Morpho-physiological and molecular responses of turmeric genotypes subjected to water stress

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By
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Under the guidance of

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Kozhikode-673 012, Kerala, India
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DECLARATION

I hereby declare that the thesis entitled '**Morpho-physiological and molecular responses of turmeric genotypes subjected to water stress**' submitted for the award of the degree of Doctor of Philosophy in Botany at University of Calicut, is a bonafide record of research work carried out at **ICAR-Indian Institute of Spices Research**, Kozhikode, Kerala under the guidance of **Dr. K. S. Krishnamurthy**, Principal Scientist, Division of Crop Production and Post-Harvest Technology, ICAR-Indian Institute of Spices Research. This thesis or part of it has not been submitted to any other university for the award of another degree or diploma previously. The plagiarism has been checked at CHMK library, University of Calicut and the values are well within the acceptable limits.

Place: Kozhikode **(Nazmin Banu CV)**
Date:

(Dr. K.S. Krishnamurthy)

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Nazmin Banu C.V

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ABBREVIATIONS AND SYMBOLS

%	:	Percentage
&	:	And
/	:	Per
@	:	At the rate of
°C	:	Degree Celsius
μg	:	Microgram
μl	:	Microlitre
μM	:	Micromolar
ABA	:	Abscissic acid
Acc.	:	Accession
ANOVA	:	Analysis of variance
APX	:	Ascorbate peroxidase
ASTA	:	American Spice Trade Association
BDMC	:	Bisdemethoxycurcumin
Bp	:	Base pair
BSA	:	Bovine Serum Albumin
Ca	:	Calcium
CAT	:	Catalase
CD	:	Critical Difference
cDNA	:	Complimentary deoxyribonucleic acid
cm	:	Centimeter
cm^2	:	Square centimeter
Cu	:	Copper
CURS	:	Curcumin synthase
$CuSO_4$	:	Copper sulphate
CV	:	Coefficient of Variance
DAP	:	Days After Planting
DCS	:	Diketide CoA synthase
DEPC	:	Diethylpyrocarbonate
DHAR	:	Dehydro ascorbate reductase
DMC	:	Demethoxycurcumin
DNase	:	Deoxyribonuclease
dNTP	:	Deoxynucleotide triphosphate
DTPA	:	Diethylene triamine pentaacetic acid
EDTA	:	Ethylenediamine tetra acetic acid
EF-1α	:	Elongation factor 1α
et al.,	:	And his coworkers

ET	:	Ethylene
Fe	:	Iron
Fig.	:	Figure
FW	:	Fresh weight
g	:	Gram
GAE	:	Gallic acid equivalent
GC-MS	:	Gas chromatography
GR	:	Glutathione reductase
H	:	Hour
H_2O_2	:	Hydrogen peroxide
H_2SO_4	:	Sulphuric acid
H_3BO_3	:	Boric acid
HCl	:	Hydrochloric acid
HPLC	:	High-performance liquid chromatography
i.e.	:	That is
ICAR	:	Indian council of agricultural research
IISR	:	Indian Institute of Spices Research
K	:	Potassium
K_2SO_4	:	Potassium sulphate
kg	:	Kilogram
l	:	Litre
Ltd	:	Limited
m	:	Meter
M	:	Molarity
MDA	:	Malondialdehyde
MDHAR	:	Mono Dehydro Ascorbate Reductase
Mg	:	Magnesium
mg	:	Milligram
min	:	Minute
ml	:	Millilitre
mM	:	Millimolar
Mn	:	Manganese
mRNA	:	Messenger RNA
MSL	:	Mean Sea Level
N	:	Normality
N	:	Nitrogen
Na_2CO_3	:	Sodium carbonate
NAGS	:	National Active Germplasm Site
NaOH	:	Sodium hydroxide
No	:	Number
ºC	:	Degree Celsius

OD	:	Optical density
P	:	Phosphorus
PAGE	:	Polyacrylamide gel electrophoresis
PCA	:	Principal Component Analysis
PCR	:	Polymerase chain reaction
PEG	:	Polyethylene glycol
pH	:	Potential of hydrogen
Plant^{-1}	:	Per plant
POD	:	Peroxidase
ppm	:	Parts per million
PPO	:	Polyphenol oxidase
PVP	:	Polyvinylpyrrolidone
Pvt.	:	Private
qRT-PCR	:	Quantitative real time PCR
CRD	:	Completely Randomized Design
RBD	:	Randomized Block Design
RH	:	Relative Humidity
RNA	:	Ribonucleic acid
RNase	:	Ribonuclease
rpm	:	Revolutions per minute
rRNA	:	Ribosomal RNA
SDS	:	Sodium dodecyl sulphate
SE	:	Standard error
Sec	:	Second
SOD	:	Superoxide dismutase
TBARS	:	Thiobarbituricacid reactive substances
TCA	:	Trichloroacetic acid
TFs	:	Transcription factors
Tris	:	Tris(hydroxymethyl)aminomethane
U	:	Unit
U/g	:	Units per gram
UV	:	Ultraviolet
v/v	:	volume in volume
VIS	:	Visible
viz.,	:	Namely
W	:	Watt
w/v	:	Weight in volume
Zn	:	Zinc
α	:	Alpha
β	:	Beta
γ	:	Gamma

H : Hour
HCl : Hydrochloric acid
HPLC : High-performance liquid chromatography
IISR : Indian Institute of Spices Research
IPTG : Isopropyl β-D-1-thiogalactopyranoside
Kb : Kilobase
M : Molar
Mg : Milligram
Min : Minute
ml : Millilitre
mM : Millimolar
mm : Millimeter
mRNA : Messenger RNA
NaCl : Sodium chloride
NaOH : Sodium hydroxide
Ng : Nanogram
nm : Nanometer
OD : Optical density
ORF : Open reading frame
PAGE : Polyacrylamide gel electrophoresis
PAL : Phenylalanine ammonia lyase
PCR : Polymerase chain reaction
PEG : Polyethylene glycol
pH : Potential of hydrogen

ABSTRACT

Morpho-physiological and molecular responses of turmeric genotypes subjected to water stress

During the turmeric crop growth, fluctuations in precipitation and seasonal changes in the crop cycle play a major role, especially water shortage, leading to yield reduction under extreme weather conditions which has become more frequent due to global climate change. However, there are no reports on the crop response and adaptation to such changes, more specifically to limited water availability during rhizome growth stage in turmeric. Understanding the mechanism by which the crop responds to drought stress will facilitate in identification/developing drought-tolerant varieties. The objective of the present study was to identify drought tolerant genotypes which produce sustainable yield under drought condition and to elucidate the physiological, biochemical and molecular mechanism involved in drought response. Morpho-physiological evaluation of 50 elite turmeric genotypes revealed that genotypes (SL 10, SL 5, IISR Prabha, IISR Prathibha, IISR Pragati, NDH 1, Suguna, Suvarna and Rajendra Sonia) with lower leaf area, higher relative water content, lesser electrolyte leakage, higher wax content and fewer stomata than other genotypes and were shortlisted as tolerant. Conversely, genotypes (IISR Alleppey Supreme, IISR Kedaram and Acc 66) with contrasting characters were selected as susceptible ones.

To study the mechanism underlying drought tolerance based on physiological, biochemical and molecular characterization, these selected genotypes were maintained in pots under different soil moisture regimes viz. 100% (control), 50% (moderate stress) and 25% (severe stress) field capacities. The genotypes IISR Pragati, SL 5, Suguna, and Suvarna showed higher relative water content (RWC), enzyme activities, accumulated more free proline, sugar, phenol and protein content with lower reduction in chlorophyll, carotenoids and starch as well as lower MDA, H_2O_2 content and membrane leakage compared to other genotypes. Relative gene expression analysis by q-RT PCR revealed that the TFs *ABF, bHLH, bZIP, NAC*, *DREB* and *WRKY* were upregulated under stress condition with a higher fold change exhibited by tolerant ones. *AP2* was upregulated but did not show a tolerance related variation. *AQP* gene was relatively more down regulated in drought tolerant genotypes under water deficit stress. These genotypes also had better yield compared to others.

These four genotypes with tolerance traits and higher yield along with two susceptible genotypes (IISR Alleppey Supreme and IISR Kedaram), were further evaluated in field conditions. The results indicated that, in terms of yield and desirable tolerance traits, the identified tolerant genotypes significantly outperformed the susceptible ones, showcasing superior drought tolerance traits. With respect rhizome quality, a notable variation was identified among different turmeric genotypes under water stress. The identified genotypes (IISR Pragati, SL 5, Suguna, and Suvarna) with better tolerance traits with sustainable yield under drought conditions can be cultivated under rainfed cultivation or areas with limited water availability during the rhizome development stage to obtain better yield with assured quality.

Keywords: Turmeric, water deficit stress response, gene expression analysis, antioxidant enzymes, turmeric quality.

സംഗ്രഹം

ജല സമ്മർദ്ദത്തിന് വിധേയമായ മഞ്ഞൾ ജനിതകരൂപങ്ങളുടെ മോർഫോ-ഫിസിയോളജിക്കൽ, മോളിക്യുലാർ പ്രതികരണങ്ങൾ

മഞ്ഞൾ വിള വളർച്ചയുടെ സമയത്ത്, മഴയുടെ ഏറ്റക്കുറച്ചിലുകളും വിള ചക്രത്തിലെ കാലാനുസൃതമായ മാറ്റങ്ങളും വിള ഉൽപാദനത്തിൽ ഒരു പ്രധാന പങ്ക് വഹിക്കുന്നു, പ്രത്യേകിച്ച് ജലദൗർലഭ്യം, ആഗോള കാലാവസ്ഥാ വ്യതിയാനം കാരണം പതിവായി മാറുന്ന തീവ്രമായ കാലാവസ്ഥയിൽ വിളവ് കുറയുന്നതിന് കാരണമാകുന്നു. എന്നിരുന്നാലും, മഞ്ഞളിലെ റൈസോം വളർച്ചയുടെ ഘട്ടത്തിൽ, പരിമിതമായ ജലലഭ്യതയുടെ പശ്ചാത്തലത്തിൽ, വിള പ്രതികരണത്തെക്കുറിച്ചും അത്തരം മാറ്റങ്ങളോട് പൊരുത്തപ്പെടുന്നതിനെക്കുറിച്ചും കൂടുതൽ വ്യക്തമായ റിപ്പോർട്ടുകളൊന്നുമില്ല. വിളയുടെ വരൾച്ചയെ നേരിടാനുള്ള സംവിധാനം മനസ്സിലാക്കുന്നത് വരൾച്ചയെ അതിജീവിക്കുന്ന ഇനങ്ങളെ തിരിച്ചറിയുന്നതിനും വികസിപ്പിക്കുന്നതിനും സഹായിക്കുന്നു. വരൾച്ചയെ അതിജീവിക്കുന്ന ജനിതകരൂപങ്ങൾ തിരിച്ചറിയുകയും വരൾച്ചയെ നേരിടാൻ സഹായിക്കുന്ന ഫിസിയോളജിക്കൽ, ബയോകെമിക്കൽ, മോളിക്യുലാർ വ്യവസ്ഥകൾ വ്യക്തമാക്കുകയും ചെയ്യുക എന്നതായിരുന്നു എന്റെ പഠനത്തിന്റെ ലക്ഷ്യം. 50 എലൈറ്റ് മഞ്ഞൾ ജനിതകരൂപങ്ങളുടെ മോർഫോ-ഫിസിയോളജിക്കൽ പഠനം വഴി കോശസ്തരത്തിലൂടെയുള്ള പരിമിതമായ ഇലെക്ട്രോലൈറ്റ് ചോർച്ച, ഉയർന്ന ആപേക്ഷിക ജലത്തിന്റെ അളവ്, ഇലയിലെ ഉയർന്ന മെഴുകിന്റെ അളവ്,, മറ്റ് ജനിതകരൂപങ്ങളെ അപേക്ഷിച്ച് കുറച്ച് സ്റ്റോമാറ്റ തുടങ്ങിയ ഗുണങ്ങളുള്ള എസ് എൽ 10, എസ് എൽ 5, ഐഐഎസ്ആർ പ്രഭ, ഐഐഎസ്ആർ പ്രതിഭ, ഐഐഎസ്ആർ പ്രഗതി, എൻ ഡി എച്ച് 1, സുഗുണ, സുവർണ, രാജേന്ദ്ര സോണിയ എന്നിവയെ വരൾച്ചയോട് സഹിഷ്ണുതയുള്ള ഇനങ്ങളായി തിരിച്ചറിഞ്ഞു. എന്നാൽ, വിപരീത പ്രതികരണങ്ങളുള്ള ജനിതകരൂപങ്ങൾ (ഐഐഎസ്ആർ ആലപ്പി സുപ്രീം, ഐഐഎസ്ആർ കേദാരം, എസിസി 66) പെട്ടെന്ന് വരൾച്ച ബാധിക്കാൻ സാധ്യതയുള്ളവയായി തിരഞ്ഞെടുത്തു.

ഫിസിയോളജിക്കൽ, ബയോകെമിക്കൽ, മോളിക്യുലാർ സ്വഭാവം എന്നിവയെ അടിസ്ഥാനമാക്കി വരൾച്ച സഹിഷ്ണുതയ്ക്ക് അടിസ്ഥാനമായ മെക്കാനിസം പഠിക്കാൻ, ഈ തിരഞ്ഞെടുത്ത ജനിതകരൂപങ്ങൾ മണ്ണിലെ വ്യത്യസ്ത ഈർപ്പ വ്യവസ്ഥകൾക്ക് (100% (നിയന്ത്രണം), 50% (മിതമായ സമ്മർദ്ദം), 25% (കടുത്ത സമ്മർദ്ദം) ഫീൽഡ് ശേഷി) വിധേയമാക്കി ചട്ടികളിൽ പരിപാലിച്ചു. ഐഐഎസ്ആർ പ്രഗതി, എസ്എൽ 5, സുഗുണ, സുവർണ എന്നീ ജനിതകരൂപങ്ങൾ ഉയർന്ന ആപേക്ഷിക ജലത്തിന്റെ അംശം (ആർഡബ്ല്യുസി), എൻസൈം പ്രവർത്തനങ്ങൾ, കൂടുതൽ പ്രോലിൻ, ഷുഗർ, ഫിനോൾ, പ്രോട്ടീൻ, മറ്റ് ജനിതകരൂപങ്ങളുമായി താരതമ്യം ചെയ്യുമ്പോൾ കുറഞ്ഞ അളവിലുള്ള ഹൈഡ്രജൻ പെറോക്സൈഡ്, മെംബ്രൻ ചോർച്ചയും ഉള്ളവയായി വിലയിരുത്തപ്പെട്ടു. ക്യൂ-ആർ ടി-പിസിആർ-ന്റെ ആപേക്ഷിക ജനിതക പ്രകടന വിശകലനം, ട്രാൻസ്ക്രിപ്ഷൻ ഫാക്ടറുകളായ എ ബി എഫ്, ബി.എച്ച്.എൽ.എച്ച്, ബി.സിപ്, എൻ.എ.സി, ഡി.ആർ.ഇ.ബി, ഡബ്ല്യു.ആർ.കെ.വൈ എന്നിവ സ്ട്രെസ് അവസ്ഥയിൽ നിയന്ത്രിക്കപ്പെട്ടതായി വെളിപ്പെടുത്തി. എപി2 ക്രമീകരിച്ചെങ്കിലും സഹിഷ്ണുതയുമായി ബന്ധപ്പെട്ട വ്യതിയാനം കാണിച്ചില്ല. ജലകമ്മി സമ്മർദ്ദത്തിൻ കീഴിലുള്ള വരൾച്ചയെ പ്രതിരോധിക്കാൻ ശേഷിയുള്ള ജനിതകരൂപങ്ങളിൽ ആക്വാപോറിൻ ജീൻ താരതമ്യേന കൂടുതൽ നിയന്ത്രിക്കപ്പെട്ടിരുന്നു. ഈ ജനിതകരൂപങ്ങൾക്ക് മറ്റുള്ളവരെ അപേക്ഷിച്ച് മികച്ച വിളവ് ഉണ്ടായിരുന്നു. സഹിഷ്ണുതയുടെ സ്വഭാവവും ഉയർന്ന വിളവുമുള്ള ഈ നാല്

ജനിതകരൂപങ്ങളും, കൂടാതെ രണ്ട് സഹിഷ്ണുത ജനിതകരൂപങ്ങളും (ഐഐഎസ്ആർ ആലപ്പി സുപ്രീം, ഐഐഎസ്ആർ കേദാരം) ഫീൽഡ് സാഹചര്യങ്ങളിൽ കൂടുതൽ വിലയിരുത്തപ്പെട്ടു.

വിളവിന്റെയും അഭിലഷണീയമായ സഹിഷ്ണുതയുടെയും അടിസ്ഥാനത്തിൽ തിരഞ്ഞെടുത്ത വരൾച്ചയെ സഹിഷ്ണുതയോടെ നേരിടാൻ സാധ്യതയുള്ള ജനിതകരൂപങ്ങൾ, വരൾച്ചയിൽ പെട്ടെന്ന് തളർന്നുപോകാൻ സാധ്യതയുള്ളവയെ ഗണ്യമായി മറികടന്നുവെന്ന് ഫലങ്ങൾ സൂചിപ്പിക്കുന്നു. റൈസോമിന്റെ ഗുണനിലവാരത്തെ സംബന്ധിച്ചിടത്തോളം, ജല സമ്മർദ്ദത്തിൻ കീഴിലുള്ള വ്യത്യസ്ത മഞ്ഞൾ ജനിതകരൂപങ്ങളിൽ ശ്രദ്ധേയമായ വ്യതിയാനം തിരിച്ചറിഞ്ഞു. വരൾച്ച സാഹചര്യങ്ങളിൽ സുസ്ഥിരമായ വിളവുള്ള മെച്ചപ്പെട്ട സഹിഷ്ണുതയുള്ള, തിരിച്ചറിഞ്ഞ ജനിതകരൂപങ്ങൾ (ഐഐഎസ്ആർ പ്രഗതി, എസ് എൽ 5, സുഗുണ, സുവർണ) മഴയെ ആശ്രയിച്ച് കൃഷി ചെയ്തോ അല്ലെങ്കിൽ റൈസോം വികസന ഘട്ടത്തിൽ പരിമിതമായ ജലലഭ്യതയുള്ള പ്രദേശങ്ങളിലോ കൃഷി ചെയ്താൽ ഗുണനിലവാരമുള്ള മികച്ച വിളവ് ലഭിക്കുന്നതായിരിക്കും.

സൂചകപദങ്ങൾ: മഞ്ഞൾ, ജലസമ്മർദ്ദ പ്രതിസന്ധി പ്രതികരണം, ജനിതക സൂചന വിശകലനം, ആന്റിഓക്സിഡന്റ് എൻസൈംസ്, മഞ്ഞളിന്റെ ഗുണമേന്മ.

Dedicated to my family

CHAPTER 1
INTRODUCTION

Turmeric (*Curcuma longa* L.) belonging to the family *Zingiberaceae* is a triploid, vegetatively propagated medicinal spice crop, widely used as food preservative, natural dye, as well as medicine (Krup *et al*., 2013). In ayurvedic medicine, turmeric has long been used to treat an array of illnesses, including diabetes, allergies, arthritis, cancer, and Alzheimer's disease. Medicinal properties associated with turmeric are due to curcumin, one of the important secondary metabolites (Aggarwal *et al*., 2007). Turmeric has a higher potential for application in the production of medications and cosmetics, according to recent studies. Hence, it is important to find good practices to increase the growth parameters of such a valuable spice crop (Mohamed *et al*., 2014). Turmeric requires assured availability of irrigation water especially during the dry season (Tripathi *et al*., 2018).

Environmental factors are often the main detrimental factors affecting the growth and development of plants which are static that limit the survival and interrelated distribution of plants in nature (Zhang *et al*., 2020). A wide range of biotic and abiotic stresses that crop plants experience substantially impair their ability to grow and develop which may lead to a decline in productivity, posing serious threat to agriculture (Sharma & Lavanya, 2002). A third of the global population lives within regions where there is a scarcity of water. Climatic models have predicted that severity and duration of drought stress is expected to increase due to elevated CO_2 in atmosphere and on-going global climate change scenarios (IPCC, 2007). Thus, water shortage is one of the major limitations to productivity worldwide (Lambers *et al*., 2008). Drought stress impacts the equilibrium of water within the plant, disturbs cellular-level metabolic reactions, and diminishes both ATP synthesis and respiration (Upadhyaya *et al*., 2021).

Abiotic stress factors such as drought was found to adversely affect growth and productivity of the plants (Mostajeran *et al*., 2014). Water shortage has a considerable impact on agricultural systems, and thus the capacity of plants to resist this stress is of great economic importance (Sankar *et al*., 2007; Reddy *et al*., 2004). In light of this, creating cultivars that can withstand water shortages has become a priority. Across plant species, drought imposes various physiological and biochemical limitations and adverse effects which may lead to suppression of plant growth and development, reducing crop productivity, or even lead to plant death (Pirasteh-Anosheh *et al*., 2016). To cope with water deficit, plants have evolved physiological and biochemical adaptations and they respond to desiccation at the biochemical, physiological, cellular, and molecular levels (Shinozaki & Yamaguchi, 2007). The response to environmental stresses varies among genotypes within a species (Sakata & Higashitani, 2008).

Under drought stress, plants first respond to changes in external form such as decreased plant height, decreased number and area of leaves. Plants adopt smaller leaf areas, greater leaf thickness, and increased leaf tissue density to adapt to drought. The change of leaf area, which directly affects plant photosynthesis and yield, is one of the most easily observed features of plant leaves under drought stress (Werner *et al*., 1999; Taiz & Zeiger, 2010). Soil water scarcity leads to increased synthesis of plant hormone abscisic acid (ABA) in the roots (Thompson *et al*., 2007). The major chemical root-to-shoot signal during stress such as limited water availability has been identified as ABA. ABA production is induced in roots with soil drying and is transported in the xylem vessels to the shoot, where it restricts leaf growth and triggers stomatal closure as a major adaptation to the limited availability of water. Plants with reduced stomatal density will be able to conserve more water by reducing transpiration (Caine *et al*., 2018). The thick cuticle may enhance the plant's tolerance to drought by enhancing light reflection and lowering transpiration. For instance, the cuticular lipids content of *Arabidopsis thaliana* leaves increased significantly under water shortage which facilitated water conservation by reducing transpiration (Kosma *et al*., 2009).

Plants exposed to abiotic stresses including drought leads to elevated oxidative stress with overproduction of ROS (Farooq *et al*., 2009).The major ROS generated in plants under abiotic stresses are superoxide radicals ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), alkoxy radicals (RO), and hydroxyl radicals (OH) (Apel & Hirt, 2004).These ROS which are highly reactive, deteriorate normal plant metabolism through oxidative damage to lipids, protein, and other macromolecules (Rout & Shaw, 2001). Major sites of generation of ROS are PSI and PSII in chloroplasts and complex I, ubiquinone and complex III of electron transport chain (ETC) in mitochondria (Gill & Tuteja, 2010). At a certain level ROS work as an indicator molecule for activating acclimatory responses through signal transduction pathways utilising H_2O_2 as a secondary messenger (Miller *et al.*, 2008; Cruz De Carvalho, 2008). But at higher quantity ROS induces harmful effects such as membrane lipid peroxidation, protein denaturation, and DNA mutation on plant cells (Grover *et al.*, 2000).

Plants have developed ROS-scavenging mechanisms to minimize the deleterious effects of ROS which consists of enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) as well as the enzymes of the ascorbate-glutathione cycle (ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR)) and nonenzymatic components such as ascorbate, glutathione, carotenoids, and tocopherol (Asada, 2006; Apel & Hirt, 2004; Kaur *et al.*, 2014).These work together for detoxification of ROS. SOD catalyzes the dismutation of the superoxide anion ($O_2^{\bullet -}$) to oxygen and H_2O_2 (Gomez *et al.*, 2004). H_2O_2 was then converted into water and oxygen by CAT or APX (Asada, 2006).The enzyme APX is implicated in the oxidation of AsA to monodehydroascorbate (MDHA), which can either dismutate back to AsA, or to dehydroascorbate (DHA). The DHA is then rapidly reduced to AsA with the reducing power supplied by the oxidation of GSH to oxidized GSH (GSSG). The regeneration of GSH from GSSG is catalyzed by GSH reductase (GR) (Davey *et al.*, 1999).

Drought decreased stomatal conductance, increased stomatal resistance, and thus led to decreased photosynthetic rate (Drake *et al.*, 2017; Liu *et al.,* 2019). Plant accumulates several organic and inorganic solutes such as proline, soluble sugars, soluble proteins, and free amino acids in its cytosol to lessen its osmotic potential for maintenance of cell turgor (Hussain *et al*., 2019). Osmotic adjustment improves drought tolerance by allowing cell enlargement, plant growth, and stomata to stay partially open and by maintaining CO_2 fixation under severe water deficit (Ahmed *et al*., 2017). Which indicate a highly positive correlation between RWC and photosynthetic rate (Moayedi *et al*., 2010). Maintenance of leaf turgor is an important adaptive mechanism that plays an important role in stomatal regulation and photosynthetic activities under water-deficit conditions (Lipiec *et al.,* 2013). A number of investigations have shown that plants, including several important crops, remobilize their starch reserve to release energy, sugars and derived metabolites to help mitigate the stress (Thalmann & Santelia, 2017)

Transcription factors play a critical role in the growth and development of plants under stress by activating numerous pathways during the process of signal transduction under drought stress, hence regulating and reducing plant damage on multiple levels. Among these, *bZIP*, *AP2*, and *NAC* along with *WRKY*, *ABF, bHLH*, and *DREB* are the transcription factor gene families that are mainly associated with drought stress. However, different transcriptional factors play different transcriptional regulatory roles under drought conditions, depending on plant species and strain, developmental stage, and drought treatment intensity (Shinozaki & Yamaguchi, 2007; Nakashima *et al*., 2014). Drought tolerance is therefore a complicated interplay of physiological, biochemical, and morphological processes. However, a high yield potential under drought conditions is an obvious target for improvement strategies (Blum, 1996).

Curcuminoids and essential oils are the primary active ingredients in turmeric. According to reports, the main components of curcuminoids are curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin. Curcumin, the main yellow chemical constituent of the turmeric possess various pharmacological potential like anti-inflammatory, anti-carcinogenic, anti-diabetic, antioxidant, antibacterial, anti-protozoal, anti-fungal, antiviral, anti-fibrotic, antiulcer and hypertensive activities (Sharma *et al*., 2005). When the quantity of active oxygen species and the antioxidant defense mechanisms were out of balance and uncoordinated, oxidative damage ensued, which lead to alteration in synthesis processes of secondary metabolites as protection mechanisms for plant survival. Secondary metabolites such as phenolic compounds (e.g. curcumin), terpenoids (such as volatile oil found in turmeric), tocopherol, ascorbate and glutathione are considered as the key components of cellular antioxidant network, whose changes occur as a result of environmental stresses (Neffati & Marzouk, 2008; Taarit *et al.*, 2010).

India is recognized as world's largest producer, consumer, and exporter of turmeric (Krishnamoorthy *et al.*, 2012). The crop requires consistent supply of water and nutrients throughout the year for the sustainability of growth and production. The utilization efficiency of nutrients is closely related to the dynamics of the nutrients in the soil-water-plant system (Acharya, 2008). Reduction in soil moisture level will lower maximum potential yield. The limited availability of irrigation water during the dry season is a major constraint in increasing cultivation area of turmeric (Tripathi *et al.*, 2018). Water deficit or a drought situation is a major concern throughout the world and is especially threatening to agriculture-based in *Curcuma* private sectors (Somerville & Briscoe, 2001). In turmeric, improper nutritional management practices and inadequate irrigation during critical crop growth stages can be considered as foremost contributing to low yields (Thiyagarajan *et al.*, 2011).

Therefore, this study involved investigation of the morphological, physiological, biochemical and molecular responses of turmeric genotypes under water deficit stress and its impact on yield in order to understand the mechanisms that enable the crop to survive under drought stress and identification of drought tolerant genotypes that would offer a sustainable yield.

Aim and objectives

Drought is a major worldwide concern that severely limits crop productivity and quality. Increased severity and frequency of drought is expected under the on-going global climate change scenarios. Development of stress resistant varieties is an important objective of many breeding programs, but their success has been hindered by insufficient screening methods and a lack of genotypes demonstrating distinct differences in response to well-defined environmental stresses. This is because stress tolerance is a complex quantitative trait regulated by multiple genes, making it challenging to study and characterize.

Sustainable yield can be achieved under drought conditions through development or identification of drought tolerant genotypes with good yielding ability. This is possible through evaluation of genotypes for stress tolerance and selection of genotypes on the basis of yield performance or transferring the drought tolerance trait to high yielders through conventional/molecular breeding. During turmeric cultivation, fluctuations in precipitation leading to water shortages result in reduced crop productivity. Despite its high economic value as a food spice, coloring agent and enormous medicinal application, there is still a lack of basic information regarding the physiological, biochemical and molecular adaptation, and rhizome yield and quality traits of turmeric in water-deficient environments.

Hence, the current investigation was undertaken with the following objectives

1. Screening selected genotypes for drought tolerance based on morphological and physiological parameters.
2. To study the physiological, biochemical and molecular mechanism underlying drought tolerance.
3. Field evaluation of selected drought tolerant genotypes for yield and quality under deficit water for their suitability for rainfed cultivation

CHAPTER 2
REVIEW OF LITERATURE

2.1 Turmeric (*Curcuma longa* L.)

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Liliopsida
Order	:	Zingiberales
Family	:	Zingiberaceae
Genus	:	*Curcuma*
Species	:	*Curcuma longa*

Turmeric is an herbaceous perennial plant which has yellowish funnel-shaped blooms, green, oblong leaves, and a pseudo stem at the base. It usually reaches a height of three to five feet. The mother rhizome is changed into the pseudo stem after dividing into primary, secondary, and tertiary rhizomes (Sasikumar, 2005; Ahmad *et al.*, 2020). Turmeric has a basic chromosomal number of X=21, and is a triploid species (2n=3X=63). The chromosomal number of turmeric was also found to be inconsistent, with 2n=32, 2n=48, 2n=84, 2n=62, and 2n=64 (Raghavan & Venkatasubban, 1943; Sato, 1948; Nair & Sasikumar, 2009). Turmeric is widely cultivated in tropics of Asia which was originated from the Indo-Malayan region (Purseglove, 1974). The main turmeric producing states are Andhra Pradesh, Maharashtra, Orissa, Kerala, Tamil Nadu, West Bengal, and Karnataka (Babu, 2015). India's favourable climate, fertile soil, abundant rainfall allows successful cultivation of turmeric. Currently, India is the major producer and consumer of turmeric. Second largest supplier is china, which is followed by a number of nations in the Indian sub-continent, Southeast Asia, the Caribbean and Latin America.

About 80% of the world's turmeric is produced in India, and 60% of its exports are sourced there.

Turmeric rhizome is rich source of essential oil which is used in aromatherapy and the perfume industries (Sasikumar, 2005). According to Lee *et al.* (2014) terpenoids and curcuminoids are the principal bioactive components of turmeric of which curcuminoids are mainly responsible for the rhizome's yellow colour. The major components of curcuminoid are curcumin (77%), demethoxycurcumin (17%), and bisdemethoxycurcumin (3%) (Strimpakos & Sharma, 2008; Goel *et al.,* 2008). According to the existing literature, *C. longa* has been described as a universal remedy among herbal treatments due to its diverse pharmacological properties. The presence of various metabolites such as curcuminoid, oil content, flavonoids, phenolics, vital elements, some important amino acids, protein and high alkaloid content reveals the co-relation with its medicinal uses (Sarangthem & Haokip, 2010; Jagtap, 2015). Curcumin has a long history of usage in both ayurvedic and Chinese medicine, and as a culinary spice and food colour (Fuloria *et al.*, 2022).

Turmeric is a crop of the tropical and subtropical region which is cultivated ideally between 450 and 900 meters above mean sea level (MSL), but up to 1200 meters above MSL. The range of optimum temperature is 18.2°C to 27.4°C. The crop is raised by rainfed methods in bimodal rainfall regions and by additional irrigation methods in unimodal and low rainfall regions. Soil pH range of 4.3–7.5 is ideal for the growth of turmeric crop. Fairly heavy rainfall for the first two months after planting is essential for sprouting, root and shoot emergence, and enlargement when the crop is grown as a rain-fed one (Babu, 2015).

Duration of turmeric crop can vary between seven to ten months. The most significant monsoon in India, the southwest monsoon, begins in the State of Kerala in early June and moves progressively throughout the country's central, north, west, and eastern regions. Planting can start from as early as April, when summer showers arrive, and last up to August, when the southwest monsoon can be in full fury (Anjaneyulu & Krishnamurthy, 1979; Kumar & Gill, 2008).

Turmeric requires assured water (about 150-165 cm) and nutrient supply throughout the year (8 to 9 months) for the sustainability of growth and to produce a good yield. However, limited availability of irrigation water during the dry season is a major constraint in increasing cultivation area of turmeric (Adhate, 1958; Thyagarajan *et al.,* 2011; Tripathi *et al*., 2018). Seasonal variations, agricultural practices, soil properties, shading, irrigation schedule, and rhizome maturity have been reported to be determinant key factors of quality and quantity of turmeric rhizomes (Ishmine *et al*., 2004; Hossain *et al.,* 2009; Singh *et al*., 2013; Mohamed *et al.,* 2014; Chitra *et al.,* 2017; Sandeep *et al*., 2017).

2.2 Drought stress and plants interactions

Abiotic environmental factors are often the main detrimental factors affecting the growth and development for immobile plants (Zhang *et al.,* 2022). Abiotic stress refers to the adverse impact of any abiotic factor on a plant in a given environment, resulting in a range of responses, leading to various responses that affect biological functions like gene expression, cell metabolism, growth, and development (Zhang *et al*., 2023). Specifically, abiotic stress includes extreme temperature stress, drought stress, flooding stress, salinity stress, metal stress, and nutrient stress, and different stresses can cause different responses (Zhang *et al.,* 2020). Extreme temperatures, drought and saline soils are the main environmental factors that limit the survival and interrelated distribution of plants in nature (Zhang *et al.,* 2023). When plants experience stress, they undergo non-adaptive alterations that result in protein misfolding and cell wall disintegration, while adaptive modifications improve plant resilience. Thus, the molecular mechanism of plant response to stress is multi-level and multi-process, involving sensing, signal transduction, transcription, processing, and protein translation and modification, and is a complex response mechanism with multiple genes, signalling pathways, and metabolic processes (Swain *et al.,* 2023).

Crop plants are exposed to several environmental stresses, all affecting plant growth and development, which consequently hampers the productivity of crop plants (Seki *et al*., 2003; Farooq *et al*., 2009). Drought is considered the single most devastating environmental stress, which decreases crop productivity more than any other environmental stress (Lambers *et al*., 2008). A continuous shortfall in precipitation coupled with higher evapotranspiration demand leads to agricultural drought (Mishra & Cherkauer, 2010). It is the lack of ample moisture required for normal plant growth and development to complete the life cycle (Manivannan *et al.,* 2007). Plant growth and development are adversely affected by drought, which significantly lowers crop growth rates and biomass buildup.

Climate models have predicted increased severity and frequency of drought under the ongoing global climate change scenarios (IPCC, 2007; Walter *et al.,* 2011). The main consequences of drought in crop plants are reduced rate of cell division and expansion, leaf size, stem elongation and root proliferation, and disturbed stomatal oscillations, plant water and nutrient relations with diminished crop productivity, and water use efficiency (WUE) (Li *et al.,* 2009; Farooq *et al.,* 2009). Understanding how drought affects a plant's morphological and physiological responses is essential for overcoming such challenge (Yamaguchi-Shinozaki & Shinozaki, 2006). Abiotic stress brings about a series of morphological, physiological, biochemical and molecular alterations in plants that may have deleterious impact on growth and productivity. Plants respond to water stress or desiccation at the biochemical, physiological, cellular, and molecular levels finally affecting its morphology (Shinozaki & Yamaguchi-Shinozaki, 2007; Shao *et al.,* 2009) and as a result, the physiological and metabolic processes are reprogrammed during drought (Maruyama *et al.,* 2009; George *et al.,* 2017).

Plants defend against drought stress through the four mechanisms including avoidance, escape, tolerance, and drought recovery (Fang & Xiong, 2015). Among these mechanisms, tolerance and avoidance are two important strategies for drought resistance (Sanchez *et al.*, 2002; Dekha *et al.*, 2018). Similarly, some extremely short duration crops and even genotypes within a species exhibit drought escape, but this always taxes yield (Turner *et al.,* 2001). Drought escape is more advantageous where chances of terminal drought are more recurrent. Short-duration cultivars frequently escape terminal drought compared with late maturing cultivars (Meyre *et* *al.,* 2001). The ability of a plant to maintain high plant water status or cellular hydration during drought is known as drought avoidance. Plants accomplish this mechanism either by catching more water from the soil or by minimizing water loss through transpiration (Blum, 2005).

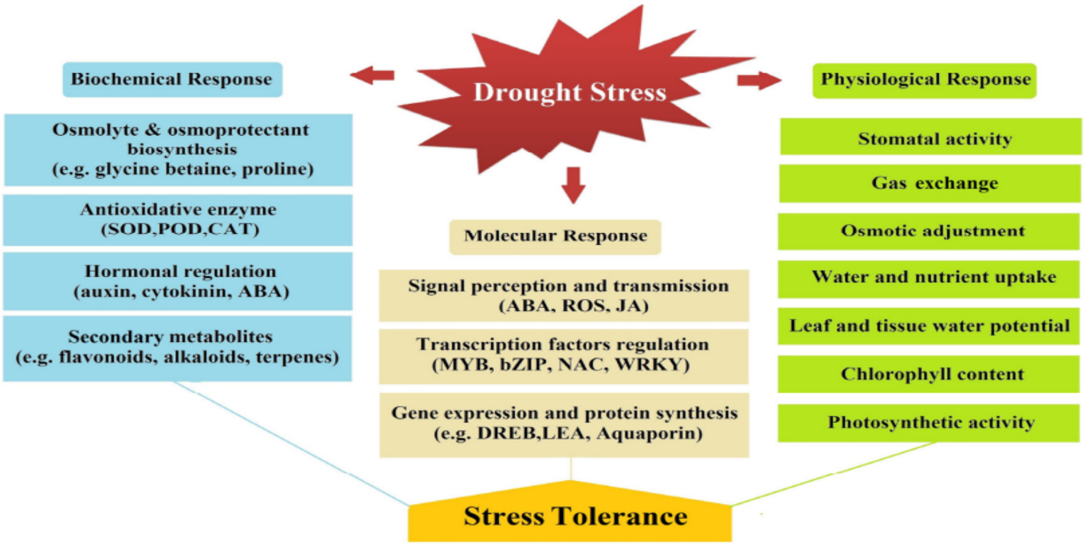


Fig. 2.1 Shematic representation of molecular, biochemical, and physiological responses to drought stress (Oguz *et al.*, 2022).

2. 2.1 Influence of drought on morphology and yield

Plant water content is lowered by drought stress, which causes cellular atrophy and apoptosis, which in turn affects morphology, growth, and development. Research has demonstrated that phenotypic plasticity allows plants to respond to environmental changes while they are under abiotic stress. The drought-resistant plants have morphological and structural characteristics that were adapted to the arid environment in terms of leaves, stems, roots, and so on (Yang *et al.,* 2021). Reduced transpirational water loss is an important adaptation of drought avoidance. To attain that, plants have developed several morphological adaptations such as higher root–shoot ratios with fewer and smaller leaves, leaf curling and increased wax deposition on the leaf surface to withstand water deficit conditions by maintaining water budget with higher uptake and minimal loss (Kim *et al.,* 2007; Ramegowda *et al.,* 2017; Kim *et al.*, 2020).

Drought decrease leaf area owing to loss of turgor and reduced leaf numbers (Farooq *et al.*, 2010). Increased stomatal and cuticular resistance, less small stomata, smaller leaf area, and vertical leaf orientation are among the major drought avoidance traits to minimize transpiration in order to save water under stress conditions (Wang & Yamauchi, 2006). However, reduced leaf area and plant stature are advantageous for restricted water use but may also result in low productivity of crop plants (Sinclair & Muchow, 2001). The reduction in plant height could be attributed to decline in the cell enlargement and more leaf senescence in the plant under water stress (Manivannan *et al.,* 2007).

An optimal leaf area is vital to photosynthesis and dry matter production. Drought stress generally reduced leaf growth in a number of plant species like populus (Wullschleger *et al*., 2005; Yang & Miao, 2010), soybean (Du *et al.,* 2020), wheat (Ian Willick *et al*., 2018), African eggplant (Mibei *et al.,* 2017) *Arabidopsis* (Clauw *et al.,* 2015) etc. Generally reduced leaf growth results in lower yield (Zhang *et al*., 2018; Ian Willick *et al*., 2018). But, under arid environmental condition, low leaf area contributed to better yield which can be attributed to a lower stomatal density thus reducing water loss (Liu *et al*., 2012; Srinivasan *et al*., 2017).

Drought screening of eighty pea varieties revealed significant ($P<0.001$) differences among treatments on growth parameters. The growth parameters decreased with a reduction in irrigation whereas the variety with better plant height led to maximum dry weight (Bodah *et al.,* 2015). Whereas cotton genotypes with medium plant height and number of nodes maintained high boll retention percentage and stomatal conductance that resulted in a higher photosynthetic rate, suggesting that moderate reduction in size led to less reduction of water content which also retained better gaseous exchange (Anwar *et al*., 2022).

According to Ghaffari *et al.* (2012) sunflower (*Helianthus annuus L.*) inbred lines with lower reduction in leaf area and plant height exhibited a higher stress tolerance index which showed a moderate positive correlation with photosynthetic efficiency and seed yield. Growth parameters, physiological and yield parameters were slightly affected under moderate stress compared to severe stress except in leaves number in turmeric varieties viz., Rajendra Sonia and Pitamber which resulted in a reduction in dry weight (Sambeta & Nageswara, 2023).

Water deficit treatment reduced mean water use by 21% due to decline in leaf area of cowpea (*Vigna unguiculata* L.) genotypes, which resulted in reduced leaf gas exchange during water deficit. Improved WUE was related to high relative water content (RWC) of leaves maintained due to a reduction in leaf area (Anyia & Herzog, 2004). A higher leaf rolling in superior wheat lines resulted in better water conservation (Ian Willick *et al.,* 2018)**.**

In wheat cultivars examined, drought (50% field capacity) resulted in a substantial reduction in growth and yield-contributing characteristics such as plant height, number of grains per spike, and grain yield per plant. AARI-11, with its superior plant height, resulted in the highest yield possible (Nawaz *et al.,* 2015). Similar reduction in plant height and yield reduction was reported in many crops such as tomato (Gupta *et al.,* 2023).Whereas moderate reduction in size led to less reduction of water content which also retained better gaseous exchange in cotton (Anwar *et al.,* 2022), sunflower (Ghaffari *et al.,* 2012) and poplar (Yang & Miao, 2010).

Maximum reduction of growth parameters resulted in greater yield reduction due to limited gaseous exchange with greatly reduced stomatal conductance in *Curcuma aroma*tica and *Curcuma domestica*. Better values of growth parameters in *C. aromatica* compared to *C. domestica* resulted in higher yield (Mohamed *et al.,* 2014). Stress tolerance also depends on stage of development.

In *Origanum vulgare* L., drought stress at anthesis phase reduced seed yield more severe than that during vegetative stage (Guo *et al.,* 2015; Morshedloo *et al.,* 2017). A significant reduction in the grain yield of barley (*Hordeum vulgare* L.) was also observed under drought conditions mainly because of less number of fertile tillers (Samarah, 2005). Tolerant wheat genotype, Faisalabad-2008 which had greater numbers of tillers/plant and spikelets/spike compared to the other tested genotypes produced higher biological yield (9.50 g plant^{-1}) and seed yield (3.39 g plant^{-1}) (Ahmad *et al.,* 2022). To minimize the water use under drought stress plants limit shoot growth by a decreased number of tillers (Soda *et al.,* 2010).

According to Li *et al.* (2021), primitive wheat genotypes, including wild, cultivated diploids, tetraploids and old hexaploids, preferentially showed a drought avoidance strategy, as evidenced by large root biomass, small leaf area, and reduced stomatal conductance under water deficit which led to lower reduction in yield under severe stress. On the other hand, contemporary hexaploid genotypes with small root systems and enhanced drought tolerance advantages, like high leaf water potential and osmotic adjustment bring about substantially greater yields and aboveground biomass under mild and moderate stress. It is the relations of phenology with the pattern of water use which makes crop to adapt against drought stress (Sekhon *et al*., 2010).

Plant size and stomatal conductance can be major factors for reduced productivity (Deikman *et al*., 2012). The interaction between the chemical signals can alter the effects of drought stress in the root zone from soil with lower moisture content. Plants growth reduction is an adaptive response of plants to stress rather than as a secondary consequence of resource margination (Rollins *et al.,* 2013). Transpiration can carry ABA from the roots, which are surrounded by dry soil, to the upper portions of the plant. ABA is an important signalling molecule for root–shoot communication and regulation of stomatal closure (Wilkinson & Davies, 2009). This type of root-generated signal could largely reduce water loss though stomata adjustment before leaf tissue senses the water deficit (Schulz *et al.,* 2021)

Stomatal density and distribution, and epidermal features may affect gas exchange quite remarkably and their relationships with key environmental factors such as light, water status, and CO_2 levels (Nilson & Assmann, 2007). Reduced stomatal density resulted in the reduced water loss and improved tolerance in *Arabidopsis* phy B mutants (Liu *et al.,* 2012). Caine *et al.,* (2019) engineered the high-yielding rice cultivar ‘IR64’ to produce fewer stomata by overexpressing the rice epidermal patterning factor *OsEPF1*, creating plants with substantially reduced stomatal density and correspondingly low stomatal conductance. Low stomatal density rice lines were more able to conserve water, using 60% of the normal amount between weeks 4 and 5 post germination but gave a lower yield compared to control because of limitation in gaseous exchange at moderate stress while a better yield under severe stress compared to other rice varieties. Low stomatal density minimize transpiration in order to save water under stress conditions (Wang & Yamauchi, 2006).Whereas extreme reduction in stomatal density impair gaseous exchange thus affect photosynthesis (Tombesi *et al*., 2015; Franks *et al*., 2015).

A higher epicuticular wax (EW) under drought stress cause to raise the leaf radiate reflection, improve water preservation, and reduce the cuticular transpiration, which helps plants to endure drought stress (Ebercon *et al*., 1977; *Ni et al.,* 2012; Bi *et al.,* 2017). EW reduces the non-stomatal water loss, shelter the plants from ultraviolet radiations and provide drought resistance (Kosma *et al*., 2009; Seo & park, 2011; Bhutta *et al.,* 2015). Higher EW content caused a lower reduction in RWC and Fv/Fm resulting in a higher photosynthetic rate and thus yield in tolerant wheat glaucous lines (Guo *et al*., 2016). Similarly, higher EW density in flag leaf led to higher relative water content in tolerant wheat varieties which resulted in better yield compared to susceptible ones (Ian Willick *et al*., 2018; Qayyum *et al.,* 2021). Thus, it is possible to use epicuticular wax content as a quantitative indicator of water stress tolerance in plants.

Drought decreased growth and productivity of sunflower (*Helianthus annuus* L.) owing to reductions in leaf water potential, rate of cell division, and enlargement primarily due to loss of turgor (Kiani *et al*., 2007; Hussain *et al.,* 2009). Under drought, all plant organs experience decreased dry matter accumulation, although the extent of the reduction varies amongst organs. For instance, drought decreased shoot and flower fresh and dry weights of marigold (*Tagetes erecta* L.) plants (Asrar & Elhindi, 2011). Both cell division and cell enlargement are affected under drought owing to impaired metabolic activities, loss of turgor, and decreased energy supply (Kiani *et al.,* 2007; Farooq *et al.,* 2009; Taiz & Zeiger, 2010).

Thus, plants undergo certain morphological changes to minimize stress-induced damages. These responses vary among crop species and even within species. Crops plants that are able to extract more water and retain it with reduced transpirational water loss can better resist drought (Liu *et al.*, 2011). Drought-related reduction in yield and yield components of plants could be ascribed to stomatal closure in response to low soil water content, which decreases the intake of CO_2 leading to decreased photosynthesis (Cornic, 2000; Flexas *et al.,* 2004). Reduction in photosynthesis not only limited the size of the source and sink tissues but also impaired the phloem loading, assimilate translocation and dry matter portioning (Farooq *et al.,* 2009). Only a genotype producing higher grain yield compared with others under drought is truly drought resistant (Kiliç & Yag˘basanlar, 2010). Genotypes that are evolved to have better morphological adaptation that reduce water loss while maintaining productivity can be considered as tolerant to drought.

2.2.2 Influence of drought on physiological parameters

Plant water balance is negatively impacted by drought stress, and regular physiological functions are impaired. In addition, plants close their stomata to reduce water loss in response to ABA signalling, which can limit the supply of CO_2 and lead to reduced photosynthesis. Whereas increased transpiration and water loss under drought stress can lead to the disorder of production and elimination of ROS in plants, and the excessive accumulation of ROS causes oxidative stress (Ben-Gal & Shani, 2002; Farooq *et al.,* 2009). It may lead to loss of integrity of biological membranes (Liu *et al.,* 2000; Anjum *et al.,* 2011). The integrity of the membrane is critical to protect the cell from external adverse environmental conditions (Xue *et al.,* 2018).

Relative water contents, leaf water potential, osmotic potential, pressure potential, and transpiration rate are the major attributes of plant water relations (Kirkham, 2005), which are significantly affected under water deficit that finally leads to the disturbances in CO_2 assimilation and photosynthetic electron transport. The exposure of plants to the drought decreases leaf water potential, relative water content and transpiration rate, while leaf temperature rises, that finally leads to the disturbances in CO_2 assimilation and photosynthetic electron transport and thus productivity (Anjum *et al.,* 2011; Feller *et al.*, 2016; Aboughadareh *et al.*, 2017).

RWC is one of the best indicators of plant water status in terms of the physiological out-turning of water-deficit in cells (Grover *et al*., 2004; Soltys-Kalina *et al.,* 2016).

Drought stress severely reduces relative water content which affects different growth traits, resulting in a lower yield. According to Nahar *et al.* (2018), water deficit negatively affected growth traits including germination index, root and shoot length, root and shoot weights in rice genotypes. But performance differed depending on the genotypes. Tolerant variety SN03 which exhibited significantly higher RWC (93.48%) showed a better performance in growth traits and yield than susceptible variety SN01 (32.88%) that had a lower RWC under drought condition.

A lower membrane permeability and retention of higher relative water content resulted in more grain yield in wheat cultivar Sids 9. While, Sakha 93 which experienced a higher RWC reduction and higher electrolyte leakage had a significant reduction in yield (Dalia *et al*., 2018). The tolerant *Amaranthus tricolor* genotype VA13 exhibited lower reduction in growth and photosynthetic pigments as a result of lower reduction in RWC and negligible increment in electrolyte leakage (EL) but contrasting responses were reported for sensitive genotype VA15 (Sarker & Oba, 2018). There is a complex and close relationship between the leaf water status and photosynthesis. A reduction in relative water content and water potential led to a decline in net photosynthetic rate, transpiration rate, and stomatal conductance levels of oak trees (Xiong *et al.,* 2022)

RWC may be affected by the interaction of severity, duration of the drought event, species and altitude. Higher reduction of RWC reduced the growth while it increased the shoot/root ratio in maize cultivars. Decrease of water content with increasing poly ethylene glycol (PEG) concentrations in susceptible was higher than tolerant, which maintained a lower yield reduction (Mohammed khani & Heidari, 2007). *Populus kangdingensis* from higher altitude which retained higher RWC (with only 16% reduction) under water deficit showed a higher membrane integrity than *Populus cathayana* that showed 23.3% reduction in RWC, which was collected from lower altitude compared to irrigated control (Yang & Miao, 2010).

Increasing water deficit stress caused a linear decline in chlorophyll contents, leaf membrane stability, relative water content and yield in all wheat varieties, while less impact was observed in variety Chakwal-50 that maintained higher RWC (Qayyum *et al.,* 2021). Similar reduction in RWC, increase in EL resulting in a lower photosynthetic activity and yield was observed with progressive drought stress in many species including maize (Guo *et al.,* 2015), cassava (Zhu *et al.,* 2020) and faba bean (Mansour *et al*., 2021) and wheat (Abid *et al.,* 2018; Qayyum *et al*., 2021; Ahmed *et al.,* 2022). Genotype that can retain higher relative water content performed better under drought condition.

Keeping higher RWC resulted in higher relative chlorophyll content (SPAD value), slight membrane damage under severe drought condition because it was less affected by oxidative stress in cassava genotypes (Zhu *et al*., 2020). Membrane lipid peroxidation (measured as malondialdehyde content) and membrane permeability significantly increased with a reduction of RWC in maize under severe stress than under moderate stress (Guo *et al.,* 2015). Higher relative water content at booting stage and anthesis stages of tolerant wheat cultivars Hashim-8, Zam-04 and Damani resulted in lower reduction of leaf area, stomatal conductance and chlorophyll fluorescence which resulted in a higher yield (Aziz *et al*., 2013). Moderate drought stress caused a reversible decline in leaf water relations and membrane stability whereas the severely stressed plants showed incomplete recovery after re-watering in wheat plants (Abid *et al.,* 2018).

Tolerant wheat genotype Faisalabad-2008 that maintained higher RWC showed a greater leaf turgor potential, membrane stability index and chlorophyll content produced higher biological yield (9.50 g plant^{-1}) and seed yield (3.39 g plant^{-1}) (Ahmad *et al*., 2022). Physiological parameters such as relative water content, membrane stability index, net photosynthetic rate and transpiration rate exhibited highly positive association with seed yield and yield contributing traits in faba bean genotypes (Mansour *et al*., 2021). The superior wheat genotypes which had higher RWC exhibited a lower drought susceptibility index values (Bayoumi *et al.,* 2008).

An earliest response of plants subjected to drought stress is stomatal closure which in general understood to be the most important reason of the drought-induced decrease in photosynthesis (Flexas *et al.,* 2008). There have been reports of hormonal imbalances brought about by drought stress in plants, which cause a decline in the concentrations of many important photosynthesis-related enzymes. Reduced RWC and water potential of leaves due to drought stress, gradually reduce stomatal conductance, which leads to a decline in CO_2 molar fraction in chloroplasts, reduction in rate of photosynthesis and decreased CO_2 assimilation (David *et al.*, 2007).

A decrease in the maximum quantum efficiency of PSII (Fv/Fm or chlorophyll fluorescence) indicates a reduction in capturing and conversion rate of excitation energy by PSII reaction centers. Fv/Fm is being used as an efficient, rapid and non-destructive tool for water deficit stress screening of plants under field condition because it indicate change in immediate plant productivity in response to fluctuation in water content. It aids in discrimination of tolerant genotype from susceptible based on chlorophyll fluorescence measurements (Ansari *et al.*, 2019). The ability of a plant to tolerate environmental stresses and the extent to which those stresses damage the photosynthetic apparatus can be examined by measuring chlorophyll fluorescence (Maxwell & Johnson, 2000). Positive correlation with photosynthetic efficiency and crop yield is reported in many crops including sunflower (Gaffari *et al.,* 2012), wheat (Guo *et al.*, 2016), straw berry (Arief *et al.,* 2023) and in *Olea europea* (Khaleghi *et al.*, 2012).

Photosynthetic machinery is resistant to a certain level of water deficit (Yi *et al.,* 2016). Smaller *Fv/Fm* decline due to lower reduction in RWC resulted in lower reduction in photosynthetic rate in tolerant wheat tolerant lines (Guo *et al.*, 2016). Reduced PSII efficiency of drought susceptible genotypes compared to drought tolerant, under water-deficit is reported in in tomato cultivars/genotypes (Bahadur *et al.*, 2011) and primary leaves of kidney beans (Miyashita *et al.*, 2005). Considerable decline in leaf osmotic potential led to chlorophyll degradation which resulted in diminution of maximum quantum yield of PSII by 23.50%, photon yield of PSII

(ΦPSII) by 29.01% in turmeric genotypes under water deficit (Chindakovid *et al*., 2022).

Scaling down of stomatal conductance led to depletion in net CO_2 assimilation rate which is indicated by relative reduction in chlorophyll fluorescence in severely stressed turmeric plants (Petridis *et al.,* 2012). Arief *et al.,* (2023) investigated the efficiency of photosynthesis in strawberry plants using chlorophyll fluorescence imaging (CFI) to maintain the quality and quantity of strawberry plants under drought stress and observed Fv/Fm values of 0.802 ± 0.0036 and 0.780 ± 0.0026, in control and drought group respectively. Under severe drought stress, a decline in the photochemical efficiency by 10.37–24.12% reduced the yield by 44.1–47.7% in naked oats. Parameters of PS II except for Fo (initial fluorescence) showed a significant positive relationship with the yield in naked oats ($P< 0.05$) (Zhang *et al.,* 2022).

In conclusion, RWC indicates the balance between supply of water to the leaf tissue and transpiration rate that exhibit highly positive association with yield and yield contributing traits. Thus it is possible to use these parameters as rapid indicators for screening for drought tolerance (Vasquez-Robinet *et al.,* 2008; Lugojan & Ciulca, 2011). The EL induced due to stress resulting from enhanced ROS, which often induce oxidative stress, ultimately lead to cell death (Demidchik *et al*., 2014). Stress-induced injury of plants is often measured through electrolyte leakage which acts as an indicator of stress tolerance in plant cells (Lee & Zhu, 2010). Elevated crop productivity is directly related to higher rate of leaf photosynthesis. Combined effect of enhanced photosynthesis and water use efficiency can have a critical role in yield improvement of crops under drought stress conditions.

2.2.3 Influence of drought on biochemical characters

Drought tolerance involves complex interaction of different stress-responsive mechanisms. Water deficit induces the formation of reactive oxygen species in plants and cause oxidative damage in plant cells. ROS signalling initiate different stress responsive molecular, biochemical, physiological, and morphological changes. ABA is produced when ROS production increases in response to stress which can regulate gene expression resulting in the production of different antioxidants. The antioxidant system and osmotic regulation are the main defence systems that provide the tolerance to plants against water deficiency stress conditions (Ahmad *et al.,* 2015; Saeidnejad *et al*., 2015; Sah *et al*., 2016; Sharma *et al*., 2021; Zou *et al*., 2021).

2.2.3.1 Oxidative stress and lipid peroxidation

Under typical conditions, plants' scavenging system and ROS production remain in equilibrium. A certain amount of ROS produced under mild stress will be used by cells as signal molecules to activate relevant active substances or defense systems, and mitigate the damage caused by abiotic stress (Orabi *et al.*, 2018; Dat *et al*., 2000; Khandaker *et al.,* 2022). However, excessive stress causes overproduction of ROS which exceeds ROS scavenging system's capacity, that will lead to the accumulation of ROS and oxidative damage.The overproduction of these reactive oxygen free radicals which are highly toxic, will lead to a variety of harmful cytological effects, such as lipid peroxidation, protein denaturation, DNA strand breakage, blocked photosynthesis due to degradation of photosynthetic pigments and inactivation of photosynthetic enzymes and other macromolecules in the absence of any protective mechanism (Rout & Shaw, 2001; Farooq *et al.,* 2009, 2011; Silva *et al*., 2012).

Superoxide radicals, single oxygen, hydrogen peroxide, alkoxy radicals, and hydroxyl radicals are among the major ROS generated in plants under abiotic stresses (Apel & Hirt, 2004). Production of ROS, especially H_2O_2, which is relatively long-lived ROS, is a major cause of oxidative stress effects (Munnebosch & Penuelas, 2003). The primary locations of ROS production are complex I, ubiquinone, and complex III of the electron transport chain in mitochondria and PSI and PSII in chloroplasts. The generation of ROS in water deficit condition results from photosynthetic apparatus, photorespiration and respiration (Gill & Tuteja, 2010).

Drought stress results in stomatal closure, which limits CO_2 influx and fixation and reduces $NADP^+$ regeneration by the Calvin cycle (Satoh & Murata, 1998). It leads to impaired activities of enzymes and damaged photosynthetic apparatus under water stress which leads to down regulation of photosynthesis. These adverse conditions increase the rate of ROS, such as hydrogen peroxide, superoxide, singlet oxygen and hydroxyl radicals, by enhanced leakage of electrons to molecular oxygen (Flexas & Medrano, 2002; Lawlor & Cornic, 2002; Anjum *et al*., 2017; Carvalho *et al*., 2023). Accumulation of foliar ABA also stimulates the production of H_2O_2 and is associated with oxygen reduction in chloroplast in leaf which is the site of H_2O_2 production (Allen *et al.,* 2000; Foyer & Hanke, 2022). Membranes of the cell or its ultra-structural organelles, apoplast, nucleolus and peroxisome are the other locations of ROS production (Gill & Tuteja, 2010).

H_2O_2 induced oxidation damage under water deficit is reported in many crops. Higher accumulation of H_2O_2 resulted in membrane leakage in shallot, rice and olive (Silalert & Pattanagul, 2021; Jira-Anunkul & Pattanagul, 2021; Gholami *et al*., 2022). Moreover, it induced peroxidation of lipids in many crops including wheat, maize and rice (Habib *et al.,* 2020; Melandri *et al*., 2020; Mansour et al., 2023). Higher H_2O_2 accumulation also led to increased degradation of chlorophyll in rice, wheat and oats (Basu *et al*., 2010; Khayatnezhad & Gholamin, 2021; El-Beltagi *et al*., 2022) and other macromolecules which ultimately resulted in lower growth and productivity.

Products of lipid peroxidation by ROS, lipid peroxides, can oxidize nearby PUFAs (polyunsaturated fatty acids), Establishing a series of reactions that may result in the disruption of biological membranes. The oxidative damage of membrane lipids by increased ROS under stress conditions leads to the formation of malondialdehyde which is indicated by TBARS which also damages biomacromolecules in cells, such as proteins and DNA (Verma & Mishra, 2005; Ozkur *et al.,* 2009; Qiu *et al*., 2008; Tartoura & Youssef, 2011). It is commonly taken as an indicator of oxidative stress that disrupts the membrane integrity of the plant cell (Blokhina *et al*., 2003; Ashraf *et al*., 2009; Noreen *et al.,* 2010;

Hasanuzzaman *et al.,* 2018). TBARS induced damage of macromolecules is reported in many crops including maize, *Macula pomifera* and wheat (Anjum *et al.,* 2017; Khalegi *et al*., 2019; Ekim *et al.,* 2024). MDA is considered to be a marker for assessing lipid peroxidation and damage to plasma membrane and organelle membranes, and its amount increases with environmental stress.

2.2.3.2 Osmotic adjustment

Abiotic stresses such as drought produce direct or indirect hyperosmotic stress in plants (Blum, 2016). The adaptation of plant cells to stressful environments by increasing the solute concentration, mainly through the accumulation of various inorganic and organic substances, thereby reducing the osmotic potential and enhancing the plant's ability to retain water, is known as osmoregulation (Serraj & Sinclair, 2002; Mahmood *et al.,* 2020; Dong *et al.,* 2020; Ozturk *et al*., 2021). It permits cell expansion and plant growth with severe water deficit by maintaining cell turgor (Loutfy *et al.,* 2012). It also permits stomata to stay partially open and CO_2 fixation to continue during water deficit by maintaining turgidity and membrane structural integrity to give tolerance against drought and cellular dehydration (Shao *et al.,* 2005). The substances involved in osmoregulation are broadly divided into two categories, namely, inorganic ions, such as K^+, Na^+, and Ca^{2+}, and organic solutes, such as proline, betaine (glycinebetaine), soluble sugars, and polyols, organic acids (Munns *et al.,* 2020; Dong *et al.,* 2020; Huseynova *et al.,* 2016; Cechin *et al*., 2006; Kiani *et al.,* 2007; Farooq *et al.,* 2008).

These solutes are known as compatible solutes since these solutes do not pose any detrimental effects on membranes or other macromolecules, even at higher concentration (Cechin *et al.,* 2006; Kiani *et al.,* 2007). These compatible solutes not only help to maintain turgor pressure but also protect the enzymes and macro molecules of cells from the damaging effects of ROS (Farooq *et al*., 2009). Among which proline, an important compatible solute which accumulates in plants exposed to dehydration stress, is an important amino acid that have high antioxidant properties and plays a role in the prevention of cell death (Sankar *et al*., 2007; Ashraf *et al.,* 2007; Jalil *et al*., 2007; Perez-Perez *et al*., 2009).

Proline accumulation played an important role in osmotic adjustment, detoxification of ROS and membrane integrity when plants encountered dehydration stress in artichoke cultivars (Zhang *et al.,* 2011). Increase in proline concentration has been used as criteria for stress tolerance study. Su & Wu (2004) reported enhanced salt and water-deficit tolerance in transgenic *Oryza sativa* over expressing P5CS cDNA, led to the accumulation of P5CS mRNA which guides the synthesis of proline.

Chickpea genotype with higher proline showed higher RWC reflecting higher osmoregulation capability to tolerate the drought stress which resulted in higher yield under multi-environment trial. The lower accumulation of proline resulted in increased electrolyte leakage because of oxidative damage in sensitive chickpea germplasm (Shah *et al.,* 2020). Exogenous proline regulated the osmotic-potential, water-holding capacity, protected antioxidant enzyme activity, chlorophyll content, and tissue structure of wheat flag leaves under drought stress, ultimately alleviating the impact of drought stress on wheat yield (Li *et al.,* 2024).

Higher proline accumulation retained water content which led to an elevated levels of antioxidant enzymes such as superoxide dismutase and a lower lipid peroxidation in *Schima superba 9* (Duan *et al.,* 2024). Proline can induce protein stabilization and conserve membrane integrity through binding to hydrogen bonds (Hossain *et al.,* 2019), in addition, protect cells via enhancing water uptake potential and enzyme activation (Burritt *et al.,* 2012), and is considered a vigorous antioxidative defense molecule as well as ROS scavenger (Dar *et al.,* 2016; Adejumo *et al.,* 2021). Foliar application with proline enhanced phenolic compounds, and antioxidant enzyme activity that decreased drought stress symptoms including enhanced ROS production, electrolyte leakage, and lipid peroxidation in sugar beet (Alkahtani *et al.,* 2021). Proline upregulated the activities of enzymes in the AsA-GSH cycle in *Arabidopsis* which indicate its protective role under dehydration stress (Cuin & Shabala, 2007; Hoque *et al.,* 2007).

The application of proline, considerably improved the concentration of leaf chlorophyll by protecting it from the oxidative damages that occurred by ROS molecules under drought in cauliflower and potato (Hayat, *et al.,* 2012; EL-Bauome *et al.,* 2022; El-Yazied *et al.,* 2022).

Research has demonstrated that drought induces proline accumulation in numerous plants and that exogenous proline application contributes significantly to ensure drought tolerance in crop plants (Yamada *et al.,* 2005; Vendruscolo *et al*., 2007; Zandalinas *et al.,* 2016; El-Beltagi *et al.,* 2020).

Mohammadkhani & Heidari (2008) showed that proline content in *Zea mays* L. leaves was 1.56–3.13 times higher than in control leaves under dehydration. In another experiment, a 56-day exposure to water deficit resulted in a 2.4-fold increase in shoot proline levels of oak shoots (Oufir *et al.,* 2009). Moreover, previous studies indicated that proline caused drought tolerance in *Arabidopsis* (Cuin & Shabala, 2007) sunflower (Manivannan *et al*., 2008; Hussain *et al*., 2008), maize (Ali *et al*., 2008), rice cultivars (Lum *et al*., 2014) and lentil (Molla *et al*., 2014; Bekka *et al.,* 2018).

Ali *et al.* (2013) reported that exogenous proline application significantly increased sugar, oil, moisture, protein, fibre and ash content in seeds under drought conditions. It also increased the concentration of antioxidants, such as flavonoids, carotenoids, phenolics, and tocopherols, as well as the level of oleic and linoleic acid in maize seeds. Exogenous proline (30 and 60 mM as foliar spray) application improved growth and development by enhancing photosynthesis rate, substomatal CO_2, stomatal conductance and photosynthetic pigment levels under dehydration stress (60% field capacity, 15 days) in maize (Ali *et al*., 2007). Higher free proline accumulation (1.5–2.1 times higher than control) reduced membrane damage in leaflets of coconut tree under drought stress (Gomes *et al*., 2010). Role of proline as a free radical scavenger is more important in alleviating stress than its role as a simple osmolyte (Hong *et al*., 2007).

Thymus daenensis demonstrated an increase in proline levels in its leaves when subjected to severe water stress (25% field capacity) which reduced chlorophyll and carotenoid degradation (Bahreininejad *et al.,* 2013). This rise in proline and sugar concentrations in various plant parts are evidence of stimulation and activation of the osmotic regulation system in response to stress. Karimi *et al.* (2012) found that, in castor bean (*Ricinus communis* L.), the highest proline content occurred under early seedling-stage under water deficit, while the lowest was observed under full irrigation.

Accumulation of protective solutes including sugars (glucose, fructose, sucrose) and sugar alcohols (mannitol, glycerol, sorbitol, pinitol, quercitol) is a unique plant response to water stress to maintain osmotic potential (Reddy *et al.*, 2004; Slabbert & Kru¨ger, 2014). Similarly, a decline in soluble sugar contents in rice anthers under drought stress impaired the water balance and further intensified oxidative stress (Guan-fu *et al.*, 2011). Sugars are the primary products of photosynthesis which have multiple roles as energy and carbon transport molecules, hormone-like signalling factors, osmolyte and the source of materials from which plants make proteins, polysaccharides, oils and woody materials (Halford *et al.,* 2010).

Stress-induced activation of starch breakdown is a typical plant response that does lead to sugar buildup (Guan-fu *et al*., 2011; Kaplan & Guy, 2004; Yano *et al.,* 2005; Thalmann *et al*., 2016). Warzecha & Bocianowski (2023) measured sugar and phenol content in Oat × Maize Addition (OMA) lines under drought and observed that on the first day of drought, plants possessed 34% more soluble sugars compared to control plants but it decreased to 41.2 % on the fourteen day of drought. Similarly the higher rate of accumulation and the higher mobilization of soluble sugar observed in the leaves, leaf sheaths and internodes of non-sequential senescence wheat cultivars improved grain weight and drought tolerance (Shi *et al.,* 2016)**.**

Proline accumulation played an important role in osmotic adjustment, detoxification of ROS and membrane integrity when plants encountered environmental stresses (Zhang *et al*., 2011). Increase in proline concentration has been used as criteria for stress tolerance study. Further, accumulation of a wide range of solutes including sugars which are known to function as compatible solute change the osmotic potential of cytoplasm to balance the osmotic potential protecting and stabilizing proteins and membranes.

2.2.3.3 Protein abundance

Drought affected protein abundance of multiple pathways in the plant. Schulz *et al.* (2021) observed significant variation in protein abundance in response to repeated drought exposure of a tolerant and a sensitive maize hybrid. Heat shock proteins were found up-regulated in both genotypes while, the proteins of starch metabolism were found to be significantly reduced upon drought especially in tolerant variety. Total soluble protein content in maize (*Zea mays* L.) cultivars (704 and 301) increased with mild to moderate dehydration but showed a downward trend with severe stress. Relatively higher accumulation of dehydrin-like proteins was observed by SDS-PAGE gel electrophoresis in the roots and leaves of cv. 704 (tolerant) than in cv. 301 (susceptible) (Mohammadkhani & Heidari, 2008). Conversely, a decline in protein concentration was reported in wheat cultivars under dehydration stress (Ullah *et al.,* 2022).

Variation in gene expression at transcript level do not always correlated with the changes at protein level. Protein abundance can be up regulated or down regulated at transcript level under drought stress. Trypsin inhibitors, xylose isomerase, sucrose synthase, asparagin synthetase, cysteine protease, alcohol dehydrogenase (ADH), cold-regulated LTCOR12, and 1, 4-α-glucan branching enzyme were all found to be up-regulated at the transcript level. In contrast, down-regulation of genes encoding proline-rich cell wall protein, aquaporin and several photosynthesis-related proteins was observed (Larcher, 2003; Triboulot *et al.,* 2007; Kosova *et al.,* 2011).

2.2.3.4 Antioxidant enzymes

Plants encounter the toxic effects of ROS by enzymatic (superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) etc.) as well as nonenzymatic anti oxidative systems (ascorbic acid (AsA), tocopherol, glutathione and phenolic compounds etc.) (Jaleel *et al.,* 2008; Shi & Zhu, 2008; Ashraf *et al.,* 2011). The primary enzymatic antioxidants in plants that protect against ROS are SOD, CAT, APX, and DHAR (dehydroascorbate reductase), MDHAR (monodehydroascorbate reductase), GR (glutathione reductase), and POD. SOD may convert O_2 − to H_2O_2 and its primary function is to eliminate O_2 −. SOD plays crucial role in the antioxidant system and is the first line of defence against ROS elimination system in plants. In organisms H_2O_2 removal is the main function of CAT and POD. Besides, APX, GR, DHAR, and MDHAR are also prominently responsible for H_2O_2 scavenging. Together, they form a second line of defence system in ROS elimination in plants.

POD plays a major role in scavenging of H_2O_2 which was produced through dismutation of O^{2-} catalyzed by SOD. CAT is a main enzyme to eliminate H_2O_2 in the mitochondrion and microbody (Hazanuzzaman *et al.,* 2017). AsA, GSH, and four enzymes viz. APX, MDHAR, DHAR, and GR which make up the Ascorbate-Glutathione (AsA-GSH) or Asada–Halliwell pathway, play a vital role in detoxifying ROS. Apart from ROS detoxification, they also interact with other defence systems in plants and protect the plants from various abiotic stress-induced damages (Noctor *et al.,* 2014). Nonenzymatic antioxidants, including the elevated level of phenolic compounds during oxidative stress potentially protects plants from oxidative damage (Kumar *et al.,* 2023).

Wang *et al.* (2019) investigated the effects of drought stress during the tillering period of drought-sensitive and drought-tolerant rice cultivars. The results showed that increased SOD, POD, CAT activity in tolerant cultivar lowered hydrogen peroxide accumulation and also resulted in higher soluble protein and sugar content as well as smaller photosynthetic affected area with the progression and intensity of drought stress to obtain similar yield to the control.

Water deficit induced lipid peroxidation and chlorophyll degradation in one year old olive cultivars (Chemlali, Meski and Picholine). While, Chemlali with Increased levels of SOD, APX and CAT activities showed less signs of oxidative stress. In contrast, it exhibited a decline in poly phenol oxidase activity (Ahmed & Rouina, 2009).

