum*. J Biomed Biotechnol Article ID 390107

Quigley F, Rosenberg JM, Shachar-Hill Y, Bohnert HJ (2001) From genome to function: the Arabidopsis aquaporins. Genome Biol 3(1): RESEARCH0001

Rahnama H, and Ebrahimzadeh H (2005) The effect of NaCl on antioxidant enzyme activities in potato seedlings. Biol Plant 49:93–97

Ramanjulu S, and Bartels D (2002) Drought- and desiccation-induced modulation of gene expression in plants. Plant Cell Environ 25:141–151

Ramanjulu S, and Sudhakar C (1997) Drought tolerance is partly related to amino acid accumulation and ammonia assimilation: A comparative study in two mulberry genotypes differing in drought sensitivity. J Plant Physiol 150: 345-350

Ramanjulu S, and Sudhakar C (2000) Proline metabolism during dehydration in two mulberry genotypes with contrasting drought tolerance. J Plant Physiol 157: 81-85

Ramel F, Sulmon C, Gouesbet G, Coue´e I (2009) Natural variation reveals relationships between pre-stress carbohydrate nutritional status and subsequent responses to xenobiotic and oxidative stress in Arabidopsis thaliana. Ann Bot 104:1323–1337

Ratnayaka H.H., Molin W.T., Sterling T.M. (2003) Physiological and antioxidant responses of cotton and spurred anoda under interference and mild drought. J Exp Bot 54:2293–2305

Rauf S, and Sadaqat HA (2007) Effects of varied water regimes on root length, dry matter partitioning and endogenous plant growth regulators in sunflower (Helianthus annuus L.). J Plant Interactions 2 (1):41–51

Rauf S, and Sadaqat HA (2008) Identification of physiological traits and genotypes combined to high achene yield in sunflower (*Helianthus annuus* L.) under contrasting water regimes. Aust J Crop Sci 1(1):23–30

Reddy KP, Subhani SM, Khan PA, Kumar KB (1995) Effect of light and benzyl adenine on dark treated growing rice (Oryza sativa) leaves-changes in peroxidase activity. Plant Cell Physiol 26: 987-994

Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. JPlant Physiol 161:1189–1202

Reddy VG, Upadhyaya HD and Gowda CLL (2006) Characterization of world's foxtail millet germplasm collections for morphological traits. J SAT Agr 47:107–109

Reiser V, Raitt D, Saito H (2003) Yeast osmosensor Sln1 and plant cytokinin receptor Cre1 respond to changes in turgor pressure. Yeast 20:S169

Rensburg LV, Kruger GHJ (1994) Evaluation of components of oxidative stress metabolism for use in selection of drought tolerant cultivars of *Nicotiana tabacum* L. J Plant Physiol 143:730-737

Reymond P, Weber H, Damond M, Farmer EE (2000) Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. Plant Cell 12:707–719

Riccardi F, Gazeau P, de Vienne D, and Zivy M (1998) Protein changes in response to progressive water deficit in maize. Quantitative variation and polypeptide identification. Plant Physiol 117: 1253-1263

Richards RA (1978) Variation between and within species of rapeseed (*Brassica campestris* and *B.napus*) in response to drought stress. III. Physiological and physicochemical characters. Australian J Agri Res 29: 491-501

Richmond T, and Somerville S (2000) Chasing the dream: plant EST microarrays.Cur Opi Plant Bio 3(2):108-116

Riechmann JL, Heard .1, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Saraaha RR, Creelman R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu G (2000) Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science 290: 2105-2110

Ritchie SW, Nguyan HT, Holaday AS (1990) Leaf water content and gas exchange parameters of two wheat genotypes differing in drought resistance. Crop Sci. 30: 105-111

Rivero RM, Kojima M, Gepstein A, Sakakibara H, Mittler R, Gepstein S, Blumwald E (2007) Delayed leaf senescence induces extreme drought tolerance in a flowering plant. Proc Natl Acad Sci USA 104:19631–19636

Robinson MJ, and Cobb MH (1997) Mitogen-activated protein kinase pathways. Curr Opin Cell Biol 9: 180-186

Romier C, Cocchiarella F, Mantovani R, and Moras D (2003) The NF-YB/NF-YC structure gives insight into DNA binding, transcription regulation by CCAAT factor NF-Y. J Biol Chem 278:1336–1345

Rushton PJ, and Sonissich IE (1998) Transcriptional control of plant genes responsive to pathogens. Curr Opin Plant Biol 1:311-315

Sadasivam S, Chandrababu R, Ravindran N, Raja Jaj (2000) Genetic variation in seed germination root traits and drought recovery in rice. Indian J Plant Physiol 5(1):73-78