Progressive level of water deficit significantly enhanced the activities of MDHAR, DHAR, APX, and GR with higher activity observed in the seedlings of drought-tolerant cv. Brown Gora compared to the sensitive cv. Malviya-36 resulted in relatively lower H_2O_2 accumulation (Pyngrope *et al.,* 2013). Similarly, Lou *et al.* (2018) found that MDHAR, DHAR, glutathione-S-transferase and GR transcripts and thus activity are predominantly induced under drought stress which efficiently scavenged H_2O_2 under drought stress in wheat.

A principal component analysis among antioxidant responses and physiological changes showed that cassava plant water status under drought treatment was closely related to higher SOD and CAT activities at 50% (medium), 20% (severe) of field capacity. However, increased GR activity was responsible for tolerance in cassava variety SC205 (Zhu *et al.,* 2020).

Elevated concentration of enzymatic antioxidants (CAT, POX, SOD and APX) lowered observed contents of H_2O_2 and O^2 •− under drought stress in tolerant faba bean genotype compared with the highly sensitive ones under moderate and severe drought stress under field condition (Siddiqui *et al.,* 2015). The tolerant genotype VA13 of *Amaranthus tricolor* exhibited higher CAT, SOD, and remarkable increment in ascorbate-glutathione cycle enzymes activity, which significantly lowered lipid peroxidation and increased membrane stability compared to sensitive genotype VA15 which showed a relatively higher H_2O_2 accumulation with higher yield reduction (Sarker & Oba, 2018). PEG induced osmotic stress induced immense accumulation of hydrogen peroxide in melon cultivars, Kırkağaç and Galia. Elevated activity of catalase, ascorbate peroxidase, and glutathione reductase in Galia resulted in a lower lipid peroxidation compared to Kırkağaç (Kavas & Baloğlu, 2013)

Arif *et al.* (2023) conducted a study in which forty upland cotton accessions were sown under different water regimes. The cotton genotype Cyto-124 with high peroxidase activity exhibited lowest H_2O_2 content and a minimum excised leaf water loss under drought conditions, which also showed a high values for root length. The AsA-GSH cycle responded considerably with excess ROS generation that resulted in stable net photosynthetic rate (P_N) and chlorophyll content as a result of higher reduction of reactive oxygen species and higher relative water content maintenance in, the spike bracts compared with the flag leaves in winter wheat (*Triticum aestivum* L.) (Lou *et al.,* 2018)

Yang *et al.* (2021) suggested that the reduction of the antioxidant defence system may result in oxidative damage to cellular components and membrane lipids that escalate lipid peroxidation by generating various ROS. Bistgani *et al.* (2017) also observed an adverse impact of reduction in antioxidant enzymes on membrane lipid peroxidation in *T. daenensis.* Doupis *et al.* (2013) investigated the effects of variable water supply in 50-year-old *Olea europaea* L. (cv Koroneiki) trees. In severely stressed leaves, there was an increase in the activities of superoxide dismutase, ascorbate peroxidase, and guaiacol peroxidase, while an inhibition of catalase activity.

Water deficit caused an enhanced production of anti-oxidative enzymes in cotton plants, while excessive activities of enzymes such as SOD, APX and POD brought down the extent of the lipid peroxidation by lowering H_2O_2 accumulation that resulted in the stability of the photosystems which enabled a rapid recovery of photosynthetic rate after rewatering (Yi *et al.*, 2016). Enhancement in activities of the overall antioxidant enzymes including SOD, GPX, CAT, GR and APX in tolerant rice cultivar, Sahbhagi Dhan protected its chlorophyll content, by reducing ROS, resulted in higher yield compared to IR64 (drought sensitive) (Nahar *et al.*, 2018).

The effects of drought stress on the development of grains and yield are contingent upon their intensity and the stage of plant development at which they arise. Among nine approved wheat cultivars, drought (50% field capacity) caused enhanced levels of SOD, POD and CAT in wheat cultivar AARI-2011 which resulted in lower efflux of K+ from cells and less degradation of photosynthetic pigments (chlorophyll “a” and “b”). Nevertheless, the cultivar AARI-11 proved to be drought tolerant cultivar with maximum productivity. Enzymes showed significantly higher activity at reproductive stage (Nawaz *et al*., 2015).

Drought stress preferentially enhanced the activities of SOD and POD activity in seedlings of oilseed rape whereas it decreased CAT activity. Licord variety with the highest level of enzyme activity under both optimum and limited irrigation regimes recorded the maximum yield and is reported as the most tolerant (Abedi & Pakniyat, 2010).

Wheat cultivar Chakwal-50 with increased antioxidation activities of superoxide dismutase, peroxidase and catalase along with higher accumulation of sugars, soluble proteins, and free amino acids resisted linear decline in chlorophyll contents and membrane stability, and maintained higher relative water content that resulted in lower susceptibility index compared with Wafaq-2001(susceptible) (Qayyum *et al.,* 2021).

The activity of antioxidant enzymes catalase, peroxidase and superoxide dismutase increased markedly in the drought-tolerant varieties was positively associated with the lower reduction in dry mass production and consequently with the drought tolerance compared to sensitive genotype in the upland rice varieties (Lum *et al*., 2014).

Plants under drought stress produce excess ROS such as $_1 O^2$, O^2 -, OH• and H_2O_2. To minimize the effects of oxidative stress, plants have evolved a complex enzymatic and non-enzymatic antioxidant system (Apel & Hirt, 2004). The enzymatic components may directly scavenge ROS or may act by producing a non-enzymatic antioxidant (Sharma & Dubey, 2005). Plant resilience to drought may be correlated with antioxidant enzymes' capacity to scavenge reactive oxygen species (ROS) and minimize their harmful effects. Enhanced concentrations and activities of antioxidant molecules under abiotic stresses confer tolerance to plants (Gill & Tuteja, 2010; Manivannan *et al.,* 2014).

2.2.3.5 Photosynthesis

Drought induced stomatal closure and ROS production impair activities of carboxylation enzymes and ATP synthesis, and cause destruction of photosynthetic apparatus lowering carbon fixation under drought (Yamance *et al.,* 2003). Leaf internal CO_2 decrease not only reduces carboxylation directly but also directs more electrons to form ROS and promotes photorespiration (Reddy *et al.*, 2004; Correia *et al.,* 2006). Plants need chlorophyll and carotenoids, the main Photosynthetic pigments for harvesting light to produce reducing powers. Both the chlorophyll a and b are prone to soil dehydration (Farooq *et al*., 2009). Whereas carotenoids safeguard the photosynthetic apparatus by dissipating excess excitation energy into heat (Yi *et al*., 2016). The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation by ROS (Kannan & Kulandaivelu, 2011).

Drought stress severely decreased chl a and chl b contents in marigold (Asrar & Elhindi, 2011). Water stress resulted in decreased chlorophyll a and b and carotenoids and an increase in polyphenols in cotton genotypes. FH-113, PB-899 and MNH-789 that had maximum amount of carotenoids [(mg/100 mL) (0.79), (0.69) and (0.66)] and polyphenols [(µg/g of leaf) (0.014), (0.012) and (0.011)] showed a lower degradation in chlorophyll a and b (Shah *et al.,* 2011).

Nahar *et al.* (2018) evaluated for 21 traditional rice varieties, along with Sahbhagi Dhan (drought tolerant) and IR64 (drought sensitive) as controls. Significant decreases in the chlorophyll a, b and total chlorophyll contents were observed. While, total chlorophyll was found the highest in SN22 (81.67%) which showed higher yield also under stress. Study indicated maximum percentage of chlorophyll a followed by chlorophyll b.

The photosynthetic activity significantly decreases with increasing drought level. The total chlorophyll decreased by 27% in the seedlings of *Catharanthus roseus* subjected to different water regimes (Amirjani, 2013). The chlorophylls and carotenoids decreased in cotton (*Gossypium hirsutum* L.) genotypes under short-term drought treatment, which resulted in a lower starch contents. Pigments tended to increase when the plants were recovered from stress. The degree of decrease in chlorophylls and carotenoids was higher in the sensitive cotton genotype (Ca/H 631) as compared to the moderately tolerant genotype (GM 090304) with higher proline, sugar and polyphenols (Parida *et al*., 2007).

The levels of chlorophyll a, b, and total chlorophyll were markedly reduced ($p<0.05$) in maize (*Zea mays* L.) cultivars -704 and 301, whereas carotenoids content increased ($p<0.05$) under water stress. Higher carotenoid content contributed to less degradation of chlorophyll in 301 var (tolerant) (Mohammed khani & Heidari, 2008). The carotenoids increased with maturity stage of the crop in African eggplant accessions. Although, the stressed crops reported significantly decreased amount of carotenes and chlorophylls and increased concentration of zeaxanthin (Mibei *et al.,* 2017).

Water Stress decreased (40% irrigation) chlorophyll “a” and total chlorophyll (chl a+b) to about 51.55% and 46.86% respectively in *Olea europea* plants (Khaleghi *et al.,* 2012). Exposure of two olive cultivars, Chemlali and Chetoui, to reduced irrigation lowered Chl (a + b) contents to about 29% and 42%, respectively (Guerfel *et al.,* 2009).

Similarly, a reduction in chlorophyll content and photosynthetic activity was reported in *Thymus daenensis* (Malekpour *et al*., 2016) and in Blue grass seedlings (Niu and Ma, 2018). Considerable decline in leaf osmotic potential led to chlorophyll degradation in turmeric that ultimately resulted in rhizome yield reduction (Chindakovid *et al.,* 2022). Drought significantly reduced relative water content and stomatal conductance (41.52%) which led to decline in maximum photosynthetic capacity (41.06%), and the chlorophyll content that resulted in significant reduction in shoot fresh (59.45%) and dry (50.83%) weights, in *Aegilops* and *Triticum* (Alireza *et al.,* 2017).

Maize genotypes 6 (BC678) and 8 (BC404) with the highest chlorophyll index contributed to a higher yield compared to other genotypes under drought stress (Majid & Roza, 2012). Rice genotypes Binadhan-13 and Nerica mutant with higher photosynthetic rate and chlorophyll content recorded the highest grain dry weight, under drought stress at grain filling stage (anthesis to maturity) and were identified as tolerant genotypes (Moonmoon *et al.,* 2020). Significant changes are observed in the ratio of chlorophyll ‘a’ and ‘b’ and carotenoids under water deficit (Farooq *et al.,* 2009). Low levels of photosynthetic pigments have an immediate impact of reducing photosynthetic potential and, consequently, primary output.

Environmental stresses have a direct impact on the photosynthetic apparatus, essentially by disrupting all major components of photosynthesis including the thylakoid electron transport, the carbon reduction cycle and the stomatal control of the CO_2 supply, together with an increased accumulation of carbohydrates, peroxidative destruction of lipids and disturbance of water balance (Allen & Ort, 2001).

2.2.3.6 Secondary metabolites

Plants synthesize a large number of secondary metabolites including flavonoids, terpenoids, and phenolic compounds that are required for survival of plants under adverse environmental conditions such as cold, desiccation, high light, metal toxicity, nutrient deprivation, pest and pathogen attack, senescence, salinity, and UV light (Davies *et al.,* 2018). Secondary metabolites, such as phenolic compounds protect plants from disease/damage and also contribute to the plant's color, aroma and flavor (Pandey, 2009).

Plants safeguard themselves from oxidative damage by phytophenols, such polyphenols, which assist ascorbate-dependent antioxidative defense systems. Polyphenols including quercetin, rutin, and catechin as well as flavanoides react with biometal ions such as (Fe (II) and Cu (I)) as a chelating agent. Polyphenols simultaneously donate H atom or transfer an electron to quickly scavenge free radical damage to biomolecules. Similarly, hydroxy groups, in the aromatic B-ring of flavonoids directly participate in ROS scavenging activity (Sakihama, 2002; Aboul-Enein, 2007; Dehghanian *et al.,* 2022).

Drought stress induces the biosynthesis of phenolic and flavonoids, modulation in the phenyl propanoids pathway, ultimately leading to enhanced plant protection (André, 2009; Rezayian, 2018; Li *et al.,* 2018). An increase in flavonoids and polyphenols has been noted in the fern *Salvia sinaloensis* (101% flavonoid and 139% total phenol) (Caser *et al.,* 2018). Quan *et al.,* (2016) reported that rice cultivar Q8 as the most tolerant with 65.3 mg gallic acid equivalent, which was significantly higher than Q2 (susceptible; 33.9 mg gallic acid equivalent) under drought.

The increased accumulation of kaempferol and quercetin in tomatoes helps them endure the drought conditions through detoxification of H_2O_2 (Miean & Mohamed, 2001). Similarly, a recent study indicated that drought stress upregulates the biosynthesis of flavonoids by regulating the heat shock factors that interacted with an AP2/ERF family transcriptional factor to activate the genes involved in the flavonoid biosynthesis in *Malus domestica* (Wang *et al*., 2020). In another study, different types of anthocyanins, such as A11, A9, A8, and A5 were found to be induced by short-term drought stress in Arabidopsis, in which A11 was the major anthocyanin (Kovinich *et al*., 2015).

Gatabazi *et al*. (2022) determined the effect of water regimes on phenolic content and yield of two ginger species (commercial ginger and African ginger) at various harvest times. Under severe stress treatment (80-85% soil moisture depletion) both species exhibited a considerable increase in total antioxidant activity as well as total flavonoid and phenolic content. Moderate stress treatment (with 40-45% soil moisture depletion) resulted in improved water use efficiency, and increased levels of flavonoids, phenolics, and antioxidants, while only causing a slight reduction in yield for both species compared to severe stress.

The phenolic compounds including phenolic alcohols, phenylpropanoids and flavonoids in Portuguese olive tree cultivar increased under deficit irrigation while showed a declining trend during maturation stage (Machado *et al.,* 2013). Whereas the rainfed regime concentrated grapes in terms of phenolic compounds like anthocyanins, flavonols, flavanols, hydroxybenzoic acids, and total phenols than in the RDI (regulated deficit irrigation) (Álvarez & Intrigliolo, 2021).

Malondialdehyde content of olive cultivars (‘Gaidourelia’, ‘Kalamon’, ‘Koroneiki’ and ‘Megaritiki’) increased due to water stress, mostly after 60 days of dehydration. While ‘Gaidourelia’ variety showing higher phenolic concentration particularly at 33% field capacity represented a lower lipid peroxidation (Petridis *et al*., 2012). The deficit irrigation treatment in sorghum grain considerably enhanced polyphenol content and antioxidant activity compared to the full irrigation treatment. Tolerant genotypes, Shawaya black short 1 and IS1311C (brown) showed the highest polyphenols levels and antioxidant activity. In response to drought, a higher concentration of endogenous secondary metabolites was observed in various medicinal plants. For instance, an increased level of phenolic compounds was observed in Trachyspermum *ammi* under drought stress (Azhar *et al*., 2011) which increased its therapeutic potential. Therefore, both irrigation treatments and genotype need to be considered by breeders during production for enough yield with the required levels of polyphenolics for targeted end-use (Wu *et al.,* 2017).

Poly phenols showed an upward trend with increasing stress levels in Oat × Maize Addition (OMA) lines under drought. In the fourteen-day drought, phenolic substances were found to be significantly higher (6% to 57%), which reduced water loss and was correlated with the high yield of OMA lines. Phenolic compound content might be used as a biochemical indicator of drought tolerance since it acted as ROS scavenger as well as signalling molecule to induce antioxidant activity which finally resulted in less water loss (Warzecha *et al*., 2023).Thus, under drought stress, SMs scavenge reactive oxygen species to safeguard plants from lipid peroxidation and other oxidative effects. Additionally, drought-induced volatile SMs alerts plant tissue to attain defensive drought stress mitigating processes via systemic induction of drought response (Idrees *et al.,* 2010; Kermani *et al*., 2019; Yadav *et al.,* 2021).

In conclusion, biochemical response to water deficit may vary with genotype, intensity, duration of stress and stage of development. Lipid peroxidation is the most reliable criterion used to determine whether a genotype is resistant or sensitive to stress. Increased accumulation of MDA has been correlated with decline in RWC and photosynthetic pigment content when plants exposed to prolonged drought (Deeba *et al*., 2012). Among osmoprotectants, proline is a key amino acid with strong antioxidant properties that helps prevent cell death. Many researchers consider stress-related proline accumulation as a biochemical marker for tolerant cultivar selection (Ashraf *et al.,* 2008). The ability of antioxidant enzymes to neutralize ROS and mitigate their harmful effects along with non- enzymatic antioxidants may correlate with the drought resistance of plants. Any alteration in environmental conditions acts as a trigger that activates the phenylpropanoid pathways to produce secondary metabolites such as polyphenols, which provide resistance to the plant by serving as ROS scavenger, while some of them such as flavonoids and anthocyanins are protective in function.

2.2.4 Molecular responses under water deficit.

In order to survive in unfavorable environment, plants coordinate the up- or down regulation of an array of stress responsive genes. Reprogramming the expression of these genes results in a recalibrated development and growth, which align with reduced water availability, ultimately enhancing stress tolerance. Various techniques are currently used to track genome-wide transcriptional changes in response to drought stress. The expression patterns of dehydration-inducible genes are intricate. Some genes react to water stress almost immediately, while others are gradually activated following the buildup of ABA. The plant growth regulator ABA is crucial for managing and enduring dehydration.

The plant growth regulator, ABA, plays an important role in the response and tolerance against dehydration. It seems that dehydration triggers production of ABA, which induces expression of genes involved in drought tolerance (Abe *et al.,* 1997; Lim *et al.*, 2013; Ding *et al.,* 2016). Analysis of the promoters of drought- and cold-inducible genes has revealed several cis-acting elements that play roles in both ABA-dependent and ABA-independent responses to water stress conditions. The molecular mechanisms that regulate plant gene responses to water stress involve several steps: detecting water or osmotic stress, adjusting stress signals to cellular signals, transmitting these signals to the nucleus, controlling the transcription of stress-inducible genes, and coordinating the function of these genes ultimately to enhance tolerance to water stress. The gene products in response to stress tolerance include functional and regulatory proteins (Shinozaki & Yamaguchi, 2007).

Functional proteins includes proteins that probably function in stress tolerance such as water channel proteins, the enzymes necessary for synthesizing various osmoprotectants (such as sugars, proline, and glycine betaine), proteins that protect macromolecules and membranes (including LEA proteins, osmotin, antifreeze proteins, chaperones, and mRNA binding proteins), proteases for protein turnover (thiol proteases, Clp protease, and ubiquitin) and the detoxification enzymes (glutathione S-transferase, soluble epoxide hydrolase, catalase, superoxide dismutase, and ascorbate peroxidase). Regulatory proteins encompass factors that further modulate signal transduction and gene expression, likely playing roles in stress responses. These include protein kinases, transcription factors, phospholipase C and 14-3-3 proteins. Understanding the functions of these regulatory proteins is now increasingly important for further understanding of plant responses to water deficit stress (Shinozaki & Yamaguchi, 1994; 2007).

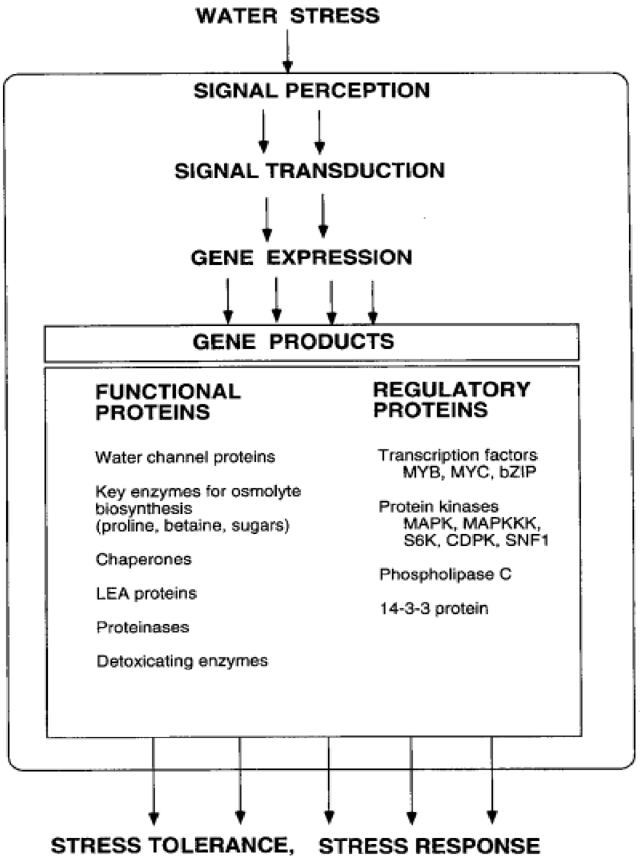


Fig. 2.2 Function of water-stress-inducible gene products in stress tolerance and stress response (Shinozaki and Yamaguchi, 2007).

2.2.4.1 Transcription factors involved drought response pathway.

Transcription factors are proteins that bind to specific regions of DNA to either initiate or inhibit the expression of particular genes. Stress-inducible transcription factors encompass several families, including the DRE-binding protein (*DREB*) family, the ethylene-responsive element binding factor (*ERF*) family, the zinc-finger family, the basic helix-loop-helix (*bHLH*) family, the basic-domain leucine zipper (*bZIP*) family, and the homeo domain transcription factor family. These transcription factors could regulate various stress inducible genes cooperatively or separately, and may constitute gene networks (Seki *et al.*, 2002).

Gene expression is majorly governed by the activation of a specific set of genes encoding transcription factors (Thakur *et al.*, 2020; Ramanjulu & Bartels, 2002). Transcription factors (TF) regulate gene expression by interacting with a pre-initiation complex of transcription by binding to the *cis*-regulatory elements present in the promoter regions of the respective genes which results in the activation or inhibition of RNA polymerase, thereby reorienting the gene expression (Nakashima *et al.,* 2007, 2014). Because TFs are regulatory in nature, they are important targets for modulating downstream gene regulatory networks, which in turn affects the development of crops that are adaptable to climate change.

In cotton (Liang *et al.*, 2016) and Arabidopsis (Fujita *et al.*, 2011) elevated expression of ABF conferred drought tolerance under drought stress. *ABF3 ABI5, ABF1, ABF2/AREB1, ABF3*, and *ABF4* of *Arabidopsis thaliana* positively regulated ABA-dependent gene expression (Choi *et al.,* 2000). Similarly, in rice, the *bZIP* TFs like *OsbZIP23, OsbZIP46, TRAB1, OsbZIP2*, *OsbZIP42* and *OsbZIP46* also play an important role in ABA signal transduction and osmotic stress responses (Lee & Song, 2019). Whereas the expression of tomato *SlbZIP38* gene was down regulated under salt stress, drought stress, and ABA application and hence, the transgenic tomato plants became drought and salt stress susceptible when *SlbZIP38* was overexpressed (Pan *et al*., 2017).

bHLH TFs conferred drought tolerance through regulating stomatal development, photosynthesis and growth in *Populus euphratica* and *Oryza sativa* (Dong *et al*., 2014; Seo *et al*., 2011). Overexpression of the *AP2/ERF* family gene, which had increased sensitivity to drought in *Arabidopsis* contributed higher relative water content, membrane stability index, wax content, osmotic potential, stomatal conductance, and transpiration rate activities in maize and in tomato (Yu *et al*., 2022; Cheng *et al*., 2023).

A comparative transcriptome analysis between the wild-type and *SNAC1*-overexpressing plants revealed that transgenic plants upregulated roughly 40 genes linked to drought stress tolerance pathways. These putative genes facilitated redox homeostasis, signal transmission, and osmolyte accumulation. Similarly, *OsNAC6* overexpression significantly improved the dehydration stress response in rice (Hu *et al*., 2006; Nakashima *et al*., 2007) and drought resistance in transgenic cotton (Saimi *et al*., 2023).

Fei *et al.* (2019) identified 38 *WRKY* transcription factors in *Zanthoxylum bungeanum*, with *ZbWRKY33* (a homolog of *AtWRKY33*) being the most significantly upregulated transcription factor under drought conditions. *BnaWRKY210* TF of *Brassica napus* was highly upregulated during drought stress (He *et al.,* 2016). The *TaWRKY44* TF positively regulated drought stress response either by eliminating ROS or by activating the expression of stress-linked genes (Wang *et al.,* 2015).

Kiranmai *et al.* (2016) observed higher mRNA transcript levels of eight *WRKY* transcription factor genes in horse gram plant under drought stress conditions which is involved in reducing the adverse effect of stress on plants. *DREB* TF interacts with a cis-acting dehydration-responsive element (*DRE*) sequence and activated the expression of genes involved in drought stress tolerance in *Arabidopsis thaliana* and in *Sorghum bicolor* (Sakuma *et al*., 2006; Akbudak *et al*., 2018).

Aquaporins (*AQP*s) are essential channel proteins that play a major role in plant growth and development, regulate plant water homeostasis, and transport uncharged solutes across biological membranes (Maurel *et al*., 2015). Aquaporin gene encodes a small integral membrane protein with six bilayer spanning domains that functions as a water channel protein. This protein permits passive transport of water along an osmotic gradient. Promoter analysis of the PaAQP genes for conserved regulatory elements suggested a greater abundance of cis-elements involved in light, hormone, and stress responses, which may reflect the differences in expression patterns of Pa*AQP*s and their various functions associated with plant development and abiotic stress responses (Li *et al*., 2021). Over-expression of aquaporin resulted in the higher water loss, low RWC and water potential, and decreased drought resistance in Arabidopsis (Li *et al*., 2015), transgenic tobacco (Aharon *et al*., 2003) and *Festuca* species, (Pawłowicz *et al*., 2017), which induced faster wilting during water stress.

2.2.4.2 Gene expression analysis using qRT-PCR

Quantitative RT-PCR is a highly sensitive and effective method for assessing differential gene expression. The alterations in gene expression triggered by abiotic stresses are intricate and multifaceted. With qRT-PCR gene expression can be compared by the most suitable reference genes, which is tissue and treatment specific (Le *et al.,* 2012). The relative differential expressions of drought responsive genes or transcription factors in susceptible and tolerant genotypes will give information about upregulation or downregulation of selected genes or transcription factors that contribute towards drought tolerance (George *et al.,* 2017).

The relative quantification analysis of 11 drought-responsive genes revealed distinct transcript profiles between drought-tolerant and drought-susceptible black pepper cultivars. Two key genes linked to drought tolerance in plants, *DHN* and *OSM*, were significantly up-regulated in both pepper genotypes under drought stress, with the tolerant genotype showing a noticeably greater transcript accumulation. *AQP* and *bZIP* genes were down-regulated in drought tolerant genotype of black pepper compared to the susceptible (George *et al.,* 2017).

The expression profiles of the marker genes *ERD1*-like, *GmaxRD20A*-like, *GmaxRD22*-like, and *GmaxRD29B*-like were analyzed by qRT-PCR in root samples from drought-sensitive soybean cultivar BR 16 and drought-tolerant cultivar Embrapa 48 under water deficit conditions. The results revealed differing gene expression responses for the *GmaxRD20A*-like and GmaxRD22-like genes between the two cultivars. *GmaxRD20A*-like increased Whereas, *GmaxRD22*-like responses decreased after abrupt water deprivation (Neves-Borges *et al*., 2012).

Comparative analysis of non-transgenic and *OsEREBP1* (AP2/ERF–type transcription factors) overexpressing genotypes revealed that it activates the jasmonate and abscisic acid signalling pathways thereby priming the rice plants for enhanced survival under abiotic or biotic stress conditions. *OsEREBP1* is thus, a good candidate gene for engineering plants for multiple stress tolerance (Jisha *et al*., 2015).

Quantitative real-time PCR in well watered and moisture stressed shoot and root tissues of sorghum revealed that PTSb00128.1 of *HAP2* family recorded 5 to 10 fold up-regulation in shoot under moisture stress regimes while it recorded 15 fold up-regulation in mild moisture stressed root tissues (Aglawe *et al.,* 2012). Expression pattern of selected conserved miRNAs was analyzed by stem-loop qRT-PCR in response to drought stress in turmeric. miR156 showed a sudden increase in 5 % PEG followed by an abrupt decrease in the expression level at 10 % PEG compared to control (Santi *et al*., 2016).

Relative gene expression of *Dhn1, SOD, POD*, and *CAT in* tolerant and sensitive genotypes of wild barley under three levels of drought stress (control, 90–95% field capacity (FC); mild stress, 50–55% FC; and severe stress, 25–30% FC) revealed that *Dhn1, SOD, POD,* and *CAT* were upregulated under drought, with the highest expression levels observed in the drought-tolerant genotype under severe stress conditions (25–30% FC) (Shirvani *et al*., 2024). Relative expression of photosynthesis related genes showed significant differential expression under drought treatment in yellow horn seedlings, which also showed decreased stomatal conductance, chlorophyll content, and fluorescence parameters. The genes related to photosynthesis were down-regulated due to lack of water which provided some candidate genes for the genetic study of photosynthesis (Hu *et al.,* 2023).

The cotton genotypes MNH-886 with higher expression of transcription factors (*DREB, MPK, MYB, NAC, HSPs*) showed tolerance to drought stress under field conditions with respect to morphological and physiological traits such as in medium plant height, high boll retention percentage, high relative water content, photosynthesis rate, stomatal conductance, transpiration rate (Anwar *et al.,* 2022).

Differential expression of genes encoding proteins, kinases, transcription factors, and phosphatase 2C proteins contributed towards higher drought tolerability at early dehydration in soya bean cultivar DT2008 compared to sensitive variety W82 (Ha *et al.,* 2015). Gene expression pattern analysis performed in maize seedlings by qRT-PCR analysis indicated that genes annotated with expansion and xyloglucan endotransglucosylase were up-regulated at re-watered stage whereas, genes involved in cell wall development were down-regulated during drought treatment stages, which resulted in the relative shorter leaves (Zhang *et al.,* 2018).

A number of genes that are affected by water stress have been identified in a wide range of plant species. The presence of various signal transduction pathways between the perception of water stress and gene expression has been discovered by analyses of stress-inducible gene expression. Comparative genomics approach is a key tool for the discovery of stress-related genes (Shinozaki *et al.,* 2003). Relative quantitation of gene expression allows quantification of differences in the expression level of a specific target (gene) between different samples (Livak & Schmittgen, 2001). Molecular analyses of these differentially expressed factors enhance our understanding of the signal transduction pathways involved in water stress. Transgenic plants that modify the expression of these genes or TFs will provide more information about the function of their gene products (Shinozaki & Yamaguchi, 2005).

2.2.5 Influence of water deficit on crop quality

Plants possess wide varieties (more than 200,000) of secondary metabolites (Fiehn, 2001). Active component or metabolites in medicinal plant species might be influenced by environmental factors, such as drought, temperature, light intensity, UV radiation, and precipitation rate (Figueiredo *et al.*, 2008; Ramakrishna & Ravishankar, 2011; Al-Gabbiesh *et al.*, 2015; Ferreira *et al.,* 2016; Isah *et al.,* 2019; Li *et al*., 2020), which generally fluctuate during seasonal variation (Kleinwächter & Selmar, 2015; Soni *et al*., 2015). Drought stress limits plant growth and consequently decreases plant biomass in turmeric. The biomass reduction is mainly due to deviation of metabolic pathway toward synthesis of defense metabolites called secondary metabolites (Mostajeran *et al.,* 2014).

Characterization of turmeric for the major biochemical constituent's viz. oleoresin, essential oil and curcumin under drought stress provides insight on the relative influence drought on the phytoconstituents. Which has led to identification of cultivars rich in one or other of these constituents, that are important determinants of the commodity's end product (Singh *et al*., 2014). The curcumin content can be influenced by geographic factors such as soil quality, climate, and environmental conditions. Curcumin content and its analogues exhibit wide variation across the genotypes (Pandey & Katiyar, 2010; Sandeep *et al*., 2016).

Growth exerted high positive significant correlation with yield and phytoconstituent content in turmeric. Tolerant turmeric variety P44 that yielded high oleoresin content (15%), high leaf oil content (1.9%) and high rhizome oil content (2%) recorded highest in leaf biomass, leaf area followed by tiller number. In GC–MS analysis it showed higher accumulation of alpha-phellandrene and ar-tumerone (Singh *et al.,* 2014).

The expression level of *CURS1*, a gene involved in curcumin production, in turmeric rhizomes was significantly increased under water-deficit conditions in the greenhouse, showing a 3.03-fold rise at 20 days and an 8.73-fold rise at 30 days of drought stress compared to the control (well-watered) condition. Also, expression levels of *CURS2, CURS3*, and *DCS* in water-deficit-stressed plants were upregulated (10-days water withholding) by 2.37-folds, 1.92-folds, and 1.38-folds over control, respectively (Chindakovid *et al*., 2022). Under water-deficit conditions in the greenhouse, the expression increased by 3.03-fold at 20 days and by 8.73-fold at 30 days.

Mohamed *et al*. (2014) investigated the Influence of three irrigation treatments (irrigation every one week, two weeks and three weeks) on growth and chemical composition of both *Curcuma aromatica* and *Curcuma domestica*. The long irrigation intervals significantly reduced growth parameters and chemical composition. Dry rhizomes of plants irrigated weekly had greater levels of total carbohydrates, volatile oil, and curcumin compared to those irrigated every two or three weeks. Furthermore, *C. aromatica* demonstrated better growth characteristics and chemical composition than *C. domestica* under all irrigation conditions.

In turmeric cv. Autumn, curcuminoid content in sub-optimum temperature (18–27 °C daily temperature) at Okinawa, Japan, was sub-optimal (only 0.2%) (Hossain *et al*., 2009). Similarly, in rain-fed cultivation, *CUR* (%), oil yield (%), and oleoresin (%) in turmeric rhizomes were strongly influenced by irrigation schedule (Tripathi *et al*., 2015). In *C. alismatifolia*, α-tocopherol in the leaf tissues of drought-stressed plants declined depending on the water withholding period (20 and 30 days) (Jungklang *et al*., 2017). Likewise, Mahmood *et al*. (2021) reported reduction in pungency of pepper varieties under drought condition.

Farahani *et al*. (2009) indicated that drought stress motivated a significant reduction in all growth parameters of *Mentha piperita* L and essential oil yield and percentage. The highest values of menthol were obtained under 70% field capacity. Hassan *et al.* (2013) found that deficit irrigation (60 and 80% of the field capacity) significantly reduced growth parameters and yield of oil in *Rosmarinus officinalis* L. compared to control (100% field capacity). Also, Hassan & Ali (2013) found that increasing the irrigation level from 40% to 120% of the potential evapotranspiration increased the volatile oil percentage of coriander plant.

Under dehydration, *S. sclarea* and *L.latifolia* plants showed significant variation in dry weight and essential oil content. Essential oil content underwater deficit conditions showed a reduction of 36% and 53% in *S. sclarea* and *L.latifolia*. In the case of *L. latifolia*, the reduction of the essential oil content (expressed in ml m^{-2}) was related to the decrease of the dry weight under drought conditions (García-Caparrós, 2019). Full irrigation results in the highest oil content in maize grains while rainfed conditions showed the lowest. Here the starch content increased and the oil content decreased with decreasing irrigation in maize (Kresović *et al*., 2018)

Under osmotic stress turmeric showed dry weight reductions of 191%, 141%, 56%, 30% in leaf, pseudo-stem, root and rhizome, respectively. The addition of curcumin of rhizome increased 2 fold under low salinity. Whereas, higher salinity (higher than 60 mM, severe osmotic stress) caused 24% reduction of curcumin compared to control plants (Mostajeran *et al.*, 2014).

The expression levels of curcuminoid-related genes (*CURS*s and *DCS*) in turmeric depends on genotypic variation (such as 4.6% for variety GNT2 whereas NDH-98 had 1.6% curcumin) (Ayer *et al.*, 2018), agroclimatic zones, seasonal variations (Sandeep *et al.*, 2017), and rhizome maturity (Santhoshkumar & Yusuf, 2020). Optimum irrigation schedule and suitable agroclimatic zone have been reported as key factors to retain the high quality and productivity in turmeric plant (Kaur & Brar, 2016; Tripathi *et al.*, 2021).

Drought generally reduces growth and productivity of crops. Nevertheless, medicinal plants cultivated in semi-arid conditions typically yield higher concentrations of active compounds compared to those grown in more moderate climates. Under drought stress, the consumption of reduction equivalents (NADPH+H+) for CO_2 fixation through the Calvin cycle significantly decreases, leading to a substantial oversupply of NADPH+H+. Accordingly, all metabolic processes are pushed towards the synthesis of highly reduced compounds, such as isoprenoids, phenols, or alkaloids (Selmar & Kleinwächter, 2013).

The growing conditions e.g. the temperature, the water regime, the nutrient supply, strongly influence synthesis and accumulation of secondary plant product (Geethanjali *et al.*, 2016). Quality characters can vary with biotic or abiotic stresses as well as -agro climatic condition in turmeric (Singh *et al.,* 2013; Prasath *et al.,* 2018) and ginger (Akshitha *et al.,* 2020). Factors affecting growth and yield parameters have significant association with bioactive constituents which aid in selection of promising turmeric genotypes with better quality.

2.2.5.1 Variation in essential oil components under drought

Essential oils, which typically contain a variety of chemical compounds, are a significant category of secondary metabolites. They include two main groups: terpenoids and phenylpropanoids, with the latter including compounds such as allyl phenol and propenyl phenol. Essential oils are generally produced during crucial metabolic processes in plants, particularly under stress conditions, where they act as a defense mechanism against water loss or biological threats. The specific metabolic pathways for some of these compounds are still not completely understood.Research has demonstrated that environmental stresses influence the chemical composition of essential oils of plants (Yadav *et al.,* 2021; Bunse *et al*., 2022; Aqeel *et al.,* 2023).

The chemical constituents of turmeric rhizome essential oil revealed a diverse range of compounds present in varying levels. Singh *et al.* (2010) reported major constituents of essential oil of dry turmeric rhizome as aromatic-turmerone (21.4%), alpha-santalene (7.2%) and aromatic-curcumene (6.6%). Naz *et al.* (2010) identified 16 constituents from the gas chromatographic analysis of turmeric oil of which, six compounds contributed 70.0 % of the total oil. The predominant components were aromatic turmerone (25.3%), α-tumerone (18.3%), and curlone (12.5%). Other constituents included caryophyllene (2.26%) and eucalyptol (1.60%). *Eucalytus camaldulensis* seedlings (young leaves) submitted to drought for four weeks developed a terpenoid blend. Drought also decreased total amount of non-oxygenated terpenes by 44%, whereas some oxygenated sesquiterpenes (globulol, epiglobulol and ledol) were doubled, and 1,8-cineole content was enhanced by 28.3% (Leicach *et al*., 2010).

Under prolonged water stress (mild stress: 60% SMC) and moderate stress (40 % SMC), *Oregano vulgare* subsp. gracile showed a significant increase in the essential oil content while, *Oregano vulgare* subsp. Virens showed no variation. The essential oil of *O. vulgare* subsp. *gracile* was predominantly composed of the phenolic monoterpene carvacrol (46.86–52.07%), while the major component in the oil of *O. vulgare* subsp. *virens* was the sesquiterpene hydrocarbon (Z)-α-bisabolene (39.17–42.64%). However, the amount of (E)-β-caryophyllene in *O. vulgare* subsp. virens was significantly increased under water stress conditions (Morshedloo *et al*., 2017).

Monoterpene and sesquiterpene variations under water deficit stress in *Rosmarinus officinalis, Pinus halepensis, Cistus albidus* and *Quercus coccifera* were studied by GC-MS. Monoterpene emissions was similar to control in *R. officinalis* and *Q. coccifera* but higher in *P. halepensis* and *C. albidus*. By contrast, sesquiterpene emissions were strongly reduced or inhibited after four days of water withholding, particularly in *R. officinalis*, thus altering terpene emission composition (Ormeño *et al*., 2007).

Polyphenol components including vanillic acid, p-coumaric acid, cinnamic acid, dihydroxybenzoic acid, quercetin 3-D-galactoside, and naringin in severe drought-stressed *Salvia officinalis* (aerial parts) were lower than control (well-watered plants) (Bettaieb *et al*., 2011). Alternatively, total soluble phenol, 1,5-dihydroxyxanthone, rutin, and betulinic acid in the shoots of *Hypericum brasiliense* were sensitive to cold temperature (10 °C constant temperature of plant growth chamber), leading to low amount of target compounds when compared with those in control (30 °C) conditions (de Abreu *et al.,* 2005).

Moreover, upregulated expression in levels of *CCOMT* (cafeoyl CoA O-methyltransferase) and *CAD* (cinnamomyl alcohol dehydrogenase) in Ocimum tenuiforum under drought stress (20% feld capacity) was evidently observed, irrespective to the phenolic compound (eugenol, methyleugenol, and caryophyllene), which declined signifcantly over control condition (Rastogi *et al.,* 2019).

Seasonal variations, soil properties, irrigation schedule, and rhizome maturity in turmeric plantation have been reported to be determinant factors of quality and quantity of turmeric rhizomes (Ishmine *et al*., 2004; Singh *et al.,* 2013; Mohamed *et al.,* 2014; Chitra *et al*., 2017).

2.2.6 Influence of drought on nutrient uptake

Drought stress greatly impacts the nutrient relations of the plants. Many important nutrients including, nitrogen, silicon, magnesium, and calcium are uptaken by roots along with water, the drought conditions limit the movement of these nutrients via diffusion and mass which leads to retarded plant growth (Barber, 1995). The soil moisture deficit at times reduce the growth of the roots and, hence, reduce the uptake of the less mobile nutrients such as phosphorus (Garg, 2003).

Nutrients used for plant growth and biomass production generally come from the internal cycling of reserve materials, which require water for their solubilisation and translocation (Singh &Singh, 2004). Reduced nutrient absorption is a common occurrence in crop plants subjected to severe water deficit. Additionally, the composition and activity of the soil microbial colonies are negatively affected by the soil water deficit which eventually disturbs the plant nutrient relations (Schimel *et al.,* 2007). The changes in mineral uptake under moisture stress differ among crop species.

Various functions of mineral nutrients include signalling, cell wall stability, interaction with osmolytes, as cofactors for enzymes, and had a central role in photosynthesis which ultimately improved the productivity (Cakmak & Kirkby, 2008; Dodd *et al*., 2010; Marschner, 2011; Herman *et al.,* 2013; Molassiotis *et al.,* 2016). The knowledge of variation in nutrient concentration of different groups of genotypes is important to support plant breeding programs to develop grain cultivars with high nutrient value as well as stress tolerance (Teixeira *et al.,* 2014).

Under drought stress condition two varieties of *Vicia faba* L. (Assiut wardy & Assiut 84) showed variation in mineral contents. Drought increased sodium content by 17.18% and 68.84% in shoot and 13.94% and 55.27% .in root in Assiut wardy and Assiut 84 respectively. Potassium decreased by 23.42% and 20.0% in shoot and 31.56% and 41.97% in Assiut wardy and Assiut 84 respectively (Dawood *et al.,* 2021). Tomato seedlings exposed to varying intensities of drought stressshowed significantly higher uptake of N and P in both roots and shoots regardless of intensities of drought stress (Subramanian *et al*., 2006). Similarly, in marigold seedlings, P content was severely reduced under dehydration (Asrar & Elhindi, 2011).

Leaf nitrogen, phosphorus, and potassium content decreased significantly in wheat genotypes under drought stress. Among the genotypes, N,P, K, was higher in genotype Faisalabad-2008 (13.50 mg g−1 DW; 4.01 mg g−1 DW; 3.78 mg g−1 DW) under the drought condition which was mainly attributed to its better morphological characters such as greater number of tillers/plant compared to the other tested genotypes (Ahmad *et al*., 2022).

D'Oria *et al.* (2022) conducted ionomic analysis in *Brassica napus* and *Triticum aestivum* that were subjected to moderate or severe water deficit.

Although the uptake of most mineral nutrients declined, the absorption of Fe, Zn, Mn, and Mo was affected more significantly and earlier. There was an upsurge in the transport of Cl and Co, a decrease in the transport of N, P, B, Mo, and Ni, and variable transport levels for S, Mg, K, Zn, Fe, Cu, and Mn.

Bista *et al.* (2018), found that decreases in grass nutrient status during drought are correlated with decreases in levels of nutrient-uptake proteins in roots and found out that drought-related decreases in nutrient concentration, especially Phosphorus, were likely caused, by decreases in the concentration of root nutrient-uptake proteins in both drought-sensitive and tolerant species.

Significant variability in mineral content was noted among 100 sorghum genotypes grown in both non-stress and water-stress conditions. Water stress led to a reduction in Mn, P, Mg, and S levels in 100%, 96%, 93%, and 56% of the genotypes. Genotype and other factors appeared to influence K, Ca, Cu, Fe, and Zn levels more than water stress did. The best two genotypes to Fe content were SC21 and SC655 (Paiva *et al.*, 2017).

Full irrigation significantly boosted the concentrations of K, Mg, Fe, Mn, and Zn in maize grains, while decreasing the Ca concentration compared to the rainfed treatment. 25% water deficit (I_{75}) positively influenced both the nutrient content and yield of maize grains. Thus treatment I_{75} can be proposed under similar soil and climate conditions (Kresović *et al.*, 2018).

Lavandula latifolia, Mentha piperita, Salvia sclarea, Salvia lavandulifolia, *Thymus capitatus* and *Thymus mastichina* plants were exposed to two different water treatments. Leaf N concentration remained unchanged in *M. piperita*, *S. lavandulifolia*, *S. sclarea*, and *T. capitatus* under drought stress, while *L. latifolia* and *T. mastichina* exhibited reduced leaf N concentration in drought conditions. Regarding leaf P concentration, a decrease was observed in all species under drought except for *S. sclarea*. There were no differences in leaf K concentration under drought conditions in all the species studied (García-Caparrós *et al.*, 2019).

With limited water supply, nutrient uptake by roots decreases because a decline in soil-water potential slows the diffusion rate of nutrients between the soil matrix and root surface (Farooq *et al*., 2009). Lower transpiration rate and impaired active transport, due to a lack of energy input and altered membrane permeability, decreases root nutrient adsorbing power (Hasanuzzaman *et al*., 2018; Sharma *et al*., 2012). While in some cases under dehydration plants increase the length and surface area of roots and change their architecture in order to capture the less mobile nutrients as seen in summer maize (Lynch & Brown, 2001; Shan & Liang, 2007; Yan *et al*., 2022).

Nutrient relations become more complicated due to interactive effects of different nutrients on each other and overall plant physiology (Morgan & Connolly, 2013). Drought stress, cause an upsurge or down turn in nutrient uptake based on their requirement for protein synthesis and maintenance of many important structures such as chlorophyll (Queiroz *et al.,* 2015; Jogaiah *et al.,* 2015). There appears to be an impairment of the nutrient contents and concentrations under drought conditions, but that might not hold true for all nutrients (Rouphael *et al.,* 2012).

Drought is a global issue that significantly limits crop production and quality, and recent climate change has exacerbated this problem. Drought stress exerts influence on the growth, dry matter, harvestable yield and quality in plants. The ever increasing threat of global climate change and declining water resources suggest germplasm identification with enhanced drought tolerance and water-use efficiency. Enhancing water use efficiency in agricultural systems is a primary focus in arable lands across many regions worldwide. Drought tolerance is a complex trait which involves cooperation of molecular, biochemical, physiological, and morphological mechanisms. Timing, duration and severity of drought, genotype variation and stage of development undoubtedly have pivotal roles in determining plant's response to water deficit. Focusing on the differences in the activation and regulation of different mechanisms will lead to identification of determinant factors for drought tolerance. A combination of genetic and molecular approaches will give more insight into the underlying mechanism of water-stress responses in plants.

CHAPTER 3
MATERIAL AND METHODS

The present investigation entitled "Morpho-physiological and molecular responses of turmeric genotypes subjected to water stress" was carried out during 2019-2024 at ICAR-Indian Institute of Spices Research, Kozhikode, Kerala. The details of material used and methods followed during the course of investigation are presented here under.

3.1 MATERIALS

This study was conducted using 50 genotypes collected from National Active Germplasm Site (NAGS) of ICAR-IISR, Kozhikode, Kerala, India (Table 1) which was used in experiment I. For biochemical and molecular characterization *i.e.,* experiment II, 12 genotypes (9 tolerant and 3 susceptible) were selected from experiment 1. For field evaluation (experiment III) six shortlisted genotypes (4 most tolerant and 2 most susceptible) were used.

Table1. List of genotypes selected for the study

S. No	Name	Type
1	Acc 66	Accessions
2	Acc 8	
3	Acc 849	
4	Amballur	released varieties
5	BSR I	
6	BSR 2	
7	BSR 2 white	
8	CO 1	
9	CO 2	
10	Duggirala Red	
11	IISR Alleppey Supreme	
12	IISR Kedaram	
13	IISR Prathibha	
14	IISR Prabha	

15	IISR Pragati	
16	Sudharsana	
17	Suguna	
18	Suvarna	
19	Kanthi	
20	Megha Turmeric	
21	NDH 1	
22	NDH 98	
23	NTC 188	
24	NTC 189	
25	Panth Peetab	
26	PH 1	
27	PH 2	
28	Rajendra Sonali	
29	Rajendra Sonia	
30	Ranga	
31	Rasmi	
32	Roma	
33	Salem Erigoor	
34	SC 61	Breeding material
35	SL 1	
36	SL 10	
37	SL 2	
38	SL 3	
39	SL-P 389_1	
40	SL 4	
41	SL 6	
42	SL 5	
43	SL 7	
44	SL 8	
45	SL 11	
46	Sobha	Released varieties
47	Sugantham	
48	Suranjana	
49	Suroma	
50	Varna	

3.2 Methods

The experimental method comprised of three parts *viz.*

1. Morphological and physiological characterization of fifty genotypes to select tolerant and susceptible genotypes under drought condition.
2. Characterization of selected turmeric genotypes to study the mechanism underlying drought tolerance through,
    a) Analysis of physiological parameters
    b) Evaluation of biochemical parameters
    c) Gene expression studies
3. Field evaluation of selected drought tolerant genotypes which involves,
    a) Analysis of physiological parameters
    b) Evaluation of biochemical parameters
    c) Gene expression studies
    d) Analysis of rhizome quality

3.3 Experiment I –Screening of 50 genotypes for drought tolerance.

3.3.1 Experimental conditions

This study was conducted during the period of 2019-2020. Planting was taken up in the month of June 2019, in a completely randomized design (CRD) with three replicates in polythene grow bags at ICAR-Indian Institute of Spices Research (IISR), Kozhikode, Kerala (North latitude 11° 17′ 58.3″; East longitude 75° 50′ 26.3″; 50 m MSL). Recommended agronomic practices were followed for crop cultivation (Prasath *et al*., 2022). Need based pest and disease management measures were taken up as and when required.

Seed rhizomes (25g) of 50 indigenous turmeric genotypes were planted in polythene grow bags (15 cm × 30 cm) containing potting mixture (2:1:1 of soil: sand: farmyard manure), which had the permanent wilting point at 7.5 to 8.0% moisture content, maintained in a greenhouse at a temperature of 30 ± 3°C (day) and relative humidity (RH) of 85 ± 5% (Banu & Krishnamurthy, 2023).

3.3.2 Treatment imposition

Plants were irrigated to field capacity (SMC of 21%, which was analysed according to the standard methods as described in AOAC (2005), till 90 days after planting (DAP). Plants were subjected to stress treatment from 90 to 120 DAP, by suspension of irrigation for 30 days. Soil moisture content was reduced to 11 % after 30 days of moisture stress (at 120 DAP). Again, the plants were irrigated to field capacity till harvest (Banu & Krishnamurthy, 2023).

3.3.3 Morphological characterization of turmeric genotypes

Observations were recorded on individual plants (replicates) of each genotype on different morphological traits viz. plant height, number of tillers, number of leaves per plant, leaf lamina length, leaf lamina width at 120 DAP *i.e.,* after one month of stress treatment (90-120 DAP) and fresh yield (g plant^{-1}) was recorded after harvest. The procedures adopted for collection of data on different characters are given below:

3.3.3.1 Plant height (cm)

Plant height was measured from soil level to tip of the top leaf of the main shoot.

3.3.3.2 Number of tillers

Total number of tillers in a single plant was counted.

3.3.3.3 Total number of leaves

Total number of leaves present in a plant was recorded.

3.3.3.4 Leaf length (cm)

Length of upper fourth leaf from the main shoot was recorded

3.3.3.5 Leaf width (cm)

Width (at the widest point) was recorded from the upper fourth leaf of the main shoot.

3.3.3.6 Leaf area (cm^2 $plant^{-1}$)

Leaf area was determined as per Panja *et al*. (2005) as follows,

$$\text{Leaf area} = 5.71 + 0.72\ (L * W)$$

Where, 5.71 and 0.72 are the intercept and regression coefficient of leaf area estimated using least-square linear regression analysis. L is leaf length (cm) and W is the maximum width of the leaf (cm).

3.3.3.7 Rhizome yield (g $plant^{-1}$)

Crop that underwent moisture stress treatment for one month (90 to 120 DAP) was harvested and the fresh rhizome yield was recorded by harvesting and weighing of the individual clump.

3.3.4 Physiological characterization

Plants were subjected to stress treatment from 90 to 120 DAP, by suspension of irrigation for 30 days. RWC (relative water content) and EL (electrolyte leakage) were determined at 10 days intervals at four different soil moisture levels viz. 21 % (control), 17.10% (10 days after treatment (DAT)-early stress), 13.5% (20 DAT-moderate stress) and 11% (30 DAT-severe stress).

Stomatal density (SD) and epicuticular wax (EW) content were determined after 30 days of stress treatment, at 120 DAP.

The youngest fully matured leaf (fourth leaf from shoot apex) of plants labelled for observation purpose was used for physiological assays.

3.3.4.1 Relative water content

RWC was determined following the method of Barrs & Weatherley (1962). For this, fresh weight of leaf discs (2 cm^2), of 150 mg, was recorded from control and stressed leaf samples. These leaf discs were washed and floated in double distilled water in a petridish for three hours to gain full turgidity and their turgid weight was determined. The turgid samples were dried in hot air oven at 80 degree centigrade to a constant weight and dry weight of the leaf discs was recorded and RWC was calculated using the formula given below.,

RWC (%) = [(FM - DM)/(TM - DM)] * 100,

Where, FM, DM, and TM are the fresh, dry and turgid masses, respectively, of the tissue.

3.3.4.2 Electrolyte leakage

Electrolyte leakage, which estimates membrane permeability, was determined at different soil moisture levels as per Blum & Ebercon (1981). Leaf discs of 1cm diameter were collected from control and 10, 20 and 30 days stressed plants. Leaf discs were washed in distilled water, blotted on filter paper and then incubated in 5 ml of distilled water for three hours. The electrical conductivity of the solution was measured using EC-TDS analyser (initial electrical conductivity).The leaf discs were then boiled for thirty minutes, cooled to room temperature and the EC of the solution was measured again (final EC). The cell electrolyte leakage was computed using the formula,

$$\text{EL (\%)} = (\text{Initial EC/ Final EC})\ \text{x}100, \text{Where, EC} = \text{Electrical conductivity}$$

3.3.5 Stomatal density

Stomatal density was determined on the abaxial and adaxial surfaces of the leaf using the method of Mahareli *et al*. (2002). Mature leaf samples were thoroughly cleaned to remove the dust adhering to the surface, then applied nail polish, the replica fluid, in a thin and uniform film by spreading a drop or two on the leaf surface and allowed it to dry completely. The replica was then carefully peeled off with the aid of cello tape, and it was positioned on the slide such that the imprinted surface was on the upper side. All the stomata that were visible in 0.9604 view field of microscope at 10X magnification were counted. The stomatal density was expressed as number of stomata mm^{-2}.

3.3.6 Leaf epicuticular wax

Leaf epicuticular wax estimation was performed as per Ebercon *et al*. (1977). A wax reagent was prepared by combining 20 grams of potassium dichromate with 40 ml of deionized water and 1 litre of concentrated H_2SO_4. The wax reagent was boiled on a hot water bath until a clear solution was obtained. Carnauba wax was used for standard graph preparation.

Leaf sample (2 cm^2 area) was immersed in 15ml of distilled chloroform for 20 seconds (to extract only EW as longer periods of immersion may extract the inner lipids). The chloroform extract was boiled on water bath to dryness. 5 ml of wax reagent was added to it and boiled for thirty minutes in hot water bath at 100°C. It was cooled to room temperature and 12 ml of deionized water was added. The mixture was filtered using Whatman No 1 filter paper and the filtrate was collected. The intensity of the colour was determined using spectrophotometer at 590nm.

3.3.7 Statistical analysis

The data were analysed using analysis of variance (ANOVA) using R software (R Core Team, 2021). This was followed by Duncan Multiple Range Test (DMRT) at 95% confidence level, for mean separation.

3.3.8 Scoring

Based on ideal genotype concept for drought tolerance such as reduced leaf area, lesser stomata, higher wax content, higher RWC and lower electrolyte leakage, cut off values for tolerant, moderate and susceptible categories were assigned for each parameter in such a way that tolerance category was assigned the highest score of 30 followed by moderate score of 20 and susceptible was assigned the least score which was 10. Based on the importance of the parameter in drought tolerance, weightage was assigned to each parameter such as 0.3 for RWC, 0.2 for electrolyte leakage, 0.10 for wax content, 0.15 for stomatal density (abaxial), 0.10 for stomatal density (adaxial) and 0.15 for leaf area and the sum total of all the weightages was equal to unity *i.e.*, one. Then, the product of the score and weightage for each parameter and its sum total for each genotype was determined which is the weighted score for each genotype. For rhizome yield, genotypes with higher yield *i.e.,* >200 g/plant, was assigned the highest score of 30 followed by moderate score of 20 for plants with 100-200 g yield per plant and the lowest score of 10 for plants with <100 g yield per plant. For yield alone, separate weightage of 0.5 was assigned. Genotypes were ranked based on weighted score of both the morpho-physiological parameters as well as yield (Banu & Krishnamurthy, 2023).

3.4 Experiment II – Characterization of selected turmeric genotypes to study the mechanism underlying drought tolerance

3.4.1 Experimental plot

This study was conducted during the period of 2021-2022. Planting was taken up in the month of June 2021, in a completely randomized design (CRD) with four replicates in polythene grow bags at ICAR-Indian Institute of Spices Research (IISR), Kozhikode, Kerala (North latitude 11° 17′ 58.3″; East longitude 75° 50′ 26.3″; 50 m MSL).

Twelve turmeric genotypes, which were selected as most tolerant and susceptible genotypes from our previous study (9 tolerant ones viz. IISR Prabha, IISR Prathibha, IISR Pragati, SL 5, SL 10, Suguna, Suvarna, Rajendra Sonia, NDH 1 and 3 susceptible genotypes viz. IISR Alleppey Supreme, IISR Kedaram and Acc 66), were used for this study. Seed rhizomes (25 g) were planted in polythene grow bags (15 cm × 30 cm) containing sterilized potting mixture (2:1:1 of soil: sand: farm yard manure), which had the permanent wilting point at 7.5 to 8.0% moisture content, maintained in a greenhouse at a temperature of 30 ± 3°C (day) and RH of 85 ± 5%. Recommended package of practices of IISR was followed for crop cultivation (Prasath *et al.*, 2022) (Fig. 3.2.1).

3.4.2 Stress imposition

Plants were maintained at different soil moisture regimes viz. 100% (control, SMC 21.5%), 50% (moderate stress, SMC 12.6%) and 25% (severe stress, SMC 10.7%) field capacities, with four replicates. Irrigation was performed with tap water. Different field capacities (FC) of 100%, 25% and 50% were maintained by weighing the grow bag on alternate days and adding the required volume of water (that was lost) manually.

Fig. 3.2.1 Plants maintained at 100% (control), 50% (moderate stress) and 25% (severe stress) field capacities with 4 replicates. .

3.4.3 Observations

Planting were evaluated for their physiological, biochemical and molecular response for assessing their drought tolerance at three growth stages *i.e.*, at 120 DAP, 150 DAP and 180 DAP and observations were recorded. Physiological parameters (RWC and EL) were analysed in leaves (third or fourth leaf), biochemical (enzyme activity, H_2O_2 content, lipid peroxidation and proline content) and molecular analyses were carried out using the leaves (third or fourth leaf) as well as the rhizomes in each genotype.

Photosynthetic synthetic pigments, sugar content and total phenol content were analysed in leaves whereas protein and starch content were analysed in rhizomes at 3 growth stages. Four replicates of each genotype were used for biochemical the analysis. Crop was harvested after nine months of planting.

3.4.4 Physiological characterization

The effect of drought on the physiology of plant was studied by EL and RWC. RWC and EL were determined at the end of growth phase, i.e., 180 days after planting in individual replicates of each genotype which were maintained at three different moisture regimes of 100% FC, 50% FC and 25% FC. The youngest fully matured leaf (fourth leaf from shoot apex) of plants labelled for observation purpose was used for physiological assays.

3.4.4.1 Relative water content

RWC of leaves was determined as mentioned in section 3.3.4.1 in control and treatments at 120, 150 and 180 DAP.

3.4.4.2 Electrolyte leakage

EL of leaves was determined as mentioned in section 3.3.4.2 in control and treatments at 120, 150 and 180 DAP.

3.4.5 Biochemical characterization.

The effect of drought on biochemical parameters was investigated through analysis of lipid peroxidation, hydrogen peroxide (H_2O_2) content, proline content, the activities of antioxidant enzymes and AsA-GSH cycle enzymes, photosynthetic pigment content, sugar, phenol and protein content as well as starch content.

3.4.5.1 Lipid peroxidation

The lipid peroxidation rate was analysed by the determination of malondialdehyde (MDA) content in leaves and rhizomes. Fresh sample of 0.2 g was weighed and homogenized in 5 ml 10% (w: v) trichloroacetic acid, and centrifuged at 12,000 g for 15 min at 4 °C. After that, 1 ml of the supernatant was mixed with 2 ml 0.5% (w: v) thiobarbituric acid in 20% (w: v) trichloroacetic acid. The mixture was then incubated at 95 °C for 30 min, and quickly cooled on ice. After centrifugation at 4,000 g for 10 min, the absorbance of the supernatant was measured at 450, 532, and 600 nm, respectively by UV/VIS spectrophotometer (Shimadzu UV spectrometer-1800). The MDA content was calculated according to the method given by Zhang *et al*. (2005) to estimate lipid peroxidation.

3.4.5.2 Endogenous hydrogen peroxide content

The H_2O_2 content in the leaf was estimated following the modified method of Zhou *et al.* (2006) and Naz *et al.* (2021). Briefly, 0.2 g sample was ground and extracted using 3 ml of ice-cold TCA (0.1%, w/v). The mixture was centrifuged at 12,000 g for 10 min followed by the addition of 0.5 ml of phosphate buffer (10 mM) at pH 7.0. Then into the mix, 0.5 ml of the supernatant and 0.7 ml of 1 M potassium iodide solution were added. The absorbance of the solution was determined at 390 nm using a spectrophotometer.

3.4.5.3 Proline content

The proline content in the leaf and rhizome samples was determined by the modified method of Mohsen *et al.* (2016). Briefly, 0.5 g of plant tissue was ground with 3% sulfosalicylic acid. The samples were centrifuged at 11,500 g for 15 min and kept in ice. A reaction mixture was prepared with 100 µl of 3% sulfosalicylic acid, 200 µl acidic ninhydrin, and 200 µl glacial acetic acid. The centrifuged filtrate (0.2 ml) was added into the reaction mixture and incubated at 95°C for 1 h followed by cooling the sample by placing in ice.

To extract and separate the proline-ninhydrin complex to avoid interference of other amino acids, 4 ml of toluene was mixed with the sample and vortexed for 20 s and left the sample for 5 min for allowing it to separate into 2 phases. The chromophore containing toluene was transferred into a fresh tube and the absorbance was read at 520 nm. Using a standard concentration curve, the concentration of proline was determined in the samples.

3.4.5.4 Assay of antioxidant enzymes and AsA-GSH cycle enzymes (APX, MDHAR, DHAR and GR) activity

3.4.5.4.1 Superoxide dismutase (SOD)

SOD activity was determined by the method described by Alici & Arabaci (2016) and Naz *et al.* (2021). The enzyme extract was prepared by grinding 1 g of fresh sample in 10 ml of cold phosphate buffer at pH 7.8. The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C and the crude enzyme extract (supernatant) was collected. To 0.1 ml of the crude extracted enzyme, 1.5 ml phosphate buffer (0.05 M at pH 7.8), 0.3 ml of 0.1 M methionine, 0.3 ml of 20 µmol/l riboflavin, 0.3 ml of 750 µmol/l nitro blue tetrazolium chlorideand 0.3 ml of 100 µmol/l EDTA were mixed to form the reaction mixture. All tubes were exposed to 400 W light for 15 minutes. The absorbance was read at 560 nm. SOD activity was expressed in U/g FW per minute.

3.4.5.4.2 Peroxidase (POD)

The method described by Ippolito *et al.* (2000) with slight modifications was used to extract and assay POD from turmeric leaves and rhizomes. The extraction buffer used was 1 ml of 0.5 M Tris-HCl buffer, pH 7.5 for 1 g of fresh leaf tissue. The samples were ground in a prechilled mortar and pestle with quartz sand. The extract was filtered and then centrifuged at 15,000 rpm at 4°C for 15 min. The supernatant collected was used as the enzyme extract. For the assay, 0.5 ml of fresh enzyme extract was mixed with 0.5 ml of 1% guaiacol, 0.5 ml of 1% H_2O_2, and 1.5 ml of 0.5 M Tris-HCl. Peroxidase activity was determined by measuring the change in absorbance at 20 second intervals for 3 min at 470 nm. Tris-HCl was used as the blank in the assay. The enzyme activity was calculated as the change in OD at 470 nm per unit time per mg protein.

3.4.5.4.3 Catalase (CAT)

The CAT activity was determined according to the method of Havir & McHale (1987) by measuring the oxidation of H_2O_2 at 240 nm. Fresh samples of 0.2 g were ground in liquid nitrogen and homogenized in 5 ml phosphate buffer solution of pH 7.8. After centrifugation at 16,000 g for 20 min at 4 °C, the supernatant was collected. The reaction mixture that consisted of 0.2 ml enzyme extract, 2 ml of 0.1 mM phosphate buffer solution of pH 6.5 and 0.5 ml of 1% H_2O_2 was prepared for monitoring the absorbance at 240 nm for 5 min in 20 second intervals, the CAT activity was calculated and expressed as the change in OD at 240 nm per unit time per mg protein.

3.4.5.4.4 AsA-GSH cycle enzymes (APX, MDHAR, DHAR and GR) activity.

The sample tissue of 0.5 g was ground with liquid nitrogen and homogenized in 5 ml of 100 mM phosphate buffer solution of pH 7 containing 1%

(w: v) polyvinyl pyrrolidone and 0.010 ml of 0.5 M EDTA. After centrifugation at 16,000 g for 20 min at 4 °C, the supernatant was used to estimate the activity of APX, MDHAR, DHAR and GR.

The activity of ascorbate peroxidase (APX) was assayed as described by Nakano &Asada (1981). Reaction mixture contained 0.1 ml enzyme extract, 1.5 ml of 0.1 M phosphate buffer solution (pH 7.0), 0.012 ml 10 mM EDTA, and 0.05 ml 0.5 mM ascorbic acid (AsA). The reaction was initiated with addition of 0.2 ml of 1% H_2O_2, and the decrease in absorbance at 290 nm was monitored in 20 second intervals for 3 minutes to indicate the rate of AsA oxidation. The activity of APX was expressed as μmol AsA oxidized min^{-1} mg^{-1} FW.

The mono dehydroascorbate reductase (MDHAR) activity was estimated using the method provided by Hossain *et al*. (1984). The reaction mixture that consisted of 1.8 ml of 0.1 M phosphate buffer solution (pH 7.0), 0.7 ml of 1 mM NADH, 0.05 ml of 0.0125% (v: v) Triton X-100, 0.2 ml 0.25 U AsA oxidase, 0.25 ml of 1 mM AsA and 0.1 ml enzyme extract was monitored for the change in absorbance at 340 nm due to NADH oxidation in 20 minute intervals for 5 min. The activity of MDHAR was expressed as μmol NADH oxidized min^{-1} mg^{-1} FW.

The activity of dehydroascorbate reductase (DHAR) was determined by the method given by Doulis *et al*. (1997) with slight modification. The reaction mixture contained 2.5 ml of 0.1 M phosphate buffer solution (pH 7.0), 0.01 ml 0.5 M EDTA, 0.1 ml 0.1 M GSH, and 0.1 ml enzyme extract was monitored. The reaction was initiated after 0.1 ml 1 M DHA was added into the reaction mix. The reduction of DHA at 265 nm was monitored for 4 min.

The glutathione reductase (GR) activity was assayed following the method of Rai *et al.* (2013). The reaction mixture which contained 2 ml of 0.1 M phosphate buffer solution (pH 7.8), 0.05 ml 10 mM NADPH, 0.05 ml of 0.5 mM GSSG, and 0.1 ml enzyme extract was monitored for change in absorbance at 340 nm. The enzyme activity was calculated as the change in optical density per unit time per mg protein.

3.4.5.5 Estimation of total phenols

The leaf tissues were ground in 1.5 ml of 80% methanol at room temperature. The extracts were centrifuged and the supernatant was used for the assay. Total phenol was estimated by Folin Ciocalteau reagent (Ainsworth & Gillespie, 2007). About 0.5 ml of the enzyme extract was mixed with 0.2 ml of Folin Ciocalteau reagent. After 3 min, 1 ml of aqueous Na_2CO_3 was added to the mixture and diluted the sample with 10 ml of distilled water. After 1 hour of incubation, the absorbance was read at 725 nm using a spectrophotometer against a standard of gallic acid.

3.4.5.6 Estimation of protein

The protein content from the fresh turmeric rhizome tissue was estimated as per the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as standard. The standard stock solution was prepared by dissolving 50 mg BSA in 50 ml distilled water and the working standard was made by diluting the stock solution to 1:5 dilutions with distilled water. For protein extraction, 500 mg of sample was ground in a prechilled mortar and pestle in 10 ml sodium phosphate buffer (0.1 M, pH 7.0) centrifuged at 10,000 rpm for 20 minutes and the supernatant was collected for protein assay.

The following reagents were prepared.

Reagent A: 1 g Na_2CO_3 in 50 ml of 0.1 (N) NaOH

Reagent B: 0.25 g $CuSO_4$ 5 H_2O in 50 ml of 1% potassium sodium tartrate

Reagent C: 50 ml reagent A + 1 ml reagent B

Reagent D: 1 (N) Folin–Ciocalteau reagent

Protein extract (0.1 ml) was taken in a test tube and its volume was made up to 1 ml using the double distilled water. Five ml of reagent C was added to it and incubated for 10 minutes. To it, 0.5 ml of reagent D was added, stirred well, and incubated at room temperature in dark for 30 minutes. The absorbance of the blue colour developed was measured at 660 nm in a UV–VIS spectrophotometer against a reagent blank without protein extract. Amount of protein present was calculated with the help of a standard curve prepared by plotting concentration of standard BSA on the X–axis versus absorbance on the Y–axis. Amount of protein present was expressed as mg g^{-1} dry weight of sample.

3.4.5.7 Estimation of starch

Fresh sample of turmeric rhizome weighing 0.1 to 0.5 g was homogenized in hot 80% ethanol to remove sugars. It was then filtered, and the residue retained. The residue was washed repeatedly with hot 80 % ethanol till the washings did not give colour with Anthrone reagent. Residue was dried over water bath. Double distilled water (5 ml) and 6.5 ml of 52% perchloric acid were added to the residue for extraction at 0 °C and the filtered supernatant was saved. Extraction was repeated using fresh perchloric acid. Supernatants were pooled, and the volume was made up to 100 ml. From this, 1:5 dilution was made using distilled water. This diluted supernatant of 0.1 and 0.2 ml was pipetted into test tubes and volume was made up to 1 ml with distilled water.

Anthrone reagent (4 ml) was added to each test tube and heated for about 8 min in boiling water bath. It was rapidly cooled and intensity of green to dark green was read at 630 nm in a Spectro photometer. A series of standards using glucose (0 to 500 µg) were run and standard curve was plotted. The glucose content in the sample was estimated using the standard graph, and then the value was multiplied by a factor nine to arrive at the starch content (Sadasivam & Manickam, 1992).

Starch content (%) = glucose content (%) x 0.9

3.4.5.8 Estimation of sugar

Fresh leaf sample of 0.1 g was homogenised in 5 ml of hot 80% methanol twice and the supernatant was collected. The supernatant was evaporated by keeping it on a water bath at 80°C. To this added 10 ml of water to dissolve the sugars. Pipetted out 1 ml of sample extracts into fresh test tubes and the volume was made up to 3 ml using distilled water. To this added 3 ml of DNS (dinitrosalicylic acid) reagent, which was made by dissolving 1 g dinitrosalicylic acid , 200 mg crystalline phenol and 50 mg sodium sulphate in 100 ml 1% NaOH. The content was heated for 5 minutes and 40% potassium sodium tartarate solution (1 ml) was added into the warm test tubes. Test tubes were cooled and the intensity of dark red colour was read at 510 nm (Sadasivam & Manickam, 1992). The sugar content in the sample was estimated using the standard graph.

3.4.5.9 Chlorophyll and carotenoid pigments

For the estimation of total chlorophyll and carotenoids, fresh leaves (25 mg) from control and stressed turmeric plants were extracted in 5 ml of 80% (v/v) acetone. The amount of total chlorophyll and carotenoids was calculated using the method described by Rai *et al.* (1999). The chlorophyll absorbance was measured at 652 and the carotenoid pigment was measured at 510 and 480 nm.

$$\text{Total chlorophyll (mg } g^{-1}) = \frac{\text{OD}_{652} \text{ x } 1000}{34.5} \times \frac{V}{1000 \text{ x W}}$$

$$\text{Carotenoids (mg } g^{-1}) = (7.6 \times \text{OD}_{480}) - (1.49 \times \text{OD510}) \times \frac{V}{1000 \text{ x W}}$$

Where

OD - Optical Density; V - Final volume of supernatant (5 ml);

W - Weight of the leaf sample taken in gram

3.4.6 Molecular characterization

Gene expression analysis of stress responsive transcription factors were conducted using qRT-PCR.

3.4.6.1 Total RNA isolation

RNA isolation was done from turmeric leaves and rhizomes by (Deepa *et al.*, 2014) following an optimised protocol developed by the modified SDS-acid phenol based method. The reagents used and the important steps involved are given below.

1. Plant tissue (about 100 mg) was ground to a fine powder in liquid nitrogen along with 2% polyvinylpyrrolidone (PVP) powder using a mortar and pestle.

2. 2 ml of pre-warmed extraction buffer (2% SDS, 100 mM Tris-HCI-pH 8, 25 mM EDTA- pH 8 and 1% B-mercaptoethanol) at 65°C was added and ground further and the contents were transferred to two, 2 ml centrifuge tubes.

3. Equal volume of acid phenol: chloroform was added and vortexed for 10 sec and the mixture was left undisturbed for 10 minutes at room temperature for separation of nucleoprotein complexes.

4. The mixture was then centrifuged at 15,000 g for 10 min at 4°C.

5. The supernatant was added to new tubes and 0.3 volumes of 5 M sodium acetate and 0.7 volume of acid phenol chloroform were added. Tubes were inverted for mixing and incubated in ice for 10 minutes.

6. The mixture was then centrifuged at 15,000 g for 10 min at 4°C and transferred the supernatant to fresh 1.5 ml centrifuge tubes.

7. Equal volumes of isopropanol was added and mixed by inverting the tubes for5 min.

8. The samples were transferred to silica gel column and centrifuged for 1 minute and discarded the flow through.

9. Wash buffer (350 µl) was added to the silica gel column and centrifuged at 10000 g for 1 minute.

10. Again centrifuged for 1 minute to remove the residual wash buffer.

11. For DNAse treatment, 10 µl of on-column DNAse enzyme and 70 µl of DNAse buffer were added to the silica gel column and incubated at room temperature for 30 minutes.

12. 300 µl of wash buffer was added and centrifuged at 10000 g for 1 minute.

13. Washing was repeated with 500 µl of wash buffer and centrifuged at 10000 g for 1 minute.

14. The column was air dried by centrifuging at 130000 g for 1 minute.

15. The column was placed in a 1.5 ml centrifuge tube and 30 µl of RNase free water was added and RNA was eluted.

RNA was quantified using nanodrop (Denovex) using 1 ul of total RNA. The concentration (ng/ul), A260/A280 ratio and A260/A230 ratio of the sample were recorded and 3 µl of RNA sample was mixed with equal volume of RNA loading dye, loaded to 2% agarose gel made in DEPC treated TAE buffer for measuring the quality of the RNA.

3.4.6.2 cDNA synthesis

Revertaid reverse transcriptase kit (Thermo Scientific) was used for first-strand cDNA synthesis. DNA contamination was removed during the last steps of RNA isolation with on column DNase (Sigma-Aldrich) as per the manufacturer's instruction. The resulting DNA-free RNA samples were used as templates for reverse transcription for synthesis of the first strand of cDNA. To 250 ng of DNA free RNA, Iul of 100 µM oligo(dT)18 was added and made up to 12.5 µl with nuclease-free water treated with DEPC and incubated at 65°C for 5 min and later incubation on ice for 5 minutes was done. Meanwhile the master mix for cDNA synthesis was prepared (4 µl 5X buffer, 0.5 µl (40 U/µl) of Ribolock, 2 ul of 10 mM dNTPs, and I µl (200 U) of reverse transcriptase) and transferred 7.5 µl to each tube containing RNA. This was then mixed carefully and incubated at 42°C for 60 minutes before terminating the reaction at 70°C for 10 minutes and the cDNA was stored at -20°C.

3.4.6.3 qRT-PCR based expression profiling

To verify expression of transcription factors involved in drought response, qRT-PCR was performed on eight selected transcription factors of the drought response pathway. An online primer designing tool viz., Primerquest, (https://eu.idtdna.com/

Primerquest), was used to design gene specific primers using the following parameters; 100-150 bp amplicon length, 60-62°C melting temperature, and 40-60% GC content (purchased from Eurofins Genomics India Private Limited). The primers were obtained in lyophilized form and a stock concentration of 100 uM was prepared by suspending in sterile water and diluted ten times to a working concentration of 10 uM (Table 2).

Table2. List of primers used in the study

<table>
<tr><th colspan="4">Quantitative real time PCR primers for candidate reference genes</th></tr>
<tr><th>Primer</th><th>Forward primer (5’-3’)</th><th>Reverse primer (5’-3’)</th><th>Function</th></tr>
<tr><td><i>EF-1α</i></td><td>GCTGACTGTGCTGTTCTCATTAT</td><td>CTCGTGTCTGTCCATCCTTTGAA</td><td>Translation</td></tr>
<tr><td><i>UBIQUITIN</i></td><td>GCACTCTCGCTGACTACAAC</td><td>GGCTTGGTGTAGGTCTTCTTC</td><td>Regulatory protein</td></tr>
<tr><td><i>GAPDH</i></td><td>AACTGTAGCCCCACTCATTG</td><td>GCATCTTAGGGTATGTGGAGG</td><td>Glycolysis, glucogenesis</td></tr>
<tr><td><i>ACTIN</i></td><td>CAACAGCAGAACGGGAAATTG</td><td>CATAATCAAGGGCGACATATGC</td><td>Cytoskeleton, structural protein</td></tr>
<tr><td><i>TUBULIN</i></td><td>GGCAGAGATCAGATGGTTCAG</td><td>TGGACAATGAAGCACTCTACG</td><td>Role in cell division</td></tr>
<tr><th colspan="4">Quantitative real time PCR primers for transcription factors</th></tr>
<tr><td><i>ABF</i></td><td>GCGAAGCAGGGATGAATAGT</td><td>CTCCTCTTCTCTTCCTGAAAGC</td><td>Transcription factors</td></tr>
<tr><td><i>bZIP</i></td><td>CCAAACAGTCCAAAGAAGCC</td><td>TCAAGGTCTCATAACTGATGCT</td><td>Transcription factors</td></tr>
<tr><td><i>DREB</i></td><td>GCGGTTCCCTCCACTCAGTC</td><td>CGTCCGTTGTCGTTCCCTGT</td><td>Transcription factors</td></tr>
<tr><td><i>AP2</i></td><td>AAATGAGTCC TGCCACCACA</td><td>GTCAACAATTAACACTAAGC</td><td>Transcription factors</td></tr>
<tr><td><i>WRKY</i></td><td>CATCTACAACGACAGCAACATC</td><td>GCCATCAGGTTCTGGAAGTA</td><td>Transcription factors</td></tr>
<tr><td><i>bHLH</i></td><td>GTGACTGATCGTGCTTCGT</td><td>GACGACGGAGACCACATTAAG</td><td>Transcription factors</td></tr>
<tr><td><i>NAC</i></td><td>GGAAGGGAGATAGATGGAAGGA</td><td>GCCATAGCTCTGGTACTGAAAC</td><td>Transcription factors</td></tr>
<tr><td><i>AQP</i></td><td>GGTATCCTATCCAACGCCATAG</td><td>GGAGACGAGGTTGTCGTTAC</td><td>Integral membrane protein</td></tr>
</table>

Sample cDNAs were diluted to a final concentration of 100 ng/µl. qRT-PCR was performed in Qiagen Real Time PCR Cap Strip (Qiagen) on Rotor-Gene Q (Qiagen) using QuantiFast SYBR Green PCR kit (Qiagen). Gene expression analysis was done in a reaction mixture consisting of 10µl of 2X SYBR Green (QuantiFast SYBR Green PCR kit (Qiagen), gene-specific primers 10 pmol each and 0.4 µl of cDNA in a total volume of 20 µl. PCR conditions were 95 °C for 5 min denaturation (for primer binding), then 40 cycles of amplification at 95°C for 10 sec and annealing at 60°C for 30 sec on Rotor-Gene Q machine (Qiagen). In order to ensure the absence of genomic DNA, corresponding reverse transcription negative controls were used. Non- specific amplification was also confirmed by including non-template control for each reaction. Ubiquitin and EF-1α genes served as internal reference genes and were used to normalize the gene expression data. A melt curve program of 65–99°C was included to check the specificity of PCR products by qRT-PCR after 40 cycles of amplification in which the presence of single peaks indicate the nonspecific amplifications. Three biological replicates were pooled together and each reaction had three technical replicates for analysing mean Ct value. The relative expression of the genes was estimated using the method $2^{-\delta Ct}$ (Livak & Schmittgen, 2001) and converted to $\log_2$ scale.

A $\log_2$ ratio of zero implies that the transcripts have equal expression values, whereas if the ratio is above zero, the transcripts are up-regulated, and if the ratio is below zero, the transcripts are down-regulated (Khraiwesh *et al*., 2015). A $\log_2$ ratio of one indicates a 2-fold change, and transcripts with a fold change of 1/-1 were considered significantly up- or down-regulated (Luo *et al*., 2005).

3.4.7 Fresh rhizome yield (g plant^{-1})

Genotypes that were grown under three water regimes after 90 DAP till harvest were harvested and the fresh rhizome yield from each of 4 replicates was recorded by weighing of the individual clump and the mean was expressed in g plant^{-1}.

3.4.8 Drought susceptibility index (DSI)

DSI was calculated to express the decrease in yield of a cultivar under drought condition with respect to the mean reduction in the yield of all the cultivars under consideration as per the equation given by Fischer & Maurer (1978).

$$DSI = [1 - (Yd/Yp)]/D$$

Where, Yd = yield in the non-irrigated treatment, Yp = yield in the irrigated control.

D = Drought intensity = 1-[(mean Yd of all genotypes in the non-irrigated treatment) / (mean Yp of all genotypes in the irrigated control)].

3.4.9 Statistical analysis

The data were analysed using analysis of variance (Factorial ANOVA), using R software (R Core Team, 2021). This was followed by Duncan Multiple Range Test (DMRT) at 95% confidence level, for mean separation.

3.5 Experiment III-Evaluation under field condition.

The field experiment was conducted during 2022-23 at ICAR-Indian Institute of Spices Research, Kozhikode, Kerala. The experimental material consisted of four shortlisted drought tolerant genotypes (IISR Pragati, SL 5, Suguna and Suvarna) and two susceptible genotypes (IISR Alleppey Supreme and IISR Kedaram) (Banu & Krishnamurthy, 2023).

3.5.1 Experimental plot

The experiment was laid out in a randomized block design (RBD) with four replications in August 2022 in ICAR-Indian Institute of Spices Research (IISR), Chelavur, Kozhikode, Kerala (North latitude 11° 18′ 04.7″; East longitude 75° 50′ 29.2″; 50 m MSL).

Each genotype was planted on the raised beds of size 1.5 × 1× 0.30 m (l×b×h) with plant to plant spacing of 30 cm and row spacing of 25 cm, accommodating 20 plants bed^{-1}. Recommended package of practices for the crop cultivation were followed.

3.5.1.1 Planting

Land was ploughed to fine tilth and beds of 1.5 m^2 (1.5 m x 1 m x 0.30 m) were prepared. Rhizome pieces of 20-25 g having one or two good buds were used as source of planting material. Before planting, rhizomes were treated with 0.3% mancozeb for 30 minutes. Rhizomes were planted at spacing of 30 x 25 cm and rhizome bits were placed in shallow pits and were covered with thin layer of soil. Mulching was done with green leaves (Jayashree *et al*., 2015). General view of experimental field during 2022-23 is given below in Fig. 3.2.2.

3.5.1.2 Aftercare

3.5.1.2.1 Fertilizer application

Fertilizer application was done as per the standard package of practice of IISR which was 70:50:50 kg/ha. Fertilizers were applied in two splits, at 45 and 90 days after planting. Foliar application of turmeric micronutrient mixture (dosage @ 5 g/l) developed by IISR was sprayed twice at 60 and 90 DAP.

3.5.1.2.2 Intercultivation

Weeding was done just before application of fertilizers and immediately after fertilizer application beds were mulched with green leaves. Earthing up which is essential to prevent exposure of rhizomes was done after weeding and fertilizer application.

Fig. 3.2.2 Turmeric cultivation in the field of ICAR-IISR, Kerala (from planting to harvest) (a) bed preparation, (b) planting of healthy turmeric rhizomes with sprouts, (c) mulching of beds, (d-e) field view (f) control, (g) treatment, (h) field before harvest.

3.5.1.2.3 Intercultivation

Weeding was done just before application of fertilizers and immediately after fertilizer application beds were mulched with green leaves. Earthing up which is essential to prevent exposure of rhizomes was done after weeding and fertilizer application.

3.5.1.2.4 Pest and disease management

Need based pest and disease management measures were taken up as and when required.

3.5.1.2.5 Preparation of the plot for planting

The experiment consists of control and treatment (Table 5.1) with 4 replications. The planting was done during the first week of August.

3.5.2 Treatment imposition

Irrigation was withheld from November (2022) to February (2023) in stress treatment for a period of four months and observations were recorded at different growth stages in both control as well as stress treatments. Soil moisture content of 18.5 to 19.0 % (field capacity) was maintained in control and soil moisture was 12.5 % (in November 2022) which reduced to 11.0 % (near wilting point) in February 2023. There were six rainy days (132 mm) in November and four (97mm) in December which did not alter the soil moisture content to a great extent (Banu & Krishnamurthy, 2023).

3.5.3 Observations

Of the nine tolerant lines studied for evaluation of mechanism underlying drought tolerance in experiment II, four genotypes (IISR Pragati, SL 5, Suguna and Suvarna) with the sustained yield and improved physico-chemical as well as molecular characteristics under drought stress, along with two susceptible ones (IISR Alleppey Supreme and IISR Kedaram) were selected for field evaluation. These genotypes were evaluated under field conditions for physiological parameters

(RWC and EL), biochemical parameters (MDA, proline content, drought-responsive enzymes), and gene expression analysis at three successive growth stages viz., 120 DAP (days after planting), 150 DAP, and 180 DAP both under control and water stress conditions.

3.5.4 Physiological characterization.

The effect of drought on the physiology of plant was studied by analysing the cell membrane permeability by measuring EL and RWC at three growth stages in three replicates for each genotype under control and treatment. The youngest fully matured leaf (fourth leaf from shoot apex) of plants labelled for observation purpose was used for physiological assays.

3.5.4.1 Relative water content

RWC of leaves was determined as explained in section 3.3.4.1

3.5.4.2 Electrolyte leakage

EL of leaves was determined as explained in section 3.3.4.2

3.5.4.3 Chlorophyll fluorescence

Chlorophyll fluorescence parameter (Fv/Fm ratio) was measured using a chlorophyll fluorometer (PEA, Hansatech Instrument Ltd., version 1.21, Norfolk, UK). Fourth leaf from the apex of plant aged 150 DAP were dark adapted for 30 min to stimulate the reaction of photosystem II. The minimum chlorophyll fluorescence (Fo) was measured using a measuring beam of 3500 µmol m^{-2} sec^{-1}). The maximum fluorescence (Fm) was determined after a 1 sec saturating pulse (>3500 µmol·m^{-2} sec^{-1}). Variable fluorescence (Fv) was determined by the difference between the maximum fluorescence and the minimum fluorescence (Fm − Fo). The maximum efficiency of PSII was determined as the ratio of (Fv) to (Fm) as follows:

$$Fv/Fm = (Fm - Fo)/Fm.$$

3.5.5 Biochemical characterization

3.5.5.1 Lipid peroxidation

The MDA content was estimated as mentioned in section 3.4.5.1

3.5.5.2 Proline content

Proline accumulation was determined as mentioned in section 3.4.5.3

3.5.5.3 Assay of antioxidant enzymes and AsA-GSH cycle enzymes (APX, MDHAR, DHAR and GR) activity.

3.5.5.3.1 Superoxide dismutase

SOD activity was measured as mentioned in section 3.4.5.4.1.

3.5.5.3.2 Peroxidase

POD activity was measured as mentioned in section 3.4.5.4.2.

3.5.5.3.3 AsA-GSH cycle enzymes (APX, MDHAR, DHAR and GR) activity.

Activity of AsA-GSH cycle enzymes was measured as mentioned in section 3.4.5.4.5

3.5.6 Molecular characterization

Gene expression analysis of stress responsive transcription factors was conducted using qRT-PCR. To verify expression of transcription factors involved in drought response, qRT-PCR was performed for four most expressed transcription factors of the drought response pathway identified from our previous study (Experiment II).

3.5.6.1 Total RNA Isolation.

RNA isolation from leaves and rhizomes was performed as explained in section 3.4.6.1

3.5.6.2 cDNA synthesis

cDNA synthesis was done as explained in section 3.4.6.2.

3.5.6.3 qRT-PCR based expression profiling

qRT-PCR were done as explained in section 3.4.6.3 in control and treatment at three different growth stages (120,150 and 180 DAP) using primers of *AP2, WRKY, DREB* and *NAC* TFs which were identified as TFs with higher fold change under drought condition from experiment II.

3.5.7 Fresh rhizome yield (g plant^{-1})

Yield of fresh rhizomes per plant were weighed in five randomly selected plants and the mean was expressed in g plant^{-1}.

3.5.8 Drought susceptibility index

DSI was calculated according to the formula given in the section 3.4.8.

3.5.9 Dry recovery (%)

Fresh turmeric was processed to obtain the dried rhizome by boiling with water followed by sun drying. One kilogram of fresh rhizomes per replication per genotype (containing 20% mother rhizomes, 60% primary rhizomes and 20% secondary rhizomes) were boiled in water for 1 hour uniformly and sun dried for approximately nine days (6-8 h/day) till constant weight was obtained and to reach a moisture level of 10%, approximately. Curing per cent was calculated by noting the differences between fresh and dry weight.

3.6 Evaluation of drought stress on nutrient uptake

Rhizome samples from control and treatment plants after harvest were evaluated for the variation in nutrient content such as nitrogen (N), phosphorus (P), potassium (K), micronutrients viz., Mg, Ca, Fe, Mn, Zn and Cu under drought stress to analyse the role of various nutrients under drought stress.

3.6.1 Determination of nitrogen (AOAC, 1999)

Reagents: Concentrated H_2SO_4, NaOH solution (40%), Mixed indicator solution:

The Mixed indicator (Bromocresol green and Methyl red): 20 g of pure H_3BO_3 was dissolved in about 700 mL of hot water, and was let to cool. The cooled solution was transferred to a 1 litre volumetric flask containing 200 ml of ethanol and 20 ml of mixed indicator solution which was prepared by dissolving 0.330 g of bromocresol green and 0.165 g of methyl red in 500 ml of ethanol. After mixing the contents of the flask, approximately 0.05 N NaOH was added cautiously until a colour change from pink to pale green was just detectable when 1 ml of the solution was treated with 1 ml of water. Then the solution was diluted to the volume with water, and was mixed thoroughly.

Catalytic mixture: 20 g $CuSO_4$. 5 H_2O, 100 g K_2SO_4, and 1 g selenium mixed and ground to a fine powder.

0.5 g finely powdered sample of dried turmeric rhizome was weighed into a 250 ml Kjeldahl digestion tube, added 2 or 3 g of digestion mixture (catalytic mixture) and about 10 ml of concentrated H_2SO_4 and kept overnight. The tubes were then heated to a temperature of 410°C for 1.5-2 hrs in a digestion block till a bluish green or clear substrate was obtained. After the digestion, the tubes were taken out of the digestion block and allowed to cool.

Then, it was distilled in Kjeldahl distillation unit (Kjeltec N distillation unit) and collected ammonia in 25 mL boric acid containing mixed indicator in 250 ml conical flask and was titrated with 0.1 N H_2SO_4 to get the original colour of boric acid mixed indicator solution. The titration value of sample (X) was noted.

Calculation:

1 mL 0.1 N H_2SO_4 = 0.0014g N = 1.4 mg N;

Therefore, X mL 0.1 N H_2SO_4= 1.4 x X mg N, where X is the titre value.

3.6.2 Determination of phosphorus by Bray-1 method (Olsen & Sommers, 1982)

Reagents:

HNO_3- Vanado Molybdate Reagent:

Solution (A)**:** Dissolved 25 g ammonium molybdate in 400 ml distilled water.

Solution (B)**:**1.25 g ammonium meta vanadate was dissolved in 300 ml boiling water, cooled and added 250 ml concentrated HNO_3 and cooled again to room temperature. The solution A was added to the solution B, mixed well and made up to one litre with distilled water.

Standard phosphorus solution (10 ppm stock)**:** 0.2132 g analar NH_2PO_4 was dissolved in 500 mL volumetric flask using distilled water to get 100 ppm phosphorus, from this 10 mL was taken and was again made up to 100 mL with distilled water to get 10 ppm and kept in the refrigerator.

Diacid digestion: Turmeric powder (1 g) was taken, in a volumetric flask, 10 ml nitric acid and 1 ml perchloric acid were added, heat refluxed till the digestion was complete and the solution was clear.

Diacid extract of plant sample (5 ml) was taken in a 25 ml volumetric flask, 10 ml distilled water and 5 ml Vanado molybdate reagent were added and made up to 25 ml using distilled water and, the absorbance was read at 470 nm in a spectrophotometer using blue filter.

3.6.3 Determination of potassium, magnesium and calcium (neutral ammonium acetate extraction) by methods of Helmke & Sparks (1996) and Suarez (1996)

For each element, 1 to 2 g finely powdered sample was digested using HNO_3-perchloric acid solution (volume ratio 10:1), and then 1 ml was diluted to 50 ml using distilled water. From this sample solution the elements -K, -Ca and -Mg were determined by Atomic Absorption Spectrophotometer (Model: Varian AA 240FS) against standard solutions of the elements.

Calculation:

Concentrations of above elements in plant tissue (as percentage) =X/W*100

Where X is the AAS/ Flame photometer reading in ppm and W is the weight of the sample

3.6.4 Determination of iron, manganese, zinc and copper (DTPA method, Lindsay & Norvell, 1978)

For each element, 1 to 2 g finely powdered sample was digested using HNO_3-perchloric acid solution (volume ratio 10:1), and then 1 ml was diluted to 50 ml using distilled water. From this sample solution the elements Fe, Mn, Zn, Cu in the sample were determined by Atomic Absorption Spectrophotometer (Model: Varian AA 240FS) against standard solutions of these elements.

Calculation:

Concentrations of above elements in plant tissue (in ppm) = (X * 100)/W,

Where X is reading of AAS (in ppm) and W is the weight of sample.

3.7 Qualitative traits

The harvested rhizomes of control and treatment were dried and powdered to analyse quality parameters.

3.7.1 Curcumin content

The dried rhizomes were powdered to uniform mesh size and used for extracting curcumin. It was estimated from the powdered and sieved sample by ASTA procedure (American Spice Trade Association) (ASTA, 2004). The absorption maxima of curcumin were measured in a Shimadzu UV-160 I spectrophotometer at 425 nm and percentage was computed based on the concentration of pure crystalline curcumin (98%).

3.7.2 Oleoresin

The ASTA method (1975) was employed to estimate the oleoresin content in turmeric rhizomes. A glass column was filled with cotton at the bottom and 10 g of finely powdered turmeric powder. 50 ml of acetone was added to this column and left overnight undisturbed. The next day, the oleoresin-acetone blend was collected in a pre-weighed empty beaker by opening the tap of the column. Another 30 ml of acetone was added to the column, and was incubated for 1 hour for complete extraction. This process was repeated until the brown colour washed off from the cotton. The total solvent obtained was evaporated to dryness by placing the beaker in a boiling water bath. The weight of the empty beaker was subtracted from the weight of the beaker containing oleoresin, and the oleoresin percentage was calculated using the following equation.

Oleoresin (%) = weight of the residue (g) / weight of the sample (g) × 100.

3.7.3 Essential oil

The essential oil was extracted using modified Clevenger apparatus following the procedure of, ASTA (1968). 25 g of the powdered sample was placed in the Clevenger apparatus's 1000 ml round bottom flask and was filled with water to half its volume. . The condenser tube and rap were positioned. Water and power were turned on and distillation was done for three hours. Since the essential oil is lighter and immiscible with water, it escapes from the sample during boiling and creates a distinct layer on top which was collected in an eppendorf tube containing anhydrous sodium sulphate. The oil was stored in a refrigerator at 4 ^{o}C for GC-MS analysis. Essential oil content was calculated using the formula,

Essential oil (%) = volume of oil extracted (ml) / mass of sample (g) × 100

3.7.4 Protein

Protein content in in dried turmeric powder samples of control and treatment was estimated as mentioned in section 3.4.5.6

3.7.5 Starch

Starch content in dried rhizome powder samples of control and treatment was estimated as mentioned in section 3.4.5.7

3.7.6 Total phenol content

Total phenol content of dried rhizome powder samples of control and treatment was measured as mentioned in section 3.4.5.5

3.7.7 Total flavonoid content

The total flavonoid content was estimated using an $AlCl_3$ colorimetric assay (Quettier *et al.,* 2000). The leaf tissues were ground in 1.5 ml of 80% methanol at room temperature. The extracts were centrifuged at 8000 rpm / g for 10 minutes and the supernatant was used for the assay. 1 mL of the extract (1 mg/mL) was mixed with 0.3 ml of 5% sodium nitrite and added to the reaction mixture. After approximately 5 min, 0.3 ml of 10% aluminum chloride was added. Kept for 6 minutes and after that another 2 ml of 1 M NaOH was added, followed by the immediate addition of 2.4 ml of distilled water to produce a total volume of 10 ml. The yellow colour intensity of the flavonoid-aluminum complex was measured at 510 nm. The total flavonoid content was determined as quercetin equivalent (QE) and was expressed as mg QE/g of turmeric powder.

3.7.8 Curcuminoid fractions

The amount of curcumin in the extract was calculated using Shimadzu High Performance Liquid Chromatography (HPLC) fitted with an SPD-10A UV-visible detector. The column used was RP C-18 and had a dimension of 5 mm. Acetonitrile with 0.1% orthophosporic acid served as the mobile phase, and measurements were conducted at 425 nm at a low flow rate of 1 ml/min. Authentic standards procured from Sigma Aldrich were used to identify the curcuminoid analogues in the samples.

3.7.9 Volatile constituents of essential oil by Gas Chromatography

The analysis was performed on a Shimadzu QP-2010 instrument with Rtx-5 column (thickness 0.25 µm, length 30 m, diameter 0.25 mm). The principle of electron ionization with ionization energy of 70 eV was used for detection. Helium was used as the carrier gas at a flow rate of 1 ml/min. The column temperature was programmed from 60°C for 5 minutes to 110°C at 5°C/min, then increased to 200°C at 3°C/min, and finally increased to 240°C at 5°C/min. Final analysis time was 55 minutes when scanning masses in the 60- 400 amu range with a 3-minute solvent delay. 1 µl of the sample was injected for analysis. Identification of the components of different samples was done by comparing the mass spectra of compounds presented in the National Institute of Standards and Technology library and the WILEY library, which were then compared to Adam’s book (Adams, 2001). The composition of the components was expressed as a relative percentage of the total peak area and calculated as follows:

Relative % of peak area = (area of specific peak/ total peak area) × 100

3.7.10 Statistical analysis

Statistical analyses were carried out using R studio (a simplified version of R statistical software) developed by the R Core Team (2023). The analysis of variance (ANOVA) for randomized block design was carried out for field experiment by following the model suggested by Fisher (1946). This was followed by Duncan Multiple Range Test (DMRT) at 95% confidence level, for mean separation. Principal component analysis (PCA) was done used for dimensionality reduction of large complex datasets to interpret without information loss (Hotelling, 1933).

CHAPTER 4
RESULTS AND DISCUSSION

The results obtained in the present investigation on “Morpho-physiological and molecular responses of turmeric genotypes subjected to water stress” are presented in this chapter.

4.1 Morphological and physiological characterization of genotypes under drought condition

4.1.1 Morphological characterization of turmeric genotypes

Morphological characters of fifty turmeric genotypes on 6 morphological traits are presented in Table 4.1.1. Genotypes varied significantly ($p < 0.05$) for all the morphological characters studied.

4.1.1.1 Plant height (cm)

Significant differences were observed in respect of plant height among the genotypes. Highest plant height was recorded in NTC 188 (167.7 cm) followed by IISR Prathibha (161.3 cm) and lowest in Acc 8 (79.00 cm).

4.1.1.2 Number of tillers

Highly significant differences were observed for number of tillers among the different genotypes. Highest number of tillers per clump was recorded in BSR 1 (7) and lowest was in Acc 66 (1) which was on par with Acc 849 and Kanthi (1). Genotypes Salem Erigoor and Rajendra Sonali (5.33) were found to be on par with BSR 1.

4.1.1.3 Number of leaves

Highly significant ($P>0.5$) differences were observed among the genotypes for number of leaves per plant. More number of leaves was observed in Salem Erigoor (15.33) and BSR 2 (15) recorded lowest number of leaves per plant with a mean of 9.97. BSR 2 (15) and BSR 2 White (14.4) were on par with Salem Erigoor.

4.1.1.4 Leaf length (cm)

Highly significant differences were observed among the genotypes for leaf length. Highest leaf length were observed in SL 2 (64.13 cm) followed by IISR Alleppey Supreme (63.75 cm) whereas NTC 189 (32.86 cm) recorded lowest leaf length. Megha turmeric (63.7 cm), SL 10 (62.9 cm), Salem erigoor (62.5cm), SL 7(62.4 cm) and SL 1(62.4 cm) were at par with IISR Alleppey Supreme.

4.1.1.5 Leaf width (cm)

Highly significant differences were observed among the genotypes for leaf width. Highest leaf width was observed in Salem Erigoor (16.4 cm) followed by IISR Alleppey Supreme (16 cm). Whereas PH 2 (8 cm) recorded lowest leaf width.

4.1.1.6 Leaf area (cm^2)

Significant differences were observed among the genotypes in respect of leaf area. Leaf area of different genotypes ranged from 11401.1 to 1847.3 cm^2. Highest leaf area of 11401.1 cm^2 was observed in Salem Erigoor which was followed by BSR 2 (9288.73 cm^2) and SL 1 (8377.9 cm^2) and lowest was in PH 2 (1847.3 cm^2). IISR Kedaram (8252 cm^2) and Amballur (8212 cm^2) were on par with SL 1.

4.1.1.7 Rhizome yield (g plant^{-1})

Rhizome yield per plant exhibited highly significant differences among the genotypes. Among the turmeric genotypes under evaluation, Suguna recorded highest per plant yield of 295 g followed by IISR Pragati (290 g plant^{-1}) followed by Suvarna (268.7 g plant^{-1}) and SL 5 (262 g plant^{-1}) and lowest yield of 62 g plant^{-1} was recorded in Acc 66 with a mean yield of 178.1g plant^{-1} (Table 4.1.1).

Drought severely affects plant growth and development with substantial reductions in crop productivity (Farooq *et al*., 2009). Plants exposed to drought experience rapid stomatal closure to reduce transpiration rate. This response has consequently decreased the carbon dioxide intake, which may lead to a decline in productivity (Cal *et al.,* 2019). In our study rhizome yield was very less due to drought treatment during the critical growth period (90 to 120 DAP) which coincides with the rhizome development. Additionally, the identified high yielders such as IISR Pragati and Suguna showed low to moderate leaf area (2998 cm^2 and 3941 cm^2 respectively), plant height (101.6 cm and 95.47 cm respectively) and number of tillers (2) under stress.

Table 4.1.1. Morphological parameters of turmeric genotypes 120 days after planting.

Genotype	**Plant height (cm)**	**Number of tillers**	**Leaf length (cm)**	**Leaf width (cm)**	**Number of leaves**	**Leaf area (cm^2 $plant^{-1}$)**	**Yield (g $plant^{-1}$)**
Acc 66	117.33^{qrs}	1.00^{k}	46.12^{p}	8.54^{wx}	14.33^{bc}	4145.5^{jklmn}	62^{y}
Acc 8	79.00^{z}	2.00^{j}	42.8^{stu}	9.73^{stu}	8.33^{mn}	2545.5^{qrst}	85^{x}
Acc 849	150.07^{cdefg}	1.00^{k}	46.78^{p}	14.9^{ef}	8.67^{lm}	4416.3^{jkl}	185^{lmn}
Amballur	123.23^{nop}	2.33^{ij}	58.35^{f}	14.9^{cde}	13^{de}	8212.13^{c}	95^{x}
BSR 2	154.33^{cd}	4.33^{cd}	59.59^{de}	14.3^{efg}	15^{ab}	9289.6^{b}	185^{lmn}
BSR I	149.33^{defg}	7.00^{a}	52^{lm}	8.6^{vwx}	14.33^{bc}	4694.2^{ij}	198^{jk}
BSR 2 White	115.33^{rs}	4.67^{c}	56.19^{hi}	12^{mno}	14.44^{abc}	7073.3^{def}	120^{uvw}
CO- 1	147.67^{efghi}	3.67^{ef}	38.07^{x}	12.3^{klm}	6.33^{p}	2170.2^{stu}	179.7^{mno}
CO- 2	117.80^{pqrs}	4.33^{cd}	46.32^{p}	9.9^{stu}	5.67^{pq}	1904.2^{u}	175^{no}
Duggirala Red	150.43^{cdefg}	3.00^{gh}	53.72^{j}	14.4^{efg}	7.67^{no}	4316.3^{jklm}	180^{mno}
IISR Alleppey Supreme	123.63^{no}	4.67^{c}	63.75^{ab}	16^{ab}	10^{ijk}	7401.1^{d}	140^{st}
IISR Kedaram	118.87^{opqr}	4.00^{de}	59.18^{ef}	13.7^{hij}	14^{c}	8253.14^{c}	125^{uv}
IISR Prathibha	161.33^{b}	4.00^{de}	51.5^{m}	12.3^{lmn}	12.67^{ef}	5851.5^{h}	243^{cd}
IISR Prabha	155.67^{bc}	4.00^{de}	46.24^{p}	12.9^{ijk}	10.44^{i}	4543.9^{ijk}	235^{def}
IISR Pragati	101.60^{vwx}	2.00^{j}	44.4^{q}	9.2^{uvw}	10^{ijk}	2998.3^{pqr}	290^{ab}
Sudarsana	130.67^{lm}	2.33^{ij}	42.25^{tu}	11.2^{pq}	8.33^{mn}	2885.9^{qr}	195^{jkl}
Suguna	95.47^{yz}	2.00^{j}	46^{p}	12.6^{jkl}	9.33^{kl}	3941.3^{klmn}	295^{a}
Suvarna	123.23^{nop}	1.33^{k}	57.1^{gh}	14.3^{efg}	10.67^{hi}	6334^{gh}	268.7^{b}
Kanthi	127.97^{mn}	1.00^{k}	55.3^{i}	14.4^{ef}	5.33^{q}	3083.3^{pq}	220^{gh}
Megha Turmeric	148.10^{efgh}	2.33^{ij}	63.7^{ab}	14.7^{de}	10.57^{hi}	7182.2^{def}	223.3^{fg}
NDH 1	128.00^{mn}	2.00^{j}	45.5^{p}	14.4^{ef}	7.44^{no}	3552.5^{nop}	250^{c}
NDH 98	150.33^{cdefg}	2.33^{ij}	46.3^{p}	8.5^{x}	7.67^{no}	2227.5^{stu}	185^{lmn}

NTC 188	167.67^{a}	2.00^{j}	52.3^{klm}	10.7^{qr}	11.44^{gh}	4677.03^{ij}	203.3^{ij}
NTC 189	118.10^{opqrs}	2.00^{j}	32.86^{z}	9.6^{stu}	8.67^{lm}	2019.41^{tu}	147^{rs}
PanthPeetab	112.33^{st}	3.00^{gh}	42.46^{tu}	8.7^{vwx}	8.87^{lm}	2409.7^{rstu}	180^{mno}
PH 1	96.00^{xyz}	3.67^{ef}	40.35^{v}	9.4^{tu}	7.33^{o}	2035.55^{tu}	130^{tu}
PH 2	97.67^{wxy}	4.00^{de}	36^{y}	8^{x}	8.67^{lm}	1849.01^{u}	188.3^{klm}
Rajendra Sonali	97.68^{wxy}	5.33^{b}	44^{qr}	11.19^{pq}	5.67^{pq}	2042.1^{tu}	230^{efg}
Rajendra Sonia	96.80^{wxyz}	2.33^{ij}	43.66^{qrs}	9.4^{uv}	10.33^{ij}	3111.7^{opq}	240^{cde}
Ranga	105.53^{uv}	2.00^{j}	43^{rst}	10^{rst}	8.67^{lm}	2735.8^{qrs}	224.67^{fg}
Rasmi	105.33^{uv}	3.33^{fg}	41.93^{u}	9.8^{stu}	8.67^{lm}	2614.85^{qrst}	200^{ijk}
Roma	136.67^{jk}	3.33^{fg}	56.82^{gh}	$12.6j^{klm}$	12.89^{de}	6718.1^{efg}	109^{w}
Salem Erigoor	134.67^{kl}	5.33^{b}	62.5^{c}	16.4^{a}	15.33^{a}	11401.9^{a}	225^{fg}
SC 61	123.30^{nop}	1.33^{k}	52.72^{kl}	13.8^{fgh}	7.33^{o}	3881.7^{lmn}	92.33^{x}
SL 1	150.63^{cdef}	4.00^{de}	62.36^{c}	14.6^{e}	12.67^{ef}	8377.5^{c}	160^{pq}
SL 10	151.33^{cdef}	3.67^{ef}	62.9^{bc}	12.7^{jklm}	11.44^{gh}	6645.1^{fg}	250^{c}
SL 2	141.97^{ij}	2.00^{j}	64.13^{a}	15.4^{bcd}	8.67^{lm}	6214.5^{gh}	118^{vw}
SL 3	153.33^{cde}	4.00^{de}	57.27^{g}	14.2^{efg}	11.89^{fg}	7030.3^{def}	150.33^{qrs}
SL 4	144.80^{ghi}	3.00^{gh}	58.45^{f}	12.6^{jklm}	13.67^{cd}	7327.3^{de}	91^{x}
SL-P389/ 1	$84.67^{(2)a}$	4.00^{de}	42.57^{tu}	14.5^{e}	8.67^{lm}	3903^{lmn}	200.33^{ij}
SL 6	150.63^{cdef}	4.00^{de}	56.55^{gh}	14.6^{e}	7.44^{no}	4465.03^{ijkl}	155^{qr}
SL 5	109.33^{tu}	3.00^{gh}	50.17^{n}	15.6^{bc}	7.67^{no}	4365.7^{jkl}	262^{bc}
SL 7	146.33^{fghi}	2.00^{j}	62.41^{c}	10.3^{rs}	10.78^{hi}	5051.01^{i}	130^{tu}
SL 8	102.33^{vw}	3.67^{ef}	53.64	11.5^{nop}	10^{ijk}	4498.3^{ijkl}	125^{uv}
SL 11	143.33^{hij}	2.33	53.12^{jk}	11.4^{opq}	9^{lm}	3975.9^{klmn}	130^{tu}
Sobha	131.30^{klm}	2.67^{hi}	53.95^{j}	10^{rst}	9.43^{jkl}	3717^{mno}	210^{hi}
Sugantham	122.33^{nopq}	1.33^{k}	52.47^{klm}	12.7^{jklm}	8.33^{mn}	4044.3^{klmn}	170^{op}
Suranjana	120.33^{opqr}	2.00^{j}	39.3^{w}	8.6^{vwx}	9^{lm}	2241.6^{stu}	162^{pq}
Suroma	123.67^{no}	3.00^{gh}	48.25^{o}	12.8^{jkl}	9.33^{kl}	4202^{jklm}	140^{st}
Varna	91.00^{z}	2.00^{j}	60.3^{d}	13.6^{ghi}	12.67^{ef}	7553.8^{d}	177^{mno}
General Mean	125.19	2.91	50.91	12.13	9.97	4722.6	178.1
CV (%)	2.88	13.76	1.24	3.71	5.90	8.21	4.06
CD (P=0.05)	5.83	0.65	1.020	0.73	0.95	628.43	11.71

The mean value (n=3) followed by different letters within a column are significantly different ($P< 0.05$) based on ANOVA test.

Significant variation in morphological characters was observed among genotypes. Similar variation in plant height, no of leaves, no of tillers, leaf length and width in turmeric was reported by many studies (Aarthi *et al*., 2018; Gupta, 2015; Ullah Jan *et al*., 2012; Bahadur *et al*., 2016; Anindita *et al*., 2020; Aswathi *et al*., 2023). Morphological characterization is the primary stepin the description and classification of any crop species (Smith & Smith,1989). It helps in identification and selection of desirable traits in crop plants (Malek *et al.,* 2014).

Leaves are the main organs of transpiration and assimilation in plants. Genotypes with lower leaf area were assumed to be tolerant as they lose less water during transpiration due to lower transpirational area. The variation in leaf area directly affects plant photosynthesis which consequently reduces the yield (Walter *et al*., 2009). In order to adapt to drought, plants often reduce leaf area, which results in fewer stomata which will reduce transpiration (Tezara *et al*., 1999).

Smaller leaf area minimised transpiration and resulted in lower reduction in yield under stress condition wheat (Wang & Yamauchi, 2006; Li *et al*., 2021). To minimize the water use plants limit number of tillers under stress conditions to maintain productivity in wild barley (Soda *et al*., 2010). While the reduced number of tillers reduced yield in barley and wheat under water stress (Samarah, 2005; Ahmad *et al.,* 2022).

Decline in the cell enlargement and increased senescence reduce plant height (Manivannan *et al.,* 2007). Reduction of growth parameters resulted in limited gaseous exchange and yield reduction in *Curcuma aromatica* and *Curcuma domestica* (Mohamed *et al.,* 2014). Similarly, Reduction in plant height resulted in yield reduction in tomato (Gupta *et al.,* 2023).Whereas moderate reduction in size led to reduced water loss and which also retained better gaseous exchange and productivity in populus (Yang & Miao, 2010), sunflower (Ghaffari *et al.,* 2012) and cotton (Anwar *et al.,* 2022).

Thus, reduced leaf area and plant stature restricts water use but may also result in low productivity (Sinclair & Muchow, 2001). But, under drought condition, low leaf area results in lower stomatal density which could reduce water loss and contribute to better yield (Anyia & Herzog, 2004; Liu *et al*., 2012; Srinivasan *et al*., 2017). Thus, optimal leaf area is important to maintain photosynthesis at an optimal rate. In this research, plants with low to moderate leaf area, plant height and number of tillers, such as IISR Pragati and Suguna, produced higher yield under drought stress, which indicate their comparatively better water retention ability under drought stress.

4.1.2 Physiological characterization of turmeric genotypes under the influence of drought.

4.1.2.1 Relative water content

An increase in stress intensity resulted in a significant reduction ($P < 0.05$) in relative water content compared to control. In control, RWC was highest in BSR 2 White (95.9%), followed by IISR Prathibha (93.49%), and lowest in SL 4 (80.17%) and Rasmi (80.26%), with a mean value of 86.735%. At early stress (10 DASI), it ranged from 87.25% (Amballur) to 65.28% (Acc 66) which was on par with 65.34% (Rasmi) with a mean of 76.36%, and at moderate stress (20 DASI), RWC varied from 76.49% (SL 5) followed by 76.18% (IISR Prabha) to 60.26% (Acc 66) with a mean of 67.48%. RWC further decreased under severe stress (30 DASI), ranging from 67.7% (IISR Prabha) to 53.32% (Acc 66), with a mean value of 61.58% (Table 4.1.2).

Leaf RWC is one of the most reliable indicators for defining drought tolerance of crop plants. Drought stress causes plant to lose water through transpiration and thus reduces its relative content (Lugojan & Ciulca, 2011). Genotypes that maintain higher RWC during water stress are supposed to have higher water stress tolerance than others. The higher maintainance of RWC by tolerant genotypes can be attributed to their their higher osmoregulation under drought stress (Keyvan, 2010). Similar results were reported by Bian & Jiang (2009) in *Poa pratensis* and Wang & Huang (2003) in Kentucky bluegrass. Maintenance of leaf turgor plays an important role in stomatal regulation and photosynthetic activities under water-deficit conditions (Lipiec *et al*., 2013). It indicates a positive correlation between RWC and photosynthetic rate (Moayedi *et al*., 2010) which may influence the yield.

The exposure of plants to the drought decreases relative water content which leads to the disturbances in photosynthetic electron transport and reduce productivity (Anjum *et al.,* 2011; Pour-Aboughadareh *et al*., 2017). Reduction in RWC and increased electrolyte leakage led to yield reduction with prolonged drought stress in drought sensitive varieties of maize (Guo *et al.,* 2015), cassava (Zhu *et al.,* 2020), faba bean (Mansour *et al*., 2021) and wheat (Abid *et al.,* 2018; Qayyum *et al*., 2021; Ahmed *et al.,* 2022). Whereas higher RWC and reduced the EL maintained the yield in tolerant varieties of cassava, *Amaranthus tricolor*, wheat and faba bean (Guo *et al*., 2016; Sarkar *et al.,* 2018; Dalia *et al*., 2018; Zhu *et al*., 2020; Mansour *et al*., 2021). A decline in RWC led to reduction in chlorophyll content and photosynthetic rate in oak trees and wheat (Qayyum *et al.,* 2021; Xiong *et al.,* 2022).

In our study, genotypes with higher relative water content including IISR Prabha (67.7%, IISR Prathibha (66.6%) and SL 5 (65%) produced higher yield as compared to genotypes with lower RWC. It may be attributed to the higher osmoregulation capacity and maintanaince of cell turgidity of these genotypes under drought stress.

4.1.2.2 Electrolyte leakage

A significant increase in membrane permeability of leaf tissue with increased drought stress was observed. In the untreated plants, EL was lowest in NTC 188 (9.82%) which was on par with Amballur (10%) and highest in Suranjana (15%) followed by SL 11(14.4%). At early stress it ranged from 13 % (NTC 188) to 19.1% (IISR Alleppey Supreme) with a mean of 15.8%. Under moderate stress there was an increment in EL from 14% (Amballur) to 22% (Acc 66). Under severe stress it was highest in Roma (15.7%) and lowest in Acc 66 (24.3%) with a mean value of 19.36% (Table 4.1.2).

Elevated levels of drought intensity are accompanied by an increase in EL due to increased cell permeability (Blum & Ebercon, 1981). Increase of EL% with development of drought stress has been reported in crop plants including rice and Kentucky bluegrass (Guo *et al.*, 2006; Liu *et al.*, 2008). Genotypes with lower EL% under drought condition are supposed to be drought tolerant as reported in rice (Larkunthod *et al.*, 2018) and in wheat (Selim *et al.*, 2019). Under water deficit, the cell membrane is subjected to changes such as penetrability which decreased photosynthetic rate and sustainability (Blokhina *et al.*, 2003). Therefore, genotypes with lower EL during water stress are supposed to have higher water stress tolerance than others.

Table 4.1.2 Relative water content and electrolyte leakage of turmeric genotypes 120 days after planting.

Genotype	**RWC (%)**				**EL (%)**			
	Control	**10 DAT**	**20 DAT**	**30 DAT**	**Control**	**10 DAT**	**20 DAT**	**30 DAT**
Acc 66	80.79^{d}	65.28^{p}	60.26^{l}	**55.4^{g}**	12.4^{l}	18.4^{c}	22^{a}	24.3^{a}
Acc 8	83.99^{x}	76.77^{u}	60.58^{k}	58.53^{y}	14^{d}	17.4^{g}	21.4^{c}	22.1^{g}
Acc 849	86.67^{p}	77.73^{s}	64.5^{yz}	63.75^{jk}	14.35^{b}	18^{d}	19.5^{h}	20.2^{mn}
Amballur	92.95^{c}	87.25^{a}	75.43^{c}	65.25^{f}	10^{y}	13.4^{z}	14^{z}	16.8^{b}
BSR 2	90.87^{g}	80.86^{m}	73.43^{h}	65.57^{e}	11.93^{no}	15.3^{q}	15.9^{u}	16.3^{c}
BSR I	92.95^{c}	82.55^{i}	74.16^{f}	65.01^{g}	10.9^{stu}	13.7^{wx}	15.2^{x}	18.2^{w}
BSR 2 white	95.9^{a}	82.38^{j}	72.6j	63.7^{jk}	10.8^{uv}	14.2^{u}	16.6^{s}	17.2^{a}
CO 1	91.48^{f}	74.95^{z}	68.076^{p}	63.75^{jk}	13.21^{f}	17.2^{h}	19.4^{h}	20.5^{k}
CO 2	87.16^{n}	83.08^{g}	73.18^{i}	62.86^{n}	10.7^{v}	13.6^{xy}	14.7^{z}	17.5^{yz}
Duggirala Red	83.3^{y}	70.13^{l}	68.08^{op}	58.93^{x}	10.84^{tuv}	14^{v}	17.1^{qr}	18.4^{uv}
IISR Alleppey Supreme	86.67^{p}	70.28^{k}	62.53^{g}	57.97^{a}	12.6^{k}	19.1^{a}	21.2^{d}	22.5^{e}
IISR Kedaram	83.38^{y}	68.17^{n}	61.54^{i}	56.33^{e}	11.8^{op}	16.8^{j}	20^{f}	20.9^{j}
IISR Prathibha	93.49^{b}	81.09^{l}	74.39^{e}	66.67^{b}	12.9^{hi}	16^{o}	17^{r}	18.3^{vw}
IISR Prabha	90.42^{i}	81^{l}	76.18^{b}	67.7^{a}	12.64^{k}	16^{o}	16.2^{t}	18.4^{uv}
IISR Pragati	86.31^{q}	73.52^{f}	68.37^{n}	66.4^{c}	13^{gh}	16.3^{lm}	18.7^{j}	19.7^{p}
Sudharsana	83.3^{y}	70.12^{l}	62.97^{f}	57.77^{b}	11.8^{op}	14^{v}	17.8^{m}	21.1^{i}
Suguna	80.79^{d}	73.87^{e}	67.61^{q}	63.8^{j}	11.7^{p}	14.4^{t}	17.3^{op}	19^{s}
Suvarna	86.63^{p}	76.81^{u}	65.3^{w}	63.99^{i}	12.7^{jk}	16.2^{mn}	17.5^{n}	18.8^{t}
Kanthi	85.24^{u}	75.13^{y}	64.69^{x}	59.55^{v}	10.9^{stu}	14.4^{t}	17.4^{no}	18.3^{vw}
Megha Turmeric	86.69^{p}	73.83^{e}	64.32^{ab}	59.4^{w}	12.4^{l}	16.4^{l}	18.6^{j}	19.9^{o}
NDH 1	85.85^{st}	73.36^{g}	64.38^{za}	63.4^{m}	12.84^{ij}	16.2^{mn}	18.3^{k}	19.6^{p}

NDH 98	90.88^{g}	73.15^{h}	63.33^{e}	60.14^{u}	13.19^{f}	16.3^{lm}	19.8^{g}	20.2^{mn}
NTC 188	84.37^{w}	74.23^{ab}	65.3^{w}	57.59^{c}	9.82^{z}	13^{z}	16.2^{t}	17.4^{z}
NTC 189	80.94^{c}	72.84^{i}	63.73^{d}	61.39^{r}	13.2^{f}	16.9^{ij}	17.4^{no}	20.3^{lm}
PanthPeetab	89.54^{j}	75.12^{y}	65.47^{v}	63.99^{i}	10.7^{v}	14^{v}	17.1^{qr}	19^{s}
PH 1	80.63^{e}	70.52^{j}	62.07^{h}	57^{d}	12.2^{m}	16.2^{mn}	17.3^{op}	19.4^{q}
PH 2	82.13^{a}	74.3^{a}	64.56^{xy}	58.97^{x}	13.44^{e}	16.6^{k}	17^{r}	20.9^{j}
Rajendra Sonali	85.24^{u}	73.98^{d}	63.64^{d}	59.5^{vw}	13.2^{f}	16.3^{lm}	17.5^{n}	19.1^{rs}
Rajendra Sonia	92.76^{d}	78.93^{p}	70.3^{m}	66.33^{c}	13.1^{fg}	17.6^{f}	19.8^{g}	20.1^{n}
Ranga	82.91^{z}	75.93^{w}	68.21^{o}	60.71^{t}	11.4^{q}	15.6^{p}	17^{r}	19.6^{p}
Rasmi	80.26^{f}	65.34^{p}	63.93^{c}	60.65^{t}	11.7^{p}	14.7^{s}	17.5^{n}	20.4^{kl}
Roma	91.5^{f}	85.03^{b}	66.25^{u}	63.67^{k}	10.2^{wx}	13.2^{a}	14.5^{a}	15.7^{e}
Salem Erigoor	90.35^{i}	74.17^{bc}	70.93^{k}	63.56^{l}	13^{gh}	16.6^{k}	19.7^{g}	20.8^{j}
SC 61	84.94^{v}	78.09^{r}	63.276^{e}	53.32^{h}	12^{n}	16.3^{lm}	17.2^{pq}	15.9^{d}
SL 1	90.74^{h}	83.25^{f}	70.27^{m}	65.6^{e}	11.7^{p}	14.7^{s}	15.5^{w}	17.6^{xy}
SL 10	91.62^{e}	83.8^{d}	74.75^{d}	66.15^{d}	11.4^{q}	15.2^{qr}	16.2^{t}	17.7^{x}
SL 2	85.95^{s}	78.36^{q}	72.486^{j}	64.07^{i}	10.1^{xy}	13.2^{a}	15.7^{v}	16.8^{b}
SL 3	87.03^{o}	82.75^{h}	73.71^{g}	62.86^{n}	12^{n}	15.1^{r}	18.3^{k}	19.2^{r}
SL 4	80.17^{f}	76.1^{tu}	67.11^{s}	62.66^{o}	11.2^{r}	14.7^{s}	15.3^{x}	16^{d}
SL-P 389_1	87.79^{m}	77.58^{t}	63.29^{e}	59.4^{w}	10.95st	13.8^{w}	17.4^{no}	18.5^{u}
SL 6	88.74^{k}	83.36^{e}	74.46^{e}	61.75^{q}	11^{s}	14.6^{s}	18.1^{l}	19.25^{qr}
SL 5	92.8^{d}	84.09^{c}	76.49^{a}	65.29^{f}	11.2^{r}	14.3^{tu}	15^{y}	17.1^{a}
SL 7	88.35^{l}	80.51^{n}	70.63^{l}	64.29^{h}	13.11^{fg}	17^{i}	18^{l}	19.7^{p}
SL 8	83.28^{y}	74.11^{c}	66.47^{t}	61.24^{s}	10.3^{w}	13.5^{yz}	15.3^{x}	16^{d}
SL 11	85.95^{s}	81.25^{k}	74.63^{d}	62.467^{p}	14.4^{b}	17.8^{e}	20.7^{e}	21.9^{h}
Sobha	80.17^{f}	75.48^{x}	63.2^{e}	57.03^{d}	14.29^{bc}	18^{d}	20^{f}	22.9^{c}
Sugantham	87^{o}	76.61^{v}	61^{j}	57.92^{a}	12^{n}	16.1^{no}	19^{i}	20.2^{mn}
Suranjana	86.17^{r}	69.4^{m}	67.4^{r}	61.37^{r}	15^{a}	18.8^{b}	20^{f}	23.5^{b}
Suroma	82.91^{z}	70.07^{l}	60.66^{k}	56.14^{f}	14.2^{c}	18.7^{b}	21.6^{b}	22.7^{d}
Varna	81.2^{b}	67.78^{o}	64.24^{b}	58.23^{z}	13.4^{e}	18^{d}	21.2^{d}	22.3^{f}
General Mean	86.74	76.37	67.49	61.58	12.16	15.76	17.8	19.36
CV (%)	0.08	0.08	0.12	0.10	0.75	0.60	0.57	0.59
CD (P=0.05)	0.11	0.10	0.13	0.10	0.15	0.15	0.17	0.18

The mean value (n=3) followed by different letters within a column are significantly different (P< 0.05) based on ANOVA test**.**

Membrane integrity is crucial to protect the cell from external adverse environmental conditions (Xue *et al.,* 2018). Excessive accumulation of ROS leads to loss of integrity of biological membranes, due to the oxidative damage (Farooq *et al.,* 2009; Anjum *et al.,* 2011).

A higher electrolyte leakage resulted in a significant reduction in yield in wheat (Dalia *et al*., 2018), in cassava genotypes (Zhu *et al*., 2020). Whereas a greater membrane stability index resulted in higher biological yield in tolerant wheat and faba bean genotypes (Ahmad *et al*., 2022; Mansour *et al*., 2021). Increasing water deficit stress caused a linear decline in leaf membrane stability and yield in wheat varieties **(**Qayyum *et al.,* 2021). Membrane permeability significantly increased with a reduction of RWC in maize (*Zea mays* L.) under severe stress than under moderate stress (Guo *et al.,* 2015). Moderate drought stress caused a reversible decline in membrane stability in wheat plants (Abid *et al.,* 2018).

Lower reduction in growth and photosynthetic pigments as a result of lower reduction in RWC and negligible increment in EL resulted in higher productivity in tolerant variety of maize, populus and *Amaranthus tricolor* (Mohammed khani & Heidari, 2007; Yang & Miao, 2010; Sarkar *et al.,* 2018).

In the present study, elevated levels of drought intensity were accompanied by an increase in EL because of increased cell permeability which indicates oxidation under drought stress. Genotypes with low to moderate EL % under severe stress including, SL 5 (17%), SL 10 (17.7%), Suvarna (18.8%), Suguna (19%), NDH 1 (19.6%), IISR Pragati (19.7%) and Rajendra Sonia (20%) provided higher yield as they exhibited a greater level of membrane stability under drought stress.

4.1.3 Stomatal density

Genotypes showed significant ($P>0.05$) variation in stomatal density among genotypes. Stomatal density was highest in PH 1 (8.67 mm^{-2}) and lowest in CO 2 (4.33 mm^{-2}) with an average of 6.38 mm^{-2}on adaxial side. It ranged from 26.3 mm^{-2} (Amballur) to 36.67 mm^{-2} (Sugantham) with an average of 32.76 mm^{-2} on abaxial side (Table 4.1.3). SD was more on abaxial side (fivefold) than on the adaxial side. Genotypes with fewer stomata were assumed to be tolerant as they reduce the rate of transpiration, thus maintaining higher leaf water status. Adaptation to drought in plants can be found in the form of reduction of stomatal density and size (Ouyang *et al*., 2017). Reduced number of stomata helps to prevent the rapid rate of water loss in plants (Chaves *et al.,* 2009).

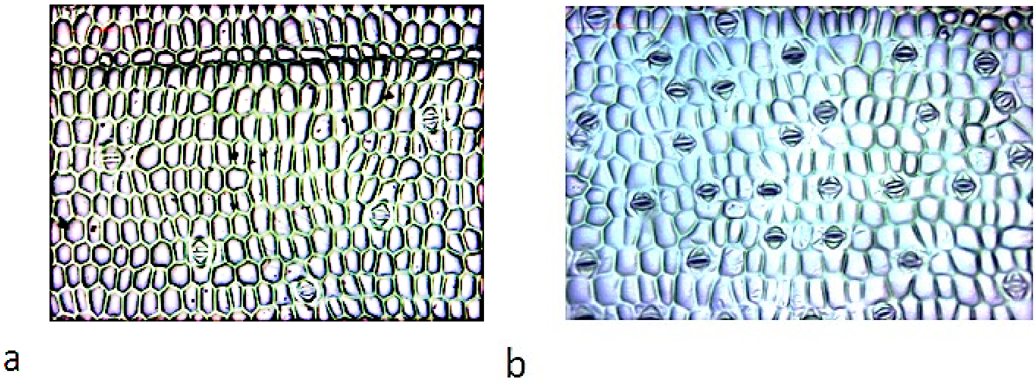


Fig. 4.1.1 Image of stomata a) abaxial side and b) adaxial side of turmeric leaf at 10X magnification with an image size of 391.634µm x 522.517 µm.

In this study there was more number of stomata on abaxial side than that on adaxial side. Windarsih *et al.*(2022) observed a higher number of stomata on abaxial surface of *Zingiberaceae* leaves. Genotypes with sustainable yield under drought are likely to have less number of stomata compared to susceptible ones. Similar findings were reported in *Arabidopsis* (Hepworth *et al*., 2015) and in rice (Caine *et al.,* 2019). Under arid environmental condition, lower stomatal density contributed to better yield (Liu *et al*., 2012; Srinivasan *et al*., 2017), which can be attributed to a reduced water loss by transpiration.

Conversely, the extreme reduction in number of stomata which contributes to drought resistance may lead to reduction in photosynthesis and hence the productivity (Cal *et al.,* 2019; Yang *et al.,* 2010). Drought response based on stomatal morphology may vary with different genotypes (Liu *et al.,* 2006).

Stomatal density and distribution affect gas exchange and CO_2 levels (Nilson & Assmann, 2007). Reduced stomatal density resulted in the reduced water loss and improved tolerance in *Arabidopsis* phy B mutants (Liu *et al.,* 2012). Presence of lower number of stomata with reduced size minimize transpiration in order to save water under stress conditions (Wang & Yamaguchi, 2006).Whereas rice cultivar 'IR64' with low stomatal density rice lines were more able to conserve water, but gave a lower yield compared to control under moderate stress while a better yield under severe stress compared to other rice varieties (Caine *et al.,* 2019). Extreme reduction in stomatal density impairs gaseous exchange thus affect photosynthesis (Tombesi *et al*., 2015; Franks *et al*., 2015).

Thus, under water-limiting drought conditions, extreme reduction of stomatal density and stomatal closure slows down water loss, with potential trade-offs being reduced carbon assimilation. On the other hand, a moderate reduction in stomatal density increased water use efficiency without much altering photosynthetic capacity (Hu *et al*., 2006; Kollist *et al*., 2014; Franks *et al*., 2015; Tombesi *et al*., 2015; Engineer *et al*., 2016; Xu *et al*., 2016; Urban *et al*., 2017). In this research, genotypes with least as well as higher number of stomata showed a lower yield compared to high yielders such as Suguna (33 mm^{-2}) and IISR Pragati (33.3 mm^{-2}) which had a moderate number of stomata which may be due to their capability of lower transpirational loss of water while maintaining an optimum gaseous exchange.

4.1.4 Leaf epicuticular wax

Significant variation was observed in epicuticular wax **(**EW) content in turmeric genotypes. Epicuticular wax content was the highest in BSR 2 (17.2 ug cm^{-2}) and the lowest in Acc66 (6.24 ug cm^{-2}) with an average of 12.11ug cm^{-2}(Table 4.1.3). In general, higher EW content provides greater tolerance to drought.The outermost covering with EW content on leaves reduces surface transpiration and improves crop water use efficiency (Ni *et al.,* 2012) and thus tolerance to water stress. The positive relationship between higher EW content and drought resistance is reported in maize (Meeks *et al.,* 2012), wheat (Bi *et al.,* 2017) and rice (Islam *et al.,* 2009). In our study the genotypes IISR Kedaram, Acc 66 and IISR Alleppey Supreme had a lower EW content and hence are likely to be susceptible to drought.

Table 4.1.3 Stomatal density and epicuticular wax content of turmeric genotypes 120 days after planting.

Genotype	Stomatal density (mm^{-2})		Wax content (µg cm^{-2})
	Abaxial	Adaxial	
Acc 66	36.33^{ab}	8.67^{a}	6.24^{q}
Acc 8	35.67^{bcd}	7.67^{bcd}	10.55^{lmnop}
Acc 849	32.67^{klm}	7^{defg}	11.18^{klmno}
Amballur	26.33^{w}	6.33^{ghi}	$12.03^{fghijklm}$
BSR 2	31^{pqr}	5.67^{ijk}	17.20^{a}
BSR I	28.67^{uv}	5^{klm}	$12.80^{defghijk}$
BSR 2 White	30.33^{rs}	5.67^{ijk}	14.22^{bcde}
CO- 1	35.33^{cde}	5^{klm}	14.20^{bcde}
CO- 2	31.67^{nop}	4.33^{m}	13.85^{cdefg}
Duggirala Red	31.33^{opq}	6^{hij}	13.60^{defghi}
IISR Alleppey Supreme	35.33^{cde}	7.67^{bcd}	10.41^{lmnop}
IISR Kedaram	34.67^{efg}	8^{abc}	8.99^{p}
IISR Prathibha	31.67^{nop}	5.33^{jkl}	$13.41^{defghij}$
IISR Prabha	27^{w}	5^{klm}	$11.92^{ghijklm}$
IISR Pragati	33.33^{ijk}	6^{hij}	$11.97^{ghijklm}$
Sudarsana	35^{def}	7.33^{cdef}	9.29^{op}
Suguna	33^{jkl}	6.67^{fgh}	11.37^{jklmn}
Suvarna	33.33^{ijk}	5.33^{jkl}	$13.08^{defghijk}$
Kanthi	34^{ghi}	7.33^{cdef}	11.40^{jklmn}
Megha Turmeric	32.33^{lmn}	7.33^{cdef}	10.60^{lmnop}
NDH 1	31.67^{nop}	6.33^{ghi}	10.62^{lmnop}
NDH 98	35.33^{cde}	6.67^{fgh}	10.35^{lmnop}
NTC 188	31.33^{opq}	5^{klm}	13.54^{defghi}
NTC 189	35^{def}	8.33^{ab}	10.06^{mnop}
PanthPeetab	33.33^{ijk}	7.33^{cdef}	$12.83^{defghijk}$
PH 1	36.33^{ab}	8.67^{a}	11.68^{ijklm}
PH 2	34^{ghi}	7^{defg}	9.51^{nop}
RajendraSonali	31^{pqr}	6.33^{ghi}	$12.24^{efghijkl}$
Rajendra Sonia	31.67^{nop}	5.67^{ijk}	14.03^{bcdef}

Ranga	36^{abc}	7.67^{bcd}	10.16^{mnop}
Rasmi	36.33^{ab}	6^{hij}	10.34^{lmnop}
Roma	28.67^{uv}	4.67^{lm}	10.15^{mnop}
Salem Erigoor	28^{v}	5.67^{ijk}	13.12^{defghijk}
SC 61	33.67^{hij}	5.33^{jkl}	11.77^{hijklm}
SL 1	29.33^{tu}	5^{klm}	16.03^{ab}
SL 10	32.67^{klm}	4.67^{lm}	13.01^{defghijk}
SL 2	33.44^{ij}	7.67^{bcd}	13.64^{defghi}
SL 3	30st	5^{klm}	14.36^{bcd}
SL 4	32^{mno}	6^{hij}	15.96^{ab}
SL-P389/ 1	32.67^{klm}	8^{abc}	16.65^{a}
SL 6	30st	5^{klm}	13.71^{cdefghi}
SL 5	30.67^{qrs}	5.67^{ijk}	13.81^{cdefgh}
SL 7	36.33^{ab}	5.33^{jkl}	15.71^{abc}
SL 8	31^{pqr}	6.33^{ghi}	13.61^{defghi}
SL 11	34.67^{efg}	8^{abc}	9.15^{op}
Sobha	34^{ghi}	5.44^{jk}	12.11^{efghijkl}
Sugantham	36.67^{a}	7.44^{cde}	8.78^{p}
Suranjana	34^{ghi}	8^{abc}	11.24^{klmno}
Suroma	34.33^{fgh}	6.89^{efg}	9.19^{op}
Varna	35^{def}	7^{defg}	10.60^{lmnop}
General Mean	32.76	6.38	12.11
CV (%)	1.43	7.08	1.56
CD (P=0.05)	0.76	0.73	0.31

The mean value (n=3) followed by different letters within a column are significantly different (P< 0.05) based on ANOVA test**.**

Drought stress triggers the increment of EW deposition on the aerial surface in plants (Jordan *et al*., 1984; Meeks *et al*., 2012; Bennett *et al*., 2012). Increased accumulation of EW under drought stress is reported in plants, such as tobacco, alfalfa, rice and wheat (Butler, 1996; Zhang *et al*., 2005; Cameron *et al*., 2006; Islam *et al*., 2009; Adamski *et al*., 2013; Wang *et al*., 2014), indicating its relation to drought tolerance.

Increased wax deposition on the leaf surface is a morphological adaptation for drought avoidance (Kim *et al.,* 2007). A higher EW content under drought stress cause to raise the leaf radiate reflection, shelter the plants from ultraviolet radiations and reduce the cuticular transpiration by reducing non stomatal water loss to improve water preservation which helps plants to endure drought stress (Ni *et al.,* 2012; Bhutta *et al.,* 2015; Bi *et al.,* 2017). Whereas reduced amount of EW content on plant surface leads to higher rate of transpiration in crop plants under arid environment (Kosma *et al*., 2009; Seo & park, 2011). Higher EW content caused a lower reduction in RWC and resulted in a higher photosynthetic rate in tolerant wheat glaucous lines (Guo *et al*., 2016) and tolerant wheat varieties which resulted in better yield compared to susceptible ones (Ian Willick *et al*., 2018; Qayyum *et al.,* 2021).

In the present study, genotypes with higher wax content maintained an optimum yield which suggests lower transpirational loss of water in these genotypes under drought which would have helped them to maintain the water content and optimum photosynthetic activity. Under drought stress, the high yielders including Suguna and IISR Pragati exhibited a higher wax content (11.37 and 11.97 cm^{-2} respectively) compared to low yielders such Acc 66 and IISR Kedaram (6.24 and 8.9 cm^{-2} respectively).

4.1.5 Scoring

Genotypes were scored based on all the morpho physiological parameters studied as well as yield and their weighted scores were determined as detailed in the materials and methods. Weighted score ranged from 39.5 to 16.5 and nine genotypes with higher weighted score (≥33.5) were identified as tolerant ones with sustainable yield. They included SL 10, SL 5, IISR Prabha, IISR Prathibha, IISR Pragati, NDH 1, Suguna, Suvarna and Rajendra Sonia. Genotypes with weighted score of ≤ 25.5 were classified as water stress susceptible genotypes. IISR Alleppey Supreme, IISR Kedaram and Acc 66 belonged to this category (Table 4.1.4).They showed higher yield reduction with respect to the highest yielder (Suguna) compared to other genotypes.

Table 4.1.4 Scoring and stress response of selected turmeric genotypes with contrasting characters

S No.	Genotype	Weightage *score (total)	Yield (g $plant^{-1}$)	Drought response
1	IISR Prabha	39.5	235	Tolerant
2	IISR Pragati	39.5	290	Tolerant
3	IISR Prathibha	39.0	243	Tolerant
4	SL5	37.5	263	Tolerant
5	Rajendra Sonia	36.5	240	Tolerant
6	SL 10	36.5	250	Tolerant
7	Suguna	36.0	295	Tolerant
8	Suvarna	33.5	268	Tolerant
9	NDH 1	33.5	250	Tolerant
10	IISR Kedaram	25.5	125	Susceptible
11	IISR Alleppey Supreme	22.0	140	Susceptible
12	Acc 66	16.5	62	Susceptible

4.2 Experiment II–Characterization of selected turmeric genotypes to study the mechanism underlying drought tolerance.

Twelve turmeric genotypes, which were selected as most tolerant and susceptible genotypes from our previous study which included tolerant ones, IISR Prabha, IISR Prathibha, IISR Pragati, SL 5, SL 10, Suguna, Suvarna, Rajendra Sonia, NDH 1 and 3 susceptible genotypes which included IISR Alleppey Supreme, IISR Kedaram and Acc 66, were used for this study.

Plants maintained at soil moisture regimes viz. 100% (control), 50% (moderate stress) and 25% (severe stress) field capacities (soil moisture content - SMC 21.5%,12.6% and10.7%), with four replicates were assessed by physiological, biochemical and molecular parameters for their drought response.

4.2.1 Effect of different water regimes on physiological parameters

Variations in physiological parameters under different soil moisture regimes viz. 100% (control), 50% (moderate stress) and 25% (severe stress) field capacities were analysed by relative water content and cell membrane permeability in leaf at 120 DAP are presented here.

4.2.1.1 Relative water content.

RWC decreased significantly (P>0.05) with stress intensity. RWC was higher in control samples. But it decreased with moderate stress and further declined with stress intensity. Both tolerant and susceptible genotypes had shown decrease in RWC under drought stress. But the decline was higher in susceptible one.

The RWC under control (100% FC) was maintained at around 82 % (IISR Kedaram) to 86.4% in IISR Prabha and 85.6 % in IISR Prathibha followed by 85.3% in suguna. RWC ranged from 55.7 % (Acc 66) to 64.8% (IISR Prathibha) under moderate stress. SL 10 (64.26%) and IISR Prabha (64.35 %) were found to be on par with ISR Prabha. Under severe stress, RWC was found to be highest in SL 10 (55.61%) followed by IISR Prathibha (55.4%) and lowest in Acc 66 (42.64%) (Table 4.2.1).

Water deficient stress induced significant decrease in RWC of leaves in bean (Zlatev *et al*., 2006) and wheat cultivars (Tambussi *et al.,* 2007). Reduction in RWC with increasing stress levels (under moderate and severe stress) was reported in rice (Srivalli *et al*., 2003) and Prunus mongolica seedlings (Guo *et al*., 2015). Balouchi (2010) found that water stress could reduce the RWC of the Australian wheat cultivars with clear differences between cultivars. Kraus *et al.* (1995) noted that there is a difference in RWC between drought-resistant and drought-susceptible wheat cultivars. Drought-tolerant plant species keep high RWC compared with drought-sensitive species in cultivars of sugarcane (Silva *et al*., 2007).

In our study, water deficient stress induced significant decrease in RWC of leaves in all genotypes with significant variation. With increasing stress levels, significant reduction in percentage of RWC in the drought group was observed i.e., from 23.9% (SL 10) to 36% (Acc 66) at moderate stress and from 34.2% (SL 10) to 48.25 % (Acc 66) under severe stress. Also, the tolerant genotypes showed lower reduction in RWC compared to susceptible ones.

4.2.1.2 Electrolyte leakage

Cell membrane permeability was very high under severe stress than in control and moderate stress. There was significant variation in EL% under drought stress compared to control. In control EL was maximum for Suguna (13.3 %) and IISR Pragati (12.6 %) and minimum for Acc 66 (10.6%). Under moderate stress condition (50% FC) it ranged from 13.6 % for IISR Prabha and 14.1% for IISR Prathibha to 18.9% (IISR Alleppey supreme). Acc 66 (18.6%) was on par with IISR Alleppey supreme followed by IISR Kedaram (17.88 %). Under severe stress, EL increase was least for IISR Prathibha (15.7%) and IISR Prabha (16%) followed by SL 10 (17.2%) and there was higher increase of electrolyte leakage in IISR Alleppey Supreme (22.5%) (Table 4.2.1). Genotypes showed an increase in EL from 17% (IISR Prabha) to 75.5% (Acc 66) and 36.5 % (IISR Prathibha) to 98% (Acc 66) under moderate and severe stress respectively compared to control.

Compared with well watered conditions, moderate stress significantly increased the relative conductivity ($P < 0.05$) of the leaves and severe stress further significantly increased ($P < 0.001$) leaf relative conductivity. These findings were in agreement with drought response in maize (Li-Ping *et al.,* 2006; Guo *et al.,* 2015), cassava (Zhu *et al.,* 2020), faba bean (Mansour *et al*., 2021) and wheat (Ahmed *et al.,* 2022).