Sadeghian SY, and Yavari N (2004) Effect of water-deficit stress on germination and early seedling growth in sugar beet. J Agron Crop Sci 190:138-144

Saijo Y, Hata S, Kyozuka J, Shimaraoto K, Izui K (2000) Over-expression o f a single Ca^{2+}- dependent protein kinase confers both cold and salt/drought tolerance on rice plants. Plant J 23: 319-327

Sakamoto S (1987) Origin and dispersal of common millet and foxtail millet. Japan Agr Res Quart 21:84–89

Sakamoto H, Maruyama K, Sakuma Y, Meshi T, Iwabuchi M, Shinozaki K, Yamaguchi-Shinozaki K (2004) Arabidopsis Cys2/ His2-type zinc-finger proteins function as transcription repressors under drought, cold and high-salinity stress conditions. Plant Physiology 136:2734–2746

Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi-Shinozaki K (2002) DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration- and coldinducible gene expression. Bioch Bioph Res Co 290: 998–1009

Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K, Yamaguchi-Shinozaki (2006) Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression. The Plant Cell 18:1292-309

Sakurai J, Ishikawa F, Yamaguchi T, Uemura M, Maeshima M (2005) Identification of 33 rice aquaporin genes and analysis of their expression and function. Plant Cell Physiol 46(9):1568-1577

Samarah NH (2005) Effects of drought stress on growth and yield of barley. Agron Sustain Dev 25:145–149

Sang Y, Zheng S, Li W, Huang B, Wang X (2001) Regulation of plant water loss by manipulating the expression of phospholipase D. Plant J 28:135–144

Schäffner AR (1998) Aquaporin function, structure, and expression: are there more surprises to surface in water relations? 204:131-139

Schontz D, and Rether B (1998) Genetic variability in foxtail millet, [Setaria italica (L.) P. Beauv.] using a heterologous rDNA probe. Plant Breed 117:231–234

Schontz D, Rether B (1999) Genetic variability in foxtail millet, Setaria italica (L.) P. Beauv.: Identification and classification of lines with RAPD markers. Plant Breed 118:190–192

Schummer M, Ng W, Nelson PS, Bumgarner RE, Hood dL (1997) Inexpensive hand held device for the construction of high-density nucleic acid arrays. BioTechniques 23:1087-1092

Schuurmans JA, van Dongen JT, Rutjens BP, Boonman A, Pieterse CM, Borstlap AC (2003) Members of the aquaporin family in the developing pea seed coat include representatives of the PIP, TIP, and NIP subfamilies. Plant Mol Biol 53(5):633-645

Schwartz SH, Qin X, Zeevaart JAD (2003) Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes, and enzymes. Plant Physiol 131:1591–1601

Seki M, Narusaka M, Abe H, Kasuga M, Yamaguchi-Shinozaki K, Caminci P, Hayashizaki Y, Shinozaki K (2001) Monitoring the expression pattern of 1300 Arabidopsis genes under drought and cold stresses by using a full-length cDNA microarray. Plant Cell 13: 61-72

Seki M, Ishida J, Narusaka M, Fujita M, Nanjo T, Umezawa T, Kamiya A, Nakajima M, Enju A, Sakurai T, Satou M, Akiyama K, Yamaguchi-Shinozaki K, Carninci P, Kawai J, Hayashizaki Y, Shinozaki K (2002[b]) Monitoring the expression pattern of around 7,000 Arabidopsis genes under ABA treatments using a full-length cDNA microarray. Funct Integr Genomics 2: 282-291

Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T, Satou M, Akiyama K, Taji T, Yamaguchi-Shinozaki K, Carninci P, Kawai J, Hayashizaki Y, Shinozaki K (2002[a]) Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J 31: 279-292

Seki M, Kamei A, Yamaguchi-Shinozaki K, Shinozaki K (2003) Molecular responses to drought, salinity and frost: common and different paths for plant protection. Curr Opin Biotechnol 14:194–199

Sgherri CLM, Pinzino C and Navari-Izzo F (1996) Sunfl ower seedlings subjected to increasing stress by water deficit: changes in O_2 production related to the composition of thylakoid membranes. Physiol Plant 96: 446–452

Shahbazi H, Taeb M, Bihamta MR and Darvish F (2010) Inheritance of antioxidant activity of bread wheat under terminal drought stress. American- Eurasian J Agric and Environ Sci 8(6): 680-684

Shamsi K (2010) The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. J Anim and Plant Sci 8(3): 1051-1060

Sharma P, and Dubey RS (2005) Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant Growth Regul 46:209–221

Sheen J (1996) Ca^{2+}-dependent protein kinases and stress signal transduction in plants. Science 274:1900-1902

Shinozaki K, and Yamaguchi-Shinozaki K (1996) Molecular responses to drought and cold stress. Curr Opin Biotechnol 7:161–167

Shinozaki K, and Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: differences and cross talk between two stress signalling pathways. Curr Opin Plant Biol 3:217–233

Shinozaki K, and Yamaguchi-Shinozaki K (2007) Gene networks involved in drought stress tolerance and response. Journal of Experimental Botany 58: 221-227.

Shinozaki, K. and Yamaguchi-Shinozaki, K. (1997) Gene expression and signal transduction in water-stress response. Plant Physiol 115: 327-334

Sicher RC, Timlin D, Bailey B (2012) Responses of growth and primary metabolism of water-stressed barley roots to rehydration. J Plant Physiol 169:686–695

Siddique MRB, Hamid A, Islam MS (2001) Drought stress effects on water relations of wheat. Bot Bull Acad Sinica 41:35–39

Siddique MRB, Hamid A, and Islam MS (2004) Drought stress effects on water relations of wheat. Botanical Bulletin of Academia Sinica 41:35-39

Siefritz F, Biela A, Eckert M, Otto B, Uehlein N, Kaldenhoff R (2001) The tobacco plasma membrane aquaporin NtAQP1. J Exp Bot 52(363):1953-1957

Singh RK, and Choudhury BD (1979) Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi, India, pp 211-215

Singh K, and Africa BS (1985) Evaluation of moisture stress tolerance in castor cultivars during germination seedling growth and emergence. Proc Indian Nat Sci Acad Biol Sci 51:364-368

Sivamani E, Bahieldin A, Wraith JM, Al-Niemi T, Dyer WE, Ho T-HD QuR (2000) Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley HVA1 gene. Plant Sci 155:1–9

Smart LB, Moskal WA, Cameron KD, and Bennett AB (2001) MIP genes are down-regulated under drought stress in Nicotiana glauca. Plant Cell Physiol 42: 686–693

Smirnoff N (1993) The role of active oxygen in the response of plants to water defi cit and desiccation. New Phytol 125:27–58

Smirnoff N (1998) Plant resistance to environmental stress. Curr Opin Biotechnol 9:214–219

Sneddon WA, and Fromm H (1998) Calmodulin, calmodulin-regulated proteins and plant responses to the environment. Trends Plant Sci 3:299–304

Sneddon WA, and Fromm H (2001) Calmodulin as a versatile calcium signal transducer in plants. New Phytol 151:35–66

Sreenivasulu N, Ramanjulu S, Ramachandra-Kini K (1999) Total peroxidase activity and peroxidase isoforms as modified by salt stress in two cultivars of foxtail millet with differential salt tolerance. Plant Science 141:1–9

Sreenivasulu N, Grimm B, Wobus U, Weschke W (2000) Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (Setaria italica). Physiol.Plant 109: 435–442.

Sreenivasulu N, Miranda M, Prakash HS, Wobus U, Weschke W (2004) Transcriptome changes in foxtail millet genotypes at high salinity: identification and characterization of a PHGPX gene specifically upregulated by NaCl in a salt-tolerant line. J Plant Physiol 161: 467–477

Stockinger EJ, Gilmour SJ, Thomashow MF (1997) Arabidopsis thaliana CBF1 encodes an AP2 domain-containing transcription activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc Natl Acad Sci USA 94:1035–1040

Stone RL, and Dixon JE (1994) Protein-tyrosine phosphatases. J Biol Chem 269: 31323-31326

Subrahmanyam D, Subash N, Haris A, Sikka AK (2006) Influence of water stress on leaf photosynthetic characteristics in wheat cultivars differing in their susceptibility to drought. Photosynthetica 44:125-129

Suga S, Imagawa S, and Maeshima M (2001) Specificity of the accumulation of mRNAs and proteins of the plasma and tonoplast aquaporins in radish organs. Planta 212: 294–304

Sun SJ, Guo SQ, Yang X, Bao YM, Tang HJ, Sun H, Huang J, Zhang HS. (2010) Functional analysis of a novel Cys2/His2-type zinc finger protein involved in salt tolerance in rice. J Exp Bot 61: 2807–281

Suresh BV, Muthamilarasan M, Mishra G, and Prasad M (2013) FmMDb: a versatile database of foxtail millet markers for millets and bioenergy grasses research. PLoS ONE 8:e71418

Szabados L, and Savoure A (2010) Proline: a multifunctional amino acid. Trends in Plant Science 15: 89–97

Tahir MHN, Muhammad I, Hussain MK (2002) Evaluation of sunflower (Helianthus annuus L.) inbred lines for drought tolerance. Int J Agri and Biol 4(3):398–400

Tahkokorpi M, Taulavuori K, Laine K, and Taulavuori E (2007) After-effects of drought related winter stress in previous and current year stems of *Vaccinium myrtillus* L. Environ and Experim Bot 61: 85–93

Taiz L, and Zeiger E (2006) Plant Physiology, 4th Ed., Sinauer Associates Inc. Publishers, Massachusetts.