According to George *et al.* (2017), tolerant black pepper genotype maintained significantly higher RWC and lower cell membrane leakage after stress induction than the sensitive genotypes. Cell membrane stability has been widely used to characterize plant tolerance to stress (Tartoura & Youssef, 2011). It is possible to use these parameters as rapid indicators for screening for drought tolerance (Vasquez-Robinet *et al.,* 2008; Lugojan & Ciulca, 2011).

In this study, EL varied significantly among genotypes. Both the tolerant and susceptible showed increased EL with stress. But susceptible genotypes viz. Acc 66, IISR Alleppey Supreme and IISR Kedaram showed more than 80% membrane permeability at severe stress compared to control.

Table 4.2.1 RWC and EL content of turmeric genotypes 120 days after planting.

Genotype	RWC (%)			EL (%)		
	100%FC	50%FC	25%FC	100%FC	50%FC	25%FC
IISR Prabha	86.4^{a}	64.34^{h}	54.68^{nop}	11.6^{pq}	13.6^{kl}	16^{i}
IISR Prathibha	85.6^{ab}	64.77^{h}	55.44^{mn}	11.5^{pq}	14.1^{jk}	15.7^{i}
IISR Pragati	85.1^{bcd}	62.305^{ij}	53.95^{pqr}	12.6^{mn}	15.7^{i}	19.26^{cd}
SL 5	84.2^{ef}	61.54^{jk}	53.78^{qr}	12^{nop}	14.5^{j}	17.76^{f}
SL 10	84.5^{cde}	64.26^{h}	55.61^{m}	11.6^{pq}	14.8^{j}	17.2^{fg}
Suguna	85.3^{bc}	61.79^{jk}	53.45^{rs}	13.31^{m}	16.83^{gh}	19.3^{cd}
Suvarna	83.8^{ef}	60.44^{lm}	52.04^{tu}	12.5^{no}	16^{i}	18.9^{cd}
Rajendra Sonia	84.4^{de}	63.07^{i}	54.53^{opq}	12.4^{no}	16.1^{hi}	19^{cd}
NDH 1	84^{ef}	61.03^{kl}	51.7^{1u}	11.8^{opq}	16^{i}	19.4^{c}
IISR Allepey Supreme	83.5^{f}	55.2^{mno}	45.4^{v}	12.4^{no}	18.9^{cd}	22.5^{a}
IISR Kedaram	82^{g}	53.6^{r}	43.52^{w}	11.1^{qr}	17.83^{ef}	21.3^{b}
Acc66	82.4^{g}	52.7^{st}	42.64^{x}	10.6^{r}	18.6^{de}	21^{b}
General mean	65.4			15.65		
CV (%)	0.76			2.91		
CD (P=0.05)	82.4			0.74		

The mean value (n=3) followed by different letters within a column are significantly different (P< 0.05) based on ANOVA test**.**

RWC which reflects the balance between water supply to the leaf tissue and transpiration rate, exhibited highly positive association with rhizome yield. Water stress enhance reactive oxygen species synthesis which leads to degradation of proteins, membrane lipids and photosynthetic pigments as well as cell membrane damages resulting in leakage of electrolytes which lead to cell death (Clua, *et al*., 2009; Alireza *et al.,* 2017; Sachdev *et al*., 2021). Plants which can maintain higher RWC can reduce ROS production thus resulting in less membrane damage (Kar *et al*., 2011).

Genotype that can retain higher relative water content and lower membrane leakage performed better under drought condition. Maintenance of higher RWC resulted in better yield in tolerant genotypes of maize, populus, wheat and rice (Mohammed khani & Heidari, 2007, Yang and Miao, 2010; Anjum *et al*., 2011, Ian Willick *et al*., 2018, Nahar *et al.*, 2018).

RWC may be affected by the interaction of severity, duration of the drought event genotype and altitude. Progressive stress led to successive reduction in water content which resulted in a linear decline in chlorophyll contents, leaf membrane stability, relative water content that led to yield reduction in wheat (Mohammed khani & Heidari, 2007; Qayyum *et al.,* 2021).

RWC reduction led to higher Membrane lipid peroxidation in maize (Guo *et al.,* 2015). Maintaining higher relative water content at booting stage and anthesis stages of tolerant wheat cultivars resulted in lower reduction of yield (Aziz *et al*., 2013). Similarly, Moderate drought stress caused a reversible decline in leaf water relations and membrane stability in wheat plants whereas severely stressed plants showed incomplete recovery after re-watering (Abid *et al.,* 2018). Physiological parameters such as relative water content, membrane stability index, net photosynthetic rate exhibited highly positive association with seed yield and yield contributing traits in wheat and faba bean genotypes (Bayoumi *et al.,* 2008; Mansour *et al*., 2021). Reduced RWC and water potential of leaves under water deficit, gradually reduce stomatal conductance, which leads to reduction in CO_2 assimilation and thus productivity (David *et al*., 2007). In this study tolerant genotypes which generated a higher rhizome yield under water deficit had higher RWC and showed a lower rise in EL compared to susceptible genotypes which helped them limit the overproduction of ROS due to better water balance.

4.2.2 Biochemical response of turmeric genotypes under different water regimes

The effect of drought on the biochemical characters at different growth stages were investigated through analysis of parameters such as lipid peroxidation, H_2O_2 content, proline content, the activities of antioxidant enzymes (POD, SOD and

CAT) and AsA-GSH cycle enzymes in both leaves and rhizome, photosynthetic pigment content, sugar content, total phenol content in leaf and protein as well as starch content in rhizome.

4.2.2.1 Accumulation of stress factors (MDA, Proline and H_2O_2)

The consequence of water stress in the turmeric plants was analyzed by the level of stress indicators like MDA, proline and H_2O_2 concentration in the turmeric leaves and rhizomes under conditions without drought and at moderate and severe stress. The study showed that the level of MDA content depicting lipid peroxidation, the concentration of the amino acid proline, and the reactive oxygen species- H_2O_2 level increased drastically with the stress intensity and later decreased from 120 DAP to 180 DAP in both leaf and rhizome. Still it was higher at early stages (120 DAP) with higher amount of accumulation in leaf. MDA content and H_2O_2 were less increased in tolerant ones but increased highly in susceptible ones. Proline accumulation was higher in tolerant ones under drought stress. The lower proline accumulation was seen in susceptible ones. Accumulation of stress factors followed similar pattern in both leaf and rhizome.

4.2.2.1.1 Lipid peroxidation

There was significant variation ($P>0.05$) among genotypes in MDA content. Among the genotypes, sensitive genotype registered higher TBARS at all the stages in both rhizome and leaves in irrigated and drought-stressed plants. At 120 DAP, MDA ranged from 1.6 µmol/g FW (IISR Prathibha) followed by 1.86 µmol/g FW (IISR Prabha) to 3.69 µmol/gFW (IISR Kedaram) in control. It ranged from, 2.38 µmol/g FW (IISR Prathibha) to 8.4 µmol/g FW (IISR Alleppey Supreme) under moderate stress. Whereas at severe stress MDA content ranged from 3.21 µmol/g FW (IISR Prabha) followed by 3.28 µmol/g FW (IISR Prathibha) to 9.1 µmol/gFW (IISR Kedaram) under severe stress in leaf.

At 150 DAP MDA ranged from 1.29 µmol/g FW (IISR Prabha) followed by 1.48 µmol/g FW (SL 10) to 2.85 µmol/g FW IISR Alleppey Supreme in control; from 1.82 µmol/g FW (IISR Prabha) and 2.13 µmol/g FW (SL 10) to 6.55 µmol/g

FW (IISR Alleppey Supreme) and 2.1 µmol/g FW (IISR Prabha) followed by 2.52 µmol/g FW (IISR Prathibha) and 2.6 µmol/g FW (SL 10) to 6.68 µmol/g FW (IISR Alleppey Supreme) moderate and severe stress respectively.

It ranged from 1.13 µmol/g FW (SL 10) and 1.3 µmol/g FW (IISR Prabha) to 2.8 µmol/g FW (IISR Alleppey Supreme); 1.51 µmol/g FW (SL 10) to 5.86 µmol/g FW IISR Alleppey Supreme and 1.68 µmol/g FW in SL 10 followed by 2.09 µmol/g FW (IISR Prathibha) to 6.2 µmol/gFW IISR Kedaram at 180 DAP in control, moderate and severe stress respectively in leaf (Fig. 4.2.1 a).

In rhizome, MDA content was lowest in SL 10 (1.2 µmol/g FW) followed by IISR Prabha (1.29 µmol/g FW) and higher in IISR Kedaram (2.47 µmol/g FW); lowest in SL 10 (1.74 µmol/g FW) and highest in IISR Kedaram (5.4 µmol/g FW); lowest in SL 10 (1.92 µmol/g FW) and highest in IISR Kedaram (6.3 µmol/g FW) at 120 DAP in control, moderate and severe stress respectively.

At 150 DAP, MDA was lowest in IISR Prabha (1.1 µmol/g FW) and SL 10 (1.12 µmol/g FW) and highest in IISR Alleppey Supreme (1.98 µmol/g FW); lowest in SL 10(1.45 µmol/g FW) followed by IISR Prathibha (1.53 µmol/g FW) and highest in IISR Alleppey Supreme (4.07 µmol/g FW) and lowest in IISR Prathibha (1.7 µmol/g FW) followed by IISR Prabha (1.72 µmol/g FW) and highest in IISR Alleppey Supreme (4.46 µmol/g FW) in control, moderate and severe stress respectively.

Similarly at 180 DAP in control lowest MDA content was observed in IISR Prathibha (0.82 µmol/g FW) followed by IISR Prabha (0.95 µmol/g FW) and highest in IISR Alleppey Supreme (1.94 µmol/g FW); at moderate stress MDA content was lowest in IISR Prathibha (1.08 µmol/g FW) and IISR Prabha (1.24 µmol/g FW) and highest in IISR Alleppey Supreme (3.71 µmol/g FW) and at severe stress, least MDA content was found to be 14 µmol/g FW in IISR Prathibha and highest in IISR Alleppey Supreme (18 µmol/g FW) (Fig. 4.2.1 b)

In leaf the MDA content at 120 DAP increased from 48 % (IISR Prathibha) to 128% (IISR Alleppey Supreme), and 72.6 % (IISR Prabha) to 167.7 % (IISR Kedaram) in moderate and severe stress respectively.

At 150 DAP it increased from 41% (IISR Prabha) to 130% (IISR Alleppey Supreme), at moderate stress; 55.6% (IISR Prathibha) to 142 % (IISR Kedaram) at severe stress and at 180 DAP 34 % (SL 10) to 109 % (IISR Alleppey Supreme) and 42% (IISR Prathibha) to 138.5% (IISR Kedaram) under moderate and severe stress respectively.

In rhizome, the MDA content at 120 DAP increased from 42 % (IISR Prabha) to 119 % (IISR Kedaram), and from 60 % (SL 10) to 155 % (IISR Kedaram) in moderate and severe stress respectively. At 150 DAP it increased from 30 % (SL 10) to 105 % (IISR Alleppey Supreme & from 47 % (IISR Prabha) to 132 % (IISR Kedaram) in moderate and severe stress respectively.

At 180 DAP, an increase in MDA content was observed from 27 % (SL 10) to 91% (IISR Alleppey Supreme) and from 31 % (IISR Prabha) to 116% (IISR Alleppey Supreme) under moderate and severe stress respectively.

The oxidative damage of membrane lipids by increased ROS under stress conditions leads to the formation of malondialdehyde which is indicated by TBARS content (Verma & Mishra, 2005; Tartoura & Youssef, 2011). In the present study, TBARS content in turmeric varieties increased under water deficit stress, which was consistent with other reports such as in *Maclura pomifera* (Khaleghi *et al*., 2019), wheat (Vuković *et al.*, 2022) under drought stress. Compared with moderate stress (50% FC), severe water deficit (25% FC) increased TBARS content in *C. longa*, which can be attributed to the fact that, as increased ROS can oxidize more polyunsaturated fatty acids in membrane lipids to form TBARS (Verma & Mishra, 2005; Tartoura & Youssef, 2011). Not only ROS, TBARS also damages biomacromolecules in cells, such as proteins and DNA (Marnett, 1999; Verma & Mishra, 2005; Ozkur *et al.,* 2009; Qiu *et al*., 2008; Tartoura & Youssef, 2011), which may aggravate the damage under stress. In the present study, there was a decrease in protein content under severe stress which may be due to degradation of proteins. Degradation of proteins during various abiotic stresses has been reported by many researchers (Habib *et al.,* 2020; Melandri *et al*., 2020; Liu *et al*., 2021; Mansour *et al*., 2023). TBARS induced damage of macromolecules is reported in many crops including maize, Macula pomifera and wheat (Anjum *et al.,* 2017; Khalegi *et al*., 2019; Ekim *et al.,* 2024).

Overproduction of highly toxic reactive oxygen free radicals will lead to harmful cytological effects, such as lipid peroxidation, protein denaturation, degradation of photosynthetic pigments and other macromolecules (Rout & Shaw, 2001; Farooq *et al.,* 2009). Drought induced peroxidation of lipids in many crops including wheat, maize and rice (Habib *et al.,* 2020; Liu *et al*., 2020; Melandri *et al*., 2020; Mansour *et al*., 2023). Increased accumulation of MDA has been correlated with decline in RWC and photosynthetic pigment content when plants exposed to prolonged drought (Deeba *et al*., 2012). Membrane lipid peroxidation and membrane permeability significantly increased with a reduction of RWC in maize (*Zea mays* L.) (Guo *et al.,* 2015) and in olive cultivars (Petridis *et al*., 2012) under stress which increased with stress intensity. MDA is regarded as a marker for the evaluation of lipid peroxidation and/or damage to plasmalemma and organelle membranes, and its amount increases with environmental stress (Blokhina *et al*., 2003; Ashraf *et al*., 2009; Noreen *et al.,* 2010; Hasanuzzaman *et al.,* 2018).

In this research, increased oxidative stress under drought resulted in higher lipid peroxidation in all genotypes. Sensitive genotypes IISR Alleppey Supreme, IISR Kedaram and Acc 66 recorded higher MDA content compared to tolerant genotypes that had higher RWC. Increased lipid peroxidation would have resulted in increased cell membrane damage and degradation of macromolecules (including important proteins and enzymes) as well as photosynthetic pigments which ultimately resulted in significant decline in yield under drought particularly in susceptible genotypes. Therefore in turmeric also MDA content analysis can be an important drought susceptibility marker.

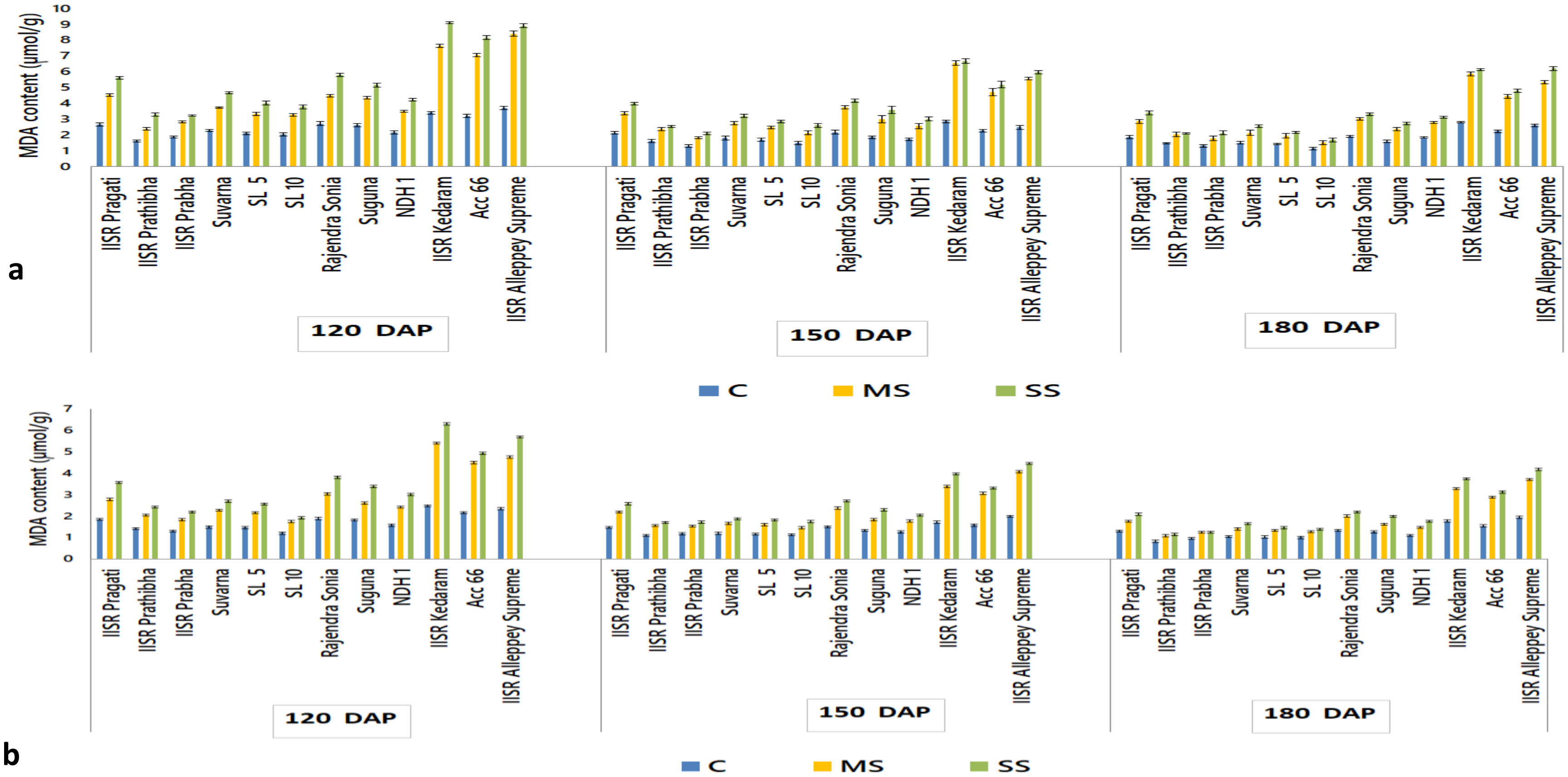


Fig. 4.2.1 Changes in the MDA production under control, moderate and severe drought treatment in (a) leaf and (b) rhizome at different developmental stages. Bars represent the mean ± SE. (C = Plants maintained under 100% field capacity; MS = Plants maintained under 50% field capacity; SS = Plants maintained under 25% field capacity)

4.2.2.1.2 Proline

Proline is an important amino acid stress indicator which synthesized excessively during biotic and abiotic stress conditions. Water deficit stress treatment significantly increased the proline content in *C. longa* genotypes compared with control (100% FC). There was a significant increase in proline content under severe stress (25 % FC) than in moderate stress (50% FC), indicating that the magnitude of increase in stress intensity with exposure to drought will increase proline accumulation. Similar results were observed at different growth stages (120 DAP, 150 DAP and 180 DAP) in both leaf (Fig. 4.2.2 a) and rhizome (Fig. 4.2.2 b). Still it was higher at early stages (120 DAP) with higher amount of proline accumulation in leaf. i.e., It peaked at 120 DAP (stage of maximum growth and rhizome development), thereafter it declined.

In leaf at 120 DAP, proline accumulation was maximum in SL 5 (2.7 µmol/g FW) and minimum in Acc 66 (1.3 µmol/g FW) in control, maximum of 5.81 µmol/g FW in IISR Prabha and minimum in Acc66 (2.26 µmol/g FW) at moderate stress condition. While under severe stress, it was highest in SL 5 (6.73 µmol/g FW) and lowest in Acc 66 (2.34 µmol/g FW). At 150 DAP, proline accumulation was highest in IISR Prabha (2.48 µmol/g FW) and lowest in IISR Kedaram (0.84 µmol/g FW) in control, it was highest in IISR Prabha (5.2 µmol/g FW) and lowest in IISR Kedaram (1.45 µmol/g FW) and highest in SL 5 (5.82 µmol/g FW) and lowest in IISR Kedaram (1.51 µmol/g FW) in moderate and severe stress respectively.

Also at 180 DAP, it ranged from 0.7 µmol/g FW (IISR Kedaram) to 1.84 µmol/g FW (SL 5); from 1.12 µmol/g FW (IISR Kedaram) to 3.67 µmol/g FW (SL 5) and from 1.22 µmol/g FW (IISR Kedaram) to 4.05 µmol/gFW (SL 5) at in control, moderate and severe stress respectively (Fig. 4.2.2 a).

In rhizome, Proline content was maximum in SL 5 (2.27 µmol/g FW) and minimum in Acc 66 (0.76 µmol/g FW) in control; maximum in SL 5 (4.67 µmol/g FW) followed by IISR Prabha (4.4 µmol/g FW) and minimum for IISR Kedaram (1.24 µmol/g FW) at moderate stress and maximum in IISR Prabha (5.32 µmol/g FW) and a minimum in IISR Kedaram (1.32 µmol/g FW) for severe stress at 120

DAP. At 150 DAP, proline content ranged from 0.68 µmol/g FW (Acc 66) to a maximum of 1.88 µmol/g FW (IISR Prabha) SL 5 (1.80 µmol/g FW) was on par with IISR Prabha; from 0.95 µmol/g FW (Acc 66) to 3.8 µmol/g FW (SL 5) and from 1.04 µmol/g FW (Acc 66) to 4.14 µmol/gFW (SL 5) under control, moderate and severe stress respectively.

At 180 DAP, proline accumulation was highest in SL 10 (1.54 µmol/g FW) and lowest in IISR Kedaram (0.45 µmol/g FW) under control; maximum in SL 10 (3 µmol/g FW) and minimum in Acc 66 (0.63 µmol/g FW) under moderate stress & a maximum of 3.2 µmol/gFW in IISR Prabha and minimum of 0.7 µmol/g FW in IISR Kedaram under severe stress (Fig. 4.2.2 b).

Proline is an important compatible solute, ROS scavenger and a vigorous antioxidative defense molecule which accumulates in plants exposed to dehydration stress (Sankar *et al.*, 2007; Ashraf *et al.,* 2007; Jalil *et al.*, 2007; Perez-Perez *et al.*, 2009). Proline accumulation maintained membrane integrity by higher osmoregulation under dehydration stress in many crops as observed in *Oryza sativa* (Su & Wu, 2004) and artichoke cultivars (Zhang *et al.,* 2011). The lower accumulation of proline resulted in increased electrolyte leakage because of oxidative damage in sensitive chickpea germplasm (Shah *et al.,* 2020). Higher proline regulated the osmotic-potential, protected antioxidant enzyme activity and chlorophyll content under drought stress, ultimately alleviating the impact of drought stress on yield as in wheat (Li *et al.,* 2024), sugar beat (AlKahtani *et al.,* 2021), cauliflower and potato (Hayat *et al.,* 2012; EL-Bauome *et al.,* 2022; El-Yazied *et al.,* 2022). Many studies demonstrated induction of proline accumulation under drought which contributes to drought tolerance in crop plants (Yamada *et al.,* 2005; Vendruscolo *et al*., 2007; Zandalinas *et al.,* 2016; El-Beltagi *et al.,* 2020).

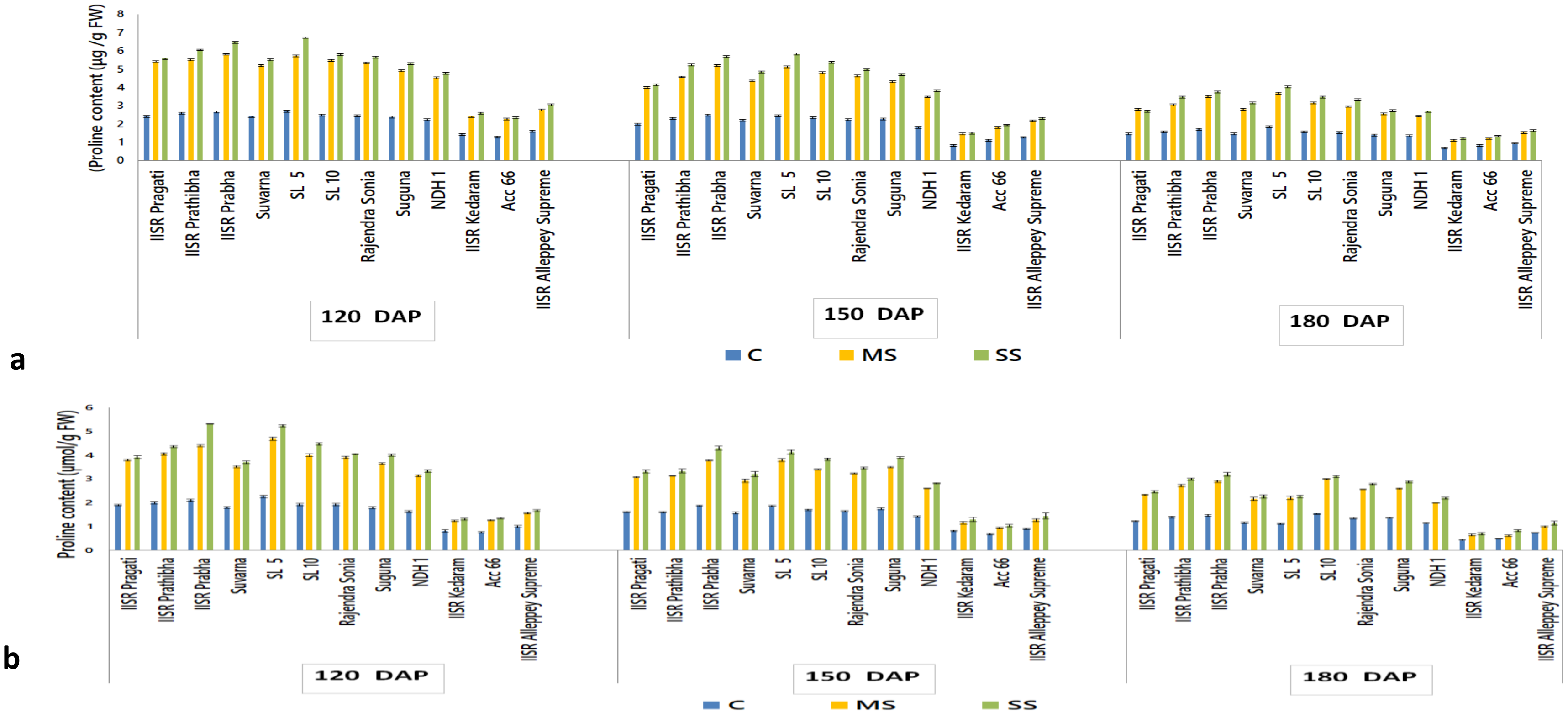


Fig. 4.2.2 Changes in the proline accumulation under control, moderate and severe drought treatment at different developmental stages in (a) leaf and (b) rhizome. Bars represent the mean ± SE. (C = Plants maintained under 100% field capacity; MS = Plants maintained under 50% field capacity; SS = Plants maintained under 25% field capacity)

In leaf, compared to control, the proline content at 120 DAP increased from 69% (IISR Kedaram) to 124% (IISR Pragati) at moderate stress and 80 % (Acc 66) to 149 % (SL 5) under severe stress. At 150 DAP the increase over control was from 65% (Acc 66) to 109.7% (IISR Prabha), and from 76 % (Acc 66) to 136.6% (SL 5) in moderate and severe stress respectively. At 180 DAP it increased from 46.3 % (Acc 66) to 105.8% (IISR Prabha) and from 63% (Acc 66) to 121% (IISR Prathibha) over control under moderate and severe stress respectively.

In case of rhizome, the proline content at 120 DAP increased from 51.2 % (IISR Kedaram) to 109.5% (IISR Prabha) and from 60.9 % (IISR Kedaram) to 153.3% (IISR Prabha) over control in moderate and severe stress respectively. At 150 DAP, it increased from 39 % (Acc 66) to 104 % (SL 5) and 52 % (Acc 66) to 129% (IISR Prabha) in moderate and severe stress respectively and at 180 DAP, the increase was from 26 % (Acc 66) to 97.2% (IISR Prabha) and from 55.4 % (IISR Alleppey Supreme) to 118% (IISR Prabha) over control under moderate and severe stress respectively.

Intracellular proline levels have been found to increased during exposure to various stresses (Handa *et al*., 1983; Verbruggen & Hermans, 2008; Hayat *et al*., 2012; Islam *et al*., 2021) including salt (Mansour *et al*., 2017), drought (Barnett & Naylor, 1966; Choudhary *et al*., 2005; Adamipour *et al*., 2020), pathogens (Fabro *et al*., 2004), and oxidative stress (Shafi *et al*., 2019). *Thymus daenensis* demonstrated an increase in proline levels in its leaves when subjected to water stress which further increased under severe stress (25% field capacity), the higher concentration of which reduced chlorophyll and carotenoid degradation in tolerant genotypes (Bahreininejad *et al.,* 2013).

Proline is well-known as an osmotic regulator that can reduce osmotic damage (Slama *et al*., 2008; Surender Reddy *et al*., 2015). Proline accumulation is associated with water deficiency induced by soil drought (Zandalinas *et al*., 2016), the higher are their proline contents, the greater the osmotic adjustment capabilities they display, resulting in stronger drought tolerance (Zu *et al*., 2017). In a study by Hare *et al*. (2003), it was shown that proline applied exogenously at a low concentration enhanced the growth in *Arabidopsis*, whereas growth was inhibited at higher concentrations which points into retarded growth at severe stress where proline was much higher compared to control. Overproduction of proline in plant cells contributes to maintaining cellular homeostasis, water uptake, osmotic adjustment and redox balance to restore the cell structures and mitigate oxidative damage (Gosh *et al* ., 2022).

Tolerant variety showed greater proline content, as in case of upland rice cultivars (Lum *et al*., 2014). A positive association between proline accumulation and drought resistance was reported in barley cultivars (Bandurska *et al*., 2017), in *Zea mays* (Ali *et al*., 2008). Proline, when applied exogenously to roots of *Arabidopsis*, resulted in a reduced level of ROS, indicating the ROS scavenging potential of proline (Cuin & Shabala, 2007).

Proline accumulation was found to be higher in young leaves compared to older leaves and rhizome in the current study. This finding was in agreement with proline accumulation in the leaves of pea at various stages of development (Zdunek-Zastocka *et al*., 2021) in which highest concentration of proline was found in the younger leaves, among leaves, while older leaves contained less proline. In case of abiotic stress such as salt stress, proline accumulated mostly in young leaves and then in pseudo-stem, old leaves and root. Higher accumulation of proline in young leaves is accompanied with higher growth rate of young leaves (Mostajeran & Gholaminejad, 2014). Wang *et al.* (2022) found that proline content dramatically increased in the leaves compared to the roots and stems under drought stress in water melon seedlings and is mainly synthesized in leaves and transported to roots in watermelon under drought stress.

In the present study, proline accumulation varied markedly in all genotypes under low water regimes. Among the genotypes, tolerant genotype exhibited higher proline accumulation at all the stages in both rhizome and leaves. Higher proline content in tolerant genotypes suggested greater osmoregulation and ROS scavenging in them which would have resulted in a lower reduction in yield.

4.2.2.1.3 Hydrogen peroxide

The water stress enhanced H_2O_2 content compared to control at all stages and it decreased with stress intensity. Tolerant genotypes showed less increase compared to the susceptible ones. Leaves had the higher content compared to rhizomes. The highest H_2O_2 content was observed at 120 DAP with successive decline at 150 DAP and 180 DAP.

In leaf H_2O_2 content ranged from 3.40 µmol/g FW (Rajendra Sonia) followed by 3.66 µmol/g FW (SL 5) to 4.36 µmol/g FW (Acc 66); from 8.34 µmol/g FW (Rajendra Sonia) and 8.96 µmol/g FW (SL 5) to 15.3 µmol/g FW (Acc 66) and from 9.18 µmol/g FW (SL 5) followed by 9.52 µmol/g FW (Suguna) to 16.6 µmol/g FW (IISR Kedaram) at 120 DAP in control, moderate and severe stress respectively.

At 150 DAP, H_2O_2 content ranged from 3.10 µmol/g FW (SL 5). Rajendra Sonia (3.21 µmol/g FW) was on par with SL 5 to 4.18 µmol/gFW in Acc66 in control, from 6.91 µmol/g FW (SL 5) followed by 7.52 µmol/g FW (Suguna) to 13.48 µmol/g FW (Acc 66) in moderate stress and from 7.23 µmol/g FW (SL 5) followed by 7.7 µmol/g FW in Rajendra Sonia to 13.8 µmol/g FW in Acc66 under severe stress at 150 DAP.

H_2O_2 content ranged from 2.64 µmol/g FW (SL 5) and 2.87 µmol/g FW (Rajendra Sonia) to 3.92 µmol/g FW in Acc 66 ; from 4.80 µmol/g FW (SL 5) and 5.05 µmol/gFW (Rajendra Sonia) to 11.9 µmol/g FW in Acc66 and from 5.9 µmol/g FW for SL 5 followed by 5.98 µmol/g FW in Rajendra Sonia to 11.75 µmol/g FW in Acc 66 at 180 DAP in control, moderate and severe stress respectively (Fig. 4.2.3 a).

In case of rhizome at 120 DAP, H_2O_2 content was lowest in SL 5 (2.63 µmol/g FW) followed by Rajendra Sonia (2.7 µmol/g FW) and highest in Acc 66 (3.84µmol/g FW) followed by 3.72 µmol/g FW for IISR Kedaram in control and lowest in SL 5 (6.12 µmol/g FW) followed by Suguna (7.4 µmol/g FW) and highest in Acc 66 (12.8 µmol/g FW) under moderate stress and it was lowest in SL 5 (6.53

µmol/g FW) followed by Rajendra Sonia (6.92 µmol/g FW) and highest in IISR Kedaram (13.9 µmol/g FW) severe stress.

At 150 DAP, H_2O_2 content ranged from SL 10 (2.43 µmol/g FW) followed by SL 5 (2.54 µmol/g FW) to highest in Acc 66 (3.64 µmol/g FW); from SL 10 (5.32 µmol/g FW) followed by Rajendra Sonia (5.7 µmol/g FW) to in Acc 66 (11.17 µmol/gFW) and from SL 10 (5.58 µmol/g FW) to IISR Alleppey Supreme (11.54 µmol/gFW) in control, moderate and severe stress respectively.

It was minimum in SL 5 (2.1 µmol/g FW) followed by Rajendra Sonia (2.2 µmol/g FW) and maximum in Acc 66 (3.24 µmol/g FW), minimum in SL 5 (3.5 µmol/g FW) followed by Suguna (4.38 µmol/g FW) and maximum in Acc 66 (9.08 µmol/g FW) and the least in SL 5 (4.8 µmol/g FW) and the most in Acc 66 (9.44 µmol/g FW) in control, moderate and severe stress respectively at 180 DAP (Fig. 4.2.3 b).

The H_2O_2 content increased to 143.5% (Suguna) to 251% (Acc 66), 152 % (Suguna) to 292.4 % (IISR Kedaram) at 120 DAP; From 119% (Suguna) to 222% (Acc 66); 133.2% (SL 5) to 230 % (Acc 66) at 150 DAP under moderate and severe stress respectively. Whereas at 180 DAP, the H_2O_2 content increased to about 76% (Rajendra Sonia) to 195 % (IISR Alleppey Supreme) and 106% (Suguna) to 203% (IISR Kedaram) at moderate and severe stress respectively in leaf.

Whereas in rhizome it increased from 133% (SL5) to 233% (Acc 66); from146.5% (Suguna) to 274% (IISR Kedaram), at 120 DAP; and from 119 %(SL 10) to 207 % (Acc 66); 128.6% (Suguna) to 238.4% (IISR Alleppey Supreme), at 150 DAP and it increased to about 66.7% (SL 10) to 180.4% (Acc 66) and 104% (SL 10) to 198% (IISR Alleppey Supreme) at 180 DAP under moderate and severe stress respectively.

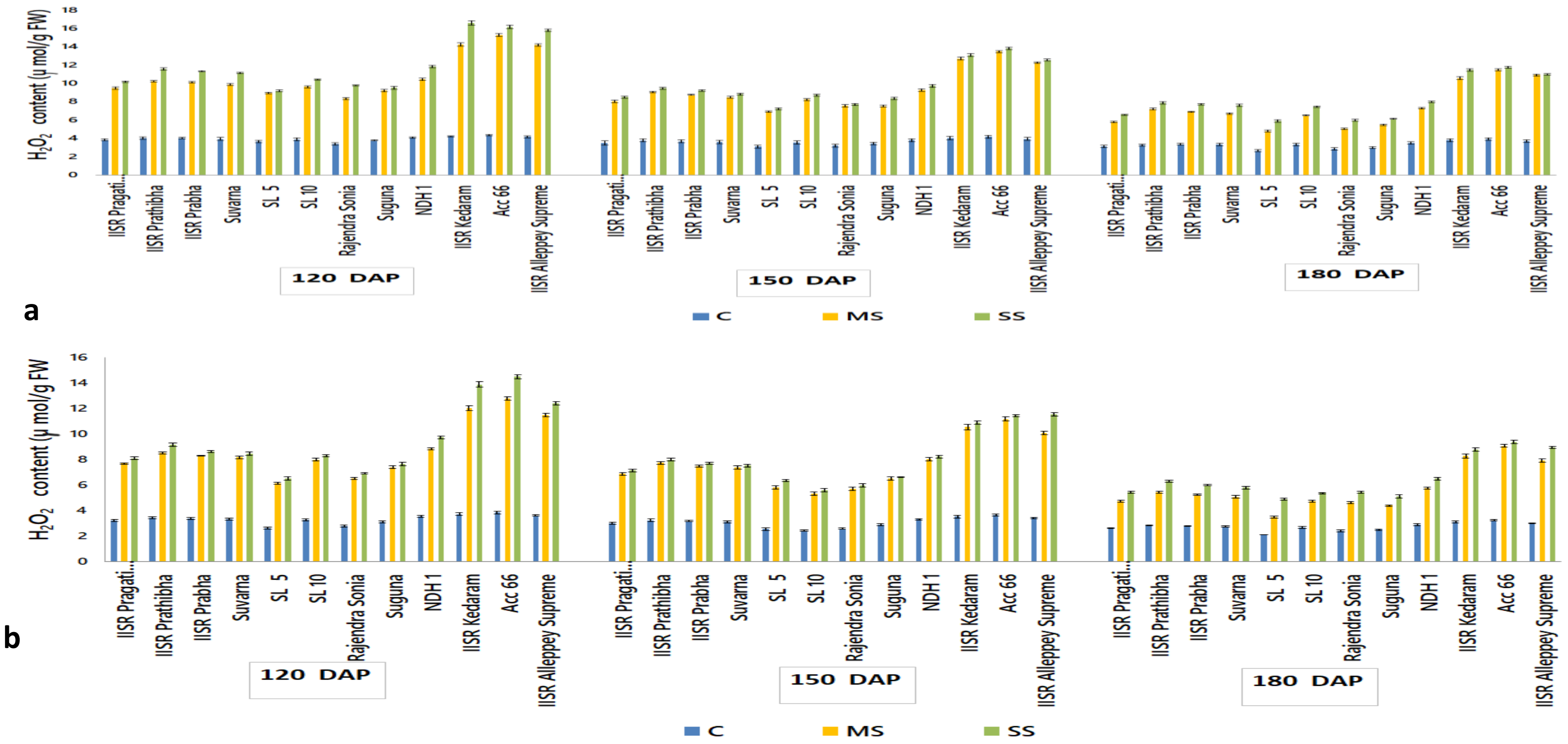


Fig. 4.2.3 Changes in the H_2O_2 content under control, moderate and severe drought treatment at different developmental stages in (a) leaf and (b) rhizome. Bars represent the mean ± SE. (C = Plants maintained under 100% field capacity; MS = Plants maintained under 50% field capacity; SS = Plants maintained under 25% field capacity)

H_2O_2 at low concentration increases the antioxidant activity (Hasanuzzaman et al., 2017). To respond to the damaging effects of ROS, the plant possesses antioxidant enzymes and thereby ameliorates the stress effects. But at higher concentration, the ROS does harm to the cell membrane and makes cells lose their function due to oxidative stress (Lee & park, 2012; Hussain *et al*., 2019).

Production of ROS, especially H_2O_2, which is relatively long-lived ROS, is a major cause of oxidative stress effects under stress (Apel & Hirt, 2004). Drought stress results in stomatal closure, which limits CO_2 influx and fixation and reduces $NADP^+$ regeneration by the Calvin cycle, impaired activities of enzymes and down regulate photosynthesis. These stress conditions increase the rate of reactive oxygen species, by enhanced leakage of electrons to molecular oxygen (Satoh & Murata, 1998, Flexas & Medrano, 2002; Lawlor & Cornic, 2002; Anjum *et al*., 2017; Cruz De Carvalho *et al*., 2023).

The accumulation of foliar ABA under stress induced by root dehydration also stimulates the production of $H_2O_{2,}$ which is associated with oxygen reduction in chloroplast, which is the sites of H_2O_2 production in leaf (Allen *et al.,* 2000; Foyer & Hanke, 2022). Chloroplast, membranes of the cell, organelles, apoplast, nucleolus and peroxisome are the locations of ROS production (Gill & Tuteja, 2010).

H_2O_2 induced oxidative damage including membrane leakage, lipid peroxidation and chlorophyll degradation was reported in shallot, rice, olive, wheat, maize and oats (Silalert & Pattanagul, 2021; Jira-Anunkul & Pattanagul, 2021; Gholami *et al*., 2022; Habib *et al.,* 2020; Melandri *et al*., 2020; Mansour *et al.,* 2023; Basu *et al*., 2010; Khayatnezhad & Gholamin, 2021; El-Beltagi *et al*., 2022) and other macromolecules which finally resulted in lower growth and productivity.

The results of present study clearly indicated that the water stress significantly increased the H_2O_2 content in all genotypes. Higher H_2O_2 content particularly in susceptible ones may have resulted in increased membrane leakage, lipid peroxidation and chlorophyll degradation which led to severe decline in yield under water stress.

4.2.2.2 Effect of drought on antioxidant enzymes

4.2.2.2.1 Superoxide dismutase

SOD enzyme activity varied significantly among the genotypes under all water regimes. In leaf it was maximum in IISR Prathibha (6.2 U/g) followed by SL 10 (6.1 U/g) and minimum in Acc 66 (3.13 U/g) in control. Under moderate stress it was highest in SL 10 (11.54 U/g FW) followed by IISR Prathibha (11.06 U/g) and minimum in Acc 66 (4.5 U/g FW). The activity ranged from 4.2 U/g FW (Acc 66) to 10.9 U/g FW (SL 10) followed by 10.27 U/g FW (IISR Prathibha) under severe stress at 180 DAP. Similar pattern of SOD enzyme activity was seen in other stages also with successive decline in 150 DAP and 120 DAP (Fig 4.2.4 a).

At 120 DAP SOD activity ranged from 2.45(Acc 66) to 4.67 U/g FW (SL 10) followed by 4.42 U/g FW (IISR Prathibha); and 3.3 (Acc 66) to 8.26 U/g FW (SL 10) followed by 7.6 U/g FW for SL 5 and from 3.08 U/g FW (Acc 66) to 7.31 U/g FW (SL 10) followed by 6.7 U/g FW (IISR Prathibha) in control, moderate and severe stress respectively.

At 150 DAP it ranged from 2.65 U/g FW in IISR Kedaram to 5.32 U/g FW for IISR Prabha, from 3.74 U/g FW (Acc 66) to 9.75 U/g FW (IISR Prabha), followed by 9.24 U/g FW for SL 10 and from 3.37 (IISR Kedaram) to 8.59 U/g FW IISR Prabha in control, moderate and severe stress respectively.

In rhizome SOD ranged from 1.92 (Acc 66) to 3.59 U/g FW (SL 10), from 2.61(Acc 66) to 6.15 U/g FW (SL 10) and from 2.3(Acc 66) to 5.52 (SL 10) at 120 DAP in control, moderate and severe stress respectively.

At 150 DAP it ranged from 2.2 U/g FW (IISR Alleppey Supreme) to 4.04 U/g FW (SL 5) closely followed by 3.9 U/g FW in SL 10; from 3.12 (IISR Alleppey Supreme) to 7.24 U/g FW (SL 5) and from 2.75 (IISR Alleppey Supreme) to 6.42 U/g FW (SL 5) in control, moderate and severe stress respectively.

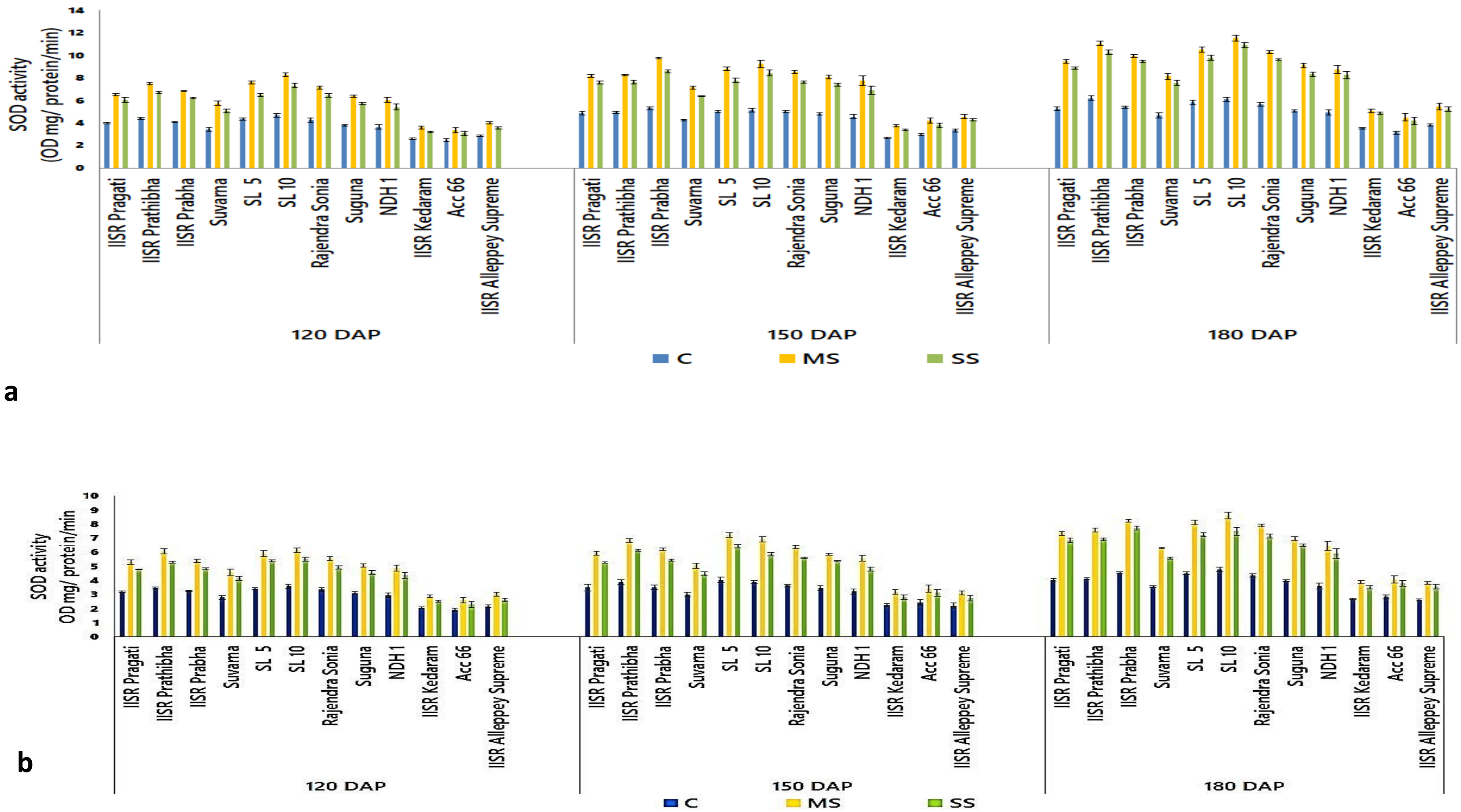


Fig. 4.2.4 Changes in the SOD activity of the turmeric under control, moderate and severe drought treatment at different developmental stages in a) leaf and b) rhizome. Bars represent the mean± SE. (C = control plants irrigated at 100% field capacity; MS = moderately stressed plants irrigated at 50% field capacity; SS = severly stressed plants irrigated at 25% field capacity)

Whereas it ranged from 2.62 (IISR Alleppey Supreme) to 4.8 U/g FW (SL 10) followed by 4.56 U/g FW (IISR Prabha); from 3.83 U/g FW (IISR Alleppey Supreme) to 8.6 U/g FW (SL 10) and from 3.52 (IISR Kedaram) to 7.75 U/g FW (IISR Prabha) at 180 DAP in control, moderate and severe stress respectively (Fig 4.2.4 b).

The increase in enzyme activity ranged from 42% (Acc 66) to 89.2% (SL 10) and from 34% (Acc 66) to 76% (IISR Prabha) under moderate and severe stress respectively in leaf. In the case of the rhizome, the increase was from 44% (Acc 66) to 83% (IISR Prathibha) and from 31% (IISR Kedaram) to 70% (IISR Pragati) under moderate and severe stress conditions.

4.2.2.2.2 Peroxidase

POD enzyme activity varied significantly among the genotypes at all the three growth stages under all water regimes. At 120 DAP, IISR Prabha (1.42 μmol/g FW) followed by IISR Prathibha (1.38 μmol/g FW) recorded maximum values and the minimum value was recorded in Acc 66 (0.68 μmol/g FW) under control condition. Under moderate stress, maximum activity was observed in IISR Prathibha (2.16 μmol/g FW) which was on par with IISR Prathibha (2.13 μmol/g FW). IISR Alleppey Supreme (0.81 μmol/g FW) showed the minimum value. SL 10 (1.9 μmol/g FW) and IISR Prabha (1.88 μmol/g FW) recorded maximum values under severe stress with a minimum value for IISR Alleppey Supreme (0.78 μmol/g FW).

Similarly at 150 DAP, SL 10 (1.65 μmol/g FW) followed by Suguna (1.60 μmol/g FW) recorded maximum values and the minimum value was observed in IISR Alleppey Supreme (0.74 μmol/gFW) under control condition. Under moderate stress, maximum activity was observed in IISR Prabha (2.73 μmol/g FW). SL 10 (2.71 μmol/g FW) and Suguna (2.59 μmol/gFW) were on par with IISR Prabha. Acc 66 (1.02 μmol/g FW) showed the minimum value. Under severe stress, SL 10 (2.27 μmol/g FW) and Suguna (2.25 μmol/g FW) recorded maximum values while minimum value was observed in IISR Alleppey Supreme (0.88 μmol/g FW).

At the stage of maximum activity, i.e. at 180 DAP, IISR Prathibha (1.90 µmol/g FW which was on par with SL 5 (1.86 µmol/g FW) followed by IISR Prabha (1.84 µmol/g FW) showed maximum activity and the minimum activity was in IISR Alleppey Supreme (0.86 µmol/gFW) under control condition. Under moderate stress, maximum activity was observed in SL 5 (3.32 µmol/g FW) which was on par with IISR Prathibha (3.26 µmol/g FW). IISR Kedaram (1.25 µmol/g FW) showed the minimum value. Under severe stress, IISR Prabha (2.68 µmol/g FW) which was on par with IISR Prathibha (2.64 µmol/g FW) followed by SL 10 (2.55 µmol/g FW) recorded maximum values and minimum activity was observed in IISR Alleppey Supreme (1.11 µmol/g FW) (Fig. 4.2.5 a).

In rhizome also, POD enzyme activity varied significantly among the genotypes at all the three growth stages under all water regimes. At 120 DAP, IISR Prabha (1.20 µmol/g FW) followed by IISR Prathibha (1.16 µmol/g FW) exhibited maximum activity and the minimum activity was recorded in Acc 66 (0.54 µmol/g FW) under control condition. Under moderate stress, maximum activity was observed in IISR Prabha (1.81 µmol/g FW) followed by SL 10 (1.75 µmol/g FW) and Acc 66 (0.67 µmol/g FW) showed the minimum value. While under severe stress, IISR Prabha (1.56 µmol/g FW) and SL 10 (1.48 µmol/g FW) recorded maximum values and the minimum activity in Acc 66 (0.61 µmol/g FW).

Similarly, at 150 DAP, IISR Prabha (1.39 µmol/g FW) recorded maximum value followed by Rajendra Sonia (1.34 µmol/g FW) and the minimum value was observed in IISR Alleppey Supreme (0.63 µmol/g FW) under control condition. Under moderate stress, maximum activity was observed in IISR Prabha (2.19 µmol/g FW) followed by Rajendra Sonia (2.10 µmol/g FW) and IISR Alleppey Supreme (0.87 µmol/g FW) showed the minimum value. Under severe stress, IISR Prabha (1.84 µmol/g FW) and Rajendra Sonia (1.79 µmol/g FW) showed the highest activity, while Acc 66 had the lowest value of 0.74µmol/g FW.

At 180 DAP, maximum activity was observed in SL 5 (1.58 µmol/g FW) followed by IISR Prathibha (1.54 µmol/g FW) and the minimum value recorded in Acc 66 (0.72 µmol/g FW) under control condition. Under moderate stress, maximum activity was observed in IISR Prathibha (2.63 µmol/g FW) followed by SL 5 (2.53 µmol/g FW) and Acc 66 (0.95 µmol/g FW) showed the minimum value. Whereas under severe stress, SL 5 (2.21 µmol/g FW) and SL 10 (2.09 µmol/g FW) recorded maximum values, while the minimum activity was noted in Acc 66 (0.85 µmol/g FW) (Fig. 4.2.5 b).

In leaf the POD activity at 120 DAP increased from 16 % (IISR Alleppey Supreme) to 56% (IISR Prathibha) and from 12 % (IISR Alleppey Supreme) to 36 % (IISR Pragati) over control in moderate and severe stress respectively. It increased from 27% (Acc 66) to 72% (IISR Prabha); from 19% (IISR Alleppey Supreme) to 41 % (Suguna), at 150 DAP. The activity increased from, 39% (IISR Kedaram) to 78.5 % (SL 5) and from 22% (Acc 66) to 46% (IISR Prabha), at 180 DAP under moderate and severe stress respectively.

While in rhizome, the POD activity at 120 DAP increased from 24% (Acc 66) to 53% (SL 10); 13% (Acc 66) to 31% (SL 10) in moderate and severe stress respectively. At 150 DAP it increased from 36% (Acc 66) to 57 % (Rajendra Sonia) and from 15% (Acc 66) to 33 % (SL 10) in moderate and severe stress respectively. At 180 DAP it increased from 32 % (Acc 66) to 74% (IISR Prathibha) and from 18 (Acc 66) to 43% (Suguna) under moderate and severe stress respectively.

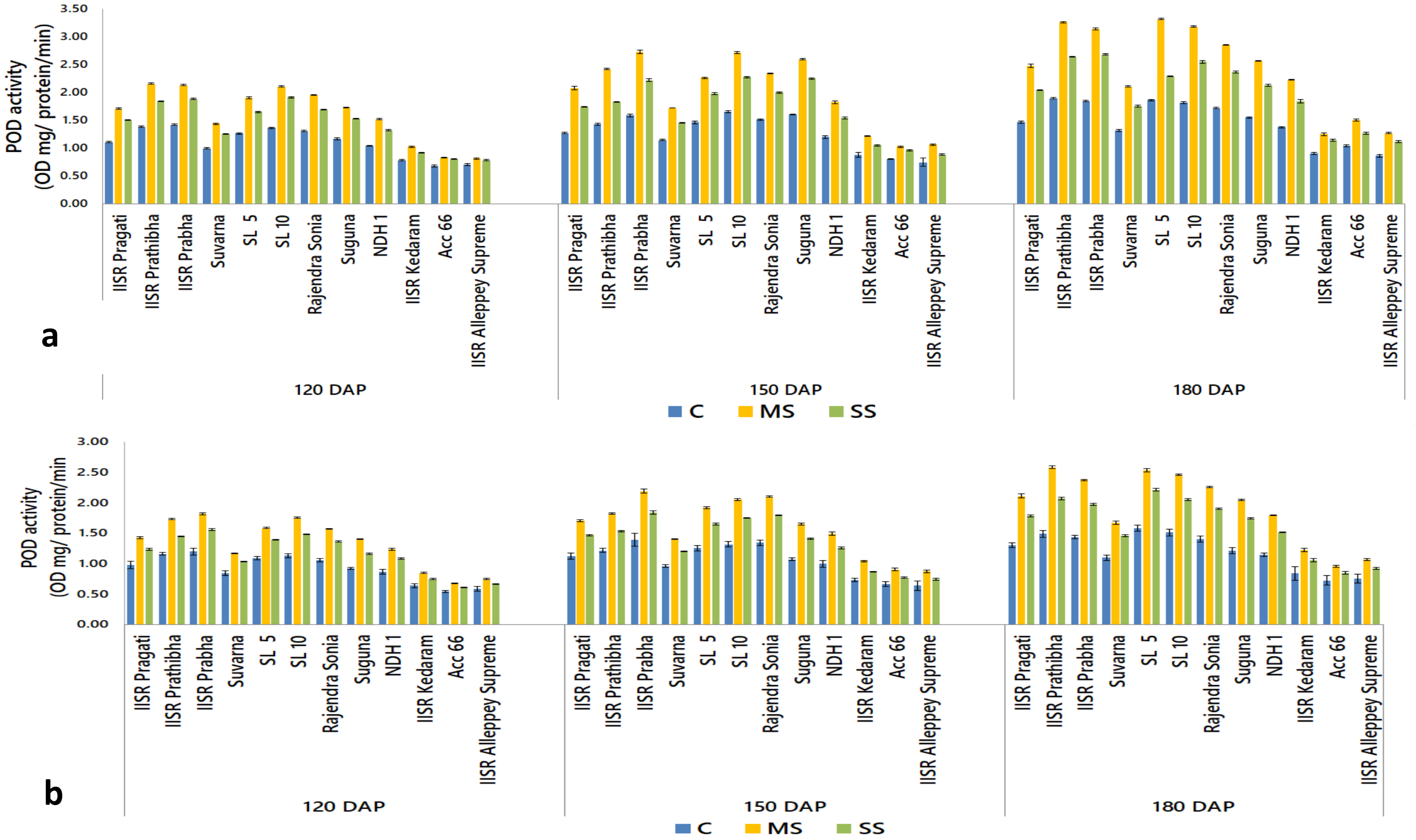


Fig. 4.2.5 Changes in the POD activity of the turmeric under control, moderate and severe drought treatment at different developmental stages in a) leaf and b) rhizome. Bars represent the mean± SE. (C = Plants maintained under 100% field capacity; MS = Plants maintained under 50% field capacity; SS = Plants maintained under 25% field capacity).

4.2.2.2.3 Catalase

CAT enzyme activity varied significantly among the genotypes. In case of leaf, at 120 DAP, IISR Prabha (1.67 µmol/g FW) followed by SL 5 (1.51 µmol/g FW) recorded maximum activity and the lowest activity was noticed in IISR Alleppey Supreme (0.96 µmol/g FW) under control condition. Under moderate stress, maximum activity was exhibited by SL 10 (2.42 µmol/g FW) followed by SL 5 (2.20 µmol/g FW) and IISR Alleppey Supreme (1.16 µmol/g FW) showed the minimum value. SL 10 (2.21 µmol/g FW) and SL 5 (1.94 µmol/g FW) recorded maximum values at severe stress and a minimum activity in IISR Alleppey Supreme (1.11 µmol/g FW).

Similarly at 150 DAP, IISR Pragati (2.01 µmol/g FW) followed by SL 5 (1.84 µmol/g FW) recorded maximum values and the minimum value was observed in Acc 66 (1.08 µmol/g FW) under control condition. Under moderate stress, maximum activity was recorded in IISR Pragati (3.05 µmol/g FW) followed by IISR Prabha (2.76 µmol/g FW) and IISR Alleppey Supreme (1.35 µmol/g FW) showed the minimum value. While under severe stress, IISR Pragati (2.72 µmol/g FW) and IISR Prabha (2.63 µmol/g FW) recorded maximum values and the minimum activity in Acc 66 (1.30 µmol/g FW).

At 180 DAP, IISR Prabha (2.27 µmol/g FW) followed by SL 10 (2.15µmol/g FW) recorded maximum values and the minimum activity in IISR Kedaram (1.23 µmol/g FW) under control condition. Under moderate stress, maximum activity was exhibited by IISR Prabha (3.59 µmol/g FW) followed by SL 5 (3.34 µmol/g FW) whereas IISR Kedaram (1.58 µmol/g FW) showed the minimum value. Maximum activity under severe stress was observed in SL 5 (3.13 µmol/g FW) and IISR Prabha (3.1 µmol/g FW) while minimum activity was shown by IISR Kedaram (1.48 µmol/g FW) (Fig. 4.2.6 a).

In rhizome, activity of CAT enzyme varied significantly among the genotypes. At 120 DAP, IISR Prathibha (1.20 µmol/g FW) followed by SL 5 (1.10 µmol/g FW) displayed maximum values and the minimum value was observed in

Acc 66 (0.70 µmol/g FW) under control condition. Under moderate stress, maximum activity was recorded in IISR Prabha (1.52 µmol/g FW) followed by IISR Prathibha (1.65 µmol/g FW) and Acc 66 (0.84 µmol/g FW) showed the minimum value. IISR Prathibha (1.57 µmol/g FW) and SL 5 (1.40 µmol/g FW) recorded highest activity at severe stress and the minimum activity was observed in Acc 66 (0.79 µmol/g FW).

Similarly at 150 DAP, SL 5 (1.47µmol/g FW) followed by IISR Prathibha (1.35 µmol/g FW) recorded maximum values and the minimum value was observed in Acc 66 (0.75 µmol/g FW) under control condition. Under moderate stress, maximum activity was recorded in SL 5 (1.47 µmol/gFW) followed by IISR Prathibha (1.35 µmol/g FW) and Acc 66 (0.75 µmol/g FW) showed the minimum value. Under severe stress, SL 5 (1.95 µmol/g FW) and IISR Prathibha (1.74 µmol/g FW) recorded maximum values while in Acc 66 (0.87 µmol/g FW) recorded the minimum value.

At 180 DAP, under control condition IISR Prathibha (1.68 µmol/g FW) followed by IISR Prabha (1.57 µmol/g FW) showed the maximum activity and the minimum was noticed in IISR Kedaram (0.92 µmol/g FW). Under moderate stress, maximum activity was observed in IISR Prathibha (2.55 µmol/g FW) followed by IISR Pragati (2.30 µmol/g FW) whereas, Acc 66 (1.19 µmol/g FW) showed the minimum value. Likewise, IISR Prathibha (2.18 µmol/g FW) and SL 5 (2.06 µmol/g FW) showed highest activity at severe stress and the minimum activity was observed in IISR Kedaram (1.11 µmol/g FW) (Fig. 4.2.6 b).

In leaves, at 120 DAP, CAT activity increased from 18% (IISR Alleppey Supreme) to 45.8% (SL 5) for moderate stress, and from 15.3% (IISR Alleppey Supreme) to 35.7% (IISR Prabha) under severe stress. At 150 DAP, CAT activity rose from 20.5% (IISR Alleppey Supreme) to 52.4% (IISR Prabha) under moderate stress, and from 17.9% (IISR Alleppey Supreme) to 45.4% (IISR Prabha) under severe stress. By 180 DAP, CAT activity increased from 27.9% (IISR Alleppey Supreme) to 59.6% (IISR Pragati) under moderate stress, and from 20.2% (IISR Kedaram) to 47.3% (SL 5) under severe stress.

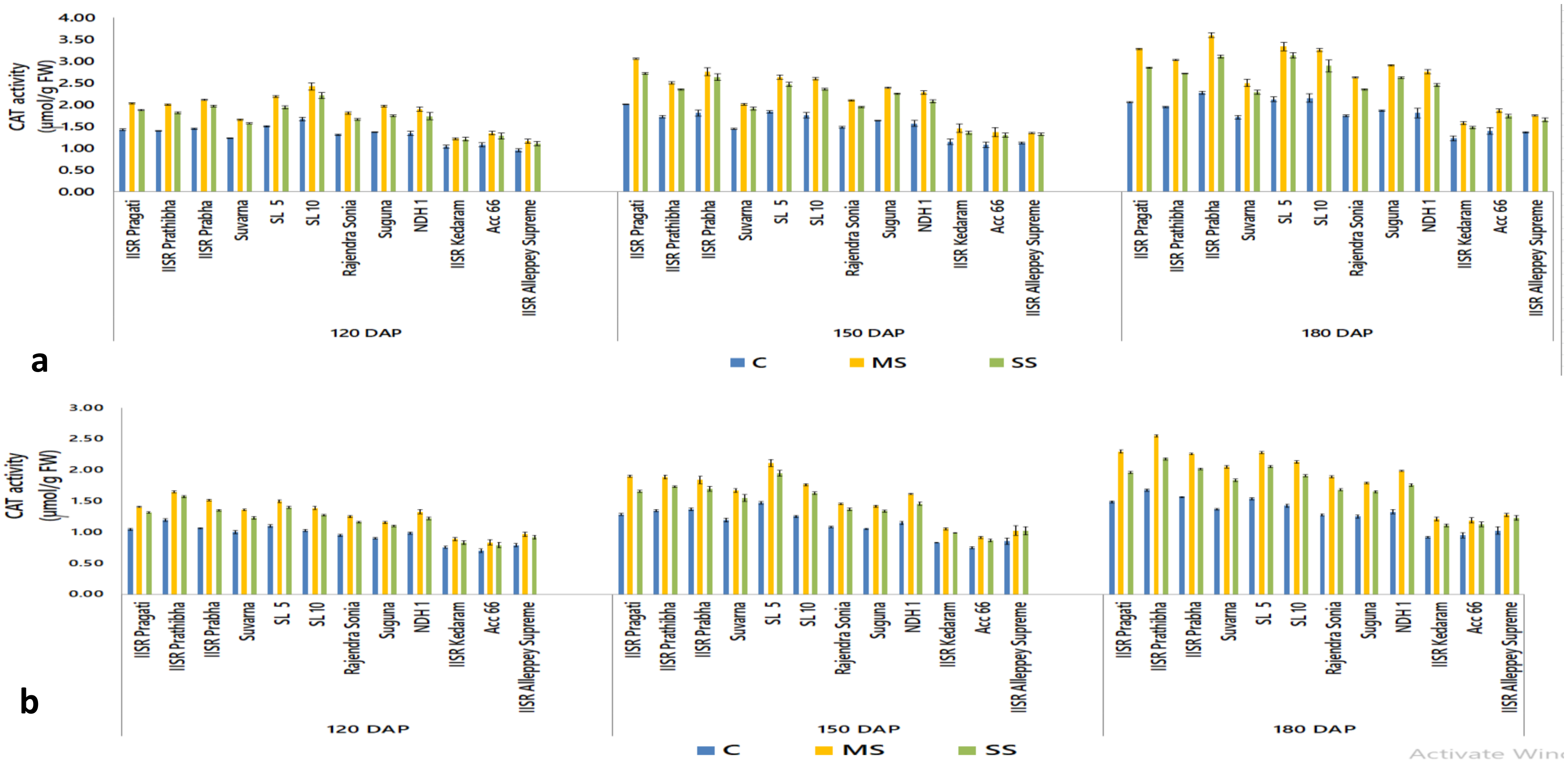


Fig. 4.2.6 Changes in the CAT activity of the turmeric under control, moderate and severe drought treatment at different developmental stages in a) leaf and b) rhizome. Bars represent the mean± SE. (C = Plants maintained under 100% field capacity; MS = Plants maintained under 50% field capacity; SS = Plants maintained under 25% field capacity)

In rhizome, the CAT activity increased from 17.3% (IISR Kedaram) to 42.8% (IISR Prabha), and from 10.2% (IISR Alleppey Supreme) to 31.2% (IISR Prathiba) at 120 DAP; from 19.7% (IISR Alleppey Supreme) to 47.6% (IISR Pragati) and from 16.3% (Acc 66) to 32.5% (SL 5) at 150 DAP; from 24.6% (IISR Alleppey Supreme) to 54.6% (IISR Pragati) and from 18.7% (Acc 66) to 33.7% (SL 10) at 180 DAP, under moderate and severe stress respectively.

Plants have developed enzymatic antioxidants (SOD, CAT, POD, APX, MDHAR, DHAR, and GR) to regulate the levels of ROS and to reduce the oxidative stress. SOD is a metalloenzyme which catalyzes the dismutation of $O_2^{\bullet-}$ to H_2O_2 and O_2 (Scandalios, 1993). In plant cells, CAT and POD are primary H_2O_2-scavenging enzymes, which catalyze the decomposition reaction of H_2O_2 to O_2 and H_2O. CAT has a high capacity to scavenge H_2O_2 but with low affinity, whereas POD has a high affinity (Mittler, 2002).

The study on the antioxidant enzymes like SOD, POD and CAT showed a significant level of increase in moderate and severe stress compared to control. The stress condition triggered the activity of SOD, POD and CAT above the control level, and antioxidant activity was much higher in tolerant ones compared to the susceptible genotypes. Moderate stress increased enzyme activity compared to control whereas severe stress showed a decrease in enzyme activity compared to moderate stress. Higher enzyme activity was observed in rhizome compared to leaf. The tolerant genotypes which showed higher SOD, POD and CAT activity exhibited lower H_2O_2 content and lipid peroxidation which resulted in a lower reduction of yield.

4.2.2.3 Effect of drought on AsA-GSH cycle enzyme activity.

Since mitochondria have very low CAT levels, the same removal of H_2O_2 is also performed by APX, MDHAR, DHAR, and GR, which are important components of AsA–GSH cycle (Holzerová & Prokisch, 2015).

In leaf, APX enzyme activity varied significantly among the genotypes at all the three growth stages under all water regimes. At 120 DAP, Rajendra Sonia (4.45

µmol/g FW) followed by SL 10 (4.28µmol/g FW) showed maximum activity and the minimum value was noticed in IISR Kedaram (1.74 µmol/g FW) under control condition. Under moderate stress, maximum activity was recorded in Rajendra Sonia (7.03 µmol/g FW) followed by SL 10 (6.56 µmol/g FW) and IISR Kedaram (2.24 µmol/g FW) showed the minimum value. Whereas under severe stress, IISR Prathibha (5.98 µmol/g FW) and SL 10 (5.92 µmol/g FW) exhibited maximum activity and the minimum activity was observed in IISR Kedaram (2.03 µmol/g FW).

Similarly at 150 DAP, Rajendra Sonia (5.24 µmol/g FW) recorded maximum activity followed by SL 10 (5.15 µmol/g FW) and lowest in IISR Alleppey Supreme (2.63 µmol/g FW) under control condition. Under moderate stress, maximum activity was observed in Rajendra Sonia (8.59 µmol/g FW) followed by SL 10 (8.3 µmol/g FW) and IISR Alleppey Supreme (3.43 µmol/g FW) showed the minimum value. Under severe stress also, Rajendra Sonia (7.50 µmol/g FW) recorded maximum values followed by IISR Prathibha (7.37 µmol/g FW) with the minimum activity in IISR Alleppey Supreme (3.21 µmol/g FW).

At 180 DAP, Rajendra Sonia (5.85 µmol/g FW) followed by SL 10 (5.65 µmol/g FW) recorded maximum values and the minimum value was noticed in IISR Kedaram (2.92 µmol/g FW) under control condition. Under moderate stress, maximum activity was observed in SL 10 (9.64 µmol/g FW) which was on par with Rajendra Sonia (9.6 µmol/g FW) while IISR Kedaram (4.02 µmol/g FW) showed the minimum activity. Under severe stress Rajendra Sonia (8.76 µmol/g FW) followed by IISR Prathibha (8.32 µmol/g FW) recorded maximum values and minimum activity in IISR Alleppey Supreme (3.8 µmol/g FW) (Fig. 4.2.7 a).

In the rhizome, at 120 DAP, the highest APX activity was recorded in Rajendra Sonia (3.53 µmol/g FW), followed by Suguna (3.37 µmol/g FW), while the lowest activity was observed in IISR Kedaram (1.33 µmol/g FW) under control conditions. Under moderate stress the highest activity was observed in Rajendra Sonia (5.40 µmol/g FW), followed by Suguna (5.09 µmol/g FW), and IISR Kedaram displayed the lowest activity (1.67 µmol/g FW). Similarly, under severe stress, Rajendra Sonia exhibited the highest activity (4.80 µmol/g FW), followed by IISR Prathibha (4.58 µmol/g FW), and IISR Kedaram showed the lowest activity (1.54 µmol/g FW).

Likewise, at 150 DAP, Rajendra Sonia showed the highest activity (4.30 µmol/g FW), followed by Suguna (4.23 µmol/g FW), while the lowest activity was observed in IISR Kedaram (1.75 µmol/g FW) under control conditions. Under moderate stress, the highest activity was observed in Rajendra Sonia (6.60 µmol/g FW), followed by Suguna (6.47 µmol/g FW), and IISR Kedaram displayed the lowest activity (2.30 µmol/g FW). Under severe stress, Rajendra Sonia and IISR Prathibha recorded the highest activities (5.92 µmol/g FW and 5.76 µmol/g FW respectively), and IISR Kedaram showed the lowest activity (2.10 µmol/g FW).

At 180 DAP, SL 5 exhibited the highest activity (4.83 µmol/g FW), followed by IISR Prathibha (4.76 µmol/g FW), while the lowest activity was observed in IISR Alleppey Supreme (2.14 µmol/g FW) under control conditions. Under moderate stress, Suguna showed the highest activity (7.84 µmol/g FW), followed by SL 5 (7.69 µmol/g FW), and IISR Alleppey Supreme displayed the lowest activity (2.93 µmol/g FW). Similarly, under severe stress, Suguna and SL 5 recorded the highest activities (6.94 µmol/g FW and 6.83 µmol/g FW respectively), while IISR Alleppey Supreme exhibited the lowest activity (2.60 µmol/g FW) (Fig. 4.2.7 b).

In leaf, the APX activities increased, from 28.7% (IISR Alleppey Supreme) to 57.8% (Rajendra Sonia) and from 16.4% (IISR Alleppey Supreme) to 44.5% (Rajendra Sonia) under moderate and severe stress respectively at 120 DAP. It increased from 30.3% (IISR Alleppey Supreme) to 63.9% (Rajendra Sonia) and from 22% (IISR Alleppey Supreme) to 48% (IISR Pragati) at 150 DAP and from 37.5% (IISR Kedaram) to 70.5% (SL 10) and from 18.4 % (IISR Alleppey Supreme) to 52% (IISR Prabha) at 180 DAP under moderate and severe stress respectively.