Tamura T, Hara K, Yamaguchi Y, Koizumi N, Sano H (2003) Osmotic stress tolerance of transgenic tobacco expressing a gene encoding a membrane-located receptor-like protein from tobacco plants. Plant Physiol 131:454–462

Tan QK, Irish VF (2006) The Arabidopsis zinc finger-homeodomain genes encode proteins with unique biochemical properties that are coordinately expressed during floral development. Plant Physiology 140:1095–1108

Tas S, and Tas B (2007) Som physiological responses of drought stress in wheat genotypes with different poliodity in turkiye. World J Agri Sci 3(2): 178-183

Testa A, Donati G, Yan P, Romani F, Huang TH, Viganò MA, et al. (2005) Chromatin immunoprecipitation ChIP on chip experiments uncover a wide spread distribution of NF-Y binding CCAAT sites outside of core promoters. J Biol Chem 280:13606–13615

Tezara W, Mitchell VJ, Driscoll SD and Lawlor DW (1999) Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature 401: 914-917

Thomashow MF (1998) Role of cold-responsive genes in plant freezing tolerance. Plant Physiol 118:1–7

Thomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Biol 50:571–599

Tian CG, Wan P, Sun SH, Li JY, Chen MS (2004) Genome-wide analysis of the GRAS gene family in rice and Arabidopsis. Plant Mol Biol 54(4):519–32

Tian ZD, Zhang Y, Liu J, Xie CH (2010) Novel potato C_2H_2-type zinc finger protein gene, StZFP1, which responds to biotic and abiotic stress, plays a role in salt tolerance.Plant Biol (Stuttg) 12: 689–697

Torok Z, Goloubinoff P, Horvath I, Tsvetkova NM, Glatz A, Balogh G, Varvasovszki V, Los DA, Vierling E, Crowe JH, Vigh L (2001) Synechocystis HSP17 is an amphitropic protein that stabilizes heat-stressed membranes and binds denatured proteins for subsequent chaperonemediated refolding. Proc Natl Acad Sci USA 98:3098–3103

Tournaire-Roux C, Sutka M, Javot H, Gout E, Gerbeau P, Luu DT, Bligny R, Maurel C (2003) Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins, Nature 425:393–397

Tran LS, Nakashima K, Sakuma Y, Simpson SD, Fujita Y, Maruyama K, Fujita M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2004) Isolation and functional analysis of Arabidopsis stress-inducible NAC transcription factors that bind to a drought-responsivecis-element in the early responsive to dehydration stress 1 promoter. The Plant Cell 16:2481–2498

Tran LS, Nakashima K, Sakuma Y, Osakabe Y, Qin F, Simpson SD, Maruyama K, Fujita Y, Shinozaki K, Yamaguchi-Shinozaki K (2007) Co-expression of the stress-inducible zinc finger homeodomain ZFHD1 and NAC transcription factors enhances expression of the ERD1 gene in Arabidopsis . Plant J 49:46–63

Tripathy JN, Zhang J, Robin S, Nguyen TH, and Nguyen HT (2000) QTL for cell-membrane stability mapped in rice (*Oriza sativa* L.) under drought stress. Theore and Appl Genet 100: 1197-1202

Trujillo LE, Sotolongo M, Menendez C, Ochogavia ME, Coll Y, Hernandez I, Borras-Hidalgo O, Thomma BPHJ, Vera P, Hernandez L (2008) SodERF3, a novel sugarcane ethylene responsive factor (ERF), enhances salt and drought tolerance when overexpressed in tobacco plants. Plant Cell Physiol 49:512–525

Tuberosa R, and Salvi S (2006) Genomics-based approaches to improve drought tolerance of crops. Trends Plant Sci 11:405–412

Tunnacliffe A, and Wise M (2007) The Continuing Conundrum of the LEA Proteins. Naturwissenschaften.94:791- 812

Turkan I, Bor M, Zdemir F, Koca H (2005) Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P*. *acutifolius* and drought-sensitive *P*. *vulgaris* subjected to polyethylene glycol mediated water stress. Plant Sci 168:223–231

Uno Y, Urao T, Yamaguchi-Shinozaki K, Kanechi M, Inagaki N, Maekawa S, and Shinozaki K (1998) Early salt-stress effects on expression of genes for aquaporin homologues in the halophyte sea aster (*Aster tripolium* L.). J Plant Res 111: 411–419

Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2000) Arabidopsis basic leucine zipper transcription factors involved in an abscisic aciddependent signal transduction pathway under drought and high-salinity conditions. Proc Nat Acad Sci USA 97:11632-11637

Urao T, Yamaguchi-Shinozaki K, Urao S, Shinozaki K (1993) An Arabidopsis myb homolog is induced by dehydration stress and its gene product binds to the conserved MYB recognition sequence. Plant Cell 5:1529–1539

Urao T, Yakubov B, Satoh R, Yamaguchi-Shinozaki K, Seki M, Hirayama T, Shinozaki K (1999) A transmembrane hybrid-type histidine kinase in *Amhidopsis* functions as an osmosensor. Plant Cell. 11: 1743-1754.

Valliyodan B, and Nguyen HT (2006) Understanding regulatory networks and engineering for enhanced drought tolerance in plants. Curr Opin Plant Biol 9:189-195

van Der Luit AH, Olivari C, Haley A, Knight MR and Trewavas AJ (1999) Distinct calcium signaling pathways regulate calmodulin gene expression in tobacco. Plant Physiol 121:705–714

van den Berg N , Crampton BG, Hein I, Birch PRJ, Berger DK (2004) High-throughput screening of suppression subtractive hybridization cDNA libraries using DNA microarray analysis. BioTechniques 37: 818-824

Van K, Onoda S, Kim MY, Kim KD, Lee SH (2008) Allelic variation of the Waxy gene in foxtail millet [Setaria italica (L.) P. Beauv.] by single nucleotide polymorphisms. Mol Genet Genomics 279: 255–266

Vavilov NI (1926) Studies on the origin of cultivated plants. Inst Appl Bot Plant Breed 16:1-248

Veeranagamallaiah G, Chandraobulreddy P, Jyothsnakumari G, and Sudhakar C (2007) Glutamine synthetase expression and pyrroline- 5-carboxylate reductase activity influence proline accumulation in two cultivars of foxtail millet (*Setaria italica* L.) with differential salt sensitivity. Environ Expt Bot 60:239–244

Velculescu VE, Zhang L, Vogelstein B, Kinzler KW (1995) Serial analysis of gene expression. Science 270: 484-487

Verbruggen N, and Hermans C (2008) Proline accumulation in plants: a review. Amino Acids 35: 753–759

Verslues PE, Bray EA (2004) LWR 1 and LWR 2 are required for osmoregulation and osmotic adjustment in Arabidopsis. Plant Physiol Prev., 1: 2831-2842

Verslues PE, and Bray EA (2006) Role of abscisic acid(ABA) and Arabidopsis thaliana ABA-insensitive loci in low water potentialinduced ABA and proline accumulation. J Exp Bot 1: 201-212

Vierling E (1991) The roles of heat shock proteins in plants. Annu Rev Plant Physiol Plant Mol Biol 42:579–620

Vinall HN (1924) Foxtail millet: its culture and utilization in the United States. USDA.Farmers Bull, Pp 793

Vinocur B, and Altman A (2005) Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr Opin in Biotech. 16:123-132

Wahid A, and Rasul E (2005) Photosynthesis in leaf, stem, flower and fruit, in: Pessarakli M. (Ed.), Handbook of Photosynthesis, 2nd ed., CRC Press, Florida, pp479–497

Wahid A (2007) Physiological implications of metabolites biosynthesis in net assimilation and heat stress tolerance of sugarcane (Saccharum officinarum) sprouts, J Plant Res 120:219–228

Wahid A, and Close TJ (2007) Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves, Biol Plantarum 51:104–109

Wang ZM, Devos KM, Liu CJ, Wang RQ and Gale MD (1998) Construction of RFLP-based maps of foxtail millet, *Setaria italica* (L.) P. Beauv. Theor Appl Genet 96:31–36

Wang C, Chen J, Zhi H, Yang L, Li W, Wang Y, Li H, Zhao B, Chen M, Diao X. (2010) Population genetics of foxtail millet and its wild ancestor. BMC Genet 11: 90

Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218:1–14

Wang R, Hong G, and Han B (2004) Transcript abundance of rml1, encoding a putative GT1-like factor in rice, is up-regulated by Magnaporthe grisea and down-regulated by light. Gene 324:105-115