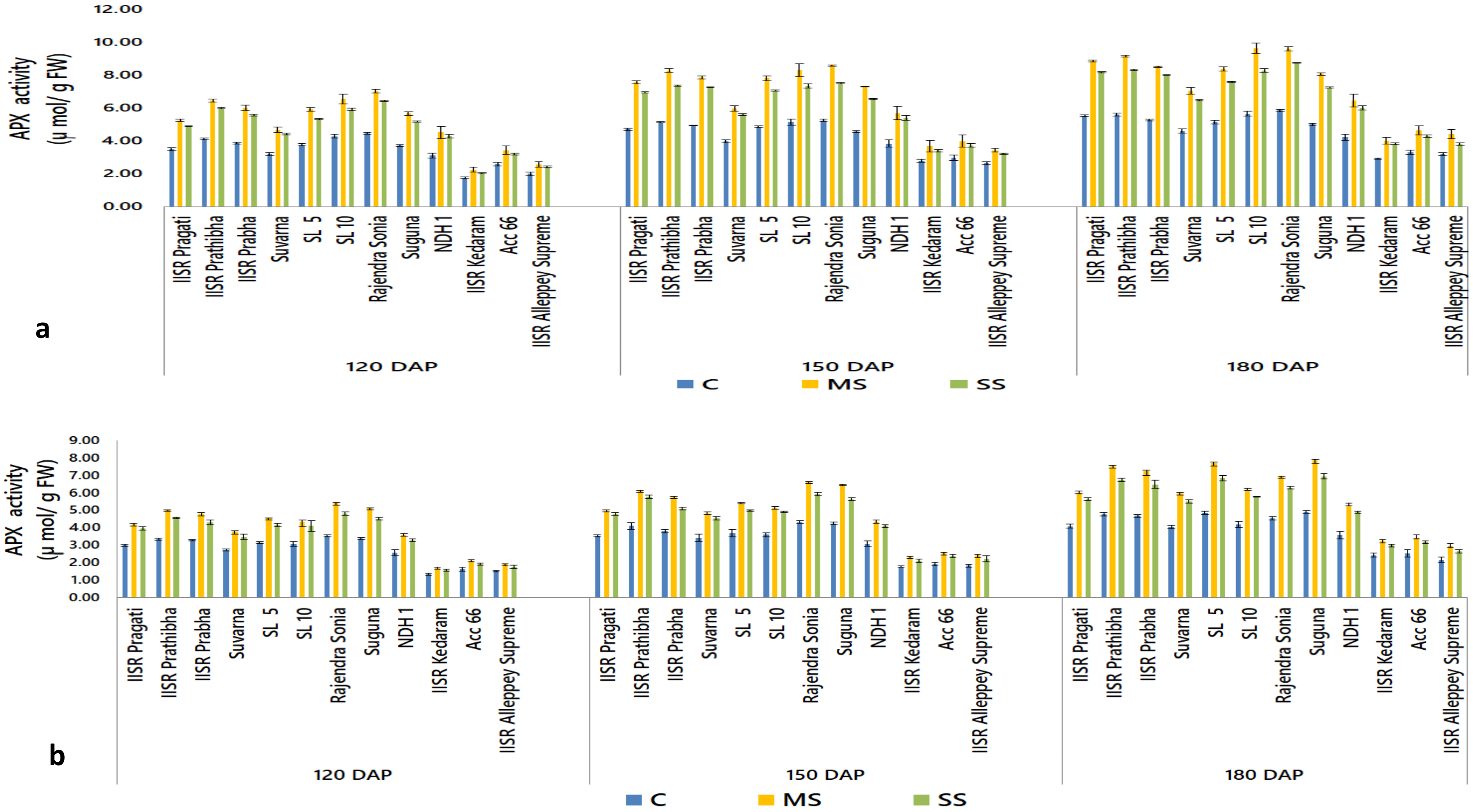


Fig. 4.2.7 Changes in the APX activity of the turmeric under control, moderate and severe drought treatment at different developmental stages in a) leaf and b) rhizome. Bars represent the mean± SE. (C = Plants maintained under 100% field capacity; MS = Plants maintained under 50% field capacity; SS = Plants maintained under 25% field capacity).

In rhizome, the APX activity at, 120 DAP increased from 25.6 % (IISR Kedaram) to 52.9 % (Rajendra Sonia) and from 15.8 % (IISR Kedaram) to 37 % (IISR Prathibha) in moderate and severe stress respectively. At 150 DAP it increased from 31.4 % (IISR Kedaram) to 53.6% (Rajendra Sonia) and from 20 % (IISR Kedaram) to 40.7 % (IISR Prathibha) while at 180 DAP, it increased from 33.5 % (IISR Kedaram) to 59.1 % (SL 5) and from 23.1% (IISR Kedaram) to 42% (Suguna) under moderate and severe stress respectively.

Highest MDHAR activity in leaf at 120 DAP, was exhibited by IISR Prathibha (0.84 µmol/g FW) followed by Rajendra Sonia (1.20 µmol/gFW), and the minimum activity was observed in IISR Kedaram (0.45 µmol/gFW) under control condition. Whereas under moderate stress, maximum activity was observed in IISR Prathibha (1.24 µmol/g FW) which was on par with Rajendra Sonia (1.20 µmol/g FW) and IISR Kedaram (0.58 µmol/g FW) showed the minimum value. Also, at severe stress, IISR Prathibha (1.13 µmol/g FW) exhibited maximum activity and the minimum activity in IISR Kedaram (0.51 µmol/g FW).

Similarly at 150 DAP, IISR Prabha (1.07 µmol/g FW) followed by IISR Prathibha (1.35 µmol/g FW) recorded highest activity and the minimum activity was observed in Acc 66 (0.52 µmol/g FW) under control condition. Under moderate stress, maximum activity was recorded in IISR Prabha (1.65 µmol/g FW) followed by IISR Prathibha (1.59 µmol/g FW) and Acc 66 (0.70 µmol/g FW) showed the minimum value. While under severe stress, IISR Prabha (1.50 µmol/g FW) and IISR Prathibha (1.45 µmol/g FW) recorded maximum values and the lowest activity in Acc 66 (0.65 µmol/g FW).

At 180 DAP, SL 10 (1.25 µmol/gFW) and IISR Prabha (1.23 µmol/g FW) recorded maximum activity and the minimum was observed in IISR Kedaram (0.63 µmol/g FW) under control condition. Under moderate stress, maximum activity was observed in IISR Prabha (2 µmol/gFW) followed by Rajendra Sonia (1.94 µmol/g FW) and IISR Kedaram (0.86 µmol/g FW) showed the minimum value. IISR Prabha (1.8 µmol/g FW) and IISR Prathibha (1.78 µmol/g FW) recorded maximum values under severe stress and the minimum value in Acc 66 (0.83 µmol/g FW) (Fig. 4.2.8 a).

In rhizome, MDHAR enzyme activity varied significantly among the genotypes at all the three growth stages under all water regimes. At 120 DAP, IISR Prathibha (0.76 µmol/g FW) followed by IISR Prabha (0.73 µmol/g FW) recorded maximum values and the minimum value was recorded in IISR Kedaram (0.34 µmol/g FW) under control condition. Under moderate stress, maximum activity was for observed in IISR Prathibha (1.08 µmol/g FW) followed by IISR Prabha (1.04 µmol/g FW) and IISR Kedaram (0.43 µmol/g FW) showed the minimum value. Under severe stress also, IISR Prathibha (0.97 µmol/g FW) and IISR Prabha (0.93 µmol/g FW) recorded maximum values and the lowest value in IISR Kedaram (0.38 µmol/g FW).

Similarly at 150 DAP, SL 5 (0.92 µmol/g FW) followed by IISR Prabha (0.89 µmol/g FW) recorded maximum values and the minimum value was showed by IISR Alleppey Supreme (0.41 µmol/g FW) under control condition. Under moderate stress, maximum activity was detected in IISR Prabha (1.34 µmol/g FW) as well as SL 5 (1.31 µmol/gFW) and IISR Alleppey Supreme (0.54 µmol/g FW) showed the minimum activity. Likewise, under severe stress, SL 5 (1.29 µmol/g FW) and IISR Prabha (1.25 µmol/g FW) recorded maximum values and the minimum value in IISR Alleppey Supreme (0.51µmol/g FW).

At 180 DAP, IISR Prathibha (1.10 µmol/g FW) followed by SL 10 (1.07 µmol/g FW) displayed maximum values and the minimum value was noted for IISR Kedaram (0.48 µmol/g FW) under control condition. Under moderate stress, maximum activity was recorded in IISR Prathibha (1.73 µmol/g FW) followed by SL 10 (1.66 µmol/g FW) and IISR Kedaram (0.64 µmol/g FW) showed the minimum value. Whereas under severe stress, maximum activity was observed in SL 10 (1.56 µmol/g FW) and IISR Prathibha (1.54 µmol/g FW) while, minimum value was observed in Acc 66 (0.60 µmol/g FW) (Fig. 4.2.8 b).

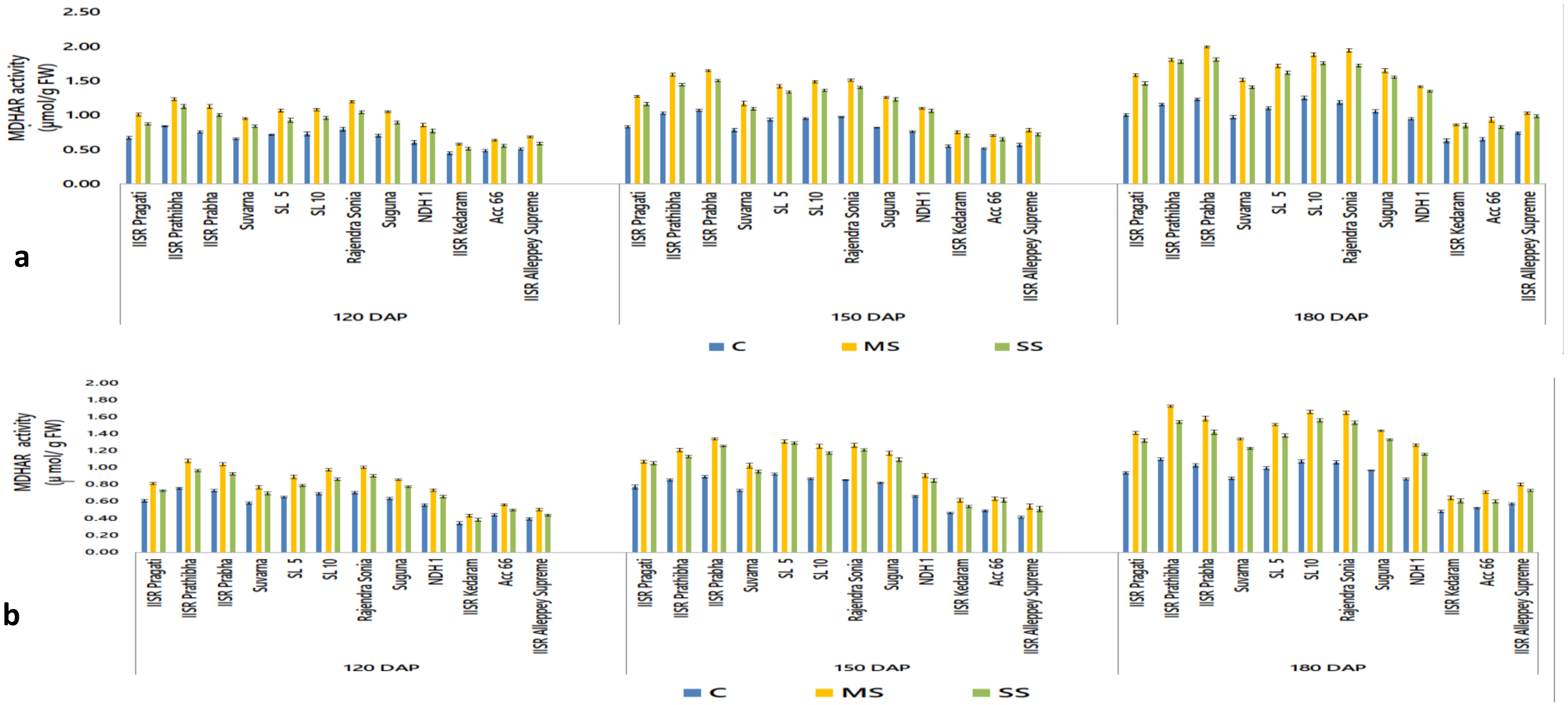


Fig. 4.2.8 Changes in the MDHAR activity of the turmeric under control, moderate and severe drought treatment in a) leaf and b) rhizome at different developmental stages. Bars represent the mean± SE. (C = Plants maintained under 100% field capacity; MS = Plants maintained under 50% field capacity; SS = Plants maintained under 25% field capacity)

In the leaf, compared with control water stress increased the MDHAR activity from 29.8% (IISR Kedaram) to 51% (Rajendra Sonia), and from 14.5% (IISR Kedaram) to 34% (IISR Prathibha) under moderate and severe stress conditions respectively at 120 DAP. At150 DAP, the activity rose from 36.05% (Acc 66) to 55% (Rajendra Sonia) and from 26.4% (Acc 66) to 55% (Suguna) under moderate and severe stress conditions respectively. At 180 DAP, the activity increased from 37% (IISR Kedaram) to 63.9% (Rajendra Sonia), and from 27.7 % (Acc 66) to 54% (IISR Prathibha) under moderate and severe stress respectively.

In rhizome, the activities of MDHAR at 120 DAP showed an increase from 26% (IISR Kedaram) to 43% (IISR Prabha) under moderate stress, and from 11.8% (IISR Kedaram) to 28.5% (Rajendra Sonia) under severe stress. At 150 DAP, it rose from 31% (in Acc 66) to 51% (IISR Prabha) under moderate stress, and from 23.2% (IISR Alleppey Supreme) to 41.4% (Rajendra Sonia) under severe stress. At 180 DAP, the enzyme activity increased from 33% (IISR Kedaram) to 57% (IISR Prathibha) under moderate stress, and from 26% (in Kedaram) to 45% (SL 10) under severe stress.

The concentration of DHAR enzyme showed significant variation among the genotypes across all three growth stages and under different water conditions. In leaf, At 120 DAP, SL 5 (0.58 µmol/g FW) exhibited the highest DHAR activity which was on par with IISR Prathibha (0.56 µmol/g FW) while Acc 66 (0.26 µmol/g FW) displayed the lowest activity under control conditions. Under moderate stress, SL 5 showed the highest activity (0.87 µmol/g FW), followed by IISR Pragati (0.83 µmol/g FW), whereas Acc 66 showed the lowest activity (0.35 µmol/g FW). Under severe stress, SL 5 and IISR Prathibha recorded the highest activities (0.75 µmol/g FW and 0.72 µmol/g FW respectively), and Acc 66 showed the lowest activity (0.30 µmol/g FW).

Similarly, at 150 DAP, IISR Pragati exhibited the highest activity (0.66 µmol/g FW), followed by IISR Prathibha (0.63 µmol/g FW), while Acc 66 showed the lowest activity (0.32 µmol/g FW) under control conditions. Under moderate stress, the highest activity was recorded in IISR Pragati (1.02 µmol/g FW), followed by IISR Prabha (0.96 µmol/g FW), with Acc 66 (0.44 µmol/g FW) displayed the lowest activity. Under severe stress, IISR Pragati and IISR Prabha recorded the highest activities (0.94 µmol/g FW and 0.86 µmol/g FW respectively), and Acc 66 exhibited the lowest activity (0.38 µmol/g FW).

At 180 DAP, IISR Prathibha showed the highest activity (0.83 µmol/g FW), followed by SL 5 (0.78 µmol/g FW), while Acc 66 displayed the lowest activity of 0.40 µmol/g FW under control conditions. Moderate stress conditions revealed the highest activity in SL 5 (1.33 µmol/g FW), and IISR Prathibha (1.31 µmol/g FW) and Acc 66 showed the lowest activity (0.55 µmol/g FW). Under severe stress, IISR Pragati and IISR Prathibha exhibited the highest activities (1.16µmol/g FW) and 1.13 µmol/g FW respectively). Acc 66 (0.49 µmol/g FW) displayed the lowest activity (Fig. 4.2.9 a).

Similar trends were observed in rhizome activity as well. At 120 DAP, SL 5 exhibited the highest activity (0.46 µmol/g FW), followed by SL 10 (0.44 µmol/g FW), while Acc 66 showed the lowest activity (0.21 µmol/g FW) under control conditions. Under moderate stress conditions saw SL 10 recorded the highest activity (0.68 µmol/g FW), followed by SL 5 (0.65 µmol/g FW), whereas Acc 66 displayed the lowest activity (0.28 µmol/g FW). Under severe stress, SL 5 and NDH 1 recorded the highest activities (0.58 µmol/g FW and 0.54 µmol/g FW respectively), and Acc 66 showed the lowest activity (0.24 µmol/g FW).

Similarly, at 150 DAP, SL 5 exhibited the highest activity (0.56 µmol/g FW), followed by SL 10 (0.54 µmol/g FW), while Acc 66 showed the lowest activity (0.25 µmol/g FW) under control conditions. Under moderate stress, SL 5 (0.89 µmol/g FW) showed the highest activity followed by SL 10 (0.83 µmol/g FW), and Acc 66 displayed the lowest activity (0.34 µmol/g FW). Under severe stress, SL 5 and SL 10 recorded the highest activities (0.78 µmol/g FW and 0.74 µmol/g FW respectively), and Acc 66 exhibited the lowest activity (0.29 µmol/g FW).

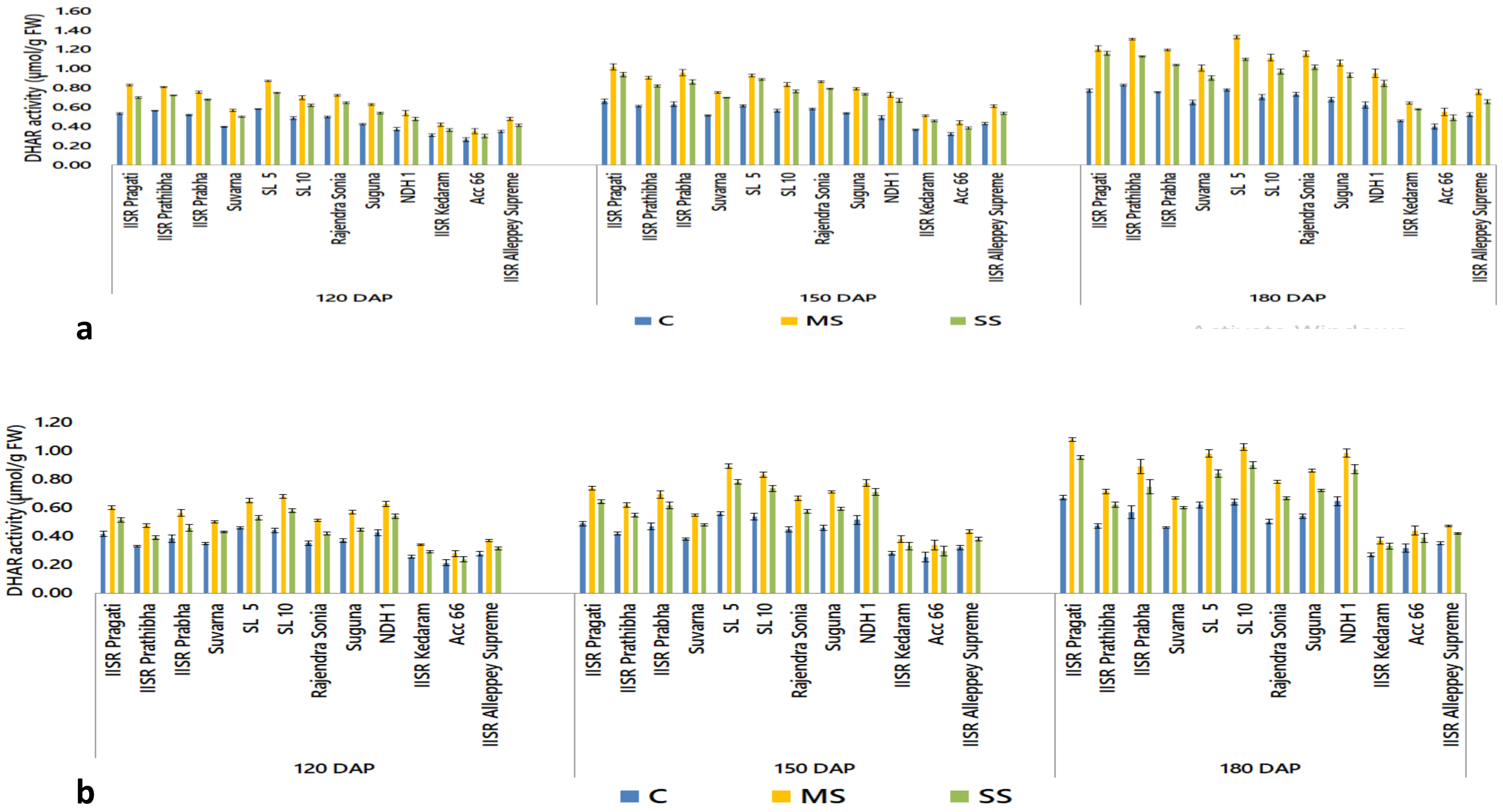


Fig. 4.2.9 Changes in DHAR activity of turmeric under control, moderate and severe drought treatment at different developmental stages in a) leaf and b) rhizome at different developmental stages. Results represent the mean± SE. (C = Plants maintained under 100% field capacity; MS = Plants maintained under 50% field capacity; SS = Plants maintained under 25% field capacity)

At 180 DAP, IISR Pragati showed the highest activity (0.67 µmol/g FW), followed by NDH 1 (0.65 µmol/g FW), while IISR Kedaram exhibited the lowest activity (0.27 µmol/g FW) under control conditions. Moderate stress conditions revealed the highest activity in IISR Pragati (1.08 µmol/g FW), followed by SL 10 (1.03 µmol/g FW), and IISR Kedaram showed the lowest activity (0.37 µmol/g FW). Under severe stress, SL 10 and NDH 1 exhibited the highest activities (0.90 µmol/g FW and 0.87 µmol/g FW respectively), and IISR Kedaram showed the lowest activity (0.33µmol/g FW) (Fig. 4.2.9 b).

In the leaf, DHAR activity, at 120 DAP, showed an increase from 34.5% (Acc 66) to 55.4% (IISR Pragati) under moderate stress, and from 15% (Acc 66) to 31% (IISR Pragati) under severe stress. At 150 DAP, it increased from 36.6% (Acc 66) to 54.2% (IISR Pragati) under moderate stress, and from 19.3% (Acc 66) to 44.7% (SL 5) under severe stress. At 180 DAP, it increased from 38.7% (Acc 66) to 65.2 % (SL 5) under moderate stress, and from 23% (Acc 66) to 49.9% (IISR Pragati) under severe stress.

In the rhizome, DHAR activity at, 120 DAP increased from 30.5% (Acc 66) to 54.2% (SL 10) under moderate stress, and from 12.2% (Acc 66) to 31.5% (SL 10) under severe stress. At 150 DAP it increased from 34.5% (Acc 66) to 58.9% (SL 5) under moderate stress, and from 16.7% (Acc66) to 39.3% (SL 5) under severe stress. At 180 DAP, it increased from 34.9% (IISR Alleppey Supreme) to 61.2% (IISR Pragati) under moderate stress, and from 20% (IISR Alleppey Supreme) to 41.8% (IISR Pragati) under severe stress.

In leaf tissues, GR enzyme activity showed significant variation among different genotypes across all growth stages and water regimes. At 120 DAP, IISR Prabha demonstrated the highest enzyme activity (1.13 µmol/g FW), followed by SL 10 (1.07 µmol/g FW), whereas the lowest activity was observed in IISR Alleppey Supreme (0.72 µmol/g FW) under control conditions. Under moderate stress, IISR Prabha (1.72 µmol/g FW) showed the highest activity and IISR Alleppey Supreme (0.85 µmol/g FW) showed the lowest. Under severe stress conditions, IISR Prabha exhibited maximum activity (1.53 µmol/g FW) followed by SL 10 (1.46 µmol/g FW), while the minimum was recorded in Acc 66 (0.82 µmol/g FW).

Similarly, at 150 DAP, the highest activity was recorded in IISR Prabha (1.36 µmol/g FW) and Rajendra Sonia (1.31 µmol/g FW) under control conditions, and the lowest was observed in Acc 66 (0.83 µmol/g FW). Under moderate stress, IISR Prabha (2.18 µmol/g FW) showed the highest activity followed by Rajendra Sonia (2.07 µmol/g FW), whereas the lowest was recorded in IISR Kedaram (1.04 µmol/g FW). Under severe stress, maximum activity was recorded in IISR Prabha (1.90 µmol/g FW) followed by Rajendra Sonia (1.82 µmol/g FW), while the minimum was observed in IISR Kedaram (0.98 µmol/g FW).

At 180 DAP, IISR Prabha displayed the highest activity (1.56 µmol/g FW), followed by SL 10 (1.51 µmol/g FW), and Acc 66 showed the lowest activity (0.92 µmol/g FW) under control conditions. Under moderate stress, IISR Prabha (2.80 µmol/g FW) showed maximum activity followed by Rajendra Sonia (2.6 µmol/g FW), while the lowest activity was recorded in Acc 66 (1.29 µmol/g FW). Under severe stress maximum activity was recorded in SL 10 (2.29 µmol/g FW) followed by Rajendra Sonia (2.23 µmol/g FW), while the minimum was observed in Acc 66 (1.17 µmol/g FW) (Fig. 4.2.10 a).

In rhizome also, GR enzyme activity varied significantly ($P > 0.05$) among the genotypes. At 120 DAP, IISR Prathibha (1.06 µmol/g FW) followed by SL 10 (1.01 µmol/g FW) recorded maximum values and the minimum value was recorded in Acc 66 (0.58 µmol/gFW) under control condition. Under moderate stress, maximum activity was observed in IISR Prathibha (1.42 µmol/g FW) followed by SL 10 (1.35 µmol/g FW) while Acc 66 (0.67 µmol/g FW) showed the minimum value. The highest activity under severe stress was observed in IISR Prathibha (1.36 µmol/g FW) followed by Rajendra Sonia (1.28 µmol/g FW), while Acc 66 (0.65 µmol/g FW) had the lowest activity.

Similarly at 150 DAP, IISR Prathibha (1.21 µmol/g FW) followed by SL 10 (1.17 µmol/g FW) recorded maximum values and the minimum value was observed in IISR Kedaram (0.64 µmol/g FW) under control condition. Under moderate stress, maximum activity was observed in IISR Prathibha (1.73 µmol/g FW) followed by SL 10 (1.64 µmol/g FW). IISR Kedaram (0.77 µmol/g FW) showed the minimum value. IISR Prathibha (1.60 µmol/g FW) followed by SL 10 (1.54 µmol/g FW) displayed maximum activity under severe stress while the minimum activity was recorded in IISR Kedaram (0.74 µmol/g FW).

Likewise, at 180 DAP, maximum activity was detected in SL 10 (1.39 µmol/g FW) followed by Rajendra Sonia (1.35 µmol/g FW) and the minimum value was observed in Acc 66 (0.71 µmol/gFW) under control condition. Under moderate stress, maximum activity was shown by SL 10 (2.18) followed by IISR Prathibha (2.11 µmol/g FW) whereas Acc 66 (0.86 µmol/g FW) showed the minimum value. Under severe stress, Rajendra Sonia (1.96 µmol/g FW) followed by IISR Prathibha (1.89 µmol/g FW) recorded maximum values and minimum value was observed in Acc 66 (0.82 µmol/g FW) (Fig. 4.2.10 b).

In the leaf, the GR activity at, 120 DAP showed an increase from 18.1% (IISR Alleppey Supreme) to 54.9% (Rajendra Sonia) under moderate stress, and from 14.4% (IISR Alleppey Supreme) to 36.4% (SL 10) under severe stress. At 150 DAP, the activity increased from 26% (IISR Kedaram) to 60.8% (IISR Prabha), and from 16.9% (Acc 66) to 40.1% (IISR Prabha) under moderate and severe stress respectively. At 180 DAP, under moderate stress, it rose from 39 % (IISR Kedaram) to 79% (IISR Prabha), and under severe stress, from 27 % (in Acc 66) to 52.7% (Rajendra Sonia).

In case of rhizome, GR activity, increased from 15.5% (Acc66) to 34% (IISR Prathibha) under moderate stress and from 12.1% (Acc 66) to 29.6% (Rajendra Sonia) under severe stress at 120 DAP. At 150 DAP, it increased from 19.7% (Acc 66) to 43.2% (IISR Prathibha), and from 12.4 % (Acc 66) to 32.5 % (IISR Prathibha) under moderate and severe stress. At 180 DAP, it increased from 21.1% (Acc 66) to 58.6 % (IISR Prathibha) and from 15.5% (Acc 66) to 45.5 % (Rajendra Sonia) under moderate and severe stress respectively.

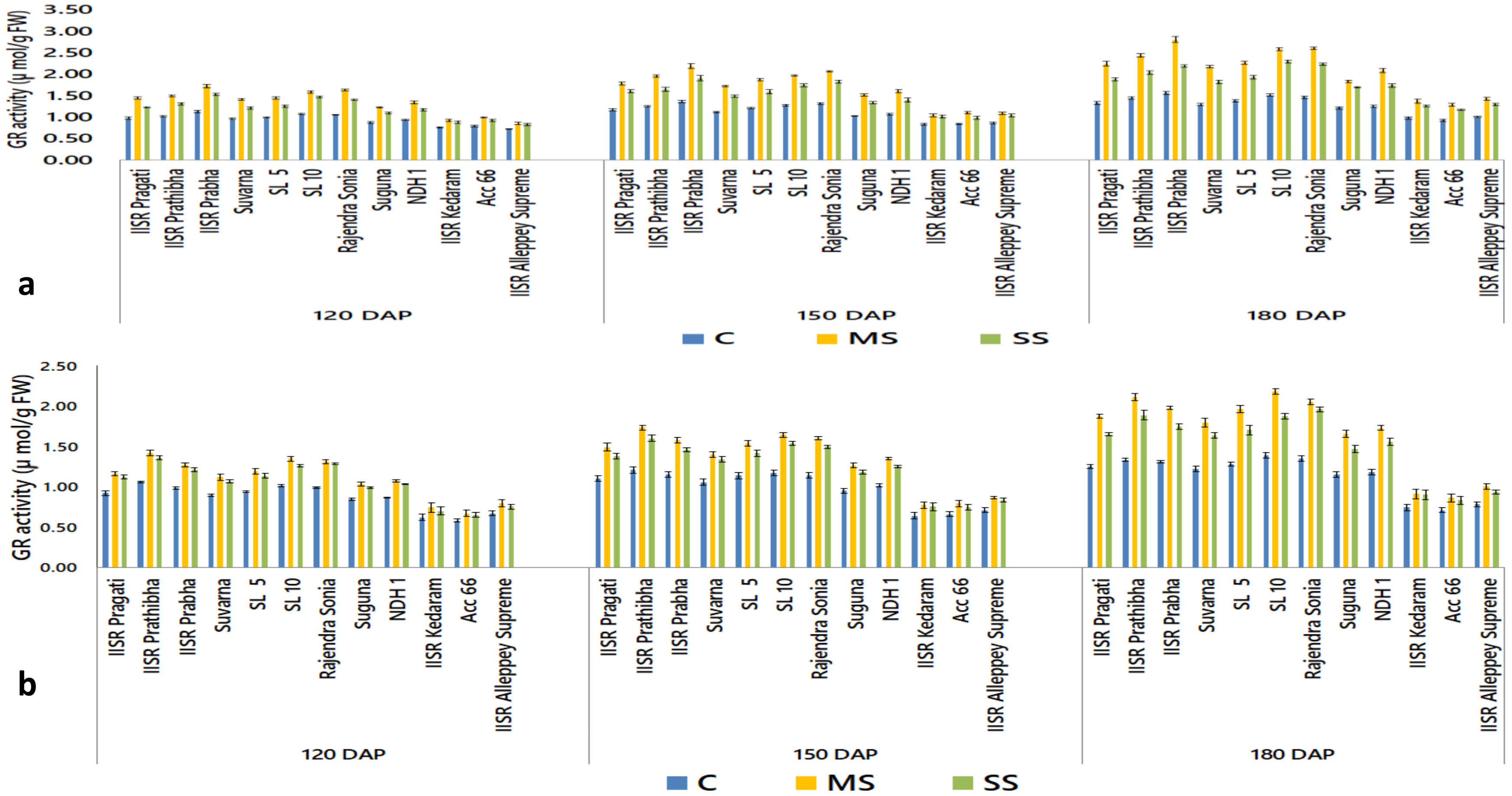


Fig. 4.2.10 Changes in the GR activity of the turmeric under control, moderate and severe drought treatment at different developmental stages in a) leaf and b) rhizome. Bars represent the mean± SE. (C = Plants maintained under 100% field capacity; MS = Plants maintained under 50% field capacity; SS = Plants maintained under 25% field capacity)

Maintaining a delicate equilibrium of antioxidant enzymes is essential for quelling harmful reactive oxygen species within cells, thereby mitigating damage caused by ROS (Apel & Hirt, 2004; Farooq *et al*., 2008). There was significant variation in the activity of AsA-GSH cycle enzymes among genotypes at all the three growth stages under all water regimes. The study on the tolerance enzymes systems APX, MDHAR, DHAR and GR showed a substantial level of difference between control and treated plants. The level of the enzyme activity was high in the stress treated as compared to the untreated plants with slight decrease in activity under severe stress compared with moderate stress in leaf. Similar results were found in rhizome also. The variation caused by drought stress was genotype dependent. The water stress escalated the activity of AsA-GSH enzymes compared to control and the tolerant genotypes showed higher activity than the susceptible genotypes.

This result was in consistent with the increase in the enzymatic activities in cucumber (Fan *et al*., 2014), in wheat (Singh *et al*., 2012) and increase in the activities of SOD and APX in bean plants (Zltev *et al*., 2006), APX and POD in mutant rice varieties (Kamarudin *et al*., 2018).This result is also supported by other reports on increased activities of antioxidant enzymes under drought stress in rice (Basu *et al*., 2010) and in pepper (Anjum *et al*., 2012) indicating that drought stress might result in increased antioxidant activities. Tolerant varieties have shown higher enzyme activities compared to susceptible ones as reported in enzyme activities in coconut (Renju *et al*., 2015), in Wheat (Singh *et al*., 2012) and in upland rice cultivars (Lum *et al*., 2014).

Increased activity of antioxidant enzymes such as SOD, CAT, GR, APX, and POX are involved in lowering oxidative injury in caper bush seedlings under drought stress (Ozkur *et al*., 2009). Improved activities of CAT, APX, and SOD decreased H_2O_2 and MDA contents in drought-prone tomato seedlings (Yuan *et al*., 2010). Which suggest that a lower antioxidant enzyme activity was closely linked to accumulation of H_2O_2 and MDA (Liu & He, 1995). Also, accumulation of high levels of H_2O_2 and an increase in lipid peroxidation inducing more damage to membranes under severe stress condition (Singh *et al*., 2012). It also suggests that plants prevent the toxic effects of ROS by enzymatic as well as nonenzymatic antioxidative systems (Jaleel *et al.,* 2008; Shi & Zhu, 2008; Ashraf *et al*., 2009; Ashraf *et al.,* 2011). Antioxidant enzymes mainly include SOD, CAT, POD, APX, DHAR, MDHAR and GR.

Main function of SOD is to removes O^2 −, and convert to H_2O_2. CAT and POD are mainly involved in the removal of H_2O_2. CAT eliminates H_2O_2 in the mitochondrion and microbody (Hazanuzzaman *et al.,* 2019). Besides, APX, GR, DHAR, and MDHAR (Ascorbate-Glutathione pathway) enzymes are also very important H_2O_2 scavenging enzymes that play a vital role in detoxifying ROS. Apart from ROS detoxification, they also interact with other defence systems in plants and protect the plants from various abiotic stress-induced damages (Noctor *et al.,* 2014).

Under water deficit conditions, increased SOD, POD, CAT and APX activity lowered H_2O_2 accumulation and protected protein and photosynthetic apparatus in tolerant rice cultivar during the tillering period (Wang *et al.* 2019). It also reduced oxidative stress and PPO activity in Olive cultivar Chemlali (Ahmed & Rouina, 2009).

Progressive level of water deficit significantly enhanced the activities of MDHAR, DHAR, APX, and GR in various crops including rice seedlings (Pyngrope *et al.,* 2013; Nahar *et al*., 2018), wheat (Lou *et al.* 2018), cassava (Zhu *et al.,* 2020), melon (Kavas & Baloğlu, 2013) and *Amaranthus tricolor* (Sarker&Oba, 2018) which resulted in relatively lower H_2O_2 accumulation and reduced membrane damage and chlorophyll degradation especially in tolerant cultivars compared to susceptible ones which resulted in sustainable productivity under water deficit.

Similarly, elevated concentration of enzymatic antioxidants (CAT, POD, SOD) lowered observed contents of H_2O_2 and O^2 •− under drought stress in tolerant faba bean genotype (Siddiqui *et al.,* 2015), *Amaranthus tricolor* (Sarker & Oba, 2018), cotton plants (Yi *et al*., 2016; Arif et al., 2023), wheat cultivar (Nawaz *et al.,* 2015; Qayyum *et al*., 2021) oilseed rape seedlings (Abedi & Pakniyat, 2010), upland rice varieties (Lum *et al*., 2014) and *Olea europaea* (Doupis *et al.,* 2013) under water deficit treatment which lowered oxidative damage by reducing H_2O_2 accumulation and protected cell structures including photosynthetic apparatus and chlorophyll pigments resulted in lower susceptibility index , improved dry matter production and yield stability.

The reduction of the antioxidant defence system may result in oxidative damage to cellular components and membrane lipids by generating various ROS (Yang *et al.,* 2021). Enhanced antioxidant enzymes reduce the damaging effects of ROS and may confer drought resistance to plants (Gill & Tuteja, 2010; Manivannan *et al.,* 2014).

Compared with moderate stress, a significant decrease was detected in the enzyme activities under severe stress. The decline of activities at severe stress compared to moderate stress condition was also found in rice seedlings (Sharma & Dubey, 2005), under severe drought stress under different growth stages in maize (Li-Ping *et al.,* 2006), where lipid peroxidation was higher demonstrating that these enzymes inactivated under extreme stress. The decline trend was adverse with the production of ROS and TBARS, demonstrating that the inactivation of enzymatic antioxidants under severe stress reduced the cleavage of ROS but promoted ROS accumulation and occurrence of more oxidation (Hasanuzzaman *et al*., 2020).

In our study, there was a significant increase in AsA-GSH enzyme activities under water deficit. Whereas severe stress resulted in a decline in enzyme activity which indicate increased ROS damage from increased oxidative stress which would have rsulted in higher degree of antioxidant enzyme degradation. Also, susceptible genotypes with lower enzyme activity showed a higher yield reduction indicating a higher oxidative damage in these genotypes under drought stress.

4.2.2.4 Effect of drought on sugar content in leaf

The sugar levels in turmeric leaf displayed considerable variation among genotypes under control, moderate stress and severe stress. The sugar contetnt decreased significantly with growth stage and the content was lowest at 180 DAP. At each growth stage, sugar content was significantly higher under stress compared to control. Maximum sugar content was recorded at early stage (120 DAP) and under severe stress. There was reduction in sugar content at successive stages as reported in wheat cultivars (Shi *et al*., 2016).

At 120 DAP, IISR Prabha (2.37 %) followed by NDH 1 (2.33%) recorded maximum sugar content and minimum value was recorded in Acc 66 (1.24%) under control; under moderate stress also, IISR Prabha (3.46 %) and NDH 1 (3.40%) had maximum sugar content and minimum in Acc 66 (1.57 %) while under severe stress, highest sugar content was observed in SL 10 (3.63 %) followed by NDH 1 (3.59 %) and lowest in Acc 66 (8.56 %).

At 150 DAP, Suvarna (2.03 %) and NDH 1(1.97 %) showed maximum values and minimum was observed in IISR Kedaram (0.98 %) under control; under moderate stress, Suvarna (2.76 %) and SL 10 (2.65 %) had maximum sugar content and minimum in Acc 66 (1.25 %). Whereas under severe stress, highest sugar content was recorded in NDH 1 (3.12 %) followed by Suvarna (3.04 %) and lowest sugar content was recorded in IISR Kedaram (1.31 %).

Similar results were observed at 180 DAP also. IISR Prabha (1.60 %) followed by NDH 1 (1.51 %) recorded maximum sugar content and minimum was observed in IISR Kedaram (0.82%) under control; under moderate stress, IISR Prabha (2.26 %) and SL 10 (2.01 %) had maximum sugar content and minimum in Acc 66 (1.08 %) whereas under severe stress, highest sugar content was recorded in IISR Prabha (2.45 %) followed by SL 10 (2.18%) and lowest in Acc 66 (1.10 %) (Table 4.2.2)

Compared to control, sugar content increased from 26.6% (Acc 66) to 46.2% (IISR Prabha) and from 38.4% (IISR Kedaram) to 59.2% (SL 10) at 120 DAP under moderate and severe stress respectively. Whereas it increased from 21.5 % (Acc 66) to 44% (SL 10) and from 34.4 % (IISR Kedaram) to 58.4% (NDH 1) at 150 DAP under moderate and severe stress respectively. At 180 DAP, sugar content increased from 20.6 % (Acc 66) to 41.7 % (IISR Prabha) and from 34% (IISR Kedaram) to 53.7% (IISR Prabha) under moderate and severe stress respectively. Tolerant genotypes accumulated more sugar compared to the susceptible stages especially under water stress at all the stages of development.

4.2.2 Sugar content (%) of turmeric under different water regimes.

Genotype	120 DAP			150 DAP			180 DAP		
	100% FC	**50% FC**	**25% FC**	**100% FC**	**50% FC**	**25% FC**	**100% FC**	**50% FC**	**25% FC**
IISR Pragati	2.09^{r}	3^{m}	3.19^{a}	1.77^{ijk}	2.53^{de}	2.69^{bcd}	1.36^{s}	1.86^{j}	2.03^{f}
IISR Prathibha	1.9^{t}	2.68^{i}	2.93^{k}	1.57^{lm}	2.2f	2.39^{e}	1.27^{v}	1.75^{l}	1.91^{i}
IISR Prabha	2.37^{o}	3.46^{l}	3.52^{g}	1.92^{ghi}	2.62^{cd}	2.85^{b}	1.6^{o}	2.26^{c}	2.45^{a}
Suvarna	2.18^{q}	3.15^{k}	3.35^{a}	2.03^{g}	2.76^{bc}	3.04^{a}	1.38^{s}	1.94^{h}	2.09^{e}
SL 5	1.84^{u}	2.56^{f}	2.8^{l}	1.48^{mn}	2.07^{fg}	2.22^{f}	1.24^{w}	1.69^{m}	1.82^{k}
SL 10	2.28^{p}	3.24^{n}	3.63^{e}	1.84^{hij}	2.65^{cd}	2.85^{b}	1.43^{r}	2.01^{fg}	2.18^{d}
Rajendra Sonia	2^{s}	2.83^{d}	3.07^{s}	1.69^{jkl}	2.4^{e}	2.6^{cd}	1.33^{t}	1.8^{k}	2^{g}
Suguna	1.79^{v}	2.45^{g}	2.68^{h}	1.42^{mno}	1.98^{kl}	2.08fg	1.14^{y}	1.55^{p}	1.65^{n}
NDH 1	2.33^{o}	3.4^{u}	3.59^{v}	1.97^{gh}	2.83^{b}	3.12a	1.51^{q}	2.1^{e}	2.29^{b}
IISR Kedaram	1.26^{y}	1.65^{b}	1.75^{w}	0.98^{q}	1.28^{op}	1.31^{op}	0.82^{D}	1.11^{z}	1.1^{zA}
Acc 66	1.24^{c}	1.57^{j}	1.78^{v}	1.03^{q}	1.25^{p}	1.44^{mno}	0.9^{C}	1.08^{A}	1.21^{x}
IISRAlleppey Supreme	1.4^{x}	1.85^{w}	1.98^{st}	1.07^{q}	1.4^{nop}	1.46^{mn}	0.96^{B}	1.23^{wx}	1.3^{u}
General mean	2.55			2.012			1.59		
CV (%)	0.94			4.92			0.958		
CD (P=0.05)	0.038			0.161			0.025		

The mean value (n=3) followed by different letters within a column are significantly different ($P < 0.05$) based on ANOVA test.

The transport and distribution of total soluble sugar and sucrose are key processes in plants response to abiotic stresses (Mathan *et al.*, 2021). Osmotic stressors, such as those brought on by drought, cold, and salt, typically result in a rise in sugar content, with tolerant types accumulating larger concentrations than the susceptible (Sarker *et al.*, 1999; Sairam *et al.*, 2002; Nayyer & Walia, 2004; Guo *et al.*, 2009). Sugars maintain cells osmotic potential, provide energy for stress defence, and act as membrane protectants and ROS scavengers (Albornoz *et al.*, 2022).

Processes such as photosynthesis and translocation seem to be the cause of accumulation of soluble sugars to adjust differences between the turgor levels required for stomatal opening and growth (Boyer, 1976). Also, it has been demonstrated that the amount of sugar varied with genotype and environmental factors as reported in maize under drought (Zaidi *et al*., 2003; Boomsma & Vyn, 2008).

Sugars have multiple roles as energy and carbon transport molecules, signalling factors, osmolyte and as the source of materials for synthesis of biomolecules (Halford *et al.,* 2010). To maintain osmotic potential plants accumulate protective solutes including sugars in response to water stress (Reddy *et al*., 2004; Slabbert & Kru¨ger *et al*., 2014). Under drought, higher rate of accumulation and mobilization of soluble sugars are observed in the leaves of wheat cultivars (Shi *et al.,* 2016) which resulted in improved grain weight and drought tolerance. Oat × Maize Addition (OMA) lines under drought possessed 34% more soluble sugars compared to control plants (Warzecha & Bocianowski, 2023).

Activation of starch degradation under stress also contribute to sugar accumulation under water deficit (Guan-fu *et al*., 2011; Kaplan *et al*., 2004; Yano *et al.,* 2005; Thalmann *et al*., 2016). Similarly, decline in soluble sugar contents in rice anthers under drought stress impaired the water balance and further intensified oxidative stress (Guan-fu *et al*., 2011). Increase in sugars under water deficit are reported in many crops including maize, castor bean, wheat, rice and cotton (Ali *et al*., 2013; Karimi *et al*., 2012; Shi *et al*., 2016; Qayyum *et al*., 2021; Wang *et al*., 2019; Parida *et al*., 2007) which contributed to higher tolerance under water deficit condition.

In our study, sugar content increased with drought intensity. Tolerant genotypes maintained higher sugar content which along with other tolerance mechanisms helped them in maintaining higher osmoregulation under drought which reduced the oxidative damage and resulted in higher yield and tolerance under drought. Whereas susceptible genotypes with lower sugar content exhibited a higher decline in yield due to lower water balance and degradation of membranes and important macromolecules.

4.2.2.5 Effect of drought on starch content of turmeric rhizome.

The starch content varied widely among the genotypes under different water regimes as well as among growth stages in turmeric leaves. It decreased under stress condition with a further decrease under severe stress. Maximum starch content was recorded at 180 DAP.

At 120 DAP, starch content was maximum in NDH 1 (32.47 %) followed by IISR Prathibha (28.4%) and minimum was recorded in Rajendra Sonia (15.04%) under control. Under moderate stress, IISR Prathibha (21%) and NDH 1 (20.74%) had maximum starch content and minimum in Rajendra Sonia (10.2%). Similarly under severe stress, highest starch content was observed in IISR Prathibha (19.5%) followed by IISR Prabha (19.37%) and lowest starch content was recorded in Acc 66 (8.56%).

At 150 DAP, Suguna (49.79%) and SL 10 (49.36%) showed maximum values and minimum was in Rajendra Sonia (27.07%) under control. Under moderate stress, NDH 1 (40.2%) and IISR Prathibha (39%) had maximum starch content and minimum for Rajendra Sonia (18.7%) while under severe stress, highest starch content was observed in IISR Prathibha (36.8%) followed by IISR Prabha (36.4%) and lowest starch content was recorded in Acc 66 (17.84%).

At 180 DAP, NDH 1 (56.72%) followed by IISR Prathibha (54.1%) recorded maximum starch content and lowest in Rajendra Sonia (28.12%) under control. Under moderate stress, NDH 1 (43.14%) followed by IISR Prathibha (42%) had maximum starch content and minimum was recorded in Acc 66 (22.6%) whereas under severe stress, highest starch content was observed in IISR Prathibha (39.3%) followed by NDH 1 (39%) and lowest in Acc 66 (20.7%) (Table 4.2.3).

Table 4.2.3 Starch content (%) in turmeric genotypes under different water regimes

Genotype	120 DAP			150 DAP			180 DAP		
	100% FC	50% FC	25% FC	100% FC	50% FC	25% FC	100% FC	50% FC	25% FC
IISR Pragati	25.5^{e}	19^{ijk}	17.8^{l}	45.2^{f}	35^{mn}	33^{p}	47.7^{f}	38.4^{l}	36^{o}
IISR Prathibha	28.4^{b}	21^{f}	19.5^{hi}	52.4^{b}	39^{h}	36.8^{j}	54.1^{b}	42^{h}	39.3^{k}
IISR Prabha	28.39^{b}	20.4^{fg}	19.37^{i}	51.1^{c}	38^{i}	36.4^{jk}	53.1^{c}	41.1^{i}	37.3^{mn}
Suvarna	19.24^{ij}	12.84^{n}	11.69^{o}	34.63^{n}	23.74^{s}	21.65^{t}	36^{o}	27^{s}	24.5^{u}
SL 5	26.89^{d}	18.84^{ijk}	17.64^{l}	48.41^{e}	36^{kl}	33.78^{o}	50.3^{e}	37.7^{m}	35.7^{o}
SL 10	27.42^{cd}	20.09^{gh}	18.46^{k}	49.36^{de}	35.5^{lm}	35.7^{klm}	51.3^{d}	38.3^{l}	36.8^{n}
Rajendra Sonia	15.04^{m}	10.2^{q}	9.42^{r}	27.07^{q}	18.7^{v}	18.2^{vw}	28.1^{r}	24.8^{u}	22.4^{vw}
Suguna	27.66^{c}	18.58^{jk}	17.52^{l}	49.79^{d}	34.5^{nm}	32.38^{p}	51.7^{d}	39.2^{k}	34.3^{p}
NDH 1	32.47^{a}	20.74^{fg}	19.23^{ij}	58.44^{a}	40.2^{g}	35.72^{klm}	56.7^{a}	43.1^{g}	39^{k}
IISR Kedaram	27.07^{cd}	14.96^{m}	12.43^{n}	48.72^{e}	27.5^{q}	24.73^{r}	50.6^{e}	30.8^{q}	26.2^{t}
Acc 66	20.45^{fg}	11.04^{op}	8.56^{s}	36.81^{jk}	20.38^{u}	17.84^{w}	40.4^{j}	22.6^{v}	20.7^{x}
IISR Alleppey Supreme	21.04^{f}	11.69^{o}	10.44^{pq}	37.88^{i}	22^{t}	20.2^{u}	39.4^{k}	25.9^{t}	21.9^{w}
General mean	18.91			34.87			37.34		
CV (%)	2.27			1.34			0.93		
CD (P=0.05)	0.702			0.7			0.57		

The mean value (n=3) followed by different letters within a column are significantly different ($P< 0.05$) based on ANOVA test**.**

Starch content decreased from 25.5% (IISR Pragati) to 46% (Acc 66) and from 30.2 % (IISR Pragati) to 58% (Acc 66) at 120 DAP under moderate and severe stress respectively. It decreased from 22.6% (IISR Pragati) to 44.6% (Acc 66) and from 27% (IISR Pragati) to 51.5% (Acc 66) at 150 DAP under moderate and severe stress respectively. At 180 DAP starch content decreased from 11.8% (Rajendra Sonia) to 44 % (Acc 66) under moderate stress and from 20.4 % (IISR Kedaram) to 48.8 % (Acc 66) under severe stress.

As the water stress intensity increased, total starch decreased significantly. With growth stages of plant, sugar content decreased while starch increased. This observation is consistent with that observed in rice (Chen *et al.*, 2023) and in sorghum (Bing *et al.*, 2014) under drought. Abiotic stress during grain filling stage is reported to cause an increase in sucrose, reducing sugars and sugar phosphates, and a reduction in starch in wheat (Jenner, 1991; Gooding *et al*., 2003).

Enhanced production of H_2O_2 under water stress induce oxidative damage to biomolecules including starch (Apel & Hirt, 2004; Kaplan *et al*., 2004; Yano *et al.,* 2005; Guan-fu *et al*., 2011; Anjum *et al.,* 2017). Reduction in photosynthetic pigments under drought treatment resulted in a lower starch contents in cotton (*Gossypium hirsutum* L.) genotypes under short-term drought treatment, the degradation was higher in the sensitive cotton genotype (Ca/H 631) as compared to the moderately tolerant genotype (GM 090304) which had higher proline, sugar and polyphenols (Parida *et al*., 2007). Whereas Kresović *et al*. (2018) reported increased starch content with decreasing irrigation in maize which may be due to reduction of proteins of starch metabolism under drought (Schulz *et al.,* 2021).

In the present study, starch content decreased significantly under drought stress which may be attributed to the enhanced production of ROS which caused increased degradation of starch with stress intensity. Also, the degradation of starch during the rhizome developmental period resulted in greater degradation of starch.

4.2.2.6 Effect of drought on protein content in leaf.

The protein content varied significantly among the genotypes between control and stress treatments and also among growth stages of turmeric. Protein content increased under moderate stress and there was a slight decrease under severe stress compared to moderate stress, but higher than control. Maximum protein content was recorded at 180 DAP.

At 120 DAP, IISR Pragati (4.08%) followed by Suguna (3.98%) recorded maximum protein content and minimum was observed in IISR Alleppey Supreme (2.76%) under control; While under moderate stress, IISR Prathibha (5.24%) followed by Suguna (5.17%) recorded maximum protein content and minimum was observed in IISR Alleppey Supreme (3.26%) itself while, under severe stress, highest protein content was detected in IISR Pragati (4.75%) followed by IISR

Prathibha (4.64%) and lowest protein content was observed in IISR Alleppey Supreme (2.94%).

At 150 DAP, IISR Prathibha (4.23%) followed by IISR Pragati (4.20%) showed maximum values and minimum was recorded in IISR Alleppey Supreme (3.20 %) under control. Under moderate stress, IISR Prathibha (5.96%) followed by IISR Prabha (5.84%) had maximum protein content and IISR Alleppey Supreme (3.84%) had the lowest. Whereas under severe stress, highest protein content was recorded in IISR Prabha (5.26%) followed by IISR Prathibha (5.20%) and lowest protein content was in Acc 66 (3.57%).

At 180 DAP, IISR Prathibha exhibited the highest protein content (4.50%), closely followed by IISR Pragati at 4.42%, while Acc 66 (3.35%) had the lowest protein content under control condition. When subjected to moderate stress, IISR Prathibha followed by SL 10 had the highest protein content of 6.79% and 6.64%, respectively, and IISR Alleppey Supreme (4.53%) recorded the lowest. Under severe stress, IISR Prathibha retained the highest protein content at 6%, followed by IISR Pragati at 5.95%, while Acc 66 (4.16%) had the lowest protein content (Table 4.2.4).

Protein content increased from 16% (Acc 66) to 41.7% (SL 5) and from 6.5 % (IISR Alleppey Supreme) to 19.4% (SL 5) at 120 DAP, At 150 DAP, it increased from 20% (IISR Alleppey Supreme) to 49.9 % (Suvarna) and from 8.8 % (Acc 66) to 29.8% (Suvarna) under moderate and severe stress respectively. At 180 DAP, protein content increased from 31.7% (IISR Alleppey Supreme) to 63.6 % (Suvarna) under moderate stress and from 23.1% (IISR Kedaram) to 36.5% (IISR Prabha) under severe stress.

This data suggests that different varieties of the crop respond differently to stress conditions in terms of protein accumulation, with tolerant genotypes generally maintaining higher protein content compared to other genotypes across all stress levels under all developmental stages. Additionally, the impact of stress is evident as the protein content tends to increase under drought stress but as the severity of stress increases there was a decline in protein concentration.

Table 4.2.4 Protein content (%) in turmeric genotypes under different water regimes.

Genotype	120 DAP			150 DAP			180 DAP		
	100% FC	50% FC	25% FC	100% FC	50% FC	25% FC	100% FC	50% FC	25% FC
IISR Pragati	4.08^{defg}	5.12^{a}	4.75^{b}	4.20^{jkl}	5.64^{abc}	5.10^{ef}	4.42^{lmno}	6.45^{bc}	5.95^{efg}
IISR Prathibha	3.93^{gh}	5.24^{a}	4.64^{bc}	4.23^{ijkl}	5.96^{a}	5.20^{cdef}	4.50^{lmn}	6.79^{a}	6.01^{defg}
IISR Prabha	3.81^{ghij}	5.14^{a}	4.45^{bcd}	4.10^{jkl}	5.84^{a}	5.26^{bcdef}	4.27^{lmno}	6.58^{abc}	5.82^{efgh}
SL 5	3.24^{klmn}	4.59^{bc}	3.87^{ghi}	3.65^{mnop}	5.36^{bcde}	4.65^{ghi}	3.84^{pq}	6.11^{de}	5.19^{i}
Suvarna	3.13^{lmno}	4.37^{cde}	3.59^{hijk}	3.46^{opq}	5.18^{def}	4.49^{hij}	3.69^{qr}	6.04^{def}	4.90^{ij}
SL 10	3.36^{klm}	4.61^{bc}	4.00^{efg}	3.84^{lmno}	5.55^{abcd}	4.94^{efg}	4.20^{no}	6.64^{ab}	5.59^{h}
Rajendra Sonia	3.52^{ijkl}	4.87^{bc}	4.12^{defg}	4.00^{klm}	5.64^{ab}	4.86^{fgh}	4.12^{op}	6.28^{cd}	5.52^{h}
Suguna	3.98^{fg}	5.17^{a}	4.54^{bc}	4.17^{jkl}	5.80^{a}	5.00 efg	4.30^{lmno}	6.63^{ab}	5.69^{gh}
NDH 1	3.47^{jklm}	4.35^{cdef}	4.05^{gh}	3.59^{pq}	4.97^{efg}	4.30^{ijk}	3.86^{pq}	5.79^{fgh}	5.12^{ij}
IISR Kedaram	2.91^{nop}	3.38^{lmno}	3.18^{lmno}	3.36^{pq}	4.10^{jkl}	3.89^{klmn}	3.57^{qrs}	4.83^{jk}	4.40^{lmno}
Acc 66	2.94^{nop}	3.41^{klm}	3.13^{mnop}	3.28^{pq}	3.98^{klm}	3.57^{nopq}	3.35^{s}	4.58^{kl}	4.16^{op}
IISR Alleppey Supreme	2.76^{p}	3.26^{klmn}	2.94^{op}	3.20^{q}	3.84^{lmno}	3.62^{mnopq}	3.44^{rs}	4.53^{klm}	4.25^{mno}
General mean	3.93			4.48			5.04		
CV (%)	5.79			5.86			3.9		
CD (P=0.05)	0.37			0.43			0.32		

The mean value (n=3) followed by different letters within a column are significantly different ($P< 0.05$) based on ANOVA test.

The molecular mechanism of plant response to stress is a complex mechanism which involves sensing, signal transduction, transcription and protein translation and modification which involve multiple genes, signalling pathways and metabolic processes (Swain *et al.,* 2023). The overproduction of toxic reactive oxygen free radicals will lead to a variety of harmful non adaptive changes effects including lipid peroxidation, protein denaturation, DNA strand breakage and inactivation of photosynthetic enzymes and other macromolecules in the cell

(Farooq *et al.,* 2009, 2011; Gholami *et al.*, 2012; Silva *et al.*, 2012; Ibrahim *et al.*, 2019).

Protein abundance of multiple pathways is differentially affected in plants under drought stress (Kosova *et al.*, 2011). Heat shock proteins were found up-regulated upon drought. Same time, the proteins of starch metabolism were found to be significantly reduced in maize cultivars Schulz *et al.* (2021). Total soluble protein content in maize (*Zea mays* L.) as well as in rice cultivars increased with mild to moderate dehydration but showed a downward trend with severe stress (Mohammad khani & Heidari, 2008; Mohammadi *et al.*, 2012) which was in agreement with our study. Conversely, a decline in protein concentration was reported in wheat cultivars under dehydration stress (Ghaffar *et al.*, 2023).

Under water deficit stress generally up-regulation of 1,4-α-glucan branching enzyme, alcohol dehydrogenase (ADH), cold-regulated LTCOR12, asparagine synthetase, cysteine protease, trypsin inhibitors, xylose isomerase, and sucrose synthase and a down-regulation of genes encoding proline-rich cell wall protein, aquaporin and several photosynthesis-related proteins was observed at transcript level (Larcher, 2003; Triboulot *et al.,* 2007, Kosova *et al.,* 2011).

In our study, there was higher accumulation of proteins under moderate stress which may include defence or regulatory proteins. While under severe stress there was significant decline in protein content because of higher degradation of proteins due to increased oxidative stress.

4.2.2.7 Effect of drought on total phenol content in leaf

There was an increase in the phenol concentration in the stress treated samples as compared to the control and it increased with stress intensity. The phenol content varied widely among the genotypes across all water regimes and all the stages in turmeric leaves. Plants to combat the abiotic stress conditions will produce a plethora of defence metabolites (Parvaiz & Satyawati, 2008; Ramakrishna & Ravishankar, 2011). Among them, the plant phenolic compounds are playing a vital role in coping with the abiotic stresses. Under stressful conditions, these phenolics are drastically accumulated in the plant for survival (Sharma *et al*., 2019).

At 120 DAP, SL 10 (5.67 mg GAE/g) followed by IISR Prathibha (5.58 mg GAE/`g) recorded maximum phenol content and minimum was observed in IISR Kedaram (4.85 mg GAE/g) under control. Under moderate stress, IISR Prathibha (8.07 mg GAE/g) and SL 10 (8.02 mg GAE/g) had maximum phenol content and the minimum in IISR Kedaram (5.54 mg GAE/g). Under severe stress also, highest phenol content was observed in IISR Prathibha (8.60 mg GAE/g) followed by IISR Pragati (8.56 mg GAE/g) and lowest concentration was detected in IISR Alleppey Supreme (6.19 mg GAE/g). At 150 DAP, SL 10 (6.19 mg GAE/g) and IISR Prathibha (6.02 mg GAE/g) showed maximum phenol content and the lowest in IISR Kedaram (5.04 mg GAE/g) under control. Under moderate stress, SL 10 (9.14 mg GAE/g) and IISR Prathibha (9.03 mg GAE/g) had maximum phenol content and the minimum in IISR Kedaram (5.90 mg GAE/g). While under severe stress it was maximum in SL 5 (9.73 mg GAE/g) followed by IISR Prathibha (9.64 mg GAE/g) and lowest phenol content was observed in IISR Kedaram (6.72 mg GAE/g).

Likewise, at 180 DAP, IISR Pragati (7.12 mg GAE/g) followed by SL 10 (7.10 mg GAE/g) recorded maximum phenol content and the minimum was observed in IISR Kedaram (5.38 mg GAE/g) under control. Under moderate stress, IISR Prathibha (10.29 mg GAE/g) and IISR Prabha (10.17 mg GAE/g) had maximum phenol content and lowest was recorded in Acc 66 (6.80 mg GAE/g) whereas under severe stress, highest phenol content was found in IISR Pragati (12.15 mg GAE/g) and IISR Prabha (12.05 mg GAE/g) and lowest phenol content was observed in IISR Kedaram (7.45 mg GAE/g) at 180 DAP (Table 4.2.5).

Phenol content showed a rise from 14% (IISR Kedaram) to 45% (IISR Prathibha) under moderate stress and from 25% (Acc 66) to 63% (IISR Pragati) under severe stress compared to control at 120 DAP. It increased from 17% (IISR Kedaram) to 50% (IISR Prathibha) and from 33% (IISR Kedaram) to 68% (SL 5) at 150 DAP under moderate and severe stress respectively compared to control. At 180 DAP, the phenol content rose from 24% (Acc 66) to 53% (SL 10) and from 39%

(IISR Kedaram) to 74% (IISR Prathibha) over control respectively under moderate and severe stress.

Table 4.2.5 Total phenol content (mg GAE/g FW) in turmeric genotypes under different water regimes

Genotype	**120 DAP**			**150 DAP**			**180 DAP**		
	100% FC	**50% FC**	**25% FC**	**100% FC**	**50% FC**	**25% FC**	**100% FC**	**50% FC**	**25% FC**
IISR Pragati	5.25^{st}	7.36^{gh}	8.56^{a}	5.71^{qrs}	8.31^{i}	9.32^{cd}	7.12^{q}	9.98^{j}	12.15^{a}
IISR Prathibha	5.58^{opq}	8.07^{bcd}	8.6^{a}	6.02^{op}	9.03^{ef}	9.64^{ab}	6.74^{t}	10.29^{gh}	11.74^{b}
IISR Prabha	5.47^{qr}	7.72^{e}	8.2^{b}	5.86^{qr}	8.48^{hi}	9.4^{bc}	6.96^{rs}	10.17^{hi}	12.05^{a}
Suvarna	5.11^{t}	6.82^{k}	7.5^{fg}	5.43^{tu}	7.35^{l}	8.65^{gh}	6.27^{vw}	8.85^{m}	10.43^{g}
SL 5	5.36^{rs}	7.6^{ef}	8.16^{bc}	5.81^{pq}	8.52^{hi}	9.73^{a}	6.82^{st}	10.14^{i}	11.32^{c}
SL 10	5.67^{op}	8.02^{cd}	8.48^{a}	6.19^{no}	9.14^{de}	9.52^{abc}	7.1^{q}	11^{d}	11.4^{c}
Rajendra Sonia	5.32^{rs}	7.48^{fg}	7.92^{d}	5.56^{rst}	7.95^{j}	8.92^{ef}	6.53^{u}	9.49^{k}	10.76^{e}
Suguna	5.14^{t}	7.04^{ij}	7.6^{ef}	5.47^{stu}	7.62^{k}	8.83^{fg}	6.34^{v}	9.23^{l}	10.62^{f}
NDH 1	5.2^{st}	6.96^{jk}	7.19^{hi}	5.39^{tu}	7.36^{l}	8.45^{hi}	6.17^{w}	8.75^{m}	9.87^{j}
IISR Kedaram	4.85^{u}	5.54^{m}	6.24^{m}	5.04^{v}	5.9^{pq}	6.72^{m}	5.38^{z}	6.8^{t}	7.45^{p}
Acc 66	5.14^{t}	5.96^{n}	6.43^{l}	5.33^{tu}	6.41^{n}	7.39^{kl}	5.76^{x}	7.12^{q}	8.16^{n}
IISR Alleppey Supreme	4.9^{u}	5.73^{o}	6.19^{m}	5.31^{u}	6.32^{n}	7.16^{l}	5.54^{y}	7.02^{qr}	7.8^{o}
General mean	6.62			7.31			8.59		
CV (%)	1.55			2.07			1.01		
CD (P=0.05)	0.17			0.25			0.14		

The mean value (n=3) followed by different letters within a column are significantly different ($P< 0.05$) based on ANOVA test**.**

Our result revealed phenol content elevation under drought stress. This result was in consistent with total phenol content elevation under drought stress in ginger (Ghasemzadeh *et al.,* 2010; Aydinşakir *et al*., 2023) and in durum wheat (Laddomada *et al*., 2021). Under drought stress, plants are producing polyphenols to cope with stress conditions by controlling the water ions flux so there is an increase in phenol content with stress intensity (Mirás *et al*., 2017; González *et al*., 2018). Also tolerant varieties had higher phenol content as reported in grapevine (Król *et al*., 2015) and in rice (Quan *et al*., 2016; Gupta & De, 2017).

In response to drought, an increased level of phenolic compounds is noticed in various plants. For instance, an increased level of anthocyanins were found to be induced by short-term drought stress in *Arabidopsis* (Kovinich *et al*., 2015), moderate stress induced higher phenolic content in two ginger species (commercial ginger and African ginger) (Gatabazi *et al*., 2022). Similar response was observed in olive, fern *Salvia sinaloensis* (101% flavonoid and 139% total phenol) and rice (Machado *et al.,* 2013; Quan *et al*., 2016; Caser *et al.,* 2018).

Plants synthesize a large number of secondary metabolites including flavonoids, terpenoids, and phenolic compounds which protect plants from disease/damage and also contribute to the plant's colour, aroma and flavour (Pandey, 2009; Davies *et al.,* 2018). The elevated level of non-enzymatic antioxidants such as phenolic compounds during oxidative stress potentially protects plants from oxidative damage (Ashraf *et al*., 2009; Ashraf *et al.,* 2011; Dehghanian *et al*., 2022; Kumar *et al.,* 2023). Polyphenols scavenge free radical damage on biomolecules through H-atom donation, electron transfer or by directly participating in ROS scavenging activity of hydroxy groups, as found in the aromatic B-ring of flavonoids (Sakihama, 2002; Aboul-Enein, 2007; Dehghanian *et al.,* 2022). Phenolic compounds also alerts plant tissue to attain defensive drought stress mitigating processes via systemic induction of drought response (Idrees *et al.,* 2010; Kermani *et al*., 2019; Yadav *et al.,* 2021).

Increased polyphenols under water stress conditions in cotton genotypes resulted in a lower degradation in Chlorophyll a and b (Shah *et al.,* 2021). Likewise, olive cultivars 'Gaidourelia' which showed higher phenolic concentration particularly at 33% field capacity resulted in a lower extend of lipid peroxidation (Petridis *et al*., 2012). Secondary metabolite production under water deficit may vary with genotype, intensity, duration of stress and stage of development. Polyphenols, which provide resistance to the plant by serving as ROS scavenger and signalling molecule are protective in function and might be used as a biochemical indicator of drought tolerance (Warzecha *et al*., 2023).

In this study there was significant increase in polyphenols with stress intensity. Its concentration was higher at late stage of development. Tolerant genotypes accumulated higher quantity of phenols compared to susceptible ones which helped them endure oxidative stress and degradation of important biomolecules and maintain cellular integrity, which indicate their higher investment in these defensive compounds to enhance their resilience and chances of survival.

4.2.2.8 Effect of drought on photosynthetic pigments

The total chlorophyll and carotenoids showed a decrease under water stress as compared to the control. The pigment concentration showed significant variation among the treatments also.

4.2.2.8.1 Chlorophyll

The highest pigment concentration was recorded in IISR Pragati (1.70 mg/g) followed by IISR Prabha (1.68 mg/g) and lowest in Acc 66 (1.27 mg/g) in control whereas under moderate stress, it was maximum in SL 10 (0.97 mg/g) followed by IISR Prabha (0.95 mg/g) while lowest chlorophyll content was observed in Acc 66 (0.45 mg/g). In case of severe stress chlorophyll pigment concentration again showed a decline when compared to moderate stress where highest concentration was shown by SL 10 (0.76 mg/g) followed by IISR Prabha (0.74 mg/g) while lowest concentration was shown by Acc 66 (0.24 mg/g) at 120 DAP.

At 150 DAP, The highest chlorophyll concentration was found in Rajendra Sonia (2.27 mg/g) followed by IISR Prabha (2.18 mg/g) while the lowest concentration was recorded in Acc 66 (1.43 mg/g) in control s whereas under moderate stress, Rajendra Sonia (1.42 mg/g) followed by IISR Prathibha (1.38 mg/g) had maximum chlorophyll content and minimum was in recorded in Acc 66 (0.73 mg/g). In case of severe stress, pigment concentration was higher in Rajendra Sonia (1.12 mg/g) followed by IISR Prabha (1.09 mg/g) and the minimum chlorophyll content was observed in Acc 66 (0.54 mg/g).

Similarly, the highest chlorophyll concentration was found in SL 10 (2.43 mg/g) followed by IISR Prabha (2.41 mg/g) and lowest in Acc 66 (1.92 mg/g) in control whereas under moderate stress, SL 10 (1.80 mg/g) recorded maximum pigment concentration followed by IISR Prathibha (1.75 mg/g) and minimum concentration was found in IISR Alleppey Supreme (1.00 mg/g). In case of severe stress, pigment concentration was highest in SL 10 (1.39 mg/g) and IISR Prabha (1.36 mg/g) and minimum value was observed in IISR Alleppey Supreme (0.86 mg/g) at 180 DAP (Table 4.2.6).

Chlorophyll content decreased from 35% (SL 5) to 65% (Acc 66) and from 51% (SL 5) to 81% (Acc 66) at 120 DAP under moderate and severe stress respectively. Whereas, it decreased from 32% (SL 5) to 52% (IISR Alleppey Supreme) and from 48% (Suvarna) to 62% (Acc 66), at 150 DAP; and from 26% (SL 10) to 53% (IISR Alleppey Supreme) and 43% (Suvarna and SL 10) to 60% (IISR Alleppey Supreme), at 180 DAP, under moderate and severe stress respectively.