Wang Y, Jiang J, Zhao X, Liu G, Yang C, Zhan L (2006) A novel LEA gene from Tamarix androssowii confers drought tolerance in transgenic tobacco. Plant Sci 171:655–662

Wang CR, Yang AF, Yue GD, Gao Q, Yin HY, Zhang JR (2008) Enhanced expression of phospholipase C 1 (ZmPLC1) improves drought tolerance in transgenic maize. Planta 227:1127–1140

Wang Y, Zhang J, Cui R, Li W, Zhi H, Li H, and Diao X (2009) Transformation of wheat with DNAj gene from foxtail millet via pollen-tube pathway. (in Chinese with English abstract) Acta Agril Boreali- Sinica

Warnasooriya SN, and Brutnell TP (2014) Enhancing the productivity of grasses under high-density planting by engineering light responses: from model systems to feedstocks. J Exp Bot 65: 2825–2834

Washburn KB, Davis EA, Ackerman S (1997) Coactivators and TAFs of transcription activation in wheat. Plant Mol Biol 35: 1037-1043

Wasilewska A, Vlad F, Sirichandra C, Redko Y, Jammes F, Valon C, Frey NFD, Leung J (2008) An update on abscisic acid signaling in plants and more. Mol Plant 1:198–217

Waters ER, Lee GJ, Vierling E (1996) Evolution, structure and function of the small heat shock proteins in plants. J Exp Bot 47:325–338

Weig A, Deswarte C, Chrispeels MJ (1997) The major intrinsic protein family of Arabidopsis has 23 members that form three distinct groups with functional aquaporins in each group. Plant Physiol 114: 1347-1357

Wessler SR (2005) Homing into the origin of the AP2 DNA binding domain. Trends Plant Sci 10: 54–56

Windhovel A, Hein I, Dabrowa R, Stockhaus J (2001) Characterization of a novel class of plant homeodomain proteins that bind to the C4 phosphoenolpyruvate carboxylase gene of Flaveria trinervia. Plant Molecular Biology 45:201–214

Wise MJ (2003) LEAping to conclusions: a computational reanalysis of late embryongenesis abundant proteins and their possible roles. BMC Bioinformatics 4:52

Wright GC, Nageswara RC, Farquhar GD (1994) Water use efficiency and carbon isotop discrimination in peanut under water deficit conditions. Crop SCI 34: 92-97

Xiao X, Xu X, Yang F (2008) Adaptive responses to progressive drought stress in two Populus cathayana populations. Silva Fennica 42:705–719

Xie ZM, Zou HF, Lei G, Wei W, Zhou QY, Niu CF, Liao Y, Tian AG, Ma B, Zhang WK, et al. (2009) Soybean trihelix transcription factors GmGT-2A and GmGT-2B improve plant tolerance to abiotic stresses in transgenic Arabidopsis. PloS One 4:e6898

Xiong L, and Ishitani M (2006) Stress signal transduction: components, pathways, and network integration. *In* Rai AK, Takabe T (eds) Abiotic stress tolerance in plants: toward the improvement of global environment and food. Springer, Dordercht, The Netherlands, pp 3–29

Xue YY, Li P, and Lin QB (2008) Research evolution on chemical component and physical character of foxtail millet. J Chinese Cereals and Oils Asso 22:51–56

Yadav RS, Hash CT, Bidinger FR, Devos KM, Howarth CJ (2004) Genomic regions associated with grain yield and aspects of postflowering drought tolerance in pearl millet across environments and tester background. Euphytica 136:265–277

Yadav C, Bonthala VS, Muthamilarasan M, Pandey G, Khan Y, and Prasad M (2014) Genome-wide development of transposable elements-based markers in foxtail millet and construction of an integrated database. DNA Research 1–12

Yadav CB, Muthamilarasan M, Pandey G, Prasad M (2015) Identification, characterization and expression profiling of Dicer-like, Argonaute and RNA-dependent RNA polymerase gene families in foxtail millet. Plant Mol Biol Rep 33, 43–55

Yamada M, Morishita H, Urano K, Shiozaki N, Yamaguchi-Shinozaki K, Shinozaki K, Yoshiba Y (2005) Effects of free proline accumulation in petunias under drought stress. J Exp Bot 56:1975–1981

Yamada S, Katsuhara M, Kelly WB, Michalowski CB, Bohnert HJ (1995) A family of transcripts encoding water channel proteins: tissue-specific expression in the common ice plant. Plant Cell 7: 1129-1142

Yamada S, Komori T, Myers PN, Kuwata S, Kubo T, and Imaseki H (1997) Expression of plasma membrane water channel genes under water stress in *Nicotiana excelsior*. Plant Cell Physiol 38: 1226–1231

Yamaguchi-Shinozaki K, and Shinozaki K (1994) A novel cw-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cll 6: 251-264

Yanagisawa S, and Sheen J (1998) Involvement of maize Dof zinc finger proteins in tissuespecific and light-regulated gene expression. Plant Cell 10: 75-89

Yanagisawa S, and Schmidt RJ (1999) Diversity and similarity among recognition sequences of Dof transcription factors. Plant J 17:209-214

Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN (1982) Living with water stress:evolution of osmolyte systems. Science 217:1214–1222

Yang T, and Poovaiah BW (2002) A calmodulin-binding/ CGCG box DNA-binding protein family involved in multiple signalling pathways in plants. J Biol Chem 277:45049–45058

Yoo JH, Park CY Kim JC, Heo WD et al. (2005) Direct interaction of a divergent CaM isoform and the transcription factor, MYB2, enhances salt tolerance in Arabidopsis. J Biol Chem 280:3697–3706

Yu J, Hu S, Wang J, Wong GK, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X, et al. (2002) A draft sequence of the rice Genome (*Oryza sativa* L. ssp. indica). Science 296: 79-92

Yu Q, Hu Y, Li J, Wu Q, Lin Z (2005) Sense and antisense expression of plasma membrane aquaporin BnPIP1 from Brassica napus in tobacco and its effects on plant drought resistance. Plant Sci 169:647–656

Zaefyzadeh M, Quliyev RA, Babayeva SM, Abbasov MA (2009) The effect of the interaction between genotypes and drought stress on the superoxide dismutase and chlorophyll content in durum wheat landraces. Turk J Biol 33:1–7

Zaifnejad M, Clark RB, Sullivan CY (1997) Aluminium and water stress effects on growth and proline of Sorghum, J. Plant Physiol. 150: 338–344

Zang X, and Komatsu S (2007) A proteomics approach for identifying osmotic-stress-related proteins in rice. Phytochem 68: 426-437

Zangre R, Nguyen-Van E, Rherissi B, and Till-Bottraud I (1992) Organisation du pool ge´nique de *Setaria italica* (L.) P. Beauv. et exploitation des ressources ge´ne´tiques d'espe`-ces spontane´es. *In* Complexes d'espe`ces, flux de ge`nes et resources ge´ne´tiques des plantes. Lavoisier e´dition, BRG, Paris,pp 87–97

Zarei S, Ehsanpour AA, and Abbaspour J (2012) The role of over expression of P5CS gene on proline, catalase, ascorbate peroxidase activity and lipid peroxidation of transgenic tobacco (Nicotiana tabacum L.) plant under in vitro drought stress. J Cell and Mol Res 4 (1):43-49

Zeid IM, and Shedeed ZA (2006) Response of alfalfa to putrescine treatment under drought stress. Biol Plant 50:635–640

Zhang B, Chen W, Foley RC, Buttner M, Singh KB (1995) Interactions between distinct types of DNA binding proteins enliance binding to ocs element promoter sequences. Plant Cell 7: 2241-2252

Zhang L, Ohta A, Takagi M, Imai R (2000) Expression of plant group 2 and group 3 LEA genes in Saccharomyces cerevisiae revealed functional divergence among LEA Proteins. J Biochem 127:611–616

Zhang L, Ma XL, Zhang Q, Ma CL, Wang PP, Sun YF, Zhao YX, Zhang H (2001) Expressed sequence tags from a NaCl-treated Suaeda salsa cDNA library. Gene 267:197-200

Zhang JZ, Creelman RA, Zhu JK (2004) From laboratory to field: Using information from Arabidopsis to engineer salt, cold, and drought tolerance in crops. Plant Physiol 135:615–621

Zhang JP, Wang MY, Bai YF, Jia JP, and Wang GY (2005) Rapid evaluation on drought tolerance of foxtail millet at seedling stage (in Chinese. English abstract). J Plant Genet Resour 6:59–62

Zhang J, Liu T, Fu J, Zhu Y, Jia J, Zheng J, Zhao Y, Zhang Y, Wang G (2007) Construction and application of EST library from Setaria italica in response to dehydration stress. Genomics 90:121–131

Zhang G, Liu X, Quan Z et al. (2012) Genome sequence of foxtail millet (Setaria italica) provides insights into grass evolution and biofuel potential. Nat Biotechnol 30:549–554

Zhang TC, and Nakajima M (2015) Advances in Applied Biotechnology. *In* (eds), Lecture Notes in Electrical Engineering, pp 332

Zhang Y, Wang Z, Chai T, Wen Z, Zhang H (2008) Indian mustard aquaporin improves drought and heavy-metal resistance in tobacco. Mol Biotechnol 40:1–13