Table 4.2.6 Effect of drought on the development of total chlorophyll content (mg g^{-1}) in turmeric plants

Genotype	120 DAP			150 DAP			180 DAP		
	100% FC	**50% FC**	**25% FC**	**100% FC**	**50% FC**	**25% FC**	**100% FC**	**50% FC**	**25% FC**
IISR Pragati	1.70^{a}	0.88^{l}	0.66^{rs}	1.90^{e}	1.15^{l}	0.89^{p}	2.35^{c}	1.67^{l}	1.26^{st}
IISR Prathibha	1.65^{b}	0.93^{k}	0.69^{pq}	2.12^{c}	1.38^{k}	1.02^{n}	2.40^{ab}	1.75^{j}	1.35^{pq}
IISR Prabha	1.68^{a}	0.95^{k}	0.74^{o}	2.18^{b}	1.36^{k}	1.09^{m}	2.41^{ab}	1.73^{j}	1.36^{p}
Suvarna	1.29^{h}	0.75^{no}	0.55^{u}	1.52^{i}	0.85^{q}	0.78^{s}	2.17^{f}	1.41^{o}	1.15^{v}
SL 5	1.39^{f}	0.90^{l}	0.68^{qr}	2.00^{d}	1.37^{k}	0.95^{o}	2.37^{bc}	1.68^{kl}	1.30^{rs}
SL 10	1.62^{c}	0.97^{j}	0.76^{n}	1.73^{f}	1.02^{n}	0.79^{rs}	2.43^{a}	1.80^{i}	1.39^{op}
Rajendra Sonia	1.65^{b}	0.85^{m}	0.64^{st}	2.27^{a}	1.42^{j}	1.12^{m}	2.28^{d}	1.60^{m}	1.23^{t}
Suguna	1.64^{bc}	0.88^{l}	0.62^{t}	1.70^{g}	0.98^{o}	0.75^{t}	2.24^{e}	1.51^{n}	1.19^{u}
NDH 1	1.47^{e}	0.71^{p}	0.46^{v}	1.52^{i}	0.81^{r}	0.66^{u}	2.06^{g}	1.32^{qr}	1.10^{w}
IISR Kedaram	1.30^{g}	0.51^{w}	0.29^{z}	1.47^{k}	0.70^{w}	0.57^{x}	1.98^{jk}	1.09^{x}	1.01^{y}
Acc 66	1.27^{i}	0.45^{x}	0.24^{A}	1.45^{i}	0.73^{w}	0.54^{y}	1.92^{l}	1.05^{xy}	0.92^{z}
IISR Alleppey Supreme	1.58^{d}	0.61^{u}	0.43^{y}	1.60^{h}	0.76^{v}	0.62^{x}	2.14^{h}	1.00^{y}	0.86^{z}
General mean	0.93			1.18			1.56		
CV (%)	1.46			1.42			1.6		
CD (P=0.05)	0.022			0.03			0.04		

The mean value (n=3) followed by different letters within a column are significantly different (P< 0.05) based on ANOVA test.

Total chlorophyll content increased from 120 DAP to 180 DAP in the current study. Similar result was reported by Padmapriya *et al.* (2007). Chlorophyll content decreased with stress intensity and it is in tune with the previous reports such as in olive plant (Khaleghi *et al.,* 2012) and rice (Saha *et al*., 2020). However, drought tolerant genotypes showed lesser reduction in chlorophyll content and it is in agreement with earlier reports (Monteoliva *et al*., 2021; Khayatnezhad & Gholamin, 2012).

Drought induced stomatal closure and reactive oxygen species production cause destruction of photosynthetic apparatus lowering carbon fixation under drought (Yamance *et al.,* 2003). Leaf internal CO_2 reduction also directs more electrons to form ROS and promotes photorespiration which further affects carbon fixation (Reddy *et al.,* 2004; Correia *et al.,* 2006). Photosynthetic pigments primarily, chlorophyll and carotenoids are important to plants for harvesting light and production of reducing powers. Additionally, carotenoids safeguard the photosynthetic apparatus by dissipating excess excitation energy into heat (Yi *et al*., 2016). The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress as a result of pigment photo-oxidation by ROS (Kannan & Kulandaivelu, 2011). Both the chlorophyll a and b are prone to soil dehydration (Farooq *et al*., 2009). Chlorophyll a and total chlorophyll were decreased to about 51.55% and 46.86% respectively, in *Olea europea* plants that received 40% irrigation compared with 100% irrigated crop (Khaleghi *et al.,* 2012). Exposure of two olive cultivars, Chemlali and Chetoui, to reduced irrigation lowered Chl (a + b) contents to about 29% and 42%, respectively (Guerfel *et al.,* 2009).

Reduction in chlorophyll content, resulted in significant decrease in shoots fresh (59.45%) and dry (50.83%) weights, in *Aegilops* and *Triticum* variety (Alireza *et al.,* 2017). Genotypes with the highest chlorophyll index contributed to a higher yield compared to other genotypes under drought stress in maize (Majid & Roza, 2012) and at grain filling stage of rice (Moonmoon *et al.,* 2020).

Drought induced reduction of chlorophyll content is reported in many plants including marigold (Asrar & Elhindi, 2011), *Catharanthus roseus* (Amirjani, 2013), *Thymus daenensis* (Malekpour *et al*., 2016), blue grass seed lings (Niu & Ma, 2018), rice (Nahar *et al.,* 2018) and cotton (Shah *et al.,* 2021) which resulted in a lower yield. Similarly, considerable decline in leaf osmotic potential led to chlorophyll degradation in turmeric that ultimately resulted in rhizome yield reduction (Chindakovid *et al.,* 2022).

In the present research, total chlorophyll content decreased with stress intensity. Tolerant genotypes exhibited lower degradation of chlorophyll pigments as compared to susceptible ones owing to their enhanced resistance mechanism which helped them maintain carbon fixation and lower the reduction in yield.

4.2.2.8.2 Carotenoids

Carotenoid content varied significantly among the genotypes between control and stress treatments but among the growth stages, there was no significant variation. Under moderate stress conditions, there was a decrease compared to control, which was further exacerbated under severe stress. Rajendra Sonia (0.37 mg/g) and IISR Prathibha (0.36 mg/g) had maximum concentration of carotenoids under control condition and minimum concentration was observed in IISR Kedaram (0.27 mg/g). It was highest in Suguna (0.29 mg/g) and SL 5 (0.27 mg/g) while lowest in IISR Alleppey Supreme (0.15 mg/g) under moderate stress and a maximum of 0.24 mg/g was found in Suguna followed by 0.23 mg/g in IISR Prathibha and a minimum of 0.12 mg/g in IISR Kedaram under severe stress at 120 DAP.

At 150 DAP, IISR Prabha (0.46 mg/g) followed by Rajendra Sonia (0.44 mg/g) had maximum amount of carotenoids, while SL 5 (0.33 mg/g) had minimum amount of carotenoids under control conditions. Under moderate stress condition, it was highest in IISR Prabha (0.38 mg/g), which was on par with IISR Prathibha (0.36 mg/g) and lowest in IISR Alleppey Supreme (0.23 mg/g) under moderate stress condition. Whereas under severe stress, highest carotenoid content was observed in IISR Prabha (0.34 mg/g) and Rajendra Sonia (0.33 mg/g) and a minimum of 0.19 mg/g was observed in IISR Alleppey Supreme.

Similarly at 180 DAP, IISR Pragati (0.53 mg/g) had maximum amount of carotenoids followed by IISR Prabha (0.50 mg/g) while IISR Kedaram (0.38 mg/g) had minimum amount of carotenoids under control. Under moderate stress condition, carotenoid concentration was highest in IISR Pragati (0.46 mg/g) and IISR Prabha (0.44 mg/g) and the minimum value was observed in IISR Kedaram (0.29 mg/g).Whereas under severe stress, it was highest in IISR Pragati (0.46 mg/g) which was on par with IISR Prabha (0.44 mg/g) and lowest concentration was found in IISR Kedaram (0.26 mg/g) (Table 4.2.7).

The carotenoid content decreased from 17.3% (Suvarna) to 52.4% (IISR Alleppey Supreme) and from 31.8% (IISR Prabha) to 60.7% (IISR Alleppey Supreme) respectively over control under moderate and severe stress at 120 DAP. Whereas it declined from 17 % (IISR Prathibha) to 36.7% (IISR Alleppey Supreme) under moderate stress and 24.8% (IISR Prabhha) to 46.7 % (IISR Alleppey Supreme) under severe stress at 150 DAP. At 180 DAP, the lowest carotenoid concentration reduction was found in IISR Prabha (13%) while maximum reduction was observed in susceptible ones such Acc 66 (26.4%) under moderate stress, and there was a reduction from 17.7% (NDH 1) to 35.3% (Acc 66) under severe stress.

This result was in consistent with carotenoid content variation under drought condition in rosemary (Munné-Bosch & Alegre, 2000) and maize (Mohammadkhani & Heidari, 2007). Carotenoids can protect photosynthetic apparatus and chlorophylls by scavenging ROS through xanthophyll cycle (Pérez-Pérez *et al.*, 2012).

Table 4.2.7 Effect of drought on carotenoid pigments (mg g^{-1}) in turmeric plants

Genotype	120 DAP			150 DAP			180 DAP		
	100% FC	**50% FC**	**25% FC**	**100% FC**	**50% FC**	**25% FC**	**100% FC**	**50% FC**	**25% FC**
IISR Pragati	0.31^{bcdef}	0.25^{hijk}	0.19^{mnop}	0.41^{abcd}	0.33^{efghi}	0.30^{hijkl}	0.53^{a}	0.46^{bcdef}	$0.42^{defghijk}$
IISR Prathibha	0.36^{ab}	0.26^{fghijk}	0.23^{jklmn}	0.43^{ab}	0.36^{cdefg}	0.31^{fghijk}	0.49^{abc}	$0.42^{defghijk}$	$0.40^{fghijklm}$
IISR Prabha	0.32^{bcde}	0.24^{ijkl}	0.22^{klmno}	0.46^{a}	0.38^{bcde}	0.34^{efgh}	0.50^{ab}	$0.44^{bcdefgh}$	$0.41^{efghijkl}$
Suvarna	$0.27^{defghij}$	0.23^{jklmn}	0.18^{nop}	0.37^{cde}	0.30^{ghijkl}	0.27^{jklmn}	$0.43^{cdefghij}$	$0.37^{hijklmnop}$	$0.34^{lmnopqr}$
SL 5	0.34^{abc}	0.27 efghijk	0.19^{lmnop}	0.33^{efghi}	0.26^{jklmn}	0.24^{mno}	$0.37^{ijklmnop}$	0.32^{opqrs}	0.30^{pqrs}
SL 10	0.28^{defghi}	0.22^{klmno}	0.17^{opq}	0.36^{cdefgh}	0.28^{ijklm}	0.25^{klmno}	$0.43^{cdefghi}$	$0.36^{jklmnopq}$	0.33^{nopqr}
Rajendra Sonia	0.37^{a}	0.26^{ghijk}	0.22^{klmno}	0.44^{a}	0.35^{defgh}	0.33^{efghi}	0.48^{abcd}	$0.41^{efghijkl}$	$0.39^{fghijklmn}$
Suguna	0.35^{ab}	0.29^{fghijk}	0.24^{ijkl}	0.41^{abc}	0.31^{hijkl}	0.30^{hijkl}	0.47^{abcde}	$0.39^{klmnopq}$	$0.37^{ghijklmno}$
NDH 1	0.30^{cdefg}	0.23^{jklm}	0.19^{mnop}	0.38^{bcde}	0.30^{ghijkl}	0.28^{ijklm}	0.44^{bcdefg}	$0.37^{ijklmnop}$	$0.36^{ijklmnop}$
IISR Kedaram	$0.27^{defghij}$	0.17^{pq}	0.12^{q}	0.35^{defgh}	0.25^{jklmno}	0.20^{o}	$0.38^{ghijklmno}$	$0.29^{ghijklmno}$	0.26^{s}
Acc 66	0.29^{cdefgh}	0.18^{mnop}	0.15^{pq}	0.34^{efgh}	0.24^{mno}	0.21^{no}	0.46^{bcdef}	0.34^{mnopqr}	0.30^{pqrs}
IISR Alleppey Supreme	0.32^{bcd}	0.15^{pq}	0.13^{q}	0.36^{cdef}	0.23^{mno}	0.19^{o}	$0.41^{defghijk}$	0.32^{opqrs}	0.28^{rs}
General mean	0.243			0.32			0.38		
CV (%)	12.7			11.33			11.08		
CD (P=0.05)	0.03			0.06			0.07		

The mean value (n=3) followed by different letters within a column are significantly different (P< 0.05) based on ANOVA test.

Reduction in carotenoid content under drought stress was reported in many crops including cotton (*Gossypium hirsutum* L.) genotypes in which short-term drought treatment caused a decrease in carotenoid content (Parida *et al*., 2007). Conversely, it was increased ($p<0.05$) in maize cultivars under water stress. Higher carotenoid content contributed to less degradation of chlorophyll in 301 var. (tolerant) of maize (Mohammed khani & Heidari, 2008). Cotton genotypes that had maximum amount of carotenoids and polyphenols showed a lower degradation in Chlorophyll a and b (Shah *et al.,* 2021). The carotenoids increased with maturity stage of the crop in African eggplant accessions (Mibei *et al.,* 2017), which was in agreement with our study.

Tolerant rice varieties, with higher total chlorophyll under dehydration stress resulted in higher yield (Nahar *et al.,* 2018). The degree of decrease in chlorophylls and carotenoids was higher in the sensitive cotton genotype (Ca/H 631) as compared to the moderately tolerant genotype (GM 090304) with higher proline, sugar and polyphenols (Parida *et al*., 2007). Variation in chlorophyll and carotenoids under water deficit directly limit photosynthetic potential by disrupting all major components of photosynthesis including the thylakoid electron transport, the carbon reduction cycle and the stomatal control of the CO_2 supply and disturbance of water balance (Allen & Ort, 2001; Farooq *et al.,* 2009).

The overall amount of chlorophyll and carotenoids in turmeric plants subjected to stress decreased compared to those in the control group. Under moderate stress conditions, there was a decrease compared to control, which was further exacerbated under severe stress. In general, tolerant genotypes lowered percentage reduction in chlorophyll and carotenoids compared to susceptible genotypes under moderate and severe stress. This could be due to the protection of photosynthetic apparatus by higher amount of antioxidant systems. Carotenoids also protect cells by ROS scavenging. Which together lead to higher photosynthate production, resulting in better partitioning and rhizome yield in tolerant genotypes under stress condition.

4.2.3 Gene expression analysis.

Drought tolerance of predicted genotypes was validated by relative gene expression analysis under different water regimes (100% FC, 50% FC and 25% FC) in different tissues (leaf and rhizome) with qRT-PCR using genotypes of contrasting response towards drought (tolerance and susceptible) (George *et al.*, 2017). Relative Gene expression analysis was conducted using important drought responsive pathway transcription factors (Shinozaki & Yamaguchi, 1994; 2006).

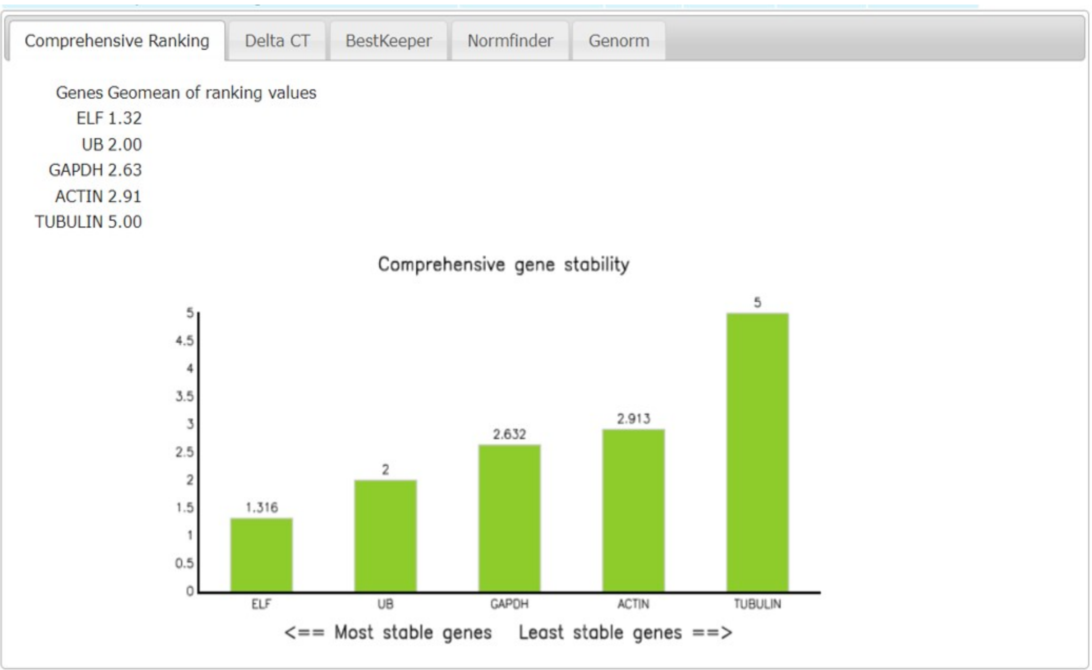
Transcription factors are proteins that regulate downstream gene expression by binding to specific sequences in DNA or other protein complexes. They are potent candidates for engineering stress tolerant plants as a single transcription factor can modulate a large set of genes. Many of drought-responsive transcription factors have been used to improve drought tolerance in different crops such as rice, wheat, soybean, and maize (Nakashima *et al.*, 2014; Krannich *et al.*, 2015). Here we have selected important dedicated pathway TFs for drought response (AP2, bZIP, ABF, bHLH, NAC, WRKY and DREB) and *AQP* gene.

4.2.3.1 Identification of stable reference gene on water stress

Among the five reference genes evaluated to identify the most stable gene (Fig. 4.2.11) *EF-1α* and ubiquitin showed less variability among samples. Analysis by different tools like Delta CT, Best Keeper, NormFinder and Genorm identified EF-1α as the most stable reference gene followed by ubiquitin (Table 4.2.8) as observed by Prashina *et al.* (2021).

Table 4.2.8 Ranking order of stable reference gene predicted using RefFinder

Method	Ranking order (Better-Good-Average)				
	1	2	3	4	5
Delta CT	*EF-1α*	*UBIQUITIN*	*ACTIN*	*GAPDH*	*TUBULIN*
BestKeeper	*EF-1α*	*ACTIN*	*GAPDH*	*UBIQUITIN*	*TUBULIN*
Normfinder	*EF-1α*	*UBIQUITIN*	*ACTIN*	*GAPDH*	*TUBULIN*
Genorm	*UBIQUITIN*	*GAPDH*	*EF-1α*	*ACTIN*	*TUBULIN*
Recommended comprehensive ranking	*EF-1α*	*UB*	*GAPDH*	*ACTIN*	*TUBULIN*



Genes Geomean of ranking values
ELF 1.32
UB 2.00
GAPDH 2.63
ACTIN 2.91
TUBULIN 5.00

Fig. 4.2.11 Expression of five candidate genes by qRT-PCR measured as Ct values and comprehensive ranking of the candidate reference genes predicted by the RefFinder under the water stress condition in turmeric.

Melt curve analysis of eight TFs and *AQP* gene by qRT-PCR after 40 cycles of amplification detected the presence of single peaks indicating that the only expected amplicons were amplified in each case.

The expression levels of each transcription factor under water deficit stress and control conditions were assessed using qRT-PCR analysis. Determining and characterizing the molecular behaviour of these drought-responsive TFs in different water-deficit stress systems is crucial not only for verifying tolerance or highlighting differences between the control and drought treatment conditions, but also for identifying reliable marker genes for studying drought stress in turmeric.

In the present study, the relative expression of the seven TFs and *AQP* gene was analysed using the Ct values obtained from amplification curve of the real time PCR. We have selected important dedicated pathway TFs for drought response (*AP2, bZIP, ABF, bHLH, NAC, WRKY* and *DREB*) leading to synthesis of important proteins involved in drought response (Schinozaki & Yamaguchi, 2006) and *AQP* gene for relative expression study under different water regimes in different genotypes such as IISR Prabha, IISR Prathibha, IISR Pragati, Suvarna, SL 5, SL 10, Rajendra Sonia, Suguna, NDH 1 (tolerant genotypes), IISR Kedaram, Acc 66 and IISR Alleppey Supreme (susceptible genotypes).

4.2.3.2 Differential expression analysis of drought responsive transcription factors

EF-1α and ubiquitin showing least variation among the samples was selected for normalizing the TFs. The differential expressions of the seven TFs were analysed using the selected reference genes *EF-1α* and ubiquitin. the use of more than one stable reference gene with low pair wise variation can give accurate data normalization (Vandesompele *et al*., 2002).The seven TFs and *AQP* gene studied were expressed under both water stress treated and control plants which were unexposed to water stress. The relative expression level of the candidate TFs in plants under different water regimes were analysed in both rhizome and leaf.

The relative expression of seven TFs (*AP2, bZIP, ABF, bHLH, NAC, WRKY* and *DREB*) increased under moderate stress (100%FC). Under severe stress there was a decline in relative expression compared to the expression level at moderate stress condition where ROS production overcomes antioxidant system capacity, and oxidative stress occurs, which results in cytotoxic protein damage, DNA damage, and lipid peroxidation (Yazici *et al*., 2007) and also cause reduction in ABA content which is necessary for signalling in drought response (De Diego *et al*., 2013). The expression was lower in rhizome compared to leaf, which indicate that the major response of crop under drought stress is initiated in leaf as reported by Yang *et al.*

(2021). Similarly the maximum upregulation of TFs studied and down regulation of AQP was at late stages.

Dehydration-responsive expression patterns by relative gene expression analysis of the selected TFs using qRT-PCR has been reported in many crops including chickpea (Borhani *et al.*, 2020) and black pepper (George *et al.*, 2017) where it revealed differential expression between tolerant and susceptible tolerant genotypes.

Abscisic acid (ABA)-responsive element (ABRE)-binding factors (ABFs): *ABF* function in ABA-dependent regulation of gene expression under drought stress (Nakashima *et al.*, 2009; Yoshida *et al.*, 2015). In leaf, At 120 DAP the maximum expression of *ABF* with 35 fold was detected in SL 10 followed by SL 5 (fold change: 27) and it was least expressed in Acc 66 (fold change: 3.4) under moderate stress. While under severe stress highest relative expression was observed in SL 10 (fold: 26.9) followed by SL 5 (fold change: 24.1) and least expression was observed in Acc 66 (fold change: 2.7).

Similarly at 150 DAP, the maximum expression of *ABF* of 46.5 fold was detected in IISR Prathibha and SL 5 (fold change: 44) followed by IISR Prabha (fold change: 38.6) and it was least expressed in Acc 66 (fold change: 8.4) at moderate stress. Under severe stress, the expression was highest in SL 10 (fold change: 35.8) followed by IISR Prathibha (fold change: 35.3) and least expressed in Acc 66 (fold change: 4.6).

The maximum expression of ABF of 76.6 fold was detected at 180 DAP in IISR Prabha followed by SL 10 (fold change: 74.5) and it was least expressed in IISR Alleppey Supreme (fold change: 12.5) under moderate stress. Likewise, expression was highest in IISR Prabha (fold change: 52.3) followed by SL 10 (fold change: 50.9) and lowest in Acc 66 (fold change: 7.6) (Fig. 4.2.12 a).

In case of rhizome, at 120 DAP the maximum expression of *ABF* was detected in SL 10 (fold change: 20.7) followed by SL 5 (fold change: 18.5) and the least expression was observed in Acc 66 (fold change: 2.3) under moderate stress; While, under severe stress highest expression was observed in SL 5(fold change:

14.4) followed by SL 10 (fold change: 11.2) and least expression was detected in Acc 66 (fold change: 1.7).

At 150 DAP, the expression of *ABF* was increased and it was maximum in SL 10 (fold change: 34.1) and SL 5 (fold change: 26.9) followed by IISR Prabha (fold change: 24.6) and it was least expressed in Acc 66 (fold change: 5.2) at moderate stress, while under severe stress condition, highest expression was exhibited by SL 10 (fold: 20.4) followed by IISR Prabha (fold:19.8) and lowest in IISR Alleppey Supreme (fold: 2.5). At 180 DAP, the maximum expression of *ABF* was detected in IISR Prabha (fold change: 41.1) followed by SL 10 (fold change: 39.9) and it was lowest in Acc 66 (fold change: 7.5) under moderate stress. Under severe stress highest expression was in SL 10 (fold change: 25.1) followed by IISR Prathibha (fold change: 21.9) while lowest expression was observed in Acc 66 (fold change: 4.8) (Fig. 4.2.12 b).

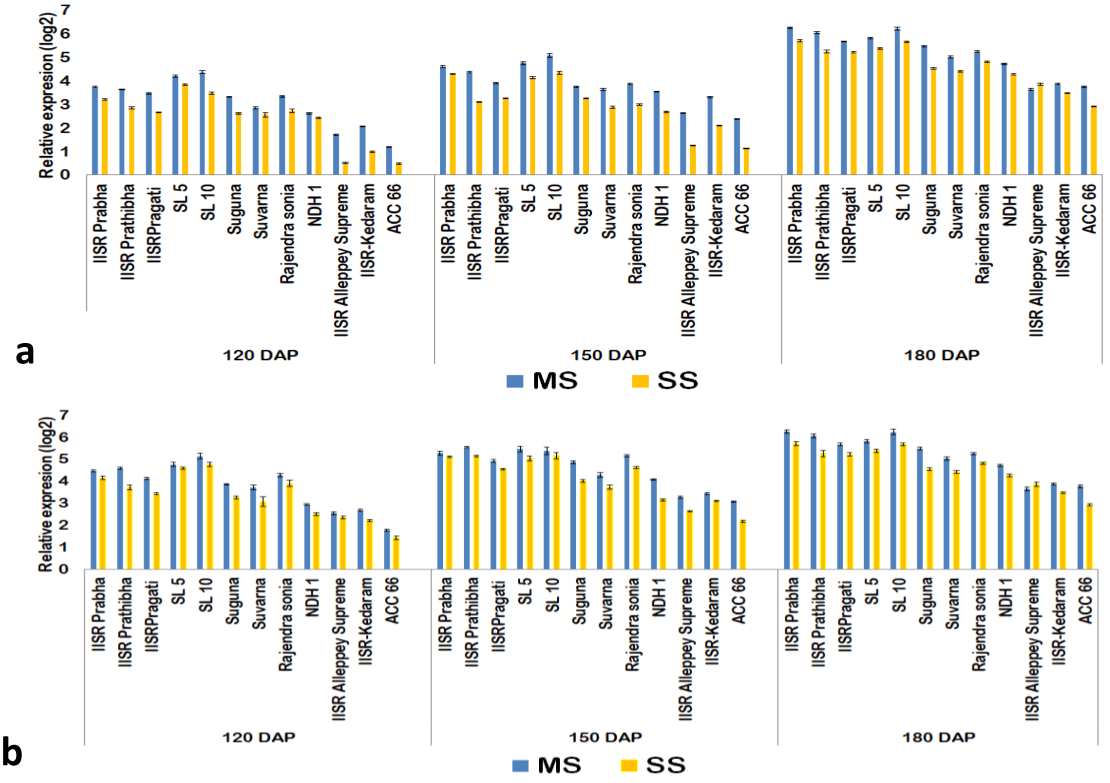


Fig. 4.2.12 Relative expression pattern of *ABF* under moderate and severe stress with respect to control condition in (a) leaf and (b) rhizome at different developmental stages; Results represent the mean ± SE.

(MS = moderately stressed plants irrigated at 50% field capacity; SS = severely stressed plants irrigated at 25% field capacity)

The elevated expression of *ABF* conferring drought tolerance under drought stress is reported in cotton (Liang *et al*., 2016) and Arabidopsis (Fujita *et al*., 2011).

The basic helix–loop–helix (bHLH) family of transcription factors: *bHLH* expression was also increased significantly under drought stress with higher expression in tolerant ones. At 120 DAP, maximum expression was detected in SL 5 (fold change: 19.7) and IISR Prathibha (fold change: 35.7) followed by IISR Prabha (fold change: 18) and it showed the lowest expression in Acc 66 (fold change: 3.18) under moderate stress. Maximum expression of *bHLH* was observed in SL 5 (fold change: 17.4) followed by SL 10 (fold change: 13.73) under severe stress which revealed minimum expression in Acc 66 (fold change: 2.22) in leaf.

At 150 DAP, the maximum expression of *bHLH* 27.47 fold in SL 10 and SL 5 (fold change: 24.4) followed by IISR Prabha (fold change: 20.7) and the least relative expression was detected in Acc 66 (fold change: 5.3). Under severe stress, highest expression was for SL 5 (fold change 19.3) followed by SL 10 (fold change: 21.4) and least relative expression was observed in Acc 66 (fold change: 3.75) in leaf.

The maximum expression of *bHLH* in leaf was detected at 180 DAP in SL 5 (fold change: 40.79) and IISR Prathibha (fold: 35.7) followed by IISR Prabha (fold change: 32.45) and it was lowest in Acc 66 (fold change: 8.51) at moderate stress. Whereas under severe stress, highest expression was in SL 5 (fold change: 36) and IISR Prabha (fold change: 25.81) followed by SL 10 (fold change: 24.76) and lowest in Acc 66 (fold change: 5.70) (Fig. 4.2.13 a).

Similarly, in rhizome, at 120 DAP the maximum expression of *bHLH* was detected in IISR Prabha (fold change: 13.27) and SL 10 (fold change: 11.9) followed by IISR Prathibha (fold change: 9.58) and the lowest expression was observed in Acc 66 (fold change: 2.66) under moderate stress. Likewise, IISR Prabha (fold change: 10.70) and SL 10 (fold change: 9.65) followed by Rajendra Sonia (fold change: 7.31) exhibited maximum relative expression under severe stress while the lowest was observed in Acc 66 (fold change: 1.80).

At 150 DAP the expression of *bHLH* was increased compared to 120 DAP. Maximum expression was detected in SL 10 (fold change: 18.51) and IISR Prabha (fold change: 16.80) followed by IISR Pragati (fold change: 16.22) while the expression was lowest in IISR Alleppey Supreme (fold change: 4.32) at moderate stress. Under severe stress, maximum expression was observed in SL 10 (fold change: 16.34) followed by IISR Prabha (fold change: 13.27) and SL 5 (fold change: 10.13) and expression was lowest in IISR Alleppey Supreme (fold change: 2.95) in rhizome.

The maximum expression of *bHLH* was detected at 180 DAP in SL 10 (fold change: 34.78) and IISR Pragati (fold change: 24.42) followed by IISR Prathibha (fold change: 20.25) and it was least in IISR Alleppey Supreme (fold change: 5.94) at moderate stress. While under severe stress the highest expression was shown by SL 10 (fold change: 19.43) and SL 5 (fold change: 13.74) followed by Suvarna (fold change: 12.55) and was lowest in Acc 66 (fold change: 3.39) in rhizome (Fig.4.2.13 b).

*bHLH t*ranscription factor conferred drought tolerance through regulating stomatal development, photosynthesis and growth in Arabidopsis and its over expression in *Populus euphratica* and *Oryza sativa* increased drought tolerance in transgenic plants (Dong *et al*., 2014; Seo *et al.*, 2011)

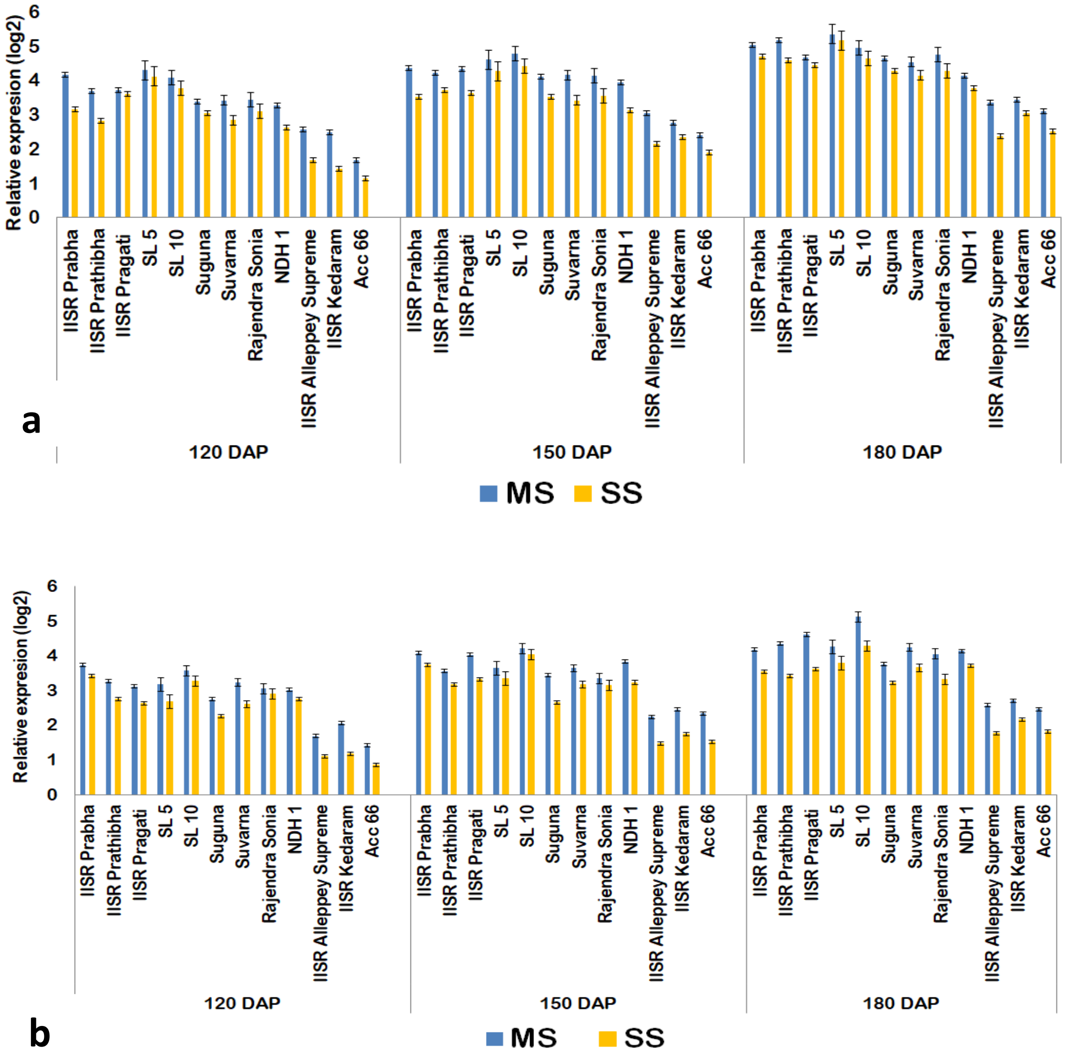


Fig. 4.2.13 Relative expression pattern of *bHLH* under moderate and severe stress with respect to control condition in (a) leaf and (b) rhizome at different developmental stages; Bars represent the mean ± SE.

(MS = moderately stressed plants irrigated at 50% field capacity; SS = severely stressed plants irrigated at 25% field capacity)

basic leucine-zipper (bZIP) superfamily of transcription factors: The up-regulation of bZIP is associated with drought tolerance in plants (Kang *et al*., 2019). In the present study, in leaf, the maximum expression of *bZIP* at 120 DAP was detected in IISR Pragati (fold change: 22) and SL 5 (fold change: 20.67) followed by IISR Prathibha (fold change: 19.03) and it was least in IISR Kedaram (fold change: 4.9) at moderate stress. At severe stress, expression was highest in SL 5 (fold change: 11.79) followed by IISR Pragati (fold change: 9.98) and least expressed in Acc 66 (fold change: 2.35) in leaf.

At 150 DAP, the maximum relative expression of *bZIP* was detected in IISR Pragati (fold change: 39.67) and SL 5 (fold change: 35.5) followed by Rajendra Sonia (fold change: 32.90) and it was lowest in Acc 66 (fold change: 6.02) at moderate stress. At severe stress, expression was highest in IISR Pragati (fold change: 28.64) followed by SL 5 (fold change: 24.76) and was least in Acc 66 (fold change: 3.43).

The maximum expression of *bZIP* was detected at 180 DAP in IISR Pragati (fold change: 71) followed by IISR Prathibha (fold change: 55.3) and it was least in IISR Alleppey Supreme (fold change: 10.5) at moderate stress. While under severe stress, expression was highest in IISR Pragati (fold change: 49.5) followed by SL 5 (fold change: 37.3) and least in Acc 66 (fold change: 5.46) in leaf (Fig. 4.2.14 a).

In case of rhizome, the maximum expression of *bZIP* at 120 DAP was detected in IISR Pragati (fold change: 15.24) followed by IISR Prathibha (fold change: 13.64) followed by SL 10 (fold change :14.12) and it was lowest in IISR Kedaram (fold change: 3.34) at moderate stress; SL 10 (fold change: 9.13) followed by IISR Prathibha (fold change: 7.31) and SL 5 (fold change:6.50) showed maximum expression under severe stress whereas Acc 66 exhibited the lowest expression (fold change: 2.39).

At 150 DAP maximum expression of *bZIP* was detected in IISR Pragati (fold change: 23.7) and SL 10 (fold change: 21.86) followed by Rajendra Sonia (fold change: 19.26) and it was lowest in Acc 66 (fold change: 4.72) under moderate stress. Whereas under severe stress, it was highest in SL 10 (fold change: 25.11) followed by IISR Pragati (fold change: 20.1) and lowest in IISR Kedaram (fold change: 2.68) in rhizome under moderate and severe stress respectively.

The maximum expression of *bZIP* was detected at 180 DAP in IISR Pragati (fold change: 37.79) and IISR Prabha (fold change: 36.50) followed by SL 10 (fold change: 34.78) while the lowest in IISR Alleppey Supreme (fold change: 8.06) at moderate stress. Under severe stress, the expression was highest in IISR Pragati (fold change: 25.11) followed by SL 10 (fold change: 20.1) whereas it was least in Acc 66 (fold change: 4.32) in rhizome (Fig. 4.2.14 b).

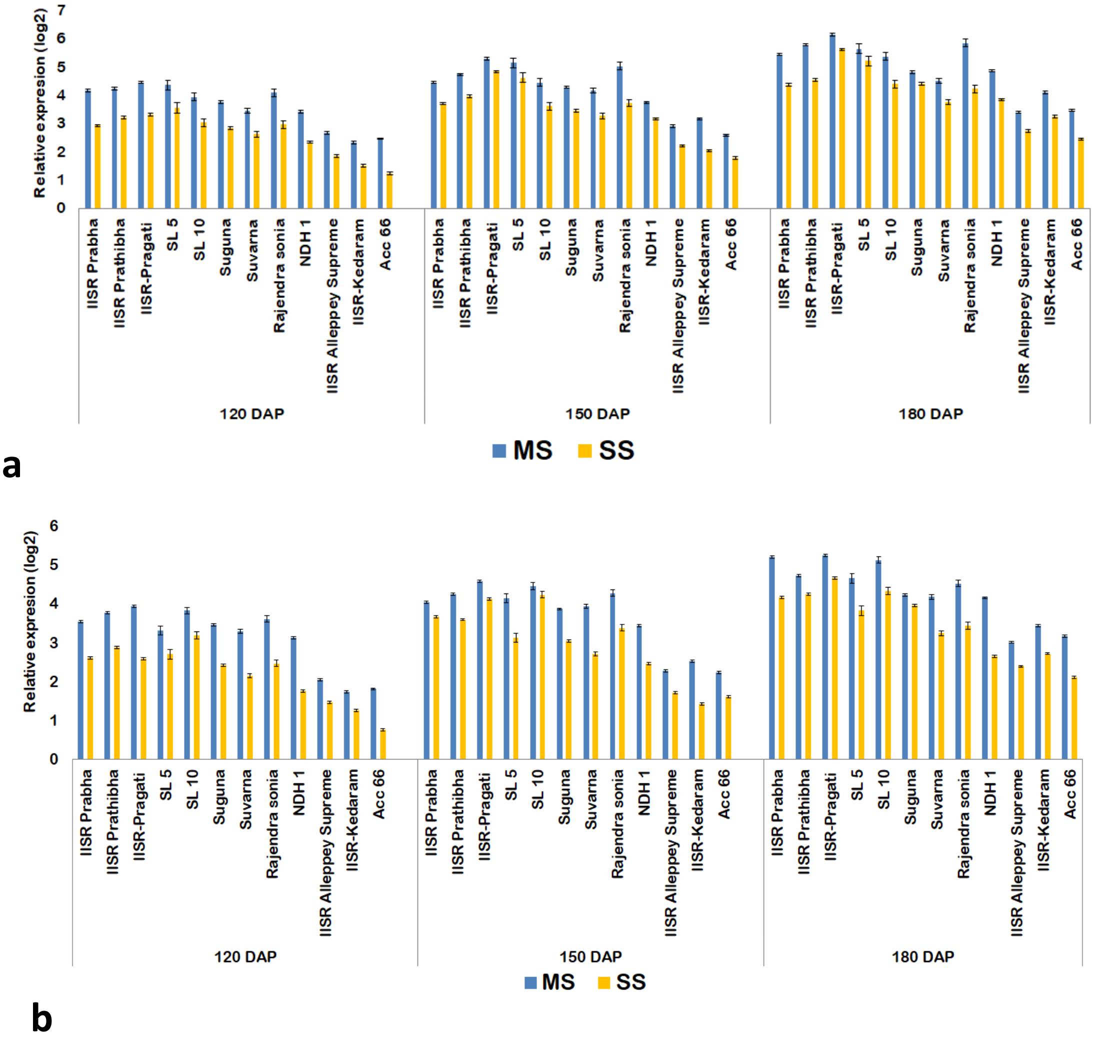


Fig. 4.2.14 Relative expression pattern of *bZIP* under moderate and severe stress with respect to control condition in (a) leaf and (b) rhizome at different developmental stages. Bars represent the mean ± SE.

(MS = moderately stressed plants irrigated at 50% field capacity; SS = severely stressed plants irrigated at 25% field capacity)

Several *bZIP* TFs from rice (Xiang *et al*., 2008), wheat (Gahlaut *et al*., 2015) and *Arabidopsis* plants (Jakoby *et al.,* 2002) reported to respond towards dehydration and increased plant tolerance.

APETALA2/ethylene response factor (AP2/ERF) transcription factor: *AP2* are involved in the regulation of hormone signalling-mediated stress response. When a plant suffers from abiotic stress, the inducing hormones (ABA and ET) and growth-promoting hormones carry out their defence against the adverse external environment through a mechanism regulated by *AP2* (Verma *et al*., 2016).

In leaf tissues, at 120 DAP the maximum expression of *AP2* was detected in IISR Pragati (fold change: 17.75) followed by Rajendra Sonia (fold change: 13.3) and it was least expressed in IISR Kedaram (fold change: 4.5) under moderate stress. Under severe stress, expression was highest in IISR Pragati (fold change: 12.6) followed by SL 5 (fold change: 10.4) and least expression in IISR Kedaram (fold change: 3.3).

At 150 DAP, the maximum expression of *AP2* was detected in IISR Pragati (fold change: 37.5) followed by SL 5 (fold change: 28.2) and it was least expressed in IISR Kedaram (fold change: 6.58) under moderate stress. Similarly under severe stress, highest expression was exhibited by IISR Pragati (fold change: 20.2) followed by Suvarna (fold change: 19.5) while minimum expression was shown by IISR Kedaram (fold change: 4.95).

The maximum expression of *AP2* was detected at 180 DAP in IISR Pragati (fold change: 43.1) and Rajendra Sonia (fold change: 38.58) followed by SL 5 (fold change; 37.8) and the lowest expression was observed in Acc 66 (fold change: 10.7) under moderate stress. Whereas under severe stress, the expression was maximum in IISR Pragati (fold change: 31.3) followed by SL 5 (fold change: 27.47) while it was lowest in Acc 66 (fold change: 6.68) (Fig.4.2.15 a).

In case of rhizome, at 120 DAP, The maximum expression of *AP2* was detected in IISR Pragati (fold change: 11.5) and SL 5 (fold change: 11) followed by Rajendra Sonia (fold change: 10.2) while it was lowest in IISR Kedaram (fold change: 3.4) at moderate stress. Whereas under severe stress highest expression was shown in IISR Pragati (fold change: 6.6) followed by Suvarna (fold change: 5.9) and Suguna (fold change: 5.6) and least expression in IISR Kedaram (fold change: 2.3).

Similarly, at 150 DAP, The maximum expression of *AP2* was detected in IISR Pragati (fold change: 22) followed by Rajendra Sonia (fold change: 19.8) and was lowest in IISR Kedaram (fold change: 5.5) under moderate stress condition. Under severe stress, IISR Pragati (fold change: 18) followed by SL 5 (fold change: 14) manifested maximum expression while the lowest expression was revealed in IISR Kedaram (fold change: 2.7).

The maximum expression of *AP2* was displayed by IISR Pragati (fold change: 32.4) followed by Suguna (fold change: 26.4) with least expression in IISR Kedaram (fold change: 6.7) at moderate stress at 180 DAP. Meanwhile under severe stress, the highest expression was recorded in SL 5 (fold change: 19.3) followed by Rajendra Sonia (fold change: 14.4) and least expression was found in IISR Kedaram (fold change: 4.1) (Fig.4.2.15 b).

Yu *et al*. (2022) found that overexpression of the *AP2/ERF* family gene increased sensitivity to drought and salt stress in *Arabidopsis*. Kumar *et al*. (2022) showed that overexpressing the *AP2* showed higher relative water content, membrane stability index, wax content, osmotic potential, stomatal conductance, and transpiration rate. In our study *AP2* was found to be upregulated under drought stress but its expression was higher in tolerant genotypes. *AP2* regulate genes involved in diverse biological processes such as growth, development, hormone and stress responses emerged as key regulators of various stress responses, in which they also respond to hormones with improved plant survival during stress conditions in *Arabidopsis* (Dietz *et al.,* 2010; Xie *et al*., 2019), in tomato (Wang *et al*., 2022) and in maize (Cheng *et al*., 2023).

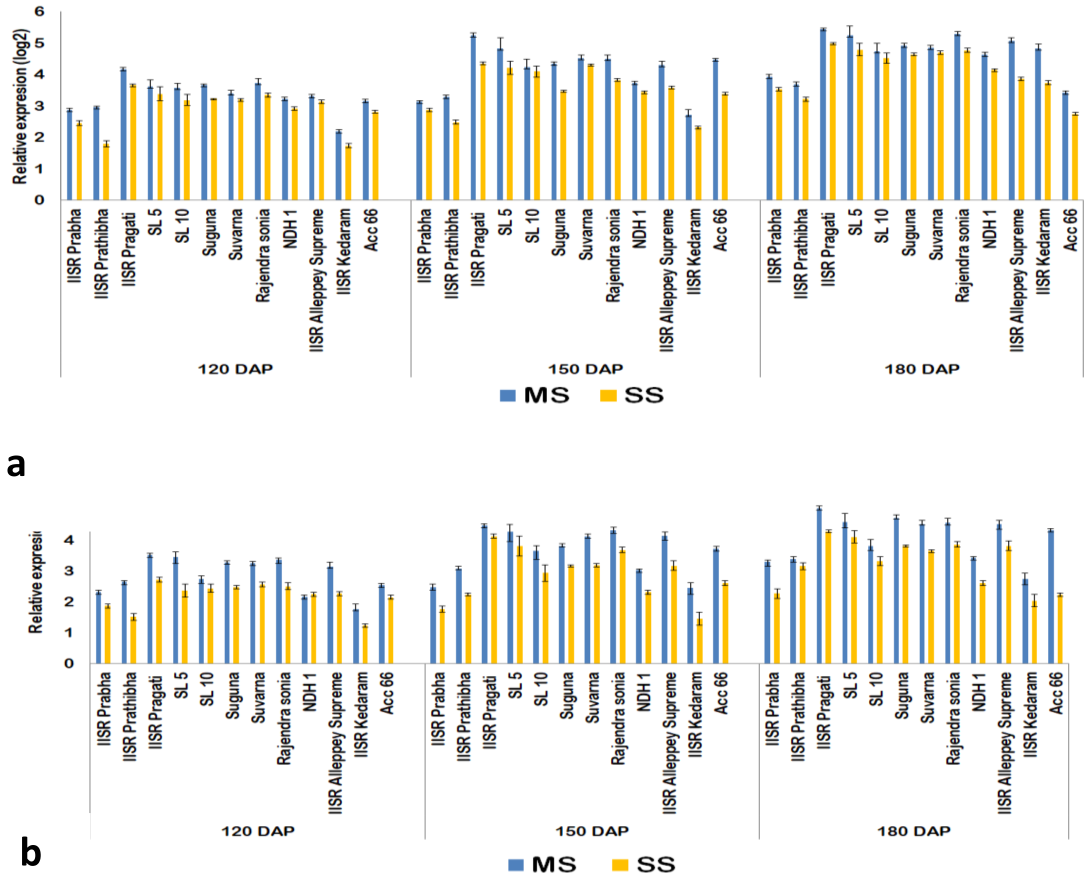


Fig. 4.2.15 Relative expression pattern of *AP2* under moderate and severe stress with respect to control condition in (a) leaf and (b) rhizome at different developmental stages. Bars represent the mean ± SE.

(MS = moderately stressed plants irrigated at 50% field capacity; SS = severely stressed plants irrigated at 25% field capacity)

Aquaporins (AQP): *AQP*s are genes responsible for precisely regulating the movement of water and therefore may play a crucial role in the drought-stress response as well as in drought-stress tolerance (Sade &Moshelion, 2017). Expression of *AQP* was down-regulated in both leaf and rhizome with higher down regulation in leaf and was highest at 180 DAP and had a lesser down regulation at 150 DAP with the lowest down regulation at 120 DAP (i.e., earlier stages). Also, there was a higher level of down regulation for *AQP* at severe stress.

In leaf, the maximum down regulation of *AQP* at 120 DAP was shown by IISR Pragati (Fold change: ‑3.66) and Rajendra Sonia (Fold change: ‑2.81) followed by Suguna (Fold change: ‑2.61) and it was least in Acc 66 (Fold change: ‑1.10) under moderate stress. Under severe stress, IISR Pragati (Fold change: ‑10.78) followed by Rajendra Sonia (Fold change: ‑7.11) and Suguna (Fold change: ‑5.66) exhibited maximum down regulation while least down regulation was in Acc 66 (Fold change: ‑1.69) itself.

At 150 DAP, the maximum down regulation of *AQP* under moderate stress was in IISR Pragati (Fold change: ‑3.78) and Suguna (Fold change: ‑2.93) followed by SL 5 (Fold change: ‑2.36) and it was least expressed in Acc 66 (Fold change: ‑1.15). Whereas under severe stress, highest down regulation was observed in IISR Prabha (Fold change: ‑12.38) and IISR Prathibha (Fold change: ‑11.47) followed by IISR Pragati (Fold change: ‑10.13) and least down regulation was found in Acc 66 (Fold change: ‑1.91).

In leaf, the maximum down regulation of *AQP* was at 180 DAP in IISR Pragati (Fold change: ‑5.03) and Suguna (Fold change: ‑4.35) followed by S L 5 (Fold change: ‑2.77) and it was lowest in Acc 66 (Fold change:: ‑1.33) at moderate stress; Whereas under severe stress, highest down regulation was observed in Suguna (Fold change‑14.3) and IISR Pragati (Fold change: ‑12.3) followed by SL 5 (Fold change: ‑ 6.19) with lowest in Acc 66 (Fold change: ‑ 2.73) (Fig.4.2.16a).

In rhizome, at 120 DAP, the maximum down regulation of *AQP* was detected in IISR Prathibha (Fold change: ‑2.20) and Suguna (Fold change: ‑2.13) followed by S L 5 (Fold change: ‑1.64) and it was least in Acc 66 (Fold change: ‑1.09) under moderate stress. Under severe stress, maximum downregulation was observed in SL 5 (Fold change: ‑5.06) followed by Suguna (Fold change: ‑3.14) and IISR Pragati (Fold change: ‑2.50) and least down regulation was exhibited by IISR Kedaram (Fold change:‑1.55).

The maximum down regulation of *AQP* at 150 DAP was detected in SL 5 (Fold change: ‑2.41) and Suguna (Fold change: ‑2.22) followed by IISR Pragati (Fold change: ‑1.79) and it was minimum in Acc 66 (Fold change: ‑1.11) under moderate stress. Severe stress caused maximum down regulation in IISR Pragati (Fold change: ‑5.82) and SL 5 (Fold change: ‑3.58) followed by Suguna (Fold change: ‑2.68) and least down regulation was in Acc 66 (Fold change: ‑1.66) followed by IISR Kedaram (Fold change: ‑ 1.25).

Maximum down regulation of relative expression of *AQP* was detected at180 DAP and it was maximum in IISR Pragati (Fold change: -2.53) followed by S L 5 (Fold change: -2.10) followed by Suguna (Fold change: -1.80) and it was lowest in Acc 66 (Fold change: -1.21) under moderate stress. Whereas under severe stress, maximum down regulation was found in S L 5 (Fold change: -6.63) followed by IISR Pragati (Fold change: -3.84) and Suguna (Fold change: -3.29) and lowest in IISR Alleppey Supreme (Fold change: -1.93) (Fig. 4.2.16b).

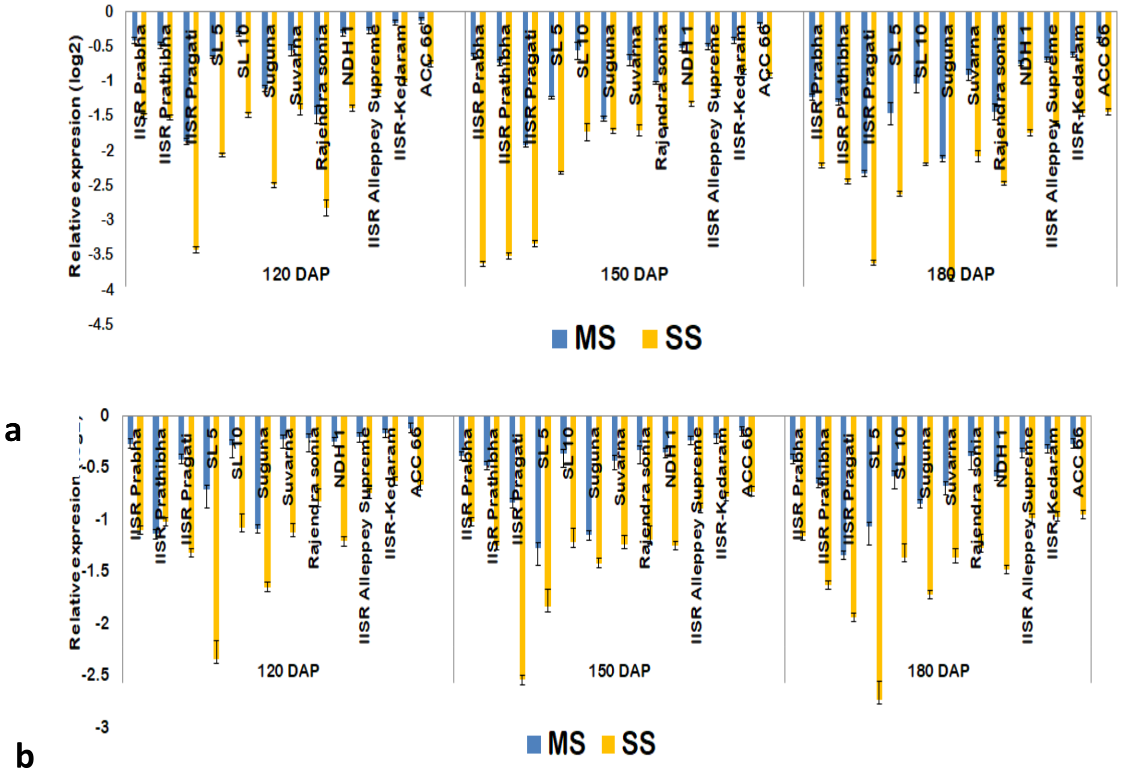


Fig. 4.2.16 Relative expression pattern of *AQP* under moderate and severe stress with respect to control condition in (a) leaf and (b) rhizome at different developmental stages; Bars represent the mean ± SE.

(MS = moderately stressed plants irrigated at 50% field capacity; SS = severely stressed plants irrigated at 25% field capacity)

Over-expression of aquaporin in Arabidopsis resulted in the higher water loss, low RWC and water potential, and decreased drought resistance (Li *et al.*, 2015). Similarly, transgenic tobacco over expressing the *AQP* gene also showed faster wilting during water stress (Aharon *et al.*, 2003). The negative regulation of the gene in the tolerant turmeric may contribute towards the reduced water transport out of the cell during drought stress and hence aid in the maintenance of water within, thus contributing to drought tolerance. Similar expression pattern has been shown by *Festuca* species, (Pawłowicz *et al*., 2017), *Nicotiana glauca* (Smart *et al*., 2001) and Arabidopsis (Alexandersson *et al*., 2005) under drought stress.

NAC (NAM, ATAF1–2 and CUC2) family of transcription factors: DNA-binding proteins interacting with cis elements involved in dehydration stress induction and in dark-induced senescence were identified as *NAC* transcription factors (Tran *et al*., 2004). In leaf, maximum expression of *NAC* at 120 DAP was detected in Suguna (fold change: 18.64) and IISR Pragati (fold change: 16.34) and lowest in Acc 66 (fold change: 4.14) under moderate stress. IISR Pragati (fold change: 13.09) followed by Suguna (fold change: 12.30) showed maximum expression and lowest expression was shown by IISR Alleppey Supreme (fold change: 2.50) under severe stress.

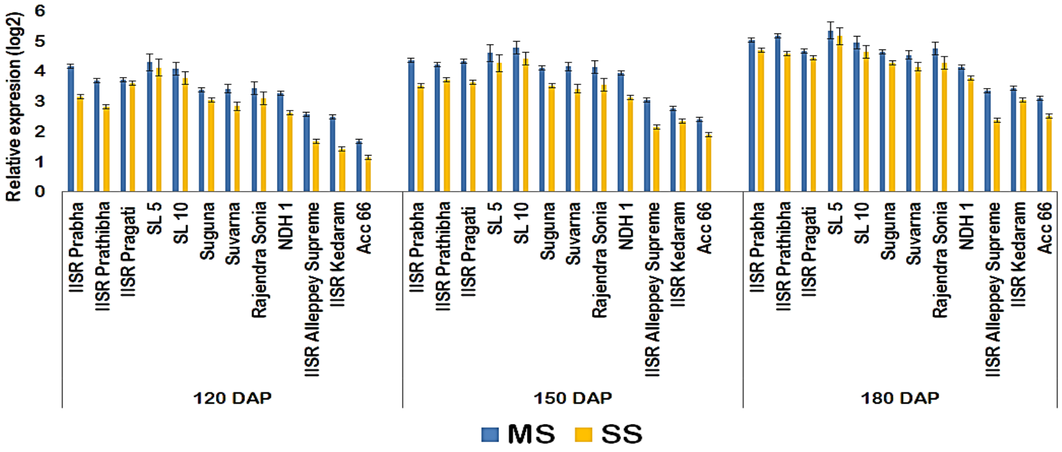
The maximum expression of NAC at 150 DAP was found in IISR Pragati (fold change: 40.5), followed by Suguna (fold change: 34.78), and the expression was least in Acc 66 (fold change: 5.77) under moderate stress. Similar expression patterns were observed under severe stress also, with maximum expression in Suguna (fold change: 26.54), followed by IISR Pragati (fold change: 19.03) and IISR Prathibha (fold change: 17.38), while the lowest expression was observed in Acc 66 (fold change: 4.34).

The maximum expression of *NAC* was detected at 180 DAP in IISR Pragati (fold change: 48.8) and NDH 1 (fold change: 45.9) followed by Suguna (fold change: 39.9) and it was lowest in Acc 66 (fold change: 8.9) under moderate stress. Whereas under severe stress, *NAC* expression was highest in IISR Pragati (fold change: 34.8) followed by Suguna (fold change: 32.4) and the least expression was observed in Acc 66 (fold change: 5.38) (Fig. 4.2.17a).

In the case of relative expression in rhizome, at 120 DAP, the highest expression of *NAC* was detected in IISR Pragati (fold change: 15.14) followed by IISR Prathibha (fold change: 12.4), and IISR Prabha (fold change: 11.24), while the lowest expression was observed in Acc 66 (fold change: 2.75) under moderate stress conditions. Similarly, under severe stress, the highest expression was observed in IISR Pragati (fold change: 6.63), followed by SL 5 (fold change: 5.70) and IISR Prabha (fold change: 5.35) and the lowest expression recorded in Acc 66 (fold change: 1.67).

At 150 DAP maximum level of expression was found in IISR Pragati (fold change: 21.86) and SL 5 (fold change: 20.25), while the lowest level was found in Acc 66 (fold change: 4.35) under moderate stress condition. The highest expression under extreme stress was observed in IISR Pragati (fold change: 14.12) followed by IISR Prathibha (fold change: 12.38) and SL 10 (fold change: 10.70) and it was the lowest in Acc 66 (fold change: 2.45).

At 180 DAP, maximum relative expression of *NAC* was detected in IISR Pragati (fold change: 28.44) followed by IISR Prabha (fold change: 26.35) and SL 5 (fold change: 22.78) and the lowest in Acc 66 (fold change: 6.59) under moderate stress. Whereas under severe stress, expression was highest in IISR Pragati (fold change: 20.25) followed by S L 5 (fold change: 18.13) and IISR Prathibha (fold change: 17.27) while the expression was lowest in Acc 66 (fold change: 2.66) in rhizome (Fig. 4.2.17b).



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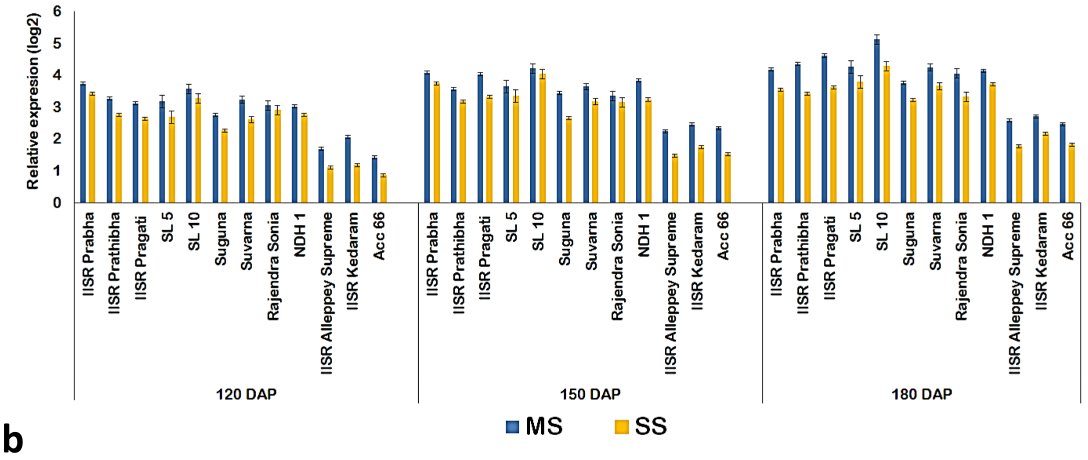


Fig. 4.2.17 Relative expression pattern of *NAC* under moderate and severe stress with respect to control condition in (a) leaf and (b) rhizome at different developmental stages; Bars represent the mean ± SE. (MS = moderately stressed plants irrigated at 50% field capacity; SS = severely stressed plants irrigated at 25% field capacity).

The *NAC* transcription factors play a pivotal role in regulating plant stress responses. In our study the expression of *NAC* was increased with drought stress especially in tolerant ones. This finding was in consistent with improved drought resistance in transgenic cotton resulting from overexpression of *NAC* (Gulisitan *et al*., 2023) and salt resistance in rice (Hu *et al*., 2006).

WRKY transcription factors: *WRKY* is also a principal TF that regulate many plant processes in response to biotic and abiotic stimuli, in response to internal signals which coordinate developmental processes (Bakshi *et al*., 2014). Here its relative expression was increased upon water stress. Moreover its expression was greater in tolerant genotypes.

In leaf tissues, the maximum relative expression of *WRKY* at 120 DAP was detected in SL 5 (fold change: 17.6) followed by IISR Prabha (fold change: 14.3) and Rajendra Sonia (fold change: 12.3) and it was lowest in Acc 66 (fold change: 4.19) under moderate stress. Under severe stress, highest expression was found in IISR Prabha (fold change: 11.08) followed by IISR Pragati (fold change: 10.78) whilst the lowest expression was exhibited by Acc 66 (fold change: 2.49).

At 150 DAP, maximum expression of *WRKY* was detected in SL 10 (fold change: 26.54) followed by SL 5 (fold change: 25.8) and IISR Prathibha (fold change: 24.76) while the expression was lowest in Acc 66 (fold change: 6.63) under moderate stress. Whereas IISR Prathibha (fold change: 21.4) followed by SL 10 (fold change: 20.39) and SL 5 (fold change: 17.87) showed maximum expression and the lowest expression was observed in Acc 66 (fold change: 3.18).

The maximum expression of *WRKY* was detected at 180 DAP in SL 10 (fold: 39.4) followed by SL 5 (fold change: 37.79) and Rajendra Sonia (fold change: 36) and minimum in Acc 66 (fold change: 8.69) at moderate stress. Under severe stress condition, SL 5 (fold change: 32.9) followed by IISR Prabha (fold change: 24.9) exhibited maximum expression while the lowest expression was observed in Acc 66 (fold change: 4.5) (Fig. 4.2.18 a).

In rhizome tissues, the maximum expression of *WRKY* at 120 DAP was detected in SL 5 (fold change: 15.1) followed by IISR Prabha (fold change: 11.1) and Suguna (fold change: 10.6) and the expression was lowest in Acc 66 (fold change: 3.3) under moderate stress. While under severe stress, highest expression was observed in SL 5 (fold change: 9.4) followed by IISR Prathibha (fold change: 6.7) whereas Acc 66 (fold change: 2.2) showed the lowest expression.

At 150 DAP, the maximum expression of *WRKY* was observed in IISR Prathibha (fold change: 22.2) followed by SL 10 (fold change: 21.9) and SL 5 (fold change: 20) whereas the lowest expression was detected in Acc 66 (fold change: 4.8) under moderate stress. Under severe stress, SL 5 (fold change: 13.4) followed by IISR Prathibha (fold change: 12.8) showed maximum expression and expression was lowest in Acc 66 (fold change: 2.7).

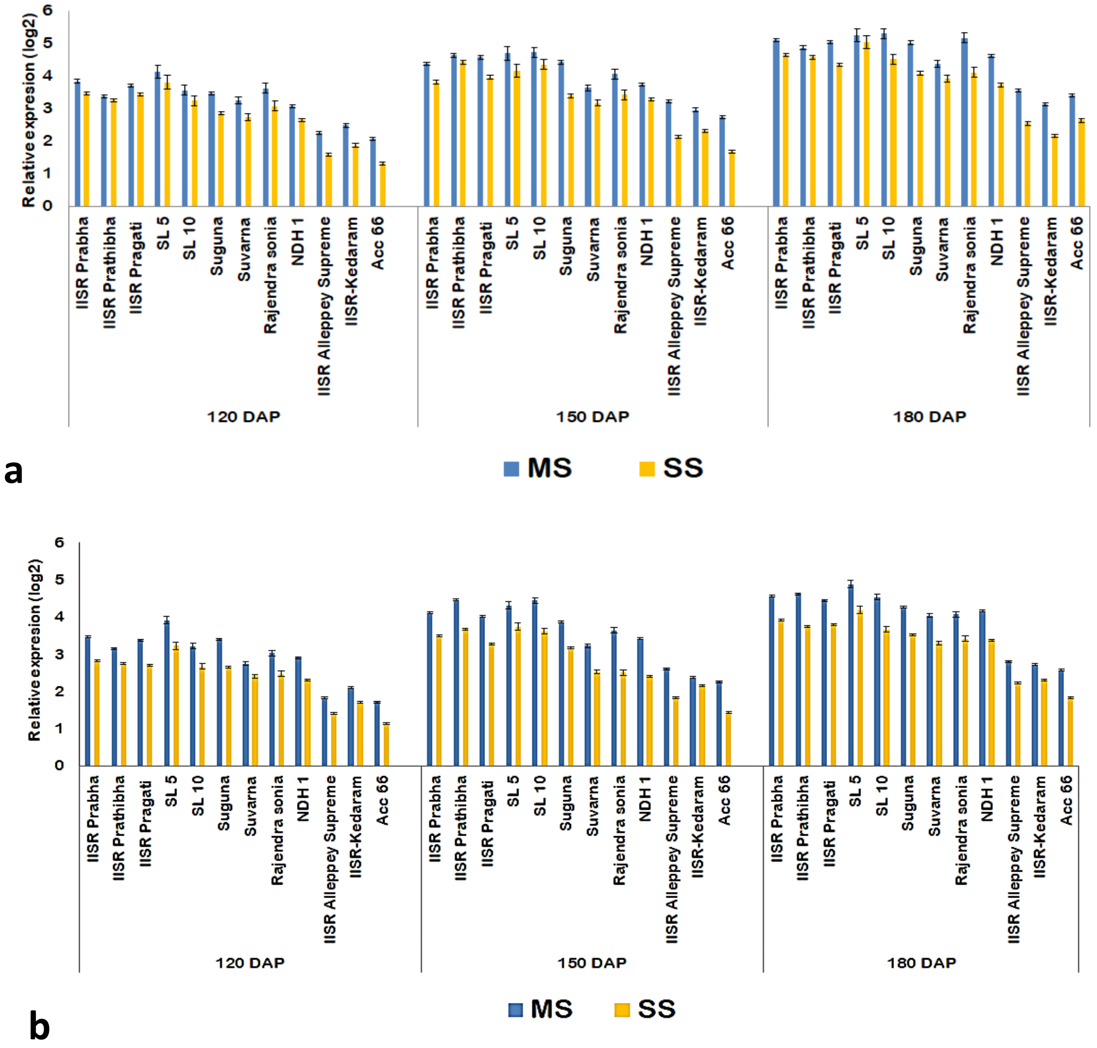


Fig. 4.2.18 Relative expression pattern of *WRKY* under moderate and severe stress with respect to control condition in (a) leaf and (b) rhizome at different developmental stages. Bars represent the mean ± SE. (MS = moderately stressed plants irrigated at 50% field capacity; SS = severely stressed plants irrigated at 25% field capacity)

At 180 DAP SL 5 showed maximum expression (fold change: 29.7) followed by IISR Prathibha (fold change: 24.8) and IISR Prabha (fold change: 23.6) while the expression was least in Acc 66 (fold change: 6) under moderate stress. Similarly under severe stress, SL 5 (fold: 18.3) followed by IISR Prabha (fold change: 15.1), and IISR Pragati (fold change: 14.0) showed maximum expression and the expression was least in Acc 66 (fold change: 3.6) (Fig. 4.2.18b).

WRKY TF over expression enhanced drought resistance in transgenic *Arabidopsis* via modulating ABA biosynthesis (Zhang *et al*., 2022; Bai *et al*., 2023). It enhanced drought tolerance in transgenic cotton also (Hu *et al*., 2021).

***Dehydration-responsive element-binding* proteins (*DREB*):** *DREB*s are vital transcription factors induced by abiotic and biotic stresses. In our study the relative expression by qRTPCR revealed its enhanced expression under drought stress particularly in tolerant ones.

In leaf tissue, at 120 DAP, the maximum expression of *DREB* was detected in SL 10 (fold change: 32.4) and SL 5 (fold change: 23.8) followed by IISR Prathibha (fold change: 21.9). The lowest expression was found in IISR Alleppey Supreme (fold change: 4.1) under moderate stress. Under severe stress, maximum expression was observed in SL 10 (fold change: 17.9) followed by SL 5 (fold change: 14.4), while the expression was least in IISR Alleppey Supreme (fold change: 2.8).

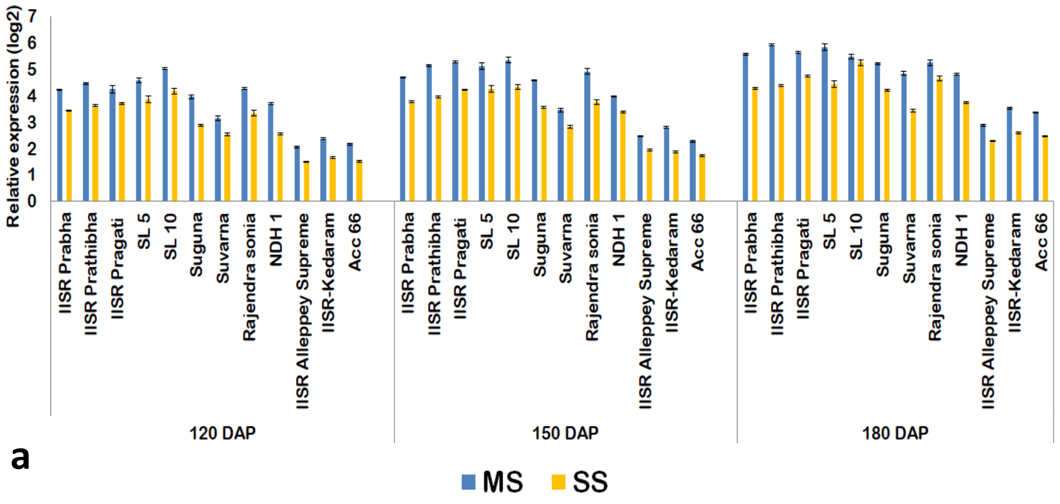
Similarly, at 150 DAP, maximum expression was manifested in SL 10 (fold change: 40.5) followed by IISR Pragati (fold change: 38.6) and IISR Prathibha (fold change: 35) and it was lowest in Acc 66 (fold change: 4.8) under moderate stress. Under severe stress expression was maximum expression in SL 10 (fold change: 37.8) followed by SL 5 (fold change: 26.5) and IISR Pragati (fold change: 18.5) while the expression was least in Acc 66 (fold change: 3.3).

Likewise, at 180 DAP, IISR Prathibha (fold change: 60) followed by SL 5 (fold change: 56.9) and IISR Pragati (fold change: 49.2) showed maximum expression while it was least expressed in IISR Alleppey Supreme (fold change: 7.3) under moderate stress. While under severe stress, maximum expression was detected in SL 10 (fold change: 37.8) followed by IISR Pragati (fold change: 26.5). Here also, the least expression was found in IISR Alleppey Supreme (fold change: 4.8) (Fig. 4.2.19 a).

With regard to rhizome, at 120 DAP, the maximum expression of *DREB* was detected in IISR Pragati (fold change: 14.2) followed by SL 10 (fold change: 13.1) and IISR Prabha (fold change: 10.8) and the expression was least in IISR Alleppey Supreme (fold change: 2.5) under moderate stress. While under severe stress, expression was highest in SL 10 (fold: 9.8) followed by IISR Pragati (fold change: 6.9), IISR Prabha (fold change: 6.1) and least expression was observed in IISR Kedaram (fold change: 1.6).

The maximum expression of *DREB* at 150 DAP in rhizome was detected in SL 10 (fold change: 21.6) followed by Suguna (fold change: 19.3) and IISR Pragati (fold change: 18.1) and its expression was lowest in IISR Alleppey Supreme (fold change: 3.3) under moderate stress. While under severe stress, highest expression was observed in SL 10 (fold change: 13.4) followed by IISR Pragati (fold change: 12.3) and SL 5 (fold change: 11.9) while the lowest expression was observed in IISR Alleppey Supreme (fold change: 2.4).

The maximum expression of *DREB* was detected at 180 DAP in SL 10 (fold change: 30.3) followed SL 5 (fold change: 29.7) and IISR Pragati (fold change: 22.2) while the expression was least in Acc 66 (fold change: 5.7) under moderate stress. Again under severe stress, the relative expression was maximum in SL 10 (fold change: 23.1) followed by IISR Prabha (fold change: 16.8) and IISR Prathibha (fold change: 15.2) and the expression was lowest in Acc 66 (fold change: 2.8) (Fig. 4.2.19 b).