Zheng X, Chen B, Lu G, Han B (2009) Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. Biochem Biophys Res Commun 379:985–989

Zheng ZL, Nafisi M, Tam A, Li HM, Crowell DN, Chary SN, Schroeder JI, Shen J, Yang Z (2002) Plasma membrane associated ROP10 small GTPase is a specific negative regulator of abscisic acid responses in *Arabidopsis*. Plant Cell 14:2787–2797

Zhou DX (1999) Regulatory mechanism of plant gene transcription by GT-elements and GT-factors. Trends Plant Sci 4:210-214

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Physiol and Plant Mol Biol 53: 247–273

Zhu XH, Song YC, Zhao ZH, Shi YS, Liu YH, Li Y and Wang TY (2008) Methods for identification of drought tolerance at germination period of foxtail millet by osmotic stress (in Chinese, English abstract). J Plant Genet Resour 9:62–67

ANNEXURE I

REAGENTS AND STOCK SOLUTIONS

i. 0.5 M EDTA (pH 8.0) (Storage at RT)

Water	:	To make 100 ml
EDTA	:	18.6 gm
pH 8.0	using NaOH	

ii. 10x TBE buffer (Storage at RT)

Water	:	To make 1 litre
Tris base	:	108.0 gm
Boric acid	:	55.0 gm
0.5 M EDTA (pH 8.0)	:	40.0 ml

iii. CTAB extraction buffer (Freshly prepared)

Water	:	To make 100 ml
1 M Tris (pH 7.5)	:	10.0 ml
5 M NaCl	:	14.0 ml
0.5 M EDTA (pH 8.0)	:	10.0 ml
CTAB	:	1.0 gm
β-mercaptoethanol	:	1.0 ml

iv. TE buffer (pH 8.0)

Tris HCl (pH 8.0)	:	10.0 mM
EDTA (pH 8.0)	:	1.0 mM

v. 10X MOPS buffer

Water	:	To make 1000 ml
MOPS	:	41.2 g
Sodium acetate	:	10.9 g
EDTA (pH 8.0)	:	3.7 g
CTAB	:	1.0 gm

vi. Agarose 6X loading dye

Bromophenol blue	:	0.25% (w/v)
Xylene cynol	:	0.25% (w/v)
Glycerol	:	30% (v/v)

vii. Phosphate buffered saline

NaCl	:	8.0 g
KCl	:	0.2 g
KH2PO4	:	0.2 g
Na2HPO4	:	1.15 g
water	:	1000 ml

ANNEXURE II

SEQUENCE SUBMISSION AT NCBI

```
Setaria italica aquaporin PIP2-7 gene, partial cds
GenBank: KX572974.1
FASTA Graphics
 LOCUS       KX572974               1280 bp    DNA     linear   PLN 04-JUN-2017
DEFINITION  Setaria italica aquaporin PIP2-7 gene, partial cds.
ACCESSION   KX572974
VERSION     KX572974.1
KEYWORDS    .
SOURCE      Setaria italica (foxtail millet)
  ORGANISM  Setaria italica
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            Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; PACMAD
            clade; Panicoideae; Panicodae; Paniceae; Cenchrinae; Setaria.
REFERENCE   1  (bases 1 to 1280)
  AUTHORS   Gawai,D., Moharil,M., Jadhav,P., Gahukar,S. and Penna,S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-JUL-2016) Biotechnology Centre, Dr.Pdkv, Krishi
            Nagar, Akola, Maharshtra 444104, India
COMMENT     ##Assembly-Data-START##
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                     /protein_id="ARU06999.1"
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Setaria italica aquaporin PIP2-7 mRNA, partial cds
GenBank: KX572973.1
FASTA Graphics

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VERSION     KX572973.1
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Tracheophyta;
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REFERENCE   1  (bases 1 to 770)
  AUTHORS   Gawai,D., Moharil,M., Jadhav,P., Gahukar,S. and Penna,S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-JUL-2016) Biotechnology Centre, Dr.Pdkv, Krishi
            Nagar, Akola, Maharshtra 444104, India
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```

VITA

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Mob. No. 7588230106
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5. Academic qualifications

SN	Name of Degree	Year	Division	Name of University	Subjects
1.	M.Sc. (DBT-JNU)	2012	First	OUAT, Bhubaneswar	Agriculture Biotechnology
2.	B.Sc.	2010	First	MPKV, Rahuri	Agriculture Biotechnology

6. Field of Interest : Biotechnology and Molecular Biology

7. Publications : 7

Place: Akola
Date:

Signature of Student