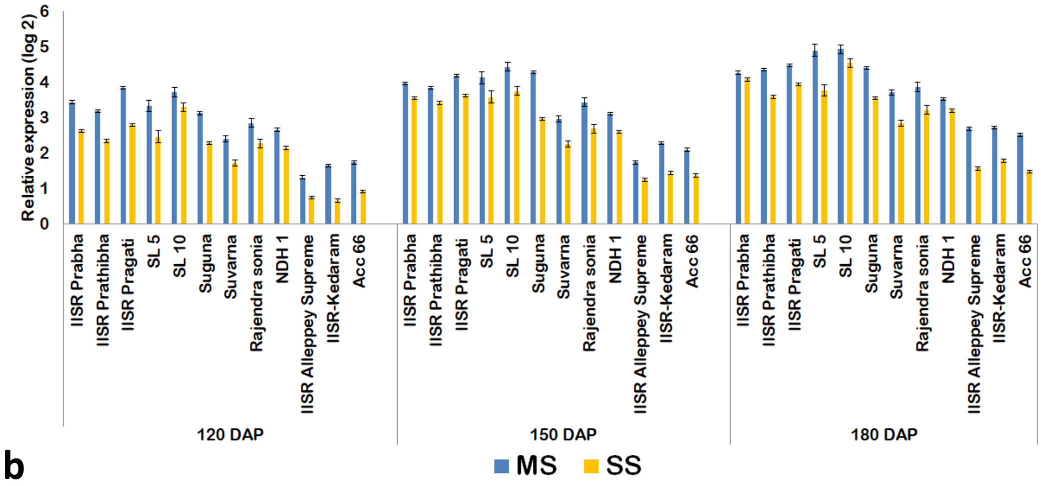


Fig. 4.2.19 Relative expression pattern of *DREB* under moderate and severe stress with respect to control condition in (a) leaf and (b) rhizome at different developmental stages; Bars represent the mean ± SE. (MS = moderately stressed plants irrigated at 50% field capacity; SS = severely stressed plants irrigated at 25% field capacity)

The relative expression of *DREB* was higher in tolerant turmeric genotypes. Transcription factor *DREB* interacts with a cis-acting dehydration-responsive element (DRE) sequence and activates expression of downstream genes involved in drought stress response in *Arabidopsis thaliana* and in *Sorghum bicolour* (Sakuma *et al*., 2006; Akbudak *et al*., 2018). It regulates expression of many water stress-inducible genes.

Transcription factors like *DREB* increased under moderate stress but decreased under severe stress due to feedback inhibition and changes in the plant's stress response strategy in *Arabidopsis* (Nakashima &Yamaguchi, 2013), rice (Fujita *et al*., 2007), soybean (Liu *et al*., 2011) and in wheat (Xue *et al*., 2008). Conversely, relative expression drought responsive genes including dehydrins in tolerant and sensitive genotypes of wild barley under three levels of drought stress (control, mild stress, and severe stress) revealed the highest expression levels in the drought-tolerant genotype under severe stress conditions (25–30% FC) (Shirvani *et al*., 2024).

In our study, the expression of the genes studied was effected by drought stress in all the genotypes of turmeric. The relative expression of seven TFs and

AQP gene followed similar pattern in the 12 genotypes studied. The expression of *AP2, bZIP, ABF, bHLH, NAC, WRKY* and *DREB* genes was found to be up regulated and that of *AQP* was down-regulated during water stress in all the genotypes when compared to their respective controls at all stages (120, 150 and 180 DAP). In contrast, there was a reduction in expression under severe stress condition compared to moderate stress in all varieties for all TFs except *AQP*. However there was higher downregulation of *AQP* under severe stress. The expression of *AQP* was found to be inversely correlated with tolerance as it was down regulated under different drought levels (moderate and severe stress) compared with control (100% FC). Among the up-regulated genes, the relative expression of TFs was higher in tolerant genotype. Similarly, the down regulation of *AQP* was higher in tolerant ones. Although the expression of *AP2* was up-regulated during drought in both tolerant and susceptible genotypes, tolerance related variation of the relative expression could not be observed. The expression of *AQP* was down-regulated during stress in both the tolerant and susceptible genotypes, with the tolerant genotype exhibiting higher down regulation.

Our evaluation of gene expression under different water regimes indicated that under water stress expression of TFs was less in susceptible genotypes like IISR Alleppey Supreme, IISR Kedaram and Acc 66 while tolerant ones showed maximum expression. This indicates the influence of TFs on water regimes. This further confirmed the tolerance levels observed in the identified genotypes. Similar results were obtained in all stages in both leaf and rhizome.

4.2.3.3 Correlation between maximum enzyme activity and normalized gene expression.

The correlation analysis between enzyme activity and the normalized gene expression was performed using Pearson's correlation coefficient. The leaves of genotypes at 180 DAP planted under control and moderate drought conditions mentioned above were used for the study. Correlations between activity of major enzymes and selected TFs was performed and are considered positive when Pearson's r > 0.6 (Wang *et al.*, 2015). There was a direct positive correlation between the gene expression levels of *ABF, BHLH, BZIP, NAC, DREB*, and *WRKY* TFs and the enzyme activity which further verified the biochemical data. Among the 6 transcription factor analysed, *WRKY* (r: 0.948085), *ABF* (r: 0.930399) and *DREB* (r: 0.92547) and *BHLH* (r: 0.920464) showed highest positive correlation (Table 4.2.9).

Table 4.2.9 Pearson's correlation of the mean enzyme activity with the mean gene expression at most expressed developmental stage (180 DAP) of 12 turmeric genotypes grown in green house.

TF	APX	MDHAR	DHAR	GR	SOD	POD
ABF	0.930	0.942	0.917	0.951	0.851	0.946
BHLH	0.920	0.969	0.857	0.929	0.820	0.937
BZIP	0.912	0.883	0.780	0.864	0.756	0.860
NAC	0.677	0.818	0.732	0.803	0.843	0.779
DREB	0.925	0.978	0.871	0.944	0.861	0.938
WRKY	0.948	0.915	0.829	0.878	0.859	0.857

In our study we have used major drought responsive TFs which are directly involved in the principal drought responsive pathways. The TFs, *ABF, BHLH, BZIP, NAC, DREB* and *WRKY* showed positive correlation with biochemical response under all experimental conditions involving different water regimes, tissues and development stages. While *AP2* showed no strict tolerance related variation and *AQP* showed negative correlation. Representative comparison of fold change of transcription factors which showed positive correlation at maximal activity (180 DAP under moderate stress condition) with drought stress response is shown in Table 4.2.10.

Table 4.2.10 Comparison of fold change of transcription factors with drought response at maximum activity

Genotype	Fold change						Drought response as observed by biochemical analysis
	ABF	*DREB*	*bZIP*	*NAC*	*WRKY*	*bHLH*	
IISR Pragati	50.9	49.2	71.01	48.84	32.9	25.46	Tolerant
IISR Prathibha	66.7	60.1	55.33	32.67	29.2	35.75	Tolerant
IISR Prabha	76.6	47.5	43.71	34.78	34.1	32.45	Tolerant
Suvarna	32.4	28.4	22.78	27.28	20.7	23.10	Tolerant
SL 5	56.5	56.9	50.21	29.65	37.8	40.79	Tolerant
SL 10	74.5	44.3	41.64	24.76	39.4	31.12	Tolerant
Rajendra Sonia	38.1	37.8	57.68	19.03	36.0	26.91	Tolerant
Suguna	44.6	37.0	28.25	39.95	32.4	25.11	Tolerant
NDH 1	26.4	27.9	29.24	45.89	24.4	17.75	Tolerant
IISR Kedaram	14.6	11.5	17.27	13.27	10.6	10.93	Susceptible
Acc 66	13.5	7.3	11.08	8.94	8.7	8.51	Susceptible
IISRAlleppey Supreme	12.5	10.2	10.56	9.71	11.8	10.13	Susceptible

Transcription factors regulate various stress inducible genes cooperatively or separately by attaching to specific sites on a DNA molecule. These stress-inducible transcription factors include members of the *DREB* family, the *ERF* family, the zinc-finger family, *bHLH* family, the basic-domain leucine zipper (*bZIP*) family and the homeo domain transcription factor family (Seki *et al.*, 2002; Thakur *et al.*, 2020; Ramanjulu & Bartels, 2002). These TFs can be key targets for modulating the downstream gene regulatory networks.

AQP gene encode a small integral membrane protein which permits passive transport of water along an osmotic gradient and play a major role in plant growth and development and transport uncharged solutes across biological membranes (Maurel *et al.*, 2015; Li *et al.*, 2021). Higher expression of aquaporin resulted in increased water loss and decreased drought resistance in Arabidopsis (Li *et al.*, 2015), transgenic tobacco (Aharon *et al.*, 2003) and *Festuca* species (Pawłowicz *et al.,* 2017) during water stress.

The relative differential expressions of drought responsive genes or transcription factors in susceptible and tolerant genotypes by quantitative RT-PCR gives information about upregulation or downregulation of selected genes or transcription factors that contribute towards drought tolerance (George *et al.,* 2017). Relative quantitation of gene expression allows quantification of differences in the expression level of a specific target (gene) between different samples (Livak & Schmittgen, 2001). Further molecular characterization of these identified differentially expressed transcription factors can provide a better understanding of the signal transduction cascades during water stress (Shinozaki & Yamaguchi, 2006).

The differential expression of drought responsive TFs and *AQP* gene in tolerant and susceptible genotypes further indicated the drought tolerance ability of the above identified genotypes suggesting that these genotypes are suitable for cultivation under water deficit condition.

4.2.4 Rhizome yield (g plant-1)

There was a reduction in yield with stress intensity compared to control. Under control (100% FC) maximum yield was recorded in IISR Pragati (305.7 g plant^{-1}). SL 5 (304 g plant^{-1}) was on par with IISR Pragati and it was minimum was in Acc 66 (132 g plant^{-1}) and IISR Prathibha (215.7 g plant^{-1}). Under moderate drought, maximum yield was in the order IISR Pragati (246 g plant^{-1}) > SL 5 (232.6 g plant^{-1}) > Suguna (210.3 g plant^{-1}) > Suvarna (198 g plant^{-1}) with lowest yield observed in IISR Alleppey Supreme (139 g plant^{-1}) followed by IISR Kedaram (127 g plant^{-1}) and Acc 66 (62 g plant^{-1}). There was a considerable reduction in yield under severe stress, highest yield was obtained in IISR Pragati (87.6 g plant^{-1}) followed by Suguna (81.9 g plant^{-1}) and Suvarna (73.7 g plant^{-1}). Whereas, lowest yield was recorded in IISR Alleppey Supreme (21.6 g plant^{-1}) followed by Kedaram (24 g plant^{-1}). But there was no yield under severe stress for Acc 66 (Table 4.2.11).

The drought susceptibility index (DSI) is used to measure yield stability of different genotypes that apprehends the changes in both drought stress and non-stress environments (Fischer & Maurer, 1978).The least value of DSI indicate yield sustainability and it was least and less than 1 in IISR Pragati ($^{-}$0.155) followed by SL 5 ($^{-}$0.099), Suguna ($^{-}$0.098) and Suvarna ($^{-}$0.071). DSI values were greater for susceptible ones such as Acc 66 (0.326), IISR Kedaram (0.218) and IISR Alleppey Supreme (0.189) under moderate stress. Under severe stress, it was least in Suguna ($^{-}$0.39) followed by IISR Pragati ($^{-}$0.34), Suvarna ($^{-}$0.30) and SL 5 ($^{-}$0.20) (Table 4.2.11).

Table 4.2.11 Yield and drought susceptibility index of turmeric genotypes under different water regimes

Genotype	Yield (g plant^{-1})			DSI (50% FC)	DSI (25% FC)
	100% FC	50% FC	25% FC		
IISR Pragati	305.7^{a}	246^{d}	87.75^{o}	-0.155	-0.34
IISR Prathibha	215.7^{g}	152.3^{l}	52.6^{t}	-0.013	-0.14
IISR Prabha	225^{f}	157.7^{k}	55.9s^{t}	-0.006	-0.16
Suvarna	265.3^{c}	198^{i}	73.71^{q}	-0.071	-0.30
SL 5	304^{a}	232.7^{e}	78.3^{pq}	-0.099	-0.20
SL 10	244.7^{d}	173.7^{j}	58.89^{rs}	-0.019	-0.13
Rajendra Sonia	223.3^{f}	162^{k}	56.1^{st}	-0.041	-0.18
Suguna	275^{b}	210.3^{h}	81.9^{p}	-0.098	-0.39
NDH 1	227^{f}	157.7^{k}	37.7^{u}	0.003	0.22
IISR-Kedaram	233^{e}	127^{n}	24^{v}	0.218	0.52
Acc66	132^{n}	62^{r}	0^{w}	0.326	1.00
IISR Alleppey Supreme	246^{d}	139^{m}	21.6^{v}	0.189	0.59
General mean	241.4	168.2	52.4		
CV (%)	2.065				
CD (P=0.05)	5.18				

The mean value (n=3) followed by different letters within a column are significantly different (P< 0.05) based on ANOVA test.

The loss in yield in response to stress treatment was least in IISR Pragati (19.5%) followed by SL 5 (23.4%), Suguna (23.5%) and Suvarna (25.4%) under moderate stress where susceptible ones suffered a greater loss IISR Alleppey Supreme (43.5%), IISR Kedaram (45.5%) and Acc 66 (53%). Under severe stress also Suguna (70.2%), IISR Pragati (71.3%), Suvarna (72.2 %) and SL 5 (74.2 %) experienced least yield loss. Conversely, susceptible ones such as Acc 66 (100%), IISR Alleppey Supreme (91%) and IISR Kedaram (89.6%) suffered the greatest loss.

Yield is the most important indicator of crop productivity. The yield all the varieties studied was affected by drought stress. However susceptible genotypes suffered a greater yield loss than the tolerant ones. The loss in yield for susceptible genotypes with the increasing intensity of drought was much higher, compared to tolerant genotypes. This was in accordance with yield response with respect to drought in chickpea cultivars (Mafakheri *et al*., 2010), rice and wheat (Zhang *et al.*, 2018; Abid *et al.,* 2018; Nawaz *et al.,* 2015) and turmeric (Sambeta & Nageswara, 2023).

In this experiment, IISR Pragati, Suguna, Suvarna and SL5 which exhibited least DSI had higher RWC, lower-medium EL, more than average enzymatic activities, accumulated more free proline and low MDA and H_2O_2 content. These genotypes which maintained a significantly higher yield than other varieties under water stress were selected as tolerant ones with sustainable yield.

All selected varieties are the popular cultivated varieties and they produced higher yields under controlled conditions. However, IISR Alleppey Supreme and IISR Kedaram experienced a significant decline in yield under drought conditions, while Acc 66 produced very little to no yield, making it unsuitable for cultivation especially under water limited environment. As a result, IISR Alleppey Supreme and IISR Kedaram, which had the lowest relative water content, low proline accumulation, and reduced enzymatic activities, as well as high levels of electrolyte leakage, malondialdehyde, and hydrogen peroxide, suffered the greatest yield loss under low soil moisture regimes. These varieties were therefore selected as susceptible genotypes for further studies to elucidate the mechanism of drought tolerance.

The results showed that tolerant genotypes significantly outperformed the susceptible ones in terms of drought tolerance traits as well as yield. The yield of all the varieties of turmeric was affected by drought stress. Genotypes produced significantly lower yield under water stress compared to the yield under non-stress.

The highest yield (under drought stress conditions) was obtained from Suguna, IISR Pragati, Suvarna and SL 5. These were selected for field analysis along with two susceptible ones (IISR Kedaram and IISR Alleppey Supreme).Thus these genotypes with contrasting characters were used in field analysis for further confirmation of drought tolerance to identify a suitable variety for cultivation under water limited environment.

4.3 Experiment III-Field analysis

Based on our study conducted in the greenhouse on physiological, biochemical, and molecular characteristics, as well as yield, four tolerant genotypes (IISR Pragati, Suguna, SL 5, and Suvarna) with sustainable yields and two susceptible genotypes (IISR Alleppey Supreme and IISR Kedaram) were identified for further field investigation. These genotypes with four replicates under control and drought treatment were evaluated for drought tolerance through physio-biochemical and molecular as well as yield and quality analysis under field condition.

4.3.1 Effect of drought on physiological characters.

Effect on physiological characters were analysed through RWC and EL. RWC and EL varied significantly among the genotypes. RWC (%) which decreased significantly ($P > 0.01$) with stress treatment had significant ($P > 0.01$) reduction from 120 to 180 DAP. The reduction in RWC under stress was lesser at 180 DAP. At 120 DAP, RWC was maximum in IISR Pragati (88.40%) which was on par with SL 5 (87.5%) and was minimum in IISR Alleppey Supreme (85.5%) in control. Under treatment it was highest in IISR Pragati (58.56%) followed by SL 5 (57.17%) and minimum was in IISR Kedaram (49.03%). At 150 DAP, it ranged from 86.5 % (IISR Pragati) to 82.6 % (IISR Alleppey Supreme) in control and 62.13% (Suguna) to 52.10 % (IISR Alleppey Supreme) in stress. Tolerant ones maintained higher RWC. At 180 DAP, It was highest in Suguna (84.2 %) which was on par with IISR Kedaram (84.17%) and IISR Pragati (83.7%) and was lowest in IISR Alleppey Supreme (81.2%) under control. Under stress it was maximum in Suguna (64.43%)

followed by IISR Pragati (62.80 %) and was lowest IISR Alleppey Supreme (52.17%) (Table 4.3.1).

At 120 DAP, RWC decreased from 33 % (IISR Pragati) to 43.3 % (IISR Kedaram). At 150 DAP, it increased from 26.6% (Suguna) to 37.8 % (IISR Kedaram) and at 180 DAP, 23.5% (Suguna) to 35.7 % (IISR Alleppey Supreme) at under water stress.

Table 4.3.1 Variation in relative water content (%) under the influence of water stress in turmeric genotypes

Genotype	120 DAP		150 DAP		180 DAP	
	control	Treatment	Control	Treatment	Control	Treatment
IISR Pragati	88.4^{a}	58.56^{c}	86.5^{a}	60.87^{g}	83.7^{a}	62.8^{e}
SL 5	87.5^{a}	57.17^{d}	84.1^{c}	61.18^{g}	82.7^{b}	62.57^{e}
Suguna	85.6^{b}	56.12^{d}	84.7^{b}	62.13^{f}	84.2^{a}	64.43^{d}
Suvarna	85.62^{b}	55.08^{e}	83.2^{d}	58.45^{h}	81.5^{c}	58.05^{f}
IISR Alleppey Supreme	85.5^{b}	50.3^{f}	82.6^{e}	52.1^{j}	81.2^{b}	52.17^{h}
IISR Kedaram	86.4^{b}	49.03^{g}	84.2^{bc}	52.36^{i}	82.2^{a}	54.4^{g}
General mean	70.43		71		71.1	
CV (%)	0.8		0.41		0.62	
CD (P=0.05)	0.96		0.49		0.75	

The mean value (n=4) followed by different letters within a column are significantly different ($P < 0.05$) based on ANOVA test.

EL (%), which increased significantly ($P > 0.05$) with stress treatment, was the highest at 180 DAP, increase of which was lesser in tolerant ones.

At 120 DAP, electrolyte leakage was minimum in IISR Alleppey Supreme (9.28%) and Suvarna (9.43%) and maximum in Suguna (10.36%) under control which was on par with SL 5 (10.2%). Under stress, it was minimum in Suvarna (11.76%) followed by SL 5 (11.9%) and maximum in IISR Alleppey Supreme (14%) and IISR Kedaram (13.62%). At 150 DAP, it ranged from 10.72 % (IISR Alleppey Supreme) to 12.2% (SL 5) under control and from 13.8 % (Suguna) followed by 15.5 % (Suvarna) to 17.6 % (IISR Alleppey Supreme) and 17.4 % (IISR Kedaram). Similarly at 180 DAP, EL was minimum in IISR Alleppey Supreme (13.6 %) followed by Suvarna (13.75 %) and maximum in SL 5 (14.6 %) and Suguna (14.4 %) in control whereas under treatment it was lowest in IISR Pragati

(19.8 %) and Suguna (20.2 %) and highest in IISR Alleppey Supreme (22.8 %). Electrolyte leakage was higher in susceptible genotypes compared to tolerant ones (Table 4.3.2).

EL increased, from 16.67 % (SL 5) to 50.86% (IISR Alleppey Supreme) at 120 DAP under stress and from 25.45 % (Suguna) to 63.57 % (IISR Alleppey Supreme) at 150 DAP. At 180 DAP it increased from 38.08 % (IISR Pragati) to 68 % (IISR Kedaram).

Table 4.3.2 Variation in electrolyte leakage (%) under the influence of water stress in turmeric genotypes

Genotype	120 DAP		150 DAP		180 DAP	
	Control	**treatment**	**control**	**Treatment**	**Control**	**Treatment**
IISR Pragati	10.12^{ef}	13.15^{abc}	12^{ef}	15.7^{bc}	14.34^{de}	19.8^{c}
SL 5	10.2^{def}	11.9^{bcd}	12.2^{e}	16.1^{b}	14.6^{d}	21.5^{b}
Suguna	10.36^{def}	12.42^{abc}	11^{h}	13.8^{d}	14.4^{de}	20.2^{c}
Suvarna	9.43^{f}	11.76^{cde}	11.6^{fg}	15.5^{c}	13.75^{de}	20.6^{c}
IISR Alleppey Supreme	9.28^{f}	14^{a}	10.76^{h}	17.6^{a}	13.6^{e}	22.8^{a}
IISR Kedaram	9.5^{f}	13.62^{ab}	11.3^{gh}	17.4^{a}	13.9^{de}	23.42^{a}
General mean	11.3		13.74		17.7	
CV (%)	9.14		2.39		2.9	
CD (P=0.05)	1.76		0.56		0.87	

The mean value (n=4) followed by different letters within a column are significantly different (P< 0.05) based on ANOVA test.

Differential response of genotypes of same species to drought stress provides us with an important tool to have an insight into the physiological and biochemical mechanisms operative in tolerant genotype. Greater RWC and lower to moderate EL are desirable characters for sustainable yield under drought condition as these characters enable crops to withstand water deficiency and maintain turgor (Jungklang *et al.*, 2017). Here in our study, higher RWC was maintained by tolerant genotypes and susceptible ones showed least RWC compared to control samples. A positive relationship was observed between yield and RWC maintenance as reported in potato cultivars (Soltys-Kalina *et al.*, 2016) and in wheat (Bayoumi *et al.*, 2015). Water deficit stress is caused by a reduction in the relative amount (or concentration) of water within plant tissues. Soltys-Kalina *et al.* (2016) reported that RWC was one of the best indicators for separating tolerant and sensitive genotypes.

In this study, enhanced electro leakage was exhibited by susceptible genotypes which affected the crop yield as reported in cucumber (Kirnak and Demirtas, 2006), squash (Abd El-Mageed *et al.,* 2016) and in pepper (Ullah *et al*., 2016). Traits like cell membrane stability (El Basyoni *et al*., 2017), a technique often used for screening drought tolerance in various crops such as sorghum (Ali *et al*., 2009) and wheat (Sallam *et al*., 2019) revealed its significant increase under drought stress with higher leakage in susceptible ones.

This study revealed that, greater RWC and lower to moderate EL enabled tolerant genotypes in attaining sustainable yield under drought condition and there was significant increase in electrolyte leakage in susceptible genotypes under drought stress compared to tolerant genotypes, which significantly affected the crop yield. Therefore in turmeric EL analysis can be used for screening for drought tolerance.

4.3.2 Effect of drought on biochemical characters.

Effect of drought on biochemical properties were analysed by stress factors such as proline and MDA and enzyme activity (POD, SOD, AsA-GSH cycle enzymes) in leaf and rhizome at different developmental stages (120, 150 and 180 DAP).

4.3.2.1 Accumulation of stress factors (Proline and MDA) under drought.

The consequence of stress condition due to water stress in the plant was analysed by the level of stress indicators like MDA and proline concentration in the turmeric leaves and rhizomes under conditions with irrigation (control) and without irrigation (drought treatment). The study showed that the level of MDA content depicting lipid peroxidation and the concentration of the amino acid proline, level increased drastically under drought stress and declined from 120 DAP to 180 DAP in both leaf and rhizome. Still both proline and MDA was maximum at early stages (120 DAP) with greater accumulation in leaf. Accumulation of stress factors followed similar pattern in both leaf and rhizome.

4.3.2.1.1 Proline

Proline accumulation increased under water stress and was maximum in tolerant ones. It was higher in leaf compared to rhizome and was highest at early stage (120 DAP) as observed under greenhouse condition. In leaf, at 120 DAP, proline content ranged from 1.73µmol/g FW (IISR Kedaram) to 3.18 µmol/gFW (IISR Pragati) followed by 2.99 µmol/g FW in SL 5 in control. Under drought treatment, it ranged from 3.03 µmol/FW (IISR Kedaram) to IISR Pragati (7.33 µmol/g FW) followed by Suguna (6.61µmol/g FW).

At 150 DAP, it ranged from 1.31 µmol/gFW (IISR Kedaram) to 2.85 µmol/g FW (IISR Pragati) followed by SL 5 (2.75 µmol/g FW). In treatment, it extended from IISR Kedaram (2.16 µmol/g FW) to maximum in IISR Pragati (6.32 µmol/g FW) followed by SL 5 (5.87 µmol/g FW).

While at 180 DAP, proline accumulation was maximum in IISR Pragati (2.28 µmol/g FW) followed by Suguna (2.17 µmol/g FW) in control and from IISR Alleppey Supreme (1.32 µmol/g FW) to Suguna (4.42 µmol/g FW) and IISR Pragati (4.37 µmol/g FW) in stress (Fig. 4.3.1a).

In the rhizome, at 120 DAP, proline accumulation was maximum in IISR Pragati (2.35 µmol/g FW) and next highest was in Suguna (2.31 µmol/g FW) while it was lowest in IISR Kedaram (1.13 µmol/g FW) in control. In the treatment, the proline content varied between1.92 µmol/g FW (IISR Kedaram) and 5.12 µmol/g FW (IISR Pragati) followed by Suguna (4.93 µmol/g FW).

At 150 DAP, it was maximum in SL 5 (2.12 µmol/g FW) followed by IISR Pragati (2.01 µmol/gFW) and minimum in IISR Kedaram (1.00 µmol/g FW) in control. While in treatment, it ranged from 1.51 µmol/g FW (IISR Kedaram) to 4.32 µmol/g FW (SL 5) followed by 3.98 µmol/g FW (IISR Pragati).

At 180 DAP, it ranged from 0.82 µmol/g FW (IISR Kedaram) to 1.66 µmol/g FW (SL 5) followed by 1.64 µmol/g FW (IISR Pragati) in control. It ranged, from 1.18 µmol/g FW (IISR Kedaram) to 3.24 µmol/g FW in SL 5 followed by IISR Pragati (3.13 µmol/g FW) under stress (Fig. 4.3.1b).

In leaf, at 120 DAP, proline content increased from 75% (IISR Kedaram) to about 130.6% (IISR Pragati) in treatment compared to control. At 150 DAP also, proline increased from 65% (IISR Kedaram) to 121.89% (IISR Pragati). Whereas at 180 DAP it had minimum increase of 55.7 % (IISR Alleppey Supreme) to 103.7 % (Suguna).

Whereas in rhizome, at 120 DAP, proline increased from 66.3% (IISR Alleppey Supreme) to 117.9 % (IISR Pragati). Similarly at 150 DAP, it increased from 50.96 % (IISR Kedaram) to 104 % (SL 5) 150 DAP. At 180 DAP it increased from 44.3 % (IISR Kedaram) to 90.8 % (IISR Pragati).

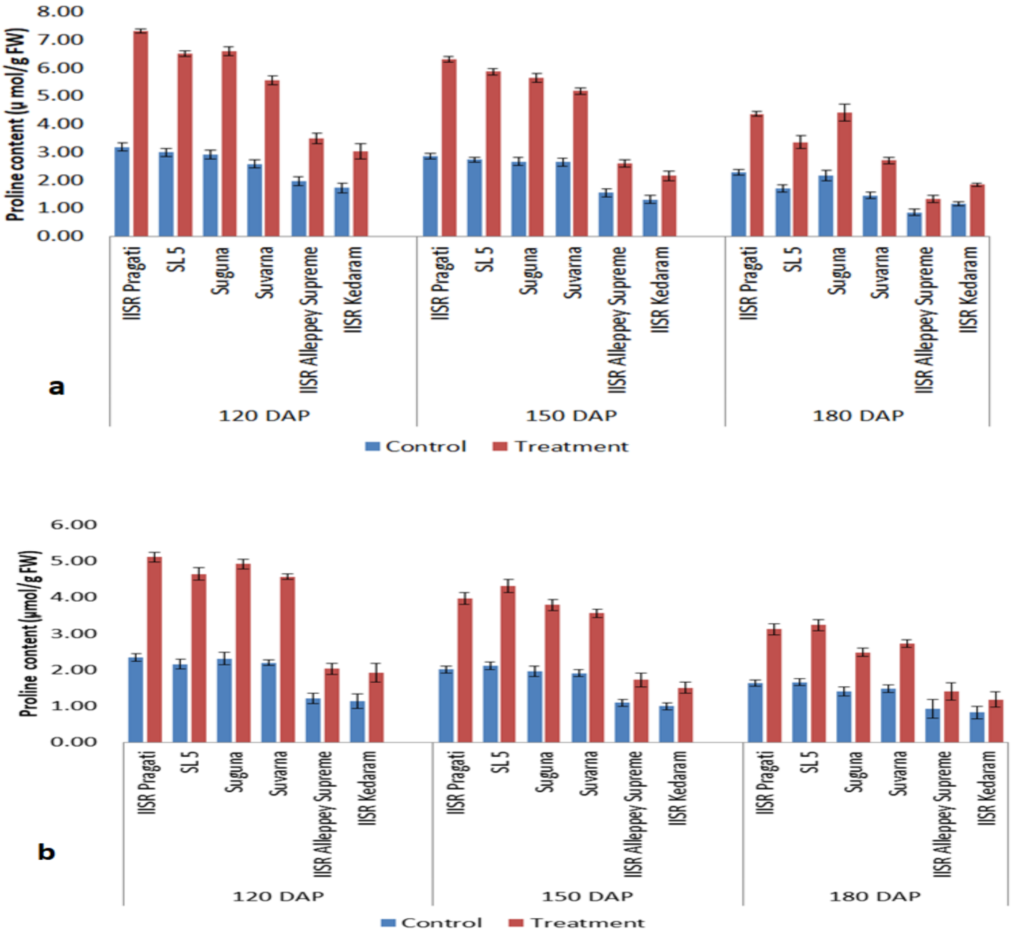


Fig. 4.3.1 Changes in the proline accumulation of the turmeric genotypes under control as well as under drought treatment in a) leaf and b) rhizome at different developmental stages. Bars represent the mean± SE (n=4).

One of the common responses to abiotic stress conditions seen in many plant species is free proline accumulation in plant tissues. A positive correlation between proline content and productivity was reported in wheat (Kamran *et al*., 2009) and maize (Špoljarević *et al*., 2011). Qasim *et al.* (2008) reported that application of the exogenous proline to *Zea mays* resulted in an enhanced growth under water deficient environment. This effect of proline is attributed to osmoregulation and membrane stabilizing potential (Webster & Leopold, 1977). Proline changed membrane from porous and leaky to stable and non-leaky (Matysik *et al.*, 2002).

Mohammadkhani & Heidari (2008) showed that proline content in *Zea mays* L. leaves was 1.56–3.13 times higher than in control leaves under dehydration. Similarly water deficit resulted in a 2.4-fold increase in shoot proline levels of drought-stressed oak shoot compared to control (Oufir *et al.,* 2009). Proline induced drought tolerance in Arabidopsis (Cuin & Shabala, 2007), sunflower (Manivannan *et al*., 2007; Hussain *et al*., 2008), maize (Ali *et al*., 2008), rice (Lum *et al*., 2014) and lentil (Molla *et al*., 2014; Bekka *et al.,* 2018).

Present study reveals that turmeric genotypes exhibited a drastic increase in proline content under water stress. Proline accumulation was higher in tolerant ones under drought stress compared to susceptible ones, which helped them maintain the productivity on account of better osmoregulation and ROS scavenging potential.

4.3.2.1.2 Lipid peroxidation

MDA accumulation increased with stress and the MDA production was less in tolerant ones. It was higher in leaf compared to rhizome and highest at early stage (120 DAP) as observed under greenhouse condition.

In leaf at 120 DAP, MDA content was minimum in 2.76 µmol/g FW (SL 5) followed by 2.82 µmol/g FW (Suvarna) and maximum in susceptible ones such as IISR Kedaram (4.52 µmol/g FW) and IISR Alleppey Supreme (4.24 µmol/g FW) in control while under stress, SL 5 (4.61 µmol/gFW) had lowest MDA content followed by and Suvarna (4.85 µmol/g FW) and was maximum in IISR Alleppey Supreme (9.83 µmol/g FW).

At 150 DAP, minimum MDA was recorded in SL 5 (2.32 µmol/g FW) followed by Suguna (2.5 µmol/g FW) though maximum was in IISR Kedaram (3.29

µmol/g FW) and IISR Alleppey Supreme (3.01 µmol/g FW) in control. Under stress, it was minimum in SL 5 (3.48 µmol/g FW) and IISR Pragati (4.17 µmol/g FW) and highest in IISR Kedaram (7.25 µmol/g FW).

At 180 DAP, under control, it ranged from 1.93 µmol/g FW (Suvarna) followed by 1.98 µmol/g FW (SL 5) to 3.41 µmol/g FW in IISR Kedaram. While under stress, it ranged from 2.81 µmol/g FW (S L 5) and 2.92 µmol/g FW (Suvarna) to 6.84 µmol/g FW in IISR Kedaram (Fig. 4.3.2 a).

In rhizome, at 120 DAP, MDA content was lowest in SL 5 (1.92 µmol/g FW) under control condition followed by Suguna (2.04 µmol/g FW) and was highest in IISR Kedaram (3.14 µmol/g FW) while under stress, lowest in SL 5 (2.95 µmol/g FW) and Suguna (3.24 µmol/g FW) and was maximum in IISR Kedaram (6.72 µmol/g FW).

At 150 DAP MDA content was lowest in IISR Pragati (1.55 µmol/g FW) and Suguna (1.65 µmol/g FW) while maximum in IISR Kedaram (2.28 µmol/g FW). Under drought, it was lowest in IISR Pragati (2.38 µmol/gFW) and SL 5 (2.47 µmol/g FW) and highest in IISR Kedaram (4.74 µmol/g FW).

Whereas at 180 DAP, MDA content was minimum in Suguna (1.35 µmol/g FW) which was on par with SL 5 (1.4 µmol/gFW) and it was maximum in IISR Kedaram (2.36 µmol/g FW) in control. Under stress, it was lowest in Suguna (1.79 µmol/g FW) and SL 5 (1.94 µmol/g FW) while it was maximum in IISR Kedaram (4.37 µmol/g FW) followed by IISR Alleppey Supreme (3.84 µmol/g FW) (Fig. 4.3.2 b).

In leaf, at 120 DAP, it increased from 67% (SL 5) to 131.8% (IISR Alleppey Supreme) and at 150 DAP, 48.4 % (IISR Pragati) to 126.2 % (IISR Alleppey Supreme). Similarly at 180 DAP the increase was from 41.9% (S L 5) to 100.6 % (IISR Kedaram).

In rhizome, at 120 DAP, MDA content accumulated 53.6% (SL 5) to 114% (IISR Kedaram) and it increased from, 42 % (SL 5) to 107.9 (IISR kedaram), 150

DAP and 32.6 % (Suguna) to 86.4% (IISR Alleppey Supreme), 180 DAP under drought condition compared to control.

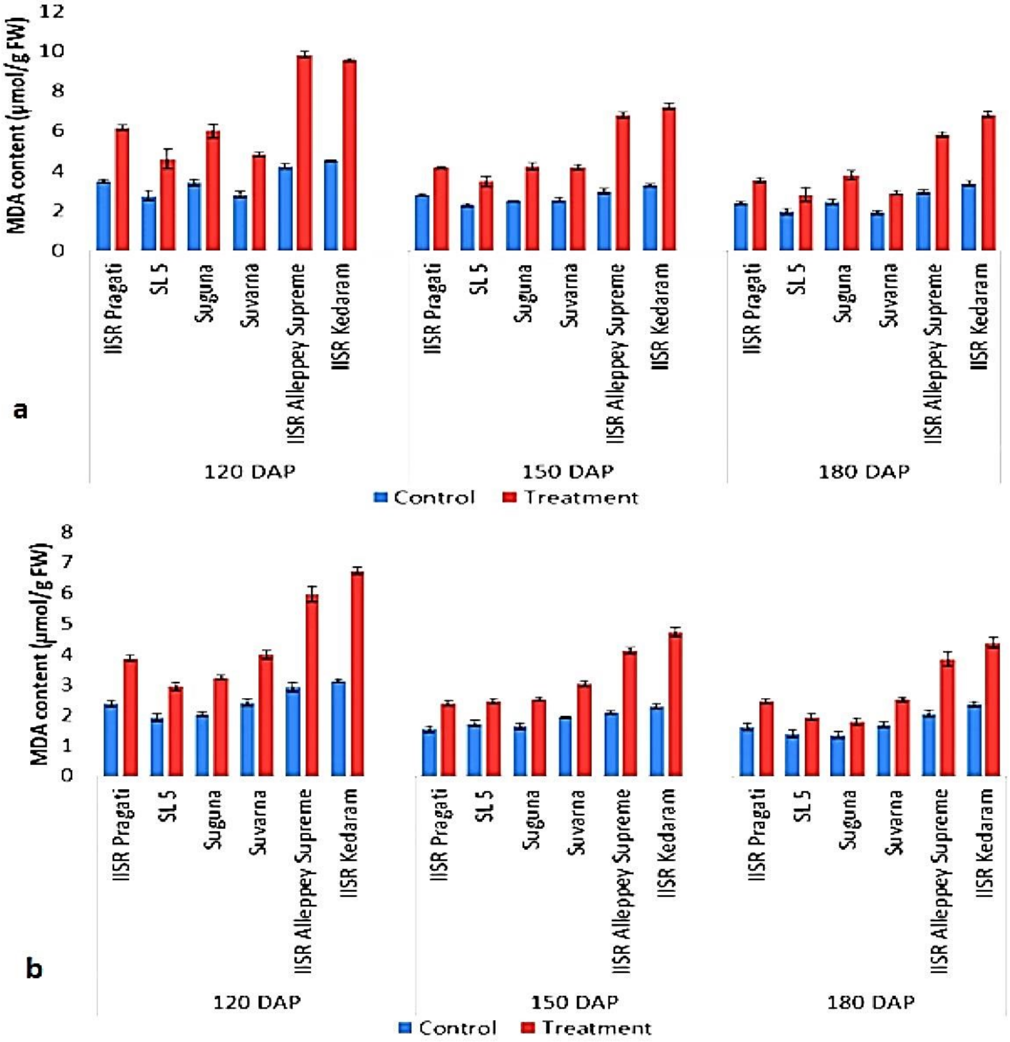


Fig. 4.3.2 Changes in the MDA accumulation of turmeric genotypes under control and under drought treatment in a) leaf and b) rhizome at different developmental stages. Bars represent the mean± SE (n=4).

Drought caused an elevated level of MDA content with higher accumulation in susceptible ones as reported in rice (Yang *et al*., 2022) whereas tolerant varieties exhibited a lower MDA level which was in accordance with reports in wheat (Upadhyay *et al*., 2020; Dvojković *et al*., 2023) and in rice (Nahar *et al*., 2018). Those genotypes with lower H_2O_2 had lower MDA content. Higher MDA concentration in drought-stressed plants was associated with higher H_2O_2 content

(Mihaljević *et al.*, 2021) which was much higher in leaf which was in line with the report by Sharma *et al.* (2012) in pea leaves.

Thus in our study, MDA content was less increased in tolerant ones as a result of better antioxidant defence and other tolerance strategies. But the rise was drastic in susceptible ones which can be attributed to higher H_2O_2 accumulation in them under drought stress. Thus MDA content can be a marker of drought sensitivity in turmeric also.

4.3.2.2 Effect of drought on antioxidant enzyme activity.

Activity of antioxidant enzymes (POD and SOD) increased more than 40 % under drought stress and the increase was maximum in tolerant ones. Higher activity was observed in leaf as compared to rhizome showing maximum activity at late stage (180 DAP) followed by 150 DAP, as observed under greenhouse condition.

4.3.2.2.1 Peroxidase

POD activity in leaf, was maximum in IISR Pragati (1.45 µmol/g FW) which was on par with SL 5 (1.41 µmol/g FW) and minimum in IISR Alleppey Supreme (0.78 µmol/g FW) and IISR Kedaram (0.85µmol/gFW) in control at 120 DAP. Under treatment also, POD was maximum in IISR Pragati (2.34 µmol/g FW) followed by SL 5 (2.21 µmol/g FW) and lowest in IISR Alleppey Supreme (1.05 µmol/gFW).

At 150 DAP it was maximum in SL 5 (1.65 µmol/g FW) and IISR Pragati (1.62 µmol/g FW) and lowest in IISR Alleppey Supreme (0.89 µmol/g FW) under control. While under drought, SL 5 (2 .77 µmol/g FW) recorded maximum activity followed by IISR Pragati (2.68 µmol/g FW) and was lowest in IISR Alleppey Supreme (1.32 µmol/g FW).

At 180 DAP, the POD activity was maximum in SL 5 (1.87 µmol/g FW) followed by Suguna (1.82 µmol/g FW) and was minimum in IISR Kedaram (1.18 µmol/g FW). Under drought, it was highest in SL 5 (3.34 µmol/g FW) which was on par with Suguna (3.22 µmol/g FW) and minimum was in IISR Kedaram (1.82 µmol/g FW) (Fig. 4.3.3a).

In rhizome also, POD enzyme concentration varied significantly among the genotypes at all the three growth stages which significantly increased under stress. At 120 DAP, IISR Pragati (1.27 µmol/g FW) followed by Suguna (1.18 µmol/g FW) recorded maximum values and the minimum activity was in IISR Kedaram (0.65 µmol/g FW) under control. Under water stress, maximum activity was observed for IISR Pragati (1.97 µmol/g FW) followed by Suguna (1.83 µmol/g FW) and IISR Kedaram (0.83 µmol/g FW) showed the minimum value.

Similarly at 150 DAP, Suguna (1.39 µmol/g FW) and SL 5 (1.35 µmol/gFW) recorded maximum activity and the lowest activity was observed in IISR Kedaram (0.78 µmol/gFW) under control. Under drought treatment, maximum activity was in SL 5 (2.23 µmol/gFW) followed by Suguna (2.19 µmol/gFW) while IISRAlleppey Supreme (1.14 µmol/g FW) showed the minimum value.

At 180 DAP, the genotype SL 5 (1.60 µmol/g FW) recorded the highest enzyme activity under control, followed by IISR Pragati (1.57 µmol/g FW). The minimum activity was observed in IISR Kedaram (0.85µmol/g FW). Under stress, SL 5 (2.76 µmol/gFW) exhibited highest enzyme activity followed by IISR Pragati (2.62 µmol/gFW), while, it was lowest in IISR Alleppey Supreme (1.29 µmol/g FW) (Fig. 4.3.3b).

In leaf the POD activity at 120 DAP increased from 35.3% (IISR Alleppey Supreme) to 60.9% (IISR Pragati), under water stress compared to control. At 150 DAP, it increased from 47.1% (IISR Kedaram) to 68.1% (SL 5) while at 180 DAP it rose from 54.2% (IISR Alleppey Supreme) to 79.8% (IISR Pragati).

In rhizome the POD activity in turmeric plants at 120 DAP increased from 29% (IISR Kedaram) to 54.8% (SL 5) with drought treatment compared to control. At 150 DAP it increased from 40.8% (IISR Alleppey Supreme) to 65 % (SL 5) and at 180 DAP there was the maximum increase from 42.9% IISR Alleppey Supreme to 72.6% (S L 5).

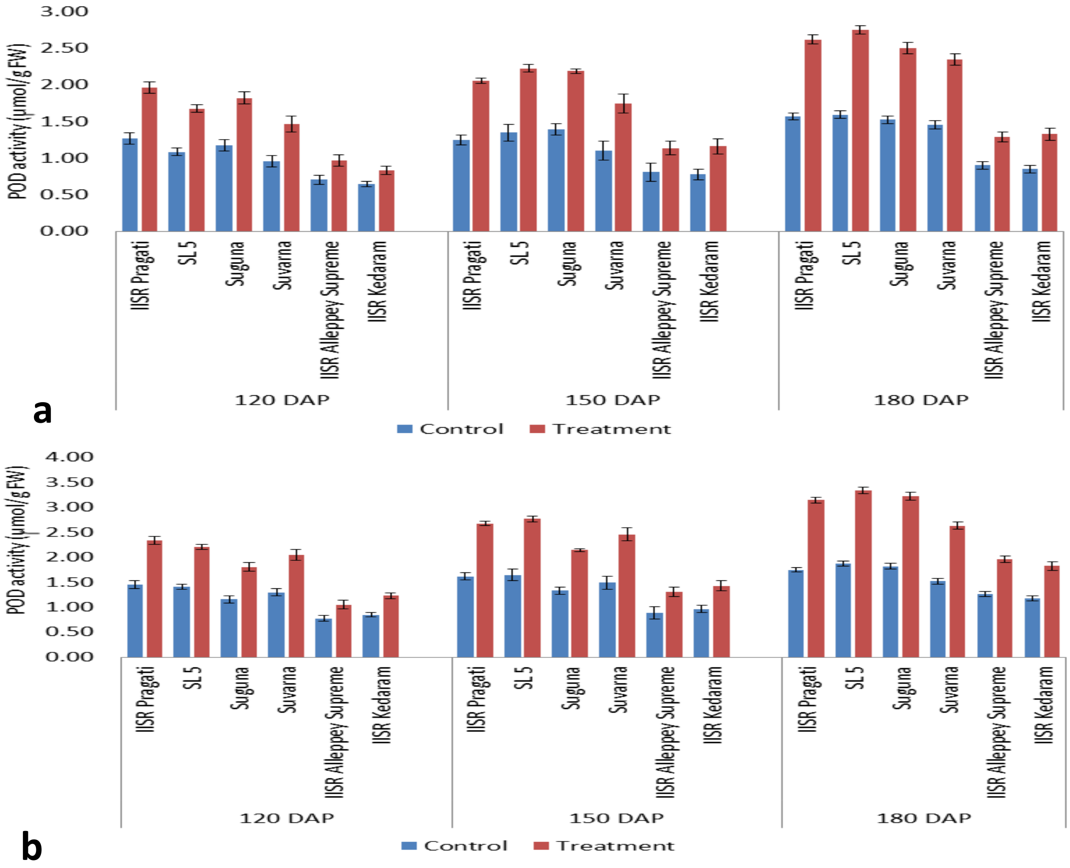


Fig. 4.3.3 Changes in the POD activity of the turmeric genotypes under control as well as drought treatment in a) leaf and b) rhizome at different developmental stages. Bars represent the mean± SE (n=4).

4.3.2.2.2 Superoxide dismutase

Drought stress enhanced the production of SOD enzyme activities in comparison with control. Superoxide dismutase enzyme dismutates superoxide into H_2O_2. Which later get decomposed by different peroxidases (Asada, 1999). In leaf, SOD activity varied significantly among the genotypes with respect to drought treatment compared to control.

At 120 DAP, IISR Pragati (5.87 µmol/g FW) recorded maximum value followed by SL 5 (5.29 µmol/g FW) and the minimum value was recorded by IISR Kedaram (3.10 µmol/g FW) under control condition. Under water stress, maximum activity was exhibited by SL5 (9.07 µmol/g FW) followed by IISR Pragati (8.31 µmol/g FW) while IISR Kedaram (4.41 µmol/g FW) showed the minimum value.

Similarly at 150 DAP, IISR Pragati (5.96 µmol/g FW) followed by SL 5 (5.72 µmol/g FW) recorded maximum SOD activity and the minimum activity was exhibited by IISR Kedaram (3.54 µmol/g FW) under control condition. Under water stress, maximum activity was exhibited by IISR Pragati (10.58 µmol/g FW) followed by SL 5 (10.26 µmol/g FW) and IISR Kedaram (5.12 µmol/g FW) showed the minimum activity.

At 180 DAP, SL 5 (6.65 µmol/g FW) and Suguna (6.29 µmol/g FW) exhibited maximum activity and it was lowest in IISR Kedaram (3.97 µmol/g FW) under control condition. Under stress, maximum activity was in SL 5 (12.48 µmol/g FW) followed by Suguna (11.90 µmol/g FW) while, IISR Kedaram (5.86 µmol/g FW) showed the minimum value (Fig. 4.3.4 a).

Thus, in leaf, the SOD content at 120 DAP increased from 42.2% (IISR Kedaram) to 71.4% (SL 5) under water stress compared to control. At 150 DAP it increased from 44% (IISR Kedaram) to 79.5% (SL 5). Likewise, there was an increase from 47.4% (IISR Kedaram) to 89.3% (Suguna) at 180 DAP.

In rhizome also, SOD enzyme concentration varied significantly among the genotypes and there was a significant increase in enzyme activity under drought compared to control. At 120 DAP, SL 5 (4.02 µmol/g FW) displayed maximum activity followed by IISR Pragati (3.90 µmol/g FW) and the minimum activity was exhibited by IISR Kedaram (2.47 µmol/g FW) under control condition. Under water stress, SL 5 displayed the highest activity at 6.76 µmol/g FW followed by IISR Pragati (6.53 µmol/g FW). Conversely, the lowest activity was observed in IISR Kedaram (3.43 µmol/g FW).

Similarly 150 DAP exposed maximum SOD activity in SL 5 (4.36 µmol/g FW) followed by Suguna (4.20 µmol/g FW) while the lowest was in IISR Kedaram (2.66 µmol/g FW) under control condition. Under water stress, maximum activity was showed by Suguna (7.52 µmol/g FW) and SL 5 (7.46 µmol/g FW) while, IISR Kedaram (3.81 µmol/g FW) showed the minimum value.

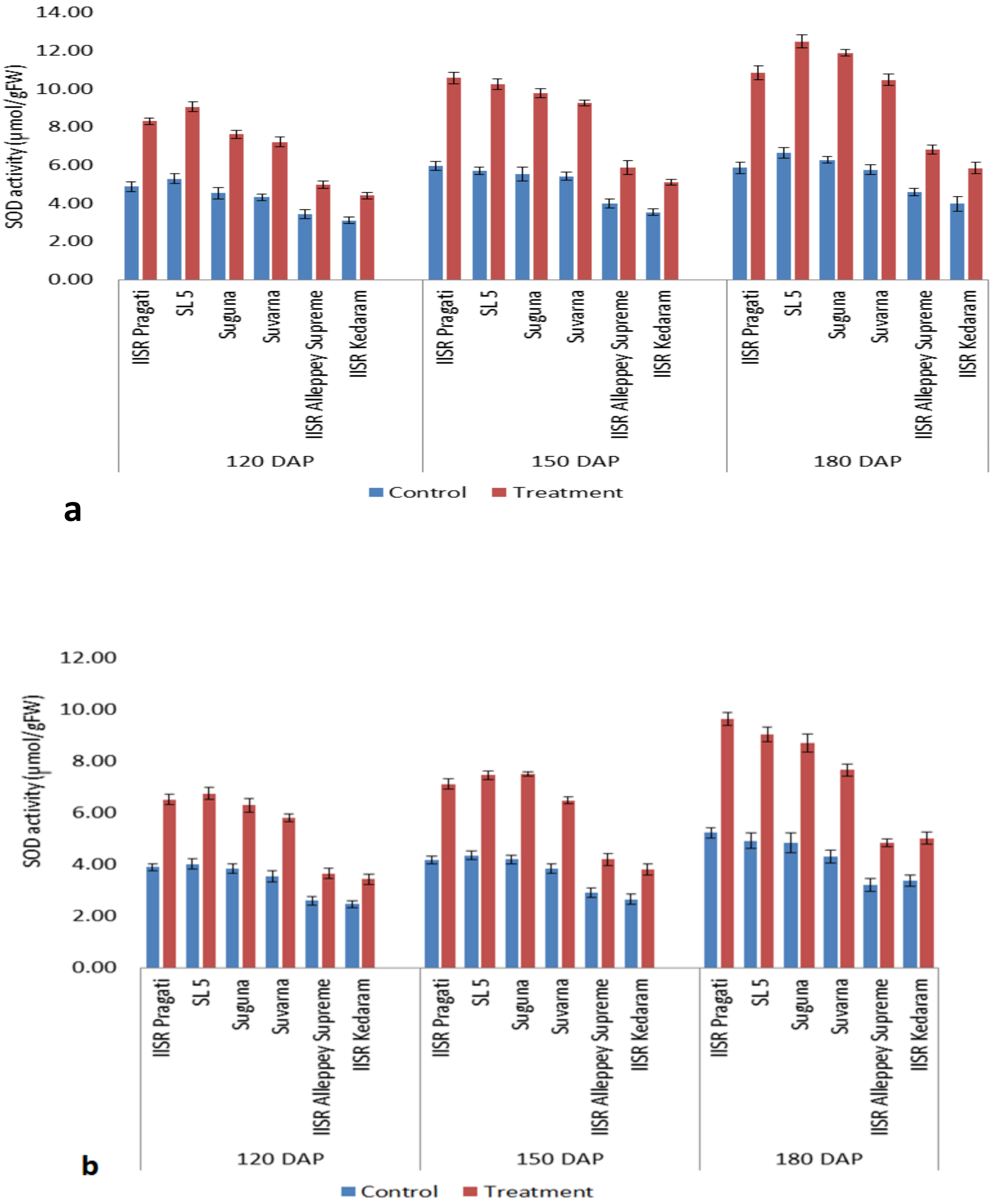


Fig. 4.3.4 Changes in the SOD activity of the turmeric genotypes under control as well as drought treatment in a) leaf and b) rhizome at different developmental stages. Bars represent the mean± SE (n=4).

At 180 days after planting (DAP), IISR Pragati exhibited the highest activity of 5.23 µmol/g FW, followed by SL 5 (4.93 µmol/g FW), under control conditions. Conversely, the lowest activity was recorded for IISR Alleppey Supreme (3.21 µmol/g FW). Under drought stress, maximum activity was observed in IISR Pragati

(9.65 µmol/g FW) followed by SL 5 (9.04 µmol/g FW) whereas IISR Alleppey Supreme (4.85 µmol/g FW) showed the lowest activity (Fig.4.3.4b).

In rhizome the SOD content at 120 DAP increased from 39.2% (IISR Kedaram) to 68.1% (SL 5) at 120 DAP and from 43.2 % (IISR Kedaram) to 78.9% (Suguna) at 150 DAP. At 180 DAP it increased from 49% (IISR Kedaram) to 84.4% (IISR Pragati) with water stress compared to control.

4.3.2.3 Effect of drought on AsA-GSH cycle enzymes.

MDHAR, DHAR, APX and GR activity augmented with stress compared to control. Higher activity was observed in the tolerant genotypes, while the increments of those enzyme activities were much lower in the sensitive genotype compared to tolerant genotype at all developmental stages. Enzyme activity was higher in leaf compared to rhizome and was maximum at late stage (180 DAP) as observed under greenhouse condition.

In leaf, APX enzyme activity varied significantly among the genotypes at all the three growth stages under control and treatment. At 120 DAP, SL 5 (4.78 µmol/g FW) and IISR Pragati (4.29 µmol/g FW) recorded maximum activity and the minimum value was observed in IISR Kedaram (2.31 µmol/g FW) under control condition. Under water stress, maximum activity was exhibited by SL 5 (7.82 µmol/g FW) followed by IISR Pragati (6.78 µmol/g FW) while IISR Kedaram (3.19 µmol/g FW) showed the minimum value.

Similarly at 150 DAP, IISR Pragati (6.16 µmol/g FW) followed by Suguna (5.30 µmol/g FW) recorded maximum activity and the minimum value was recorded in IISR Kedaram (3.22 µmol/gFW) under control condition. Under water stress, maximum activity was exhibited by IISR Pragati (10.17 µmol/g FW) followed by Suguna (8.82 µmol/g FW) and IISR Kedaram (4.54 µmol/g FW) showed the minimum activity.

At 180 DAP, Suguna (6.21 mol/g FW) and IISR Pragati (5.79 µmol/g FW) showed the maximum APX activity and the lowest activity was observed in IISR Alleppey Supreme (3.62 µmol/gFW) under control condition. Under stress, maximum activity was observed in Suguna (10.6 µmol/g FW) followed by IISR Pragati (9.92 µmol/g FW) and SL5 (9.11 µmol/g FW) while, it was lowest in IISR Alleppey Supreme (5.28 µmol/g FW) (Fig. 4.3.5a).

In leaf, the APX concentration, increased from 37.8% (IISR Kedaram) to 63.6% (SL 5), from 40.8 % (IISR Kedaram) to 66.4% (Suguna) and from 45.7% (IISR Alleppey Supreme) to 71.2% (IISR Pragati) under water stress at 120 DAP, 150 DAP and 180 DAP respectively compared to control.

In rhizome, APX enzyme activity varied significantly among the genotypes at all the three growth stages under control and treatment. At 120 DAP, maximum activity was observed in SL 5 (3.80 µmol/g FW) followed by IISR Pragati (3.62 µmol/g FW) and Suguna (3.56 µmol/g FW) and the activity was lowest in IISR Kedaram (1.82 µmol/g FW) under control condition. Under water stress, maximum activity was exhibited by SL 5 (6.03 µmol/g FW) followed by IISR Pragati (5.65 µmol/g FW) while IISR Kedaram (2.40 µmol/g FW) showed the minimum activity.

Similarly at 150 DAP, SL 5 (4.39 µmol/g FW) recorded maximum activity which was on par with IISR Pragati (4.32 µmol/g FW), while the minimum activity was observed in IISR Alleppey Supreme (2.11 µmol/g FW) µmol/gFW) under control condition. Under water stress, maximum activity was exhibited by Suguna (7.03 µmol/g FW) and SL 5 (6.74 µmol/g FW) while IISR Alleppey Supreme (2.84 µmol/gFW) showed the minimum value.

Whereas at 180 DAP, SL 5 (5.61 mol/g FW) exhibited maximum activity followed by Suguna (5.37 µmol/g FW) and the minimum value was observed in IISR Kedaram (2.80 µmol/gFW) under control condition. Under stress, maximum activity was in SL 5 (8.96 µmol/g FW) followed by Suguna (8.64 µmol/g FW) and IISR Kedaram (4.02 µmol/g FW) showed the minimum APX activity (Fig.4.3.5b).

In rhizome the APX concentration increased from 31.67% (IISR Kedaram) to 58.6% (SL 5), from 37.50 % (IISR Alleppey Supreme) to 62.6% (IISR Pragati), and from 47% IISR Alleppey Supreme to 65.4% (IISR Pragati) under water stress at 120 DAP, 150 DAP and 180 DAP respectively compared to control.

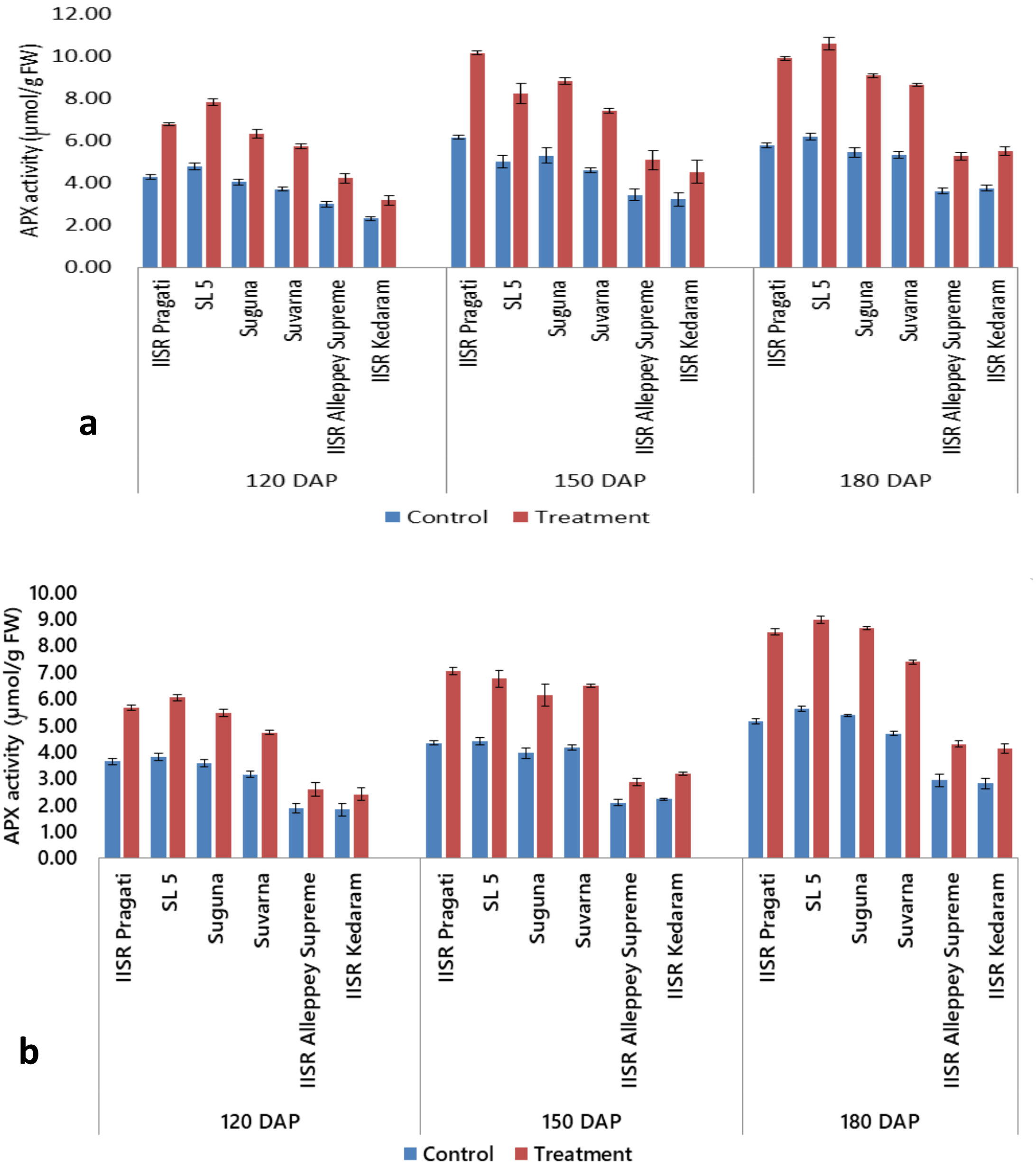


Fig. 4.3.5 Changes in the APX activity of the turmeric genotypes under control as well as drought treatment in a) leaf and b) rhizome at different developmental stages. Bars represent the mean± SE (n=4).

Significant variation was observed among genotypes for MDHAR activity under field conditions. At 120 DAP, SL 5 (0.94 μmol/g FW) recorded maximum activity followed by IISR Pragati (0.89 μmol/g FW) and the minimum value was observed in IISR Alleppey Supreme (0.61 μmol/g FW) under control condition. Under water stress, maximum activity was observed in SL 5 (1.52 μmol/g FW) followed by IISR Pragati (1.41 µmol/g FW), while IISR Alleppey Supreme (0.82 µmol/g FW) showed the minimum activity.

Similarly at 150 DAP, IISR Pragati (1.22 µmol/g FW) followed by S L 5 (1.13 µmol/g FW) recorded maximum activity and the minimum activity was shown by IISR Kedaram (0.70 µmol/g FW) under control condition. Whereas IISR Pragati (2.04 µmol/g FW) followed by S L 5 (1.83 µmol/gFW) showed maximum activity and IISR Kedaram (0.96 µmol/g FW) showed the minimum activity under water stress.

At 180 DAP, SL 5 (1.47 mol/g FW) & Suguna (1.34 µmol/g FW) showed maximum activity and the minimum activity was observed in IISR Kedaram (0.89 µmol/g FW) under control condition. Likewise under stress condition, maximum activity was observed in SL 5 (2.49 µmol/g FW) and Suguna (2.30 µmol/g FW) and IISR Kedaram (1.24 µmol/g FW) showed the minimum activity (Fig.4.3.6 a).

In leaf, the MDHAR activity increased from 33.6% (IISR Alleppey Supreme) to 61.2% (SL 5), from 37.5 % (IISR Kedaram) to 66.7% (IISR Pragati) and from 39.2% IISR Kedaram to 69.6% (SL 5) under water stress at 120 DAP, 150 DAP and 180 DAP respectively compared to control.

In rhizome tissues, MDHAR enzyme activity enhanced significantly among the genotypes under drought treatment compared to control at all the three growth stages with greater activity observed in tolerant ones. At 120 DAP, SL 5 (0.85 µmol/g FW) and IISR Pragati (0.80 µmol/g FW) exhibited maximum activity and the minimum value was observed in IISR Kedaram (0.48 µmol/g FW) under control condition. Under water stress, maximum activity was observed in SL 5 (1.27 µmol/g FW) followed by IISR Pragati (1.22 µmol/g FW) while IISR Kedaram (0.63 µmol/g FW) showed the minimum activity.

Similarly at 150 DAP, Suguna (0.97 µmol/g FW) recorded maximum activity, which was on par with S L 5 (0.92 µmol/g FW) and the minimum value was observed in IISR Kedaram (0.57 µmol/g FW) under control condition. Under water stress, maximum activity was noticed in Suguna (1.52 µmol/g FW) and S L 5

(1.48 µmol/g FW) and IISR Kedaram (0.76 µmol/g FW) showed the minimum value.

At 180 DAP, IISR Pragati (1.21 µmol/g FW) followed by Suguna (1.16 µmol/g FW) exhibited maximum activity and the minimum value was for IISR Alleppey Supreme (0.64 µmol/gFW) under control condition. Under stress, maximum activity was with IISR Pragati (1.97 µmol/g FW) followed by Suguna (1.85 µmol/g FW) and SL 5 (1.81 µmol/g FW) while IISR Alleppey Supreme (0.89 µmol/g FW) showed the minimum value (Fig. 4.3.6 b).

In rhizome, the MDHAR content at 120 DAP increased from 31.3% (IISR Kedaram) to 52.9% (IISR Pragati), from 34.7 % (IISR Kedaram) to 59.8% (SL 5) and from 40% (IISR Alleppey Supreme) to 62.8% (SL 5) under water stress at 120 DAP, 150 DAP and 180 DAP respectively compared to control.

DHAR also displayed a comparable trend of activity in both leaf and rhizome, akin to APX and MDHAR. By 120 DAP, IISR Pragati exhibited the highest activity (0.60 µmol/g FW), followed by SL 5 (0.52 µmol/g FW), while IISR Kedaram displayed the lowest activity (0.34 µmol/g FW) under control condition. Under water stress, the highest activity was noticed in IISR Pragati (0.94 µmol/g FW), followed by SL 5 (0.81 µmol/g FW) and it was lowest in IISR Kedaram (0.46 µmol/g FW).

Likewise, at 150 days after planting (DAP), IISR Pragati (0.70 µmol/g FW) and SL 5 (0.68 µmol/g FW) demonstrated the highest activity levels, while IISR Kedaram displayed the lowest (0.40 µmol/g FW) under irrigated conditions. Conversely, under water stress, SL 5 (1.14 µmol/g FW) and IISR Pragati (1.12 µmol/g FW) exhibited the highest activity, whereas IISR Alleppey Supreme showed the lowest activity (0.56 µmol/g FW).

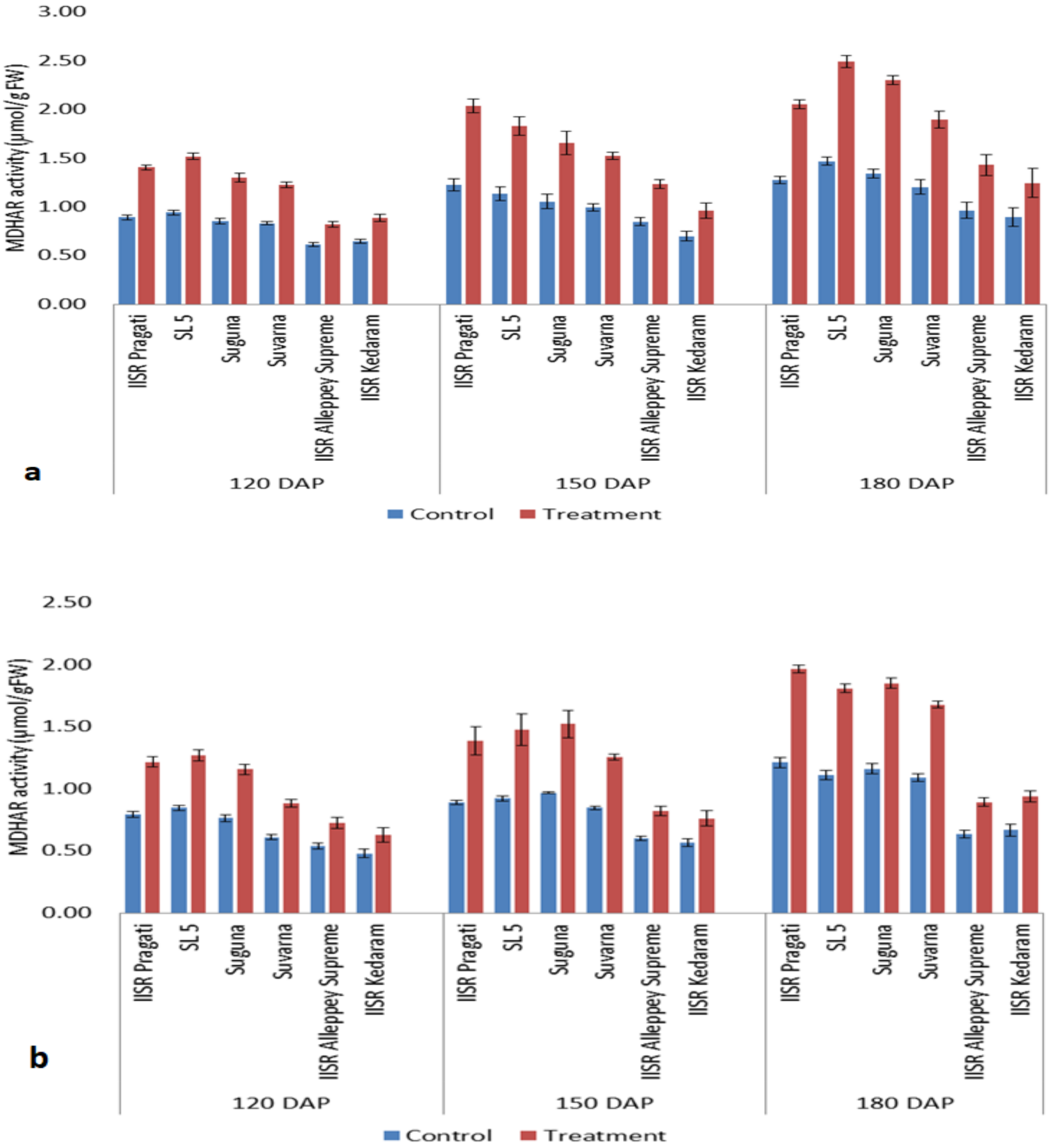


Fig. 4.3.6 Changes in the MDHAR activity of the turmeric genotypes under control as well as drought treatment in a) leaf and b) rhizome at different developmental stages. Bars represent the mean± SE (n=4).

At 180 DAP, IISR Pragati (0.84 mol/g FW) and Suguna (0.76 µmol/g FW) exhibited highest enzyme activity under control condition, while the lowest activity was observed in IISR Kedaram (0.50 µmol/g FW). Under drought stress, IISR Pragati (1.36 µmol/g FW) and Suguna (1.28 µmol/g FW) had the maximum activity, whereas lowest activity was recorded in IISR Kedaram (0.75 µmol/g FW) (Fig. 4.3.7a).

Here in leaf, the DHAR concentration increased from 36.4% (IISR Kedaram) to 56.8% (IISR Pragati), from 38.8% (IISR Alleppey Supreme) to 67.1% (SL 5) and from 47.7% IISR Alleppey Supreme to 71.5% (SL 5) under water stress at 120 DAP, 150 DAP and 180 DAP respectively compared to control.

In rhizome tissues also, DHAR enzyme activity was significantly enhanced among the genotypes under drought treatment compared to control as that observed in case of leaf. At 120 DAP, IISR Pragati (0.48 µmol/g FW) and SL 5 (0.45 µmol/g FW) recorded maximum activity and the minimum value was observed in IISR Alleppey Supreme (0.30 µmol/gFW) under control condition. Under water stress, IISR Pragati (0.73 µmol/g FW) and SL 5 (0.70 µmol/g FW) exhibited maximum activity followed by Suguna (0.64 µmol/g FW) while IISR Alleppey Supreme (0.38 µmol/g FW) displayed the lowest activity.

Similarly at 150 DAP, IISR Pragati (0.57 µmol/g FW) followed by S L 5 (0.49 µmol/g FW) showed higher activity levels while the minimum value was observed in IISR Alleppey Supreme (0.33 µmol/gFW) under control condition. Under water stress also maximum activity was exhibited by IISR Pragati (0.89 µmol/g FW) followed by SL 5 (0.76 µmol/g FW) and minimum activity was observed in IISR Alleppey Supreme (0.45 µmol/g FW).

At 180 DAP, IISR Pragati (0.62 mol/g FW) and Suguna (0.57 µmol/g FW) exhibited maximum activity and the minimum value was observed in IISR Kedaram (0.37 µmol/g FW) under control condition. Whereas under stress, maximum activity was showed by IISR Pragati (0.98 µmol/g FW) and Suguna (0.93 µmol/g FW) while, IISR Kedaram (0.52 µmol/g FW) showed the minimum activity (Fig. 4.3.7b).

In rhizome, the DHAR activity increased from 28% (IISR Kedaram) to 54.6% (SL 5), from 34.2% (IISR Kedaram) to 55% (IISR Pragati) and from 38.9% IISR Alleppey Supreme to 61.4% (Suguna) with water stress at 120 DAP, 150 DAP and 180 DAP respectively compared to control.

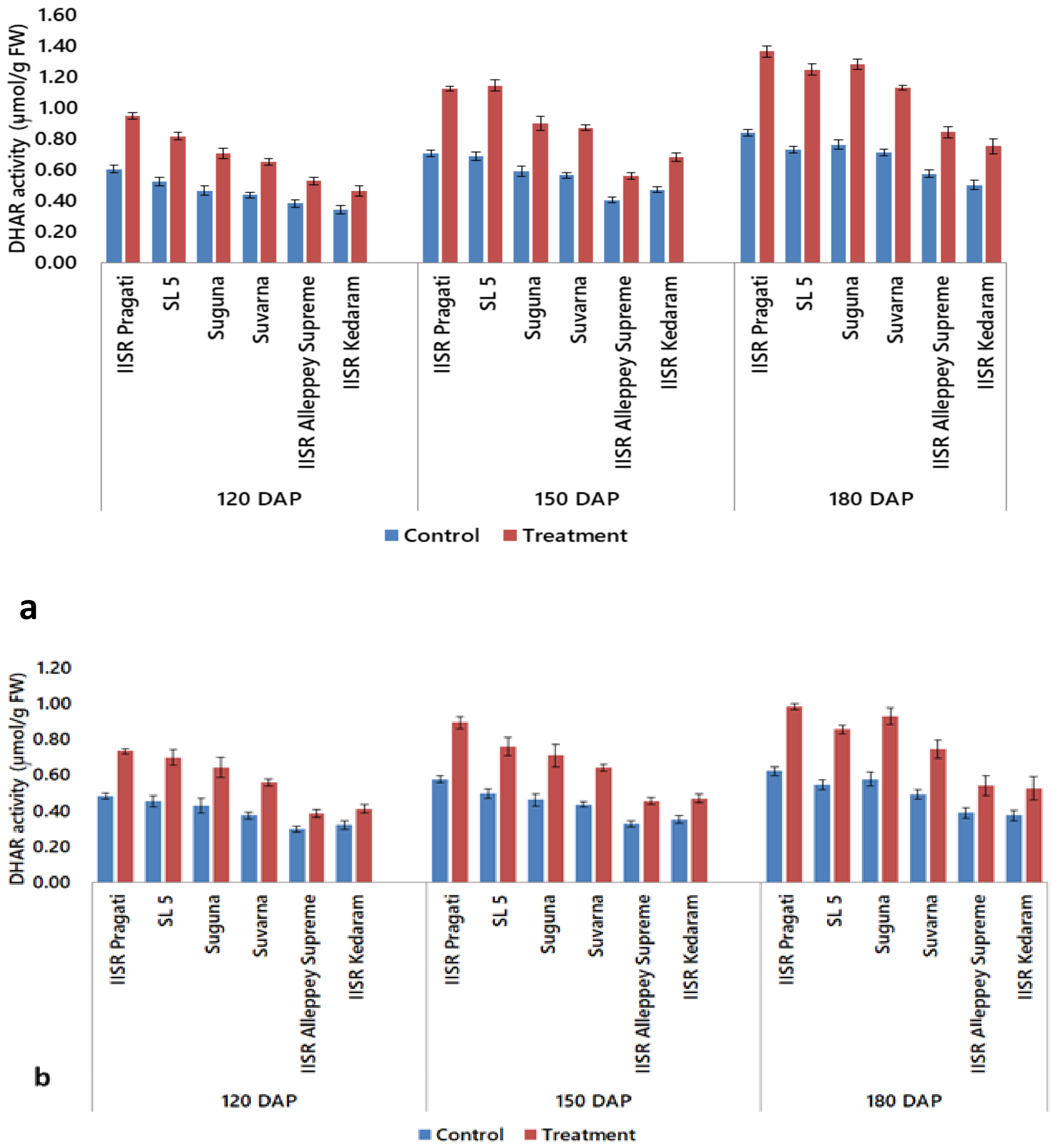


Fig. 4.3.7 Changes in the DHAR activity of the turmeric genotypes under control as well as drought treatment in a) leaf and b) rhizome at different developmental stages. Bars represent the mean± SE (n=4).

GR enzyme concentration increased significantly among the genotypes at all the three growth stages under drought treatment. In leaf, at 120 DAP, SL 5 (1.32 µmol/g FW) showed the maximum activity followed by IISR Pragati (1.25 µmol/g FW) and the minimum value was observed in IISR Kedaram (0.87 µmol/g FW) under control condition. Under water stress, maximum activity was recorded in SL 5

(1.96 µmol/g FW) followed by IISR Pragati (1.82 µmol/g FW), and IISR Kedaram (1.09 µmol/g FW) showed the minimum activity.

Similarly at 150 DAP, S L 5 (1.41 µmol/g FW) recorded maximum activity, followed by Suguna (1.33 µmol/g FW) and IISR Pragati (1.28 µmol/g FW) and it was minimum in IISR Kedaram (0.93 µmol/g FW) under control condition. Under water stress, maximum activity was exhibited by SL 5 (2.18 µmol/g FW) and Suguna (2.04 µmol/g FW), while, IISR Kedaram (1.21 µmol/g FW) and IISR Alleppey Supreme (1.32 µmol/g FW) showed the minimum activity.

At 180 DAP, maximum activity was exhibited by IISR Pragati (1.63 mol/g FW) followed by Suguna (1.52 µmol/g FW). The lowest activity was shown by IISR Kedaram (1.11 µmol/g FW) and IISR Alleppey Supreme (1.17 µmol/g FW) under control condition. Under stress condition, maximum activity was observed in IISR Pragati (2.73 µmol/g FW) which was on par with SL 5 (2.45 µmol/g FW). Whereas, IISR Kedaram (1.48 µmol/g FW) showed minimum activity (Fig. 4.3.8a)

In leaf, the GR activity increased from 25% (IISR Kedaram) to 48% (SL 5), from 29% (IISR Kedaram) to 55% (SL 5) and from 34% IISR Kedaram to 67% (IISR Pragati) under water stress at 120 DAP, 150 DAP and 180 DAP respectively compared to control.

Similar to what was seen in the case of leaf tissues, GR enzyme activity increased considerably in rhizome tissues across the genotypes experiencing drought treatment compared to control. At 120 DAP, S L 5 (1.11 µmol/g FW) followed by IISR Pragati (1.06 µmol/g FW) recorded maximum activity and it was the lowest in IISR Kedaram (0.73 µmol/gFW) under control condition. Under water stress, SL 5 (1.53 µmol/g FW) demonstrated the highest activity which was on par with IISR Pragati (1.48 µmol/g FW) and IISR Kedaram (0.86 µmol/g FW) displayed the lowest activity.

Similarly, under control conditions, IISR Pragati (1.30 µmol/g FW) and SL 5 (1.26 µmol/g FW) recorded the greatest activity at 150 DAP, followed by Suguna (1.04 µmol/g FW), while IISR Kedaram (0.82 µmol/g FW) showed the least activity.

IISR Pragati (1.92 µmol/g FW) followed by Suguna (1.83 µmol/g FW) exhibited maximum activity under water stress. While, IISR Kedaram (1.05 µmol/g FW), exhibited the minimum activity.

At 180 DAP, SL 5 (1.48 mol/g FW) and Suguna (1.45 µmol/g FW) exhibited maximum GR activity while the lowest activity was observed in IISR Alleppey Supreme (0.91 µmol/gFW) under control condition. Whereas under drought stress, maximum activity was exhibited by SL 5 (2.32 µmol/g FW) and Suguna (2.25 µmol/g FW) while IISR Alleppey Supreme (1.19µmol/g FW) displayed the lowest activity (Fig. 4.3.8b).

In rhizome, the GR content at 120 DAP increased from 18% (IISR Kedaram) to 40% IISR Pragati), from 28% (IISR Alleppey Supreme) to 49% (Suguna) and from 30% (IISR Alleppey Supreme) to 56% (SL 5) under water stress at 120 DAP, 150 DAP and 180 DAP respectively compared to control.

There was significant variation in the activity of AsA-GSH cycle enzymes among genotypes at all the three growth stages under all water regimes. APX, MDHAR, DHAR and GR activities showed significant difference between control and treated plants. It was higher in the stress treated as compared to the untreated plants with slight decrease in activity under severe stress compared with moderate stress in leaf. Similar results were found in the activity of AsA-GSH cycle enzymes in rhizome also.

Exposure of plants to drought stress cause elevated generation of ROS (e.g., $_1$ O^2, O^2 -, OH• and $H_2O_{2)}$. Enzymatic and non-enzymatic antioxidant systems minimize the effects of oxidative stress by directly scavenging ROS (Apel & Hirt, 2004; Sharma & Dubey, 2005). The extent to which antioxidant enzyme activities are heightened during drought stress varies widely among plant species (Zhang & Kirkham, 1995), and even between different genotypes of the same species (Bartoli *et al.*, 1999). Plants can respond and adapt to water stress by detecting the stimulus, producing and transmitting signals, and activating various defence mechanisms (Ming-Yi & Jian-Hua, 2004). The antioxidant system, which includes enzymes like superoxide dismutase, peroxidase and catalase, plays a crucial role in regulating levels of reactive oxygen species (Aroca *et al*., 2003). Elevated activities of SOD and POD have been observed in drought-tolerant varieties of alfalfa (Zhang *et al*., 2018) and maize (Shafiq *et al*., 2019). The AsA-GSH reduction system collaborates in ROS removal under drought stress, the greater enhancement of which has been documented in maize (Jiang *et al*., 2022) and wheat (Shan *et al*., 2015) under water stress, particularly in drought-resistant crops. In the present study, the variation in enzyme activity under drought stress was genotype dependent. Tolerant genotypes showed higher enzyme activity compared to susceptible genotypes as observed under greenhouse condition.

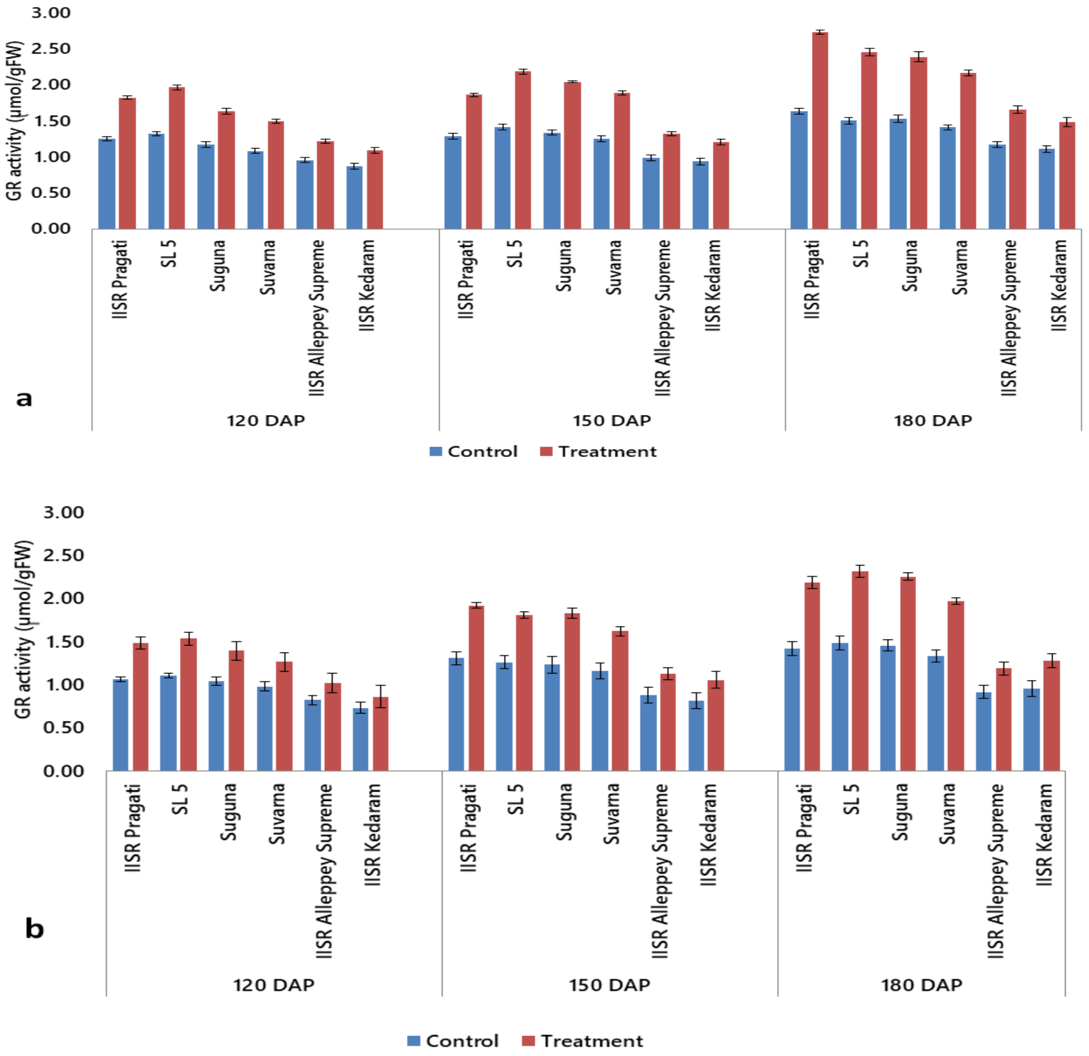


Fig. 4.3.8 Changes in the GR activity of the turmeric genotypes under control as well as drought treatment in a) leaf and b) rhizome at different developmental stages. Bars represent the mean± SE (n=4).

4.3.2.4. Effect on Chlorophyll Fluorescence Parameter.

Our results indicated that chlorophyll fluorescence parameters were affected significantly under drought conditions compared to control. The maximum efficiency of PSII (Fv/Fm) significantly reduced in turmeric genotypes under water stress compared to the control. It was highest in Suguna (0.738) followed by Suvarna (0.712) and was least in SL 5 (0.673) under control. Under water stress treatment it reduced to 0.693 in Suguna and 0.654 in Suvarna and it was least in IISR Kedaram (0.601) and IISR Alleppey Supreme (0.607). It reduced to about 6.1 % in Suguna and 7.3% in IISR Pragati and the most decrease was in IISR Alleppey Supreme which was up to 12.1%. Changes in chlorophyll fluorescence parameters of the turmeric genotypes under control as well as drought treatment at fully mature growth stage (180DAP) is represented in, Table 4.3.3.

Table 4.3.3 Variation in chlorophyll fluorescence parameters under the influence of water stress in turmeric genotypes

Genotype	Fo		Fm		Fv		Fv/Fm	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
IISR Pragati	63^{c}	57^{efg}	197^{d}	170.64^{h}	134^{cd}	107.64^{f}	0.681^{de}	0.631^{g}
SL 5	66^{b}	58^{e}	202^{c}	174.96^{g}	136^{c}	108.96^{f}	0.673^{e}	0.623^{g}
Suguna	56^{g}	52^{h}	214^{a}	182.52^{f}	158^{a}	126.52^{e}	0.738^{a}	0.693^{c}
Suvarna	57^{ef}	53^{h}	198^{d}	164.56^{i}	141^{b}	107.56^{f}	0.712^{b}	0.654^{f}
IISR-Kedaram	66^{a}	62c^{d}	209^{b}	165.24^{i}	143^{b}	99.24^{g}	0.684^{d}	0.601^{h}
IISR Alleppey Supreme	61^{d}	56^{fg}	192^{e}	155.36^{j}	131^{d}	94.36^{h}	0.682d^{e}	0.607^{h}
General mean	58.7		185		124		0.665	
CV (%)	1.27		1.17		1.55		0.885	
CD(P=0.05)	1.28		3.73		3.28		0.01	

The mean value (n=4) followed by different letters within a column indicate significant differences between the samples determined by ANOVA, Duncan´s multiple range test at 0.05 level.

Drought significantly decreased F_V/F_M ratio (photochemical efficiency of PS II) in turmeric genotypes especially in susceptible ones. Similar results were reported in maize (Efeoğlu *et al*., 2009), wheat (zlatev *et al*., 2009) and rice (Faseela *et al*., 2020).

Reduction in the maximum quantum efficiency of PSII implies a declined capturing and conversion rate of excitation energy by PSII reaction centres. It indicates change in immediate plant productivity in response to fluctuation in water content (Ansari *et al*., 2019). Chlorophyll fluorescence value reveals the extent to which stresses damage the photosynthetic apparatus (Maxwell & Johnson, 2000). Positive correlation with Fv/Fm ratio (photosynthetic efficiency) and crop yield is reported in many crops including sunflower (Gaffari *et al.,* 2012), Olea europea (Khaleghi *et al*., 2012), wheat (Guo *et al*., 2016) and straw berry (Arief *et al*., 2023). Here, turmeric genotypes exhibited a decline in Fv/Fm ratio under water stress. Sucsceptible genotypes showed higher reduction in quantum efficiency of PSII which resulted in higher reduction of rhizome yield.

4.3.3. Gene expression analysis

Of the seven transcription factors studied under greenhouse condition four TFs (*ABF, bHLH, DREB, WRKY*) that are most correlated to biochemical parameters were selected for field analysis considering Pearson's correlation study with enzyme activity.

ABF increases drought tolerance by regulating genes related to ABA (abscissic acid) under drought stress (Liang *et al*., 2016). In leaf, the maximum relative expression of ABF under drought was detected in Suguna (fold change: 38.43) followed by IISR Pragati (fold change: 25.63) and the expression was lowest in IISR Kedaram (fold change: 8.99) at 120 DAP. At 150 DAP Suguna (fold change: 69.94) was expressed greatly, followed by SL 5 (fold change: 38.91) and the least expression was exhibited by IISR Kedaram (fold change: 10.38). Furthermore, at 180 DAP also, Suguna (fold change: 71.1) and IISR Pragati (fold change: 66.17) showed maximum expression and it was least in IISR Kedaram (fold change: 19.7) (Fig. 4.3.9a).

In rhizome, the maximum expression of *ABF* was detected in SL 5 (fold change: 71) and IISR Pragati (fold change: 21.23) and it was least in IISR Kedaram (fold change: 6.48) at 120 DAP. At 150 DAP, SL 5 (fold change: 44.14) followed by IISR Pragati (fold change: 28.92) displayed maximum expression whereas least expression was in IISR Kedaram (fold change: 8.27). Also, at 180 DAP, SL 5 (fold change: 42.8) and IISR Pragati (fold change: 35.16) had maximum expression and lowest was exhibited by IISR Alleppey Supreme (fold change: 10.34) (Fig.4.3.9b).

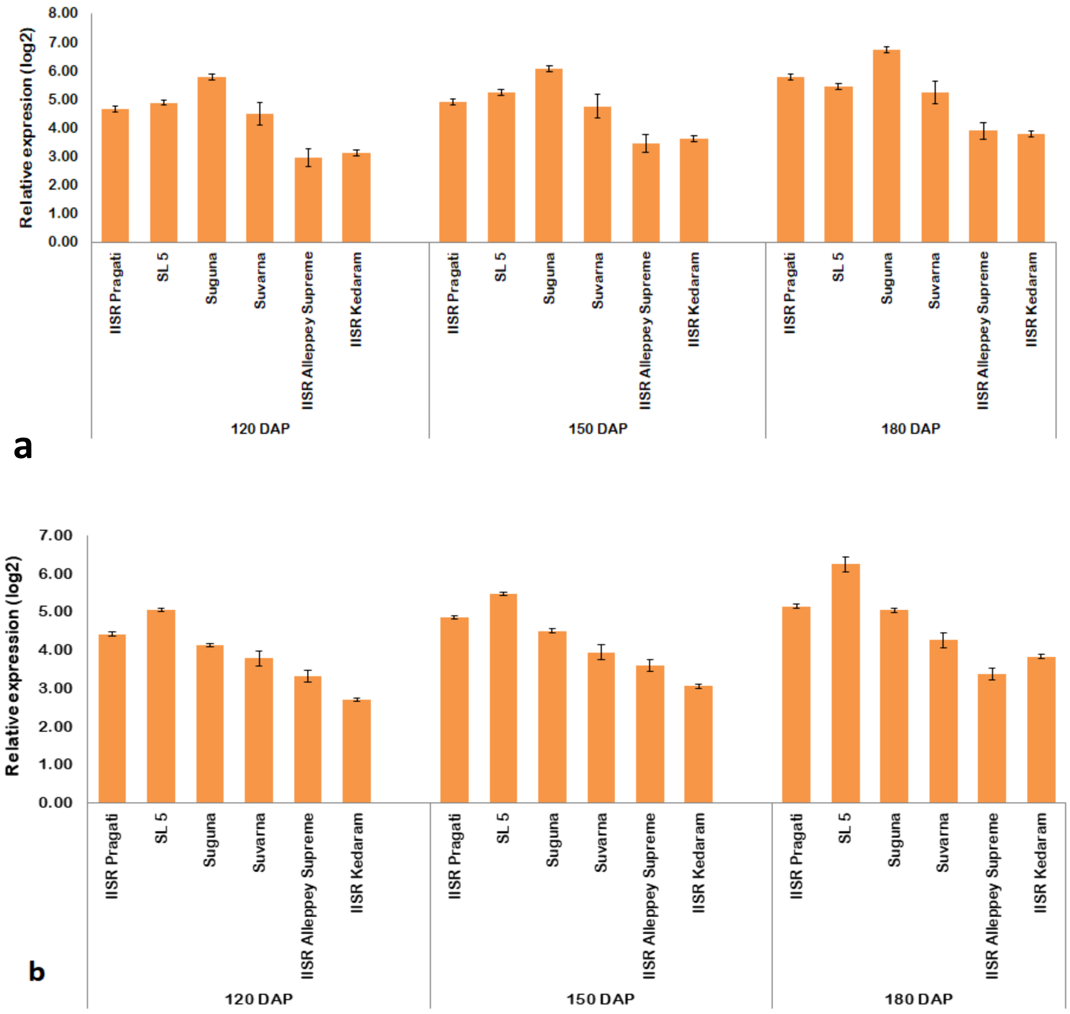
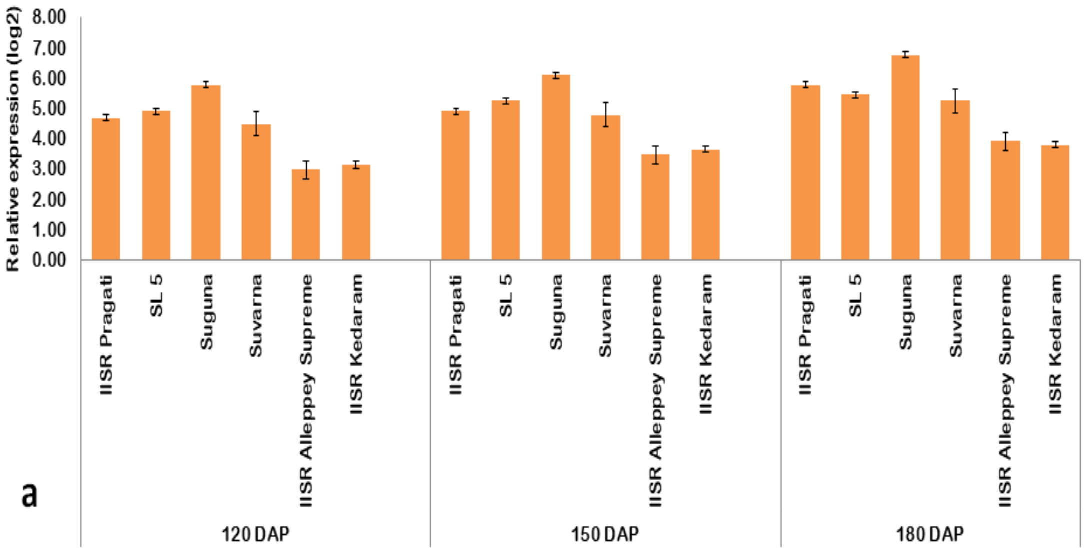


Fig. 4.3.9 Relative expression pattern of *ABF* under drought treatment with respect to irrigated condition (control) in (a) leaf and (b) rhizome. Bars represent the mean ± SE.

bHLH transcription factors regulate gene expression through interactions with specific motifs in target genes. These are involved in plant growth as well as play an important role in plant responses to abiotic stress (Li *et al.,* 2021). In the leaf, the maximum expression of *bHLH* was detected in Suguna (fold change: 55) and SL 5 (fold change: 29.9) and it was least expressed in IISR Alleppey Supreme (fold change: 7.8) at 120 DAP. Suguna (fold change: 68.4) followed by SL 5 (fold change: 38.2) exhibited maximum expression at 150 DAP, and the least expression was observed in IISR Alleppey Supreme (fold change: 11.1). Likewise at 180 DAP, Suguna (fold change: 71.5) and IISR Pragati (fold change: 59.7) showed maximum expression while least expression was shown by IISR Kedaram (fold change: 13.9) (Fig.4.3.10a).

In rhizome, the maximum expression of *bHLH* was detected in Suguna (fold change: 30.3) followed by IISR Pragati (fold change: 19.2) and it was least expressed in IISR Kedaram (fold change: 5.7) at 120 DAP. Similarly, Suguna (fold change: 42.2) followed by SL 5 (fold change: 28.2) exhibited maximum expression at 150 DAP, and lowest in IISR Alleppey Supreme (fold change: 7). While, at 180 DAP, IISR Pragati (fold change: 52.1) and Suguna (fold change: 45.1) showed maximum expression while lowest expression was observed in IISR Alleppey Supreme (fold change: 9.9) (Fig.4.3.10b).

The *DREB* transcription factor family is found in multiple plant species, and has been shown to function in enhancing plant tolerance to various abiotic stresses, including drought stress (Agarwal *et al.*, 2006). Here *DREB* had more expression in tolerant genotypes compared to susceptible ones. In leaf, the maximum relative expression of *DREB* under drought was detected in IISR Pragati (fold change: 42.58) followed by SL 5 (fold change: 27.11) and the relative expression was least in IISR Alleppey Supreme (fold change: 13.70) at 120 DAP. IISR Pragati (fold change: 56.71) followed by Suguna (fold change: 35.46) showed higher expression at 150 DAP while the expression was lowest in IISR Alleppey Supreme (fold change: 16.74). Similarly, at 180 DAP, IISR Pragati (fold change: 86.01) and SL 5 (fold change: 56.6) showed highest expression while, minimum was observed in IISR Kedaram (fold change: 23.42) (Fig. 4.3.11a).



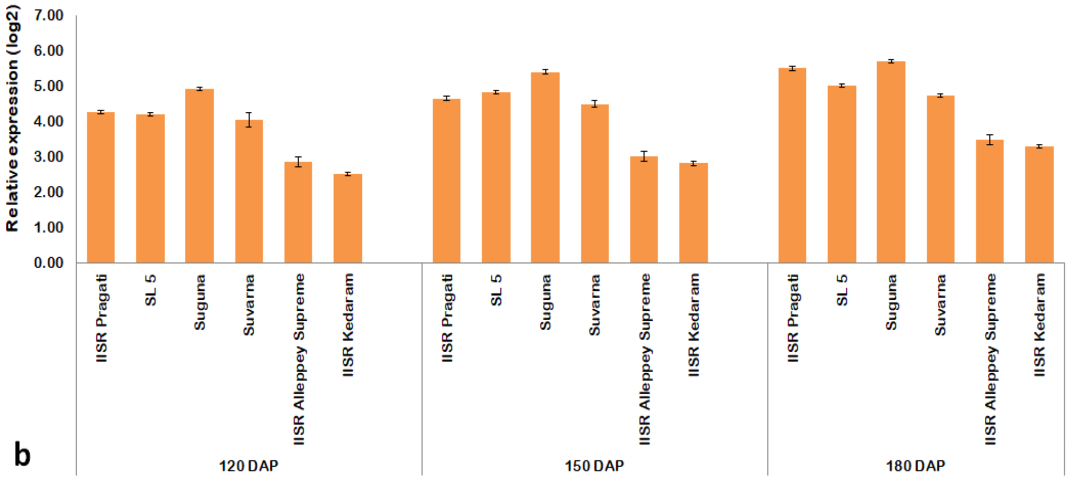


Fig. 4.3.10 Relative expression pattern of *bHLH* under drought treatment with respect to irrigated condition (control) in (a) leaf and (b) rhizome. Bars represent the mean ± SE (n=4).

In the rhizome, the maximum expression of *DREB* was detected in IISR Pragati (fold change: 27.95) followed by Suguna (fold change: 21.77) and lowest in IISR Kedaram (fold change: 8.20) at 120 DAP. Whereas at 150 DAP, highest expression was displayed by IISR Pragati (fold change: 37.00) followed by Suvarna (fold change: 30.26) and least expression was observed in IISR Kedaram (fold: 12.56). At 180 DAP, IISR Pragati (fold change: 61.39) and Suvarna (fold change:

39.1) displayed maximum expression while least expression was observed in IISR Alleppey Supreme (fold change: 17.88) (Fig.4.3.11b).

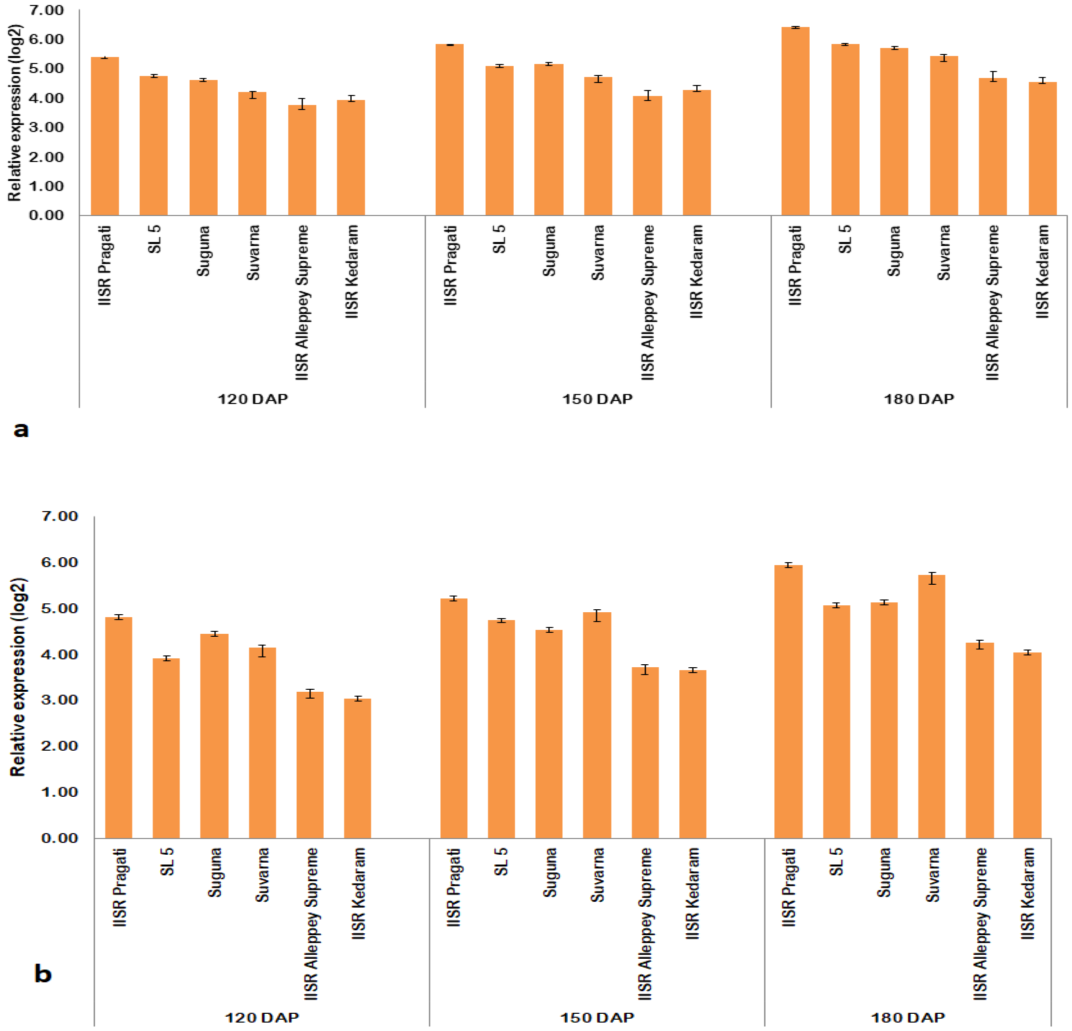


Fig. 4.3.11 Relative expression pattern of *DREB* under drought treatment with respect to irrigated condition (control) in (a) leaf and (b) rhizome. Bars represent the mean ± SE.

The *WRKY* transcription factor family not only imparts resistance to abiotic stresses but also governs plant growth and development (Jiang *et al*., 2012; Xu *et al*., 2014). *WRKY* was more expressed in tolerant genotypes than in sensitive ones. In leaf, the maximum relative expression of *WRKY* was detected in SL 5 (fold change: 35.75) and Suguna (fold change: 20.31) and it was lowest in IISR Alleppey Supreme (fold change: 7.57) at 120 DAP. At 150 DAP, SL 5 (fold change: 57.28) revealed maximum relative expression followed by Suguna (fold change: 30.95) and lowest expression was observed in IISR Kedaram (fold change: 10.76). Similarly at 180 DAP, SL 5 (fold change: 81) showed maximum expression followed by Suguna (fold change: 55.25) and lowest expression was observed in IISR Kedaram (fold change: 14.1) (Fig. 4.3.12a).

In the rhizome, the *WRKY* expression was most pronounced in SL 5 (fold change: 23.3) and IISR Pragati (fold change: 14.89) at 120 DAP, while it was lowest in IISR Kedaram (fold change: 5.93). At 150 DAP, the highest expression was observed in SL 5 (fold change: 3.58), followed by IISR Pragati (fold change: 20.20) and IISR Kedaram displayed the least expression (fold change: 6.70). At 180 DAP, the higher expression was observed in SL 5 (fold change: 44.6) followed by Suguna (fold change: 25.5), while it was lowest in IISR Alleppey Supreme (fold change: 8.6) (Fig. 4.3. 12b).

Gene expression studies indicated that expression of TFs increased under water stress. But it was lower in susceptible ones like IISR Alleppey Supreme and IISR Kedaram under drought conditions while tolerant ones gave maximum expression. This indicates the influence of TFs on water regimes. Similar results were obtained in all stages for all TFs analysed in leaf and rhizome as observed in greenhouse condition. Leaf showed higher relative expression compared to rhizome under drought.

Crop production is greatly impacted by complex environmental factors. Some studies have shown that overexpression of stress-related TFs may increase crop yield under stress condition (Yu *et al*., 2013; Zhao *et al*., 2014; Shavrukov *et al*., 2016) by enhancing the abiotic stress tolerance in transgenic plants through overexpression of some stress-responsive TFs (Saad *et al*., 2013). *AREB/ABF*s, *DREB*s and *WRKY* are the vital transcription factors regulating a large fraction of drought inducible genes involved in one or more drought responsive mechanisms (Yamaguchi-Shinozaki & Shinozaki, 2006). Over expression of *bHLH* in peanut resulted in accumulation of less ROS, accompanied by increased activity and transcript levels of antioxidant enzymes (Li *et al*., 2018).

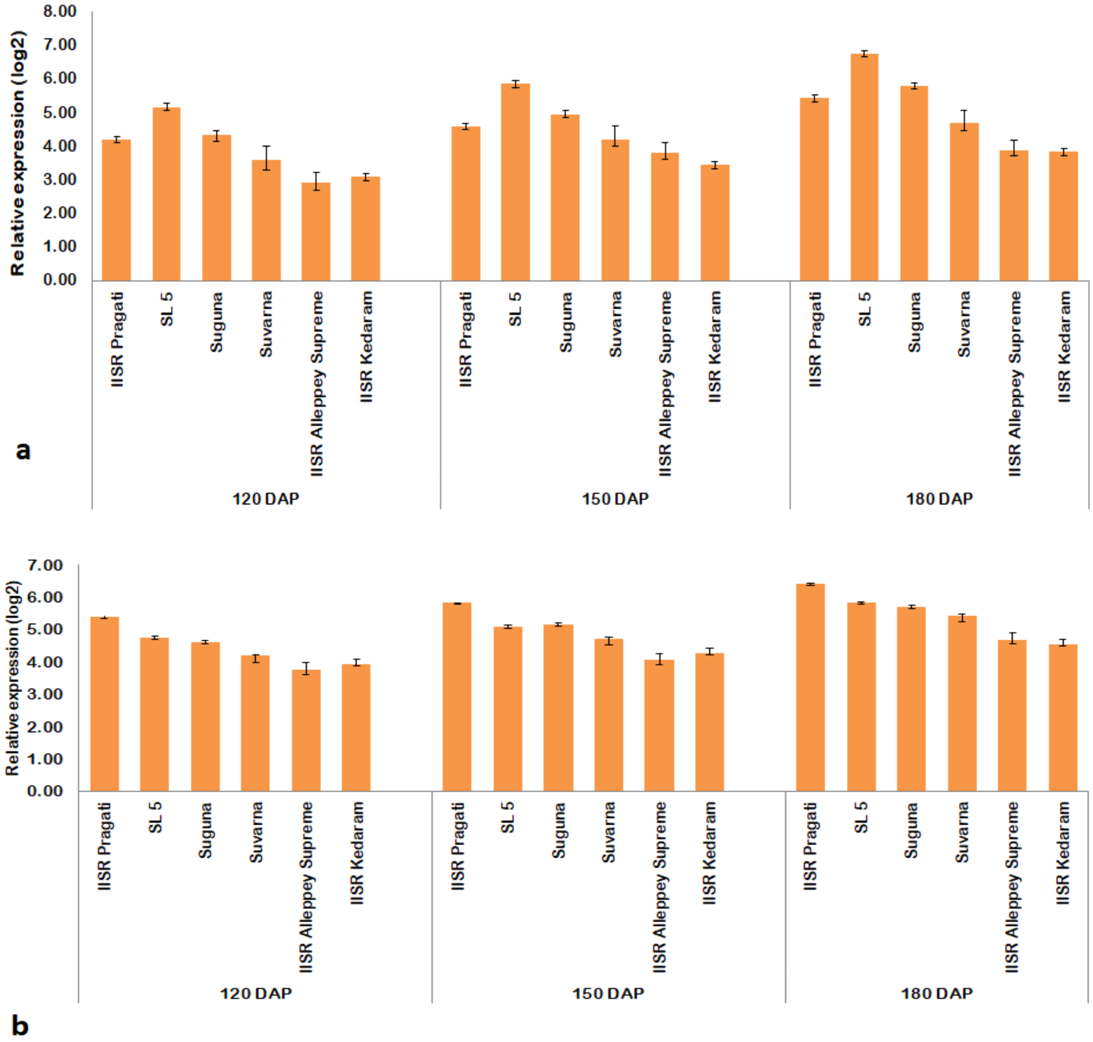


Fig. 4.3.12 Relative expression pattern of *WRKY* under drought treatment with respect to irrigated condition (control) in (a) leaf and (b) rhizome. Bars represent the mean ± SE.

The transcription factors, namely, *DREB* (Lata & Prasad, 2011) and *bHLH* (Gu *et al*., 2021) are involved in the drought responsive mechanism in rice, tobacco, and *Arabidopsis*, and influenced the drought tolerance in these plants. *DREB* conferred enhanced drought tolerance in soybean (Kidokoro *et al*., 2015), *Arabidopsis* (Sakuma *et al*., 2006) and in wheat (Zhou, *et al*., 2020). Likewise *WRKY* over expression significantly enhanced drought resistance in tobacco (Li *et al.,* 2023), soybean (Luo *et al*., 2013), wheat (Gao *et al*., 2018) and Arabidopsis (Niu *et al*., 2012).While *ABF* over expression gave drought tolerance in *Arabidopsis* (kim *et al*., 2004) and in cotton (Liang *et al*., 2016).

On account of its ability to detect relative expression level of TFs under drought treatment, qRT-PCR is used to detect resistant crops in various studies (Zhang *et al*., 2011; Li *et al*., 2023). In this study gene expression analysis in turmeric using qRT-PCR revealed relatively higher expression of drought responsive TFs such as *DREB, bHLH, WRKY* and *ABF* in tolerant genotypes which enabled them endure drought stress by enhanced protective mechanisms.

4.3.4 Yield (g plant^{-1})

The data on yield showed significant variation among the genotypes both under control as well as under water stress (Table 4.3.4). Under control, maximum yield was recorded in IISR Pragati (242 g plant^{-1}) followed by Suguna (223 g plant^{-1}) and lowest in IISR Alleppey Supreme (206 g plant^{-1}). Whereas IISR Pragati (215 g plant^{-1}) followed by Suguna (190 g plant^{-1}) and SL 5 (182 g plant^{-1}) recorded maximum rhizome yield under drought condition.

DSI was least in IISR Pragati (¯0.56) followed by SL 5 (¯0.53), Suguna (¯0.49) and Suvarna (¯0.46). Genotypes identified as susceptible showed DSI of 0.01 (IISR Alleppey Supreme) and 0.02 (IISR Kedaram). Thus least value of DSI corresponds to sustainable yield. Similar results were reported in rice (Babu *et al.,* 2011), wheat (Siahpoosh *et al*., 2011; Bayoumi *et al.,* 2008) and soybean (Kisman *et al*., 2021).

Alterations to physiological processes caused by dehydration reduce growth and yield (Anjum *et al*., 2011). The yield loss percentage under drought differs between crop species (Hussain *et al.,* 2019a).

Here the yield reduced by 11%, 14.8%, 12.5% and 16.9% in tolerant varieties, IISR Pragati, Suguna, SL 5 and Suvarna respectively, while there was a higher reduction in susceptible ones such as IISR Alleppey Supreme (43%) and IISR Kedaram (44%).

Drought interferes with growth, water relations, photosynthesis, assimilate partitioning and ultimately cause a significant reduction in crop yields (Farooq *et al*., 2009; Praba *et al*., 2009). However, the extent of damage depends upon crop growth stage and severity of the stress (Shah *et al*., 2017). Similar reduction in yield under drought was reported in rice (Raman *et al*., 2012), maize (Majid and Roza, 2012) and wheat (Alireza *et al.,* 2017; Anwaar *et al*., 2020). Here the yield reduced by 11%, 14.8%, 12.5% and 16.9% in tolerant varieties, IISR Pragati, Suguna, SL 5 and Suvarna respectively, while there was a higher reduction in susceptible ones such as IISR Alleppey Supreme (43%) and IISR Kedaram (44%).

Drought induces stomatal closure and reactive oxygen (ROS) production which cause degradation of photosynthetic system under drought (Yamane *et al.,* 2003). Reduced gaseous exchange also promotes photorespiration which further affects carbon fixation and yield (Reddy *et al.,* 2004; Correia *et al.,* 2006). Degradation of chlorophyll content under water stress resulted in significant yield reduction in rice at grain filling stage (Moonmoon *et al.,* 2020).

The exposure of plants to the drought decreases relative water content which leads to the disturbances in photosynthetic electron transport, degradation of important enzymes, increased electrolyte leakage and reduce productivity (Anjum *et al.,* 2011; Pour-Aboughadareh *et al*., 2017) as reported in maize (Guo *et al.,* 2015), cassava (Zhu *et al.,* 2020), faba bean (Mansour *et al*., 2021) and wheat (Ahmed *et al.,* 2022). Drought stress induce imbalance in hormonal level in plants, which also results in decreased CO_2 assimilation (David *et al*., 2007).

Tolerant plants with enhanced antioxidative system (such as antioxidant enzymes and phenol) and osmoregulation (such as proline and sugar) can resist stress induced damaging effects of ROS including oxidation of membranes, photosynthetic apparatus and macromolecules and maintain a better yield under drought (Su & Wu, 2004; Ashraf *et al*., 2009; Gill & Tuteja, 2010; Manivannan *et al.,* 2014; Noctor *et al.,* 2014; Li *et al.,* 2024).

In our study tolerant turmeric genotypes that showed higher RWC, enzyme activity, proline, sugar, carotenoid and phenol content and a lower membrane leakage, H_2O_2, MDA content gave higher yield compared to susceptible genotypes indicating that higher antioxidant, osmoregulatory system and better water balance enabled them withstand oxidative stress (**Fig. 4.3.13)**.

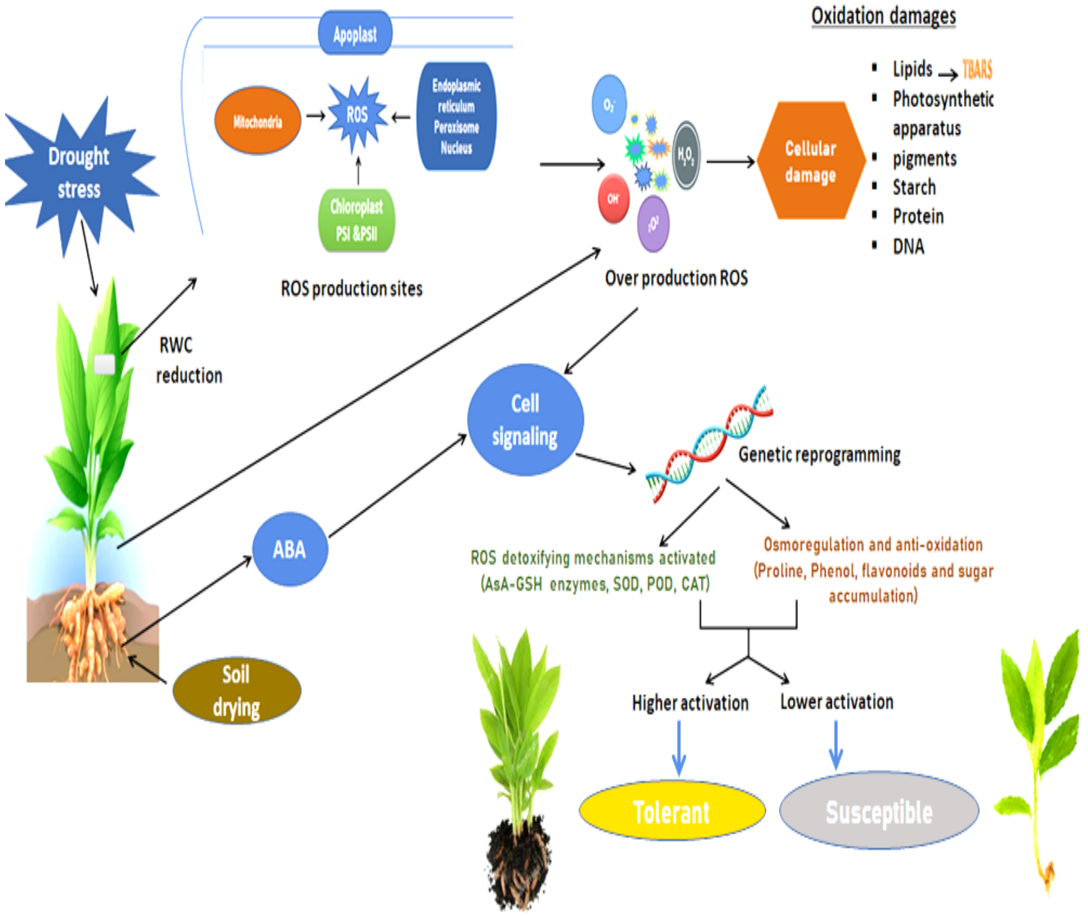


Fig. 4.3.13 Drought response pathway in turmeric as revealed by physiological, biochemical and molecular characterization. Defensive response followed similar pattern in both leaf and rhizome. The expression was lower in rhizome compared to leaf, which indicate that the major response of crop under drought stress is initiated in leaf as reported by Yang *et al.* (2021).

4.3.5 Dry recovery (%)

Recovery of cured turmeric significantly (P >0.01) varied among the genotypes under control and stress treatment, which ranged from 17.3 – 24.56% and 15.8 -21.9% in control and stress respectively (Table 4.3.4). Suvarna (24.56%) and Suguna (21%) recorded maximum recovery of cured turmeric under control and was lowest in IISR Pragati (17.3%) followed by IISR Kedaram (19.26%). While under drought treatment, Suvarna (21.9%) followed by SL 5 (19 %) and Suguna (18.8%) recorded maximum recovery of cured turmeric. Here also, it was lowest in IISR Pragati (15.8%) followed by IISR Kedaram (16.5%) and IISR Alleppey Supreme (17.1%) (Table 4.3.4).

Under drought, reduced dry matter accumulation at varying degrees as reported in marigold which experienced decreased shoot and flower fresh and dry weights (Asrar & Elhindi, 2011).Under dought, decrease in dry recovery has been reported in rice (Moonmoon *et al*., 2017), barley (Samarah *et al*., 2005) and in maize (Kamara *et al.,* 2003) under drought and in turmeric under salinity stress (Mostajeran *et al*., 2014; Harisha *et al*., 2021). Tolerant varieties which maintained better growth with reduction in irrigation showed maximum dry weight pea varieties (Bodah *et al.,* 2015). Rice genotypes with higher photosynthetic rate and chlorophyll content at grain filling stage gave the highest grain dry weight (Moonmoon *et al.,* 2020). Under osmotic stress turmeric showed 30% dry weight reduction in rhizome compared to control plants (Mostajeran *et al*., 2014). Drought stress suppresses growth and photosynthesis which results in reduction in dry matter accumulation and yield under drought (Kramer & Boyer, 1995; Kumar *et al*., 2006). In this study, relative decrease in dry recovery in treatment compared to control was higher in susceptible ones such as IISR Alleppey Supreme (12.9%) and IISR Kedaram (14.3%) whereas percentage decrease was lesser in tolerant ones such as SL 5 (5%), IISR Pragati (8.7%), Suguna (10.5%) and Suvarna (10.8%) which can be ascribed to their better growth and photosynthetic activity under drought stress.

Table 4.3.4 Changes in rhizome yield, DSI and dry recovery of turmeric genotypes subjected to water stress in field condition.

Genotype	Yield (g plant^{-1})		DSI	Dry recovery (%)	
	Control	Treatment		Control	Treatment
IISR Pragati	242^{a}	215^{bc}	-0.56	17.3h	15.8j
Suguna	223^{b}	190^{de}	-0.49	21c	18.8g
SL 5	208^{bc}	182^{ef}	-0.53	20d	19g
Suvarna	172^{f}	143^{g}	-0.46	24.56a	21.9b
IISR Alleppey Supreme	206^{cd}	117^{i}	0.01	19.63e	17.1h
IISR Kedaram	216^{bc}	121^{h}	0.02	19.26f	16.5i
General mean	211	161		19.27	
CV(%)	4			0.72	
CD (P= 0.05)	10.6			0.235	

DSI= Drought susceptibility index; the mean value (n=4) followed by different letters within a column are significantly different ($P < 0.05$) based on Duncan and ANOVA test

4.3.6. Effect of drought on plant nutrient uptake

Post-harvest estimation of nutrients was done in dried and powdered turmeric samples. Drought treatment exhibited either slight upsurge or decline in nutrient level in all the elements tested compared to control in all the genotypes, (table 4.3.5 and 4.3.6). Increase was more profound in the available concentration of Mn, Cu and K. There was decrease in concentration of Ca, Zn, N, Fe, Mg and P levels under drought compared to control.

4.3.6.1 Effect of drought on turmeric N, P and K content.

Drought treatment showed a decrease in the N and P content over control in all the genotypes. In control N content was found to be maximum in IISR Pragati (2.41ppm) followed by S L 5 (2.2 ppm) and minimum in Suguna (1.8 ppm). Under stress, it was maximum in IISR Pragati (2.1 ppm) followed by SL 5 (1.9 ppm) and was minimum in IISR Alleppey Supreme (1.4 ppm).

Phosphorus was maximum in Suvarna (0.458 ppm) which was on par with IISR Pragati (0.455 ppm) under control and was lowest in SL 5 (0.413 ppm). There was a significant reduction in P content under stress. It was maximum in IISR Kedaram (0.420 ppm) followed by IISR Pragati (0.405 ppm) while it was minimum in IISR Alleppey Supreme (0.368 ppm).

K content increased with stress, under control, potassium content was highest in IISR Pragati (1.71 ppm) followed by SL 5 (1.65 ppm) and was minimum in Suguna (1.25 ppm). Under stress also it was maximum in IISR Pragati (1.84 ppm), which was on par with SL 5 (1.79 ppm) while it was minimum in Suguna (1.43 ppm). There was no drought tolerance related variation for P and K. (Table 4.3.5).

N and P contents decreased under stress. Minimum reduction of nitrogen was found in Suvarna (11.11%) followed by Suguna (14.29%). Reduction was higher in susceptible ones such as IISR Kedaram (26.31%) and IISR Alleppey Supreme (20 %).

Minimum decrease in P content was observed in IISR Kedaram (6.87%) under stress compared to control and highest decrease was found in Suvarna (14.25%). Maximum increase in K was observed in Suguna (14.4 %) followed by Suvarna (13.7%) and the increase was minimum in IISR Pragati (7.60%).

Table 4.3.5 Effect of drought on N, P and K content of turmeric rhizome.

Genotype	N (%)		P (%)		K (%)	
	Control	Treatment	Control	Treatment	Control	Treatment
IISR Pragati	2.41^{a}	2.1^{c}	0.455^{a}	0.405^{ef}	1.71^{b}	1.84^{a}
SL 5	2.2^{b}	1.9^{d}	0.413^{de}	0.368	1.65^{bc}	1.79^{a}
Suguna	2.1^{c}	1.8^{e}	0.439^{b}	0.398^{fg}	1.25^{f}	1.43^{d}
Suvarna	1.8^{e}	1.6^{f}	0.458^{a}	0.391^{g}	1.46^{d}	1.66^{b}
IISR Alleppey Supreme	1.9^{d}	1.4^{g}	0.425^{c}	0.368^{h}	1.6^{b}	1.78^{a}
IISR Kedaram	1.95^{d}	1.58	0.451^{a}	0.42^{cd}	1.35^{e}	1.49^{d}
General mean	1.89		0.415		1.58	
CV (%)	1.88		1.39		2.6	
CD (P=0.05)	0.04		0.01		0.07	

* Values represent the mean values of replications. Superscript with different alphabets indicates that values are significantly different between treatments (P <0.05).

4.3.6.2 Effect of drought on micronutrients (Ca, Fe, Cu, Mg, Mn, and Zn) in turmeric rhizome

Significant variation was observed among the genotypes in micronutrient content under control as well as drought treatment (Table 4.3.6). Ca content decreased under stress. In control, It was maximum in SL 5 (0.15 ppm) followed by Suvarna (0.14 ppm) and minimum was observed in IISR Kedaram (0.1 ppm). Under stress, it was maximum in SL 5 (0.13 ppm) followed by Suvarna (0.12 ppm) and minimum in IISR Kedaram (0.07 ppm) and IISR Alleppey Supreme (0.08 ppm). Ca content was highest in tolerant and lowest in susceptible ones. Ca decreased up to 30% and 27% in IISR Kedaram and IISR Alleppey Supreme respectively compared to control. However, least reduction was seen in Suvarna (14.29%) followed by IISR Pragati (16.67%).

Fe content reduced under stress. Under control it was maximum in Suvarna (544.7 ppm) followed by SL 5 (529.3 ppm) and lowest in IISR Alleppey Supreme (356.3 ppm). Under stress, it was maximum in Suvarna (458.3 ppm) followed by Suguna (430 ppm) and was lowest in IISR Alleppey Supreme (306.3 ppm).

Reduction was higher in tolerant ones such as SL 5 (20.08%) and IISR Pragati (18.03%). Least reduction was noticed in IISR Kedaram (13.76%).

Cu increased markedly under stress compared to control. In control it ranged from 3.8 ppm (Suguna) to 4.7 ppm (SL 5). While under stress, it ranged from 5.2 ppm (IISR Alleppey Supreme) to a maximum of 6.4 ppm in Suguna. There was higher increase in copper content under drought stress in tolerant ones. Cu increase was more in tolerant genotypes with maximum increase in Suguna (68.42%) while there was only 25.58 % increase in IISR Kedaram.

There was a reduction in Mg content under drought stress compared to control. Under control Mg concentration ranged from 0.16 ppm in Suvarna to 0.21 ppm in SL5 followed by IISR Pragati (0.19 ppm). Under treatment it was lowest in Suvarna and IISR Kedaram (0.14 ppm) and maximum in SL 5 (0.19 ppm) followed by IISR Pragati (0.17 ppm). Minimum reduction was observed in tolerant ones such as SL 5 (9.52%) followed by IISR Pragati (10.53%) whereas maximum reduction was observed in IISR Kedaram (18%) and IISR Alleppey Supreme (16.67 %).

Mn increased significantly under drought stress compared to control. In control Mn ranged from 68.3 ppm (Suvarna) to 126 ppm (IISR Alleppey Supreme) and under stress it ranged from 134.7 ppm (Suvarna) to 161.3 ppm (IISR Pragati). Compared to control, Mn increased highly in tolerant ones to about 104.68 % in SL 5 followed by Suvarna (97.23%) and minimum increase was in observed in IISR Alleppey Supreme (8.71%).

Zn was maximum in tolerant ones and lowest in susceptible ones. It decreased under drought stress. It ranged from 43.7 ppm (Suvarna) to 54 ppm (IISR Pragati) in control while under stress, it was maximum in IISR Pragati (44ppm) followed by Suguna (37.8 ppm) and lowest in IISR Alleppey Supreme (34.7 ppm). Zn reduction was greater in susceptible ones such as IISR Kedaram (23.33%) and IISR Alleppey Supreme (21.14%) whereas lower reduction was found in Suguna (14.73%) followed by Suvarna (15.33 %).

Table 4.3.6 Effect of drought on micronutrient (Ca, Mg, Cu, Mn, Fe, and Zn) content

Genotype	Ca (%)		Mg (%)		Cu(ppm)		Mn(ppm)		Fe(ppm)		Zn(ppm)	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
IISR Pragati	0.12^{cd}	0.1^{e}	0.19^{b}	0.17^{d}	4.3^{g}	5.8^{c}	117^{f}	161.3	465.7^{d}	381.7^{g}	54^{a}	44^{c}
SL 5	0.15^{a}	0.13^{bc}	0.21^{a}	0.19^{b}	4.7^{f}	6.3^{b}	71.33^{g}	146^{c}	529.3^{b}	423^{e}	45.7^{b}	37.5^{d}
Suguna	0.13^{bc}	0.11^{de}	0.17^{d}	0.15^{f}	3.8^{j}	6.4^{a}	117.3^{f}	144.3^{c}	517.7^{c}	430^{e}	44.3^{c}	37.8^{d}
Suvarna	0.14^{ab}	0.12^{cd}	0.16^{e}	0.14^{g}	3.9^{i}	5.5^{d}	68.3^{g}	134.7^{d}	544.7^{a}	458.3^{d}	43.7^{c}	37^{d}
IISR Alleppey Supreme	0.11^{de}	0.08	0.18^{c}	0.15^{f}	4^{h}	5.2^{e}	126.3^{e}	137.3^{d}	356.3^{h}	306.3^{j}	44^{c}	34.7^{e}
IISR Kedaram	0.1^{e}	0.07	0.17^{d}	0.14^{g}	4.3^{g}	5.4^{d}	116.3^{f}	154^{b}	397.3^{f}	342.7^{i}	45.3^{b}	34.4^{e}
General mean	0.11		0.17		4.96		124.4		429.4		41.9	
CV (%)	7.97		1.84		0.92		2.17		1.09		1.21	
CD (P=0.05))	0.015		0.005		0.078		4.59		7.97		0.86	

*Values represent the mean values of four replications. Superscript with different alphabets indicates that values are significantly different between treatments (P <0.05).

Under water stress, Ca, Mg and Fe decreased significantly in all genotypes. Ca and Fe were less in susceptible ones. Likewise, Cu and Mn rise was less in susceptible ones under drought condition. Similarly, Mg, Zn and N reduction was also higher in susceptible genotypes.

Drought stress decreased the availability, uptake, transfer and metabolism of nutrients (Farooq *et al*., 2009). Drought can depress plant growth by reducing N and P uptake, transport and redistribution (Rouphael *et al*., 2012). A majority of studies have indicated that plants decrease N and P uptake with a decline in soil moisture (Cramer *et al*., 2009; Waraich *et al*., 2011) as reported in sugarcane (Boschiero *et al.,* 2019), corn and maize (Bista *et al*., 2018). Under water deficit, *Spartina alterniflora* experienced higher reduction in the uptake of Ca, Mg and Zn (Brown *et al.,* 2006). Drought downregulated the uptake of Fe, Mg and Zn in wheat and rapeseed plants (D'Oria *et al*., 2022). However, Cu, Mn and K increased under drought in soybean (Samarah *et al*., 2004) and *Cunninghamia lanceolata* (Shubin *et al*., 2023). This suggests that in response to water stress some minerals are utilised for the synthesis of proteins responsible for antioxidant activity. Ca deficiency under drought usually related to the inability of the plant to translocate adequate Ca to the affected part as observed in tomato (Peet, 2009). The impact of Ca deficiency increases as a consequence of drought because of several potential interactions with other factors, such as imbalances with other ions, especially with cations (Voogt, 1993). Here in our study Ca reduction was less in tolerant ones which may help in reduction of electrolyte leakage as reported *Camellia sinensis* L. (Upadhyaya, 2011).

Thus, nutrient uptake and allocation may play an essential role in the response of plants to drought stress.This points to the need for further studies to evaluate the regulatory mechanisms of different minerals in different organs (Li *et al*., 2023). In our study reduction in availability of Ca, Mg, Fe, Zn and N was less in tolerant ones. Also, they showed higher accumulation of Cu and Mn under water stress. This can be related to their roles in antioxidant defense, photosynthesis, and enzyme activation to help plants better cope with and adapt to water limitations.

4.3.7 Effect of drought on rhizome quality

The drought treatment showed marked variation in the quality traits of turmeric as compared to the untreated control. The quality traits studied were essential oil, oleoresin, and curcumin contents**.**

4.3.7.1 Oleoresin (%).

Significant variation was observed among genotypes in oleoresin content under field conditions for control and treatment. It showed a decline under drought stress. Oleoresin content significantly varied among the genotypes which ranged from 11.9% to 15.4% and 11.1% to 14.2% for control and stress respectively (Table 4.3.7). The maximum oleoresin content was recorded in IISR Alleppey Supreme (15.4%) followed by IISR Pragati (14.6%) and IISR Kedaram (14.1%). The lowest oleoresin was recorded in S L 5 (11.9%) under control. Under drought condition, IISR Alleppey Supreme (14.2%), followed by IISR Pragati (13.6%) and Suguna (12.9%) recorded maximum oleoresin and was minimum in SL 5 (11.1%). The results are shown in Table 4.3.7

Suguna outperformed other genotypes with only 3.7 % reduction and the second best was Suvarna (5.3%). There was a maximum reduction of 10% was observed in IISR Kedaram and 7.8% in IISR Alleppey Supreme.

Turmeric quality characters can vary with biotic or abiotic stresses (Prasath *et al*., 2018). Similar variation with different-agro climatic condition was reported in turmeric (Singh *et al*., 2013) and ginger (Akshitha *et al*., 2020). Mahmood *et al.* (2021) reported reduction in pungency of pepper varieties under drought condition.

ROS can directly attack and degrade oleoresins and their precursors reducing their overall content (Maiti & Mitra, 2017). It also can damage enzymes involved in the biosynthesis of oleoresins and disrupts processes involved in secondary metabolite synthesis (Pandey *et al*., 2012). Also, Plants reallocate resources under stress conditions, affecting the production of secondary metabolites (Saini *et al.,* 2018).

In this study, drought stress reduced oleoresin content in turmeric, which may have occurred through direct degradation of oleoresins and enzymes involved in its synthesis, disruption of metabolic processes, shifts in resource allocation, and altered gene expression. The reduction was greater in susceptible genotypes.

4.3.7.2 Essential oil (%).

Under drought stress there was a reduction in essential oil content compared to control. The genotypes varied significantly for essential oil content under both drought and control condition, which ranged from 5.2% - 6.4% and 4.6% -5.7% in control and stress respectively as presented in table 4.3.7.

In control, Suvarna (6.4%) recorded maximum essential oil followed by SL 5 (6.1%) and IISR Pragati (6%) and was lowest in IISR Kedaram (5.2%) whereas under stress, Suvarna recorded maximum essential oil (5.7%) followed by SL 5 (5.6%) and IISR Pragati (5.5%) whereas IISR Kedaram (4.6%) recorded minimum essential oil.

Variation in essential oil content of genotypes under different environments was reported earlier by Leela *et al*. (2002) and Singh *et al*. (2005). A reduction in oil content with drought stress was reported in basil (Mandoulakani *et al.,* 2017), *Rosmarinus officinalis* L (Hassan *et al*., 2013), *Mentha piperita* L (Farahani *et al*., 2009) and maize (Kresović *et al*., 2018) under drought in turmeric under salt stress (Mostajeran *et al*., 2014).

Here minimum reduction was noticed in SL 5 (8.2%) followed by IISR Pragati (8.3%) and maximum reduction was observed in IISR Kedaram (11.54%). Low essential oil yield may also be related to decreased photosynthesis and other changes in metabolism (Aziz *et al*., 2008; Costa *et al*., 2020) under stress condition.

In our study the reduction in essential oil content was higher in susceptible genotypes which indicate the significant impact of drought on its metabolic activity, whereas tolerant genotypes exhibited lower reduction in essential oil content suggesting its enhanced resistance mechanisms that collectively contributed to plant's ability to cope with and adapt to drought-induced metabolic challenges.

4.3.7.3 Curcuminoides (%)

Curcuminoides varied significantly among six turmeric genotypes under both control and stress which ranged from 3.11% - 3.98% and 3.29-4.28% respectively (Table 4.3.7). IISR Alleppey Supreme (3.98%) recorded maximum curcuminoides followed by IISR Kedaram (3.87%), whereas SL 5 (3.11%) recorded minimum curcuminoides under control condition. Under drought stress IISR Alleppey Supreme (4.28%) was found to be best for curcuminoides followed by IISR Kedaram (4.12%) recorded higher curcuminoides and minimum was recorded in SL 5 (3.29%). Thus drought stress increased the curcuminoides content in all the genotypes compared to control. IISR Kedaram outperformed all other genotypes with 7.54% increase of curcuminoides content compared to control followed by IISR Pragati (7.4 %). Suvarna (5.4 %) and SL 5 (5.79) had least increase compared to control under drought condition. Similar results were reported by Chindakovid *et al*. (2022) and Mostajeran *et al*. (2014) under drought and salt stress in turmeric.

According to Rao (2021) the curcumin content of the rhizomes under drought stress treatment doubled compared to that under stress free treatment. According to Mostajeran *et al*. (2014) extreme osmotic stress caused a reduction in curcumin content while above moderate stress level (salinity up to 60 mM) increased the curcumin content.

Table 4.3.7 Effect of drought on quality traits of turmeric

Genotype	Essential oil (%)		Oleoresin (%)		Curcumin (%)	
	Control	**Treatment**	**Control**	**Treatment**	**Control**	**Treatment**
IISR Pragati	6^{b}	5.5^{d}	14.6^{b}	13.6^{cde}	3.64^{d}	3.91^{c}
Suguna	5.8^{c}	5.2^{e}	13.4^{cdef}	12.9^{ef}	3.32^{e}	3.54^{d}
SL 5	6.1^{b}	5.6^{cd}	11.9^{gh}	11.1^{h}	3.11^{g}	3.29^{ef}
Suvarna	6.4^{a}	5.7^{cd}	13.2^{def}	12.5^{fg}	3.17^{fg}	3.34^{e}
IISR Kedaram	5.2^{e}	4.6^{f}	14.1^{bcd}	12.7^{efg}	3.87^{c}	4.12^{b}
IISR Alleppey Supreme	5.6^{cd}	5^{e}	15.4^{a}	14.2^{bc}	3.98^{c}	4.28^{a}
General mean	5.53		13.3		3.63	
CV (%)	2.15		4.27		1.9	
CD (P=0.05)	0.203		0.97		0.12	

**Superscript with different alphabets indicate that values are significantly different ($p<0.05$).

Thus in our study, water stress has significant impact on oil yield, oleoresin and curcumin content in turmeric rhizomes. According to Tripathi *et al.* (2015) quality of turmeric is strongly influenced by irrigation schedule itself.

Rhizome quality and productivity of turmeric plant is influenced by genotype, irrigation schedule, geographic factors such as soil quality, climate and environmental conditions (Sandeep *et al*., 2016; Aarthi *et al*., 2020; Pandey & Katiyar, 2010; Kaur & Brar, 2016; Hossain *et al*., 2009).Characterization of turmeric for the major biochemical constituent's viz. oleoresin, essential oil and curcumin under drought reveal the relative influence of growth and yield affecting parameters on the phytoconstituents and enable the identification of cultivars that are rich in one or other of these constituents that are important determinants of the commodity's end product (Singh *et al*., 2014).

In our study under water deficit stress led to a decrease in essential oil and oleoresin content, but significantly increased the curcumin content. IISR Alleppey Supreme (4.28%) showed the highest curcumin content, minimum oil reduction was noticed in SL 5 (8.2%) followed by IISR Pragati (8.3%) and Suguna outperformed other genotypes in oleoresin content with only 3.7 % reduction under stress.

According to Singh *et al*. (2014) turmeric plants with better growth parameters (Leaf area followed by tiller number) exerted highly significant correlation with yield and phytoconstituents content. In our study, there was no observable tolerance related variation with respect to curcumin content. But essential oil and oleoresin percentage reduction was lower in tolerant genotypes.

4.3.8 Effect of drought on protein content of rhizome

Protein content in turmeric rhizomes varied significantly among the genotypes under different water regimes. It ranged from 4.85% - 5.19% in control and 3.79% - 4.43% under drought condition. Protein was maximum in SL 5 (5.19%) and IISR Pragati (5.03%) and it was lowest in Suguna (4.86%) under control condition. Whereas, SL 5 (4.43%) and IISR Pragati (4.35%) followed by Suguna (4.29%) recorded maximum protein content under drought condition and it was lowest in IISR Alleppey Supreme (3.8%). The protein content decreased significantly (P>0.01) under drought condition in all genotypes. The results are presented in Table 4.3.8.

Suguna outperformed all other genotypes with only 11.63% reduction of protein content compared to control followed by IISR Pragati (13.50%). IISR Alleppey Supreme (22.3%) and IISR Kedaram (20.48%) had maximum reduction compared to control under drought condition.

Protein content was earlier reported to vary from 1% - 9% among turmeric genotypes by Ikpeama *et al.* (2014) and Choudhury (2019). On prolonged water stress, percentage of protein content, in plant tissues decreased in *Peganum harmala* L. (El-Azim, 2009) and Rosemary (Amirjani, 2013) and in wheat (Abid *et al.*, 2018).

According to Bogeat *et al.* (2007) under drought condition there may be upsurge or downturn of different proteins based on their function. For instance, the largest stress-responsive group of proteins included several wall hydrolases whose abundance decreased in cell wall upon stress indicating a reduced cell wall elongation activity. However, several β-D-glucosidases increased under dehydration stress. It is known that these enzymes participate in cleavage of abscisic acid (ABA) from saccharide conjugates which is a prerequisite for ABA activity (Alam *et al.*, 2010).

In this study, drought stress led to a significant decrease in protein content, with the reduction being more pronounced in susceptible genotypes. This finding highlighted that tolerant genotypes exhibited more effective resistance mechanisms, which helped to minimize protein degradation.

4.3.9 Effect of drought on starch content in turmeric rhizome

The starch content decreased markedly under drought stress compared to control. There was significant difference among the genotypes in starch content both under control as well as stress. The starch content ranged from 38.87 % to 44.57% and 25.78% to 36.12% among the genotypes under control and stress condition respectively (table 4.3.8). Under control condition, IISR Pragati (44.57%) followed by SL 5 (43.34%) and Suguna (42.66%) recorded maximum starch content and was lowest in IISR Alleppey Supreme (38.87%). Similarly, IISR Pragati (36.15%) followed by SL 5 (34.16%) and Suguna (33.75%) recorded maximum values under drought condition while, minimum starch content was observed in IISR Kedaram (25.8%).

IISR Pragati outperformed all other genotypes with least reduction of about 18.9% starch content compared to control followed by Suguna (20.9%). IISR Alleppey Supreme (28.84%) and IISR Kedaram (29.06%) had the most reduction under drought stress compared to control.

Mittal *et al.* (2018) reported wide variation in starch content among the wheat genotypes. Reduction in starch content under water scarcity was reported in many crops including rice (Chen *et al*., 2023) and wheat (Lu *et al*., 2019). Activation of starch degradation under stress is a common plant response and does contribute to sugar accumulation (Kaplan & Guy, 2004; Yano *et al.,* 2005; Thalmann *et al*., 2016) which function as osmoprotectants along with compatible solutes to mitigate the negative effect of the stress (Krasensky & Jonak, 2012). In the present study, starch content significantly decreased under drought stress, with a greater extent of degradation observed in susceptible genotypes.

4.3.10 Effect of drought on total phenol content in turmeric rhizome

The total phenol estimation indicated an increase in the phenol concentration under drought as compared to the control. Under irrigated control, the phenol concentration ranged from 10.3 to 12.6 mg GAE/g and it ranged from 12.92-17.4 mg GAE/g under drought. Under control, phenol concentration was highest in SL 5 (12.6 mg GAE/g) and IISR Alleppey Supreme (12.41 mg GAE/g) followed by Suguna (12.27 mg GAE/g). The lowest concentration was found in Suvarna (10.35 mg GAE/g). Under drought treatment, it was highest in SL 5 (17.4 mg GAE/g) followed by Suguna (16.35 mg GAE/g) and IISR Pragati (15.5 mg GAE/g) and the lowest phenol content were observed in Suvarna (12.92 mg GAE/g) (Table 4.3.8).

SL 5 outperformed all other genotypes with 38.10% increase of phenol content compared to control followed by Suguna (33.25%). IISR Kedaram (13.23%) and IISR Alleppey Supreme (17.08%) revealed minimum increase in phenol content compared to control under drought condition.

4.3.11 Effect of drought on flavonoids in turmeric rhizome

Flavonoid concentration ranged from 3.96 -5.53 mg QE/g and 4.72-7.56 mg QE/g in control and drought treatment respectively. The flavonoids showed an increase under drought as compared to the control. Under control condition the flavonoid concentration was maximum in IISR Pragati (5.53) and Suguna (4.86) and was lowest in IISR Kedaram (3.96). IISR Pragati (7.56 mg QE/g) had maximum flavonoid content under drought followed by Suguna (6.55 mg QE/g) while, IISR Kedaram (4.72 mg QE/g) showed the lowest concentration. Under drought stress, concentration increased to a maximum of 36.6 % in IISR Pragati compared to control followed by 34.7% in Suguna. The increase was minimum in IISR Kedaram (19.22%) and IISR Alleppey Supreme (24.8%) (Table 4.3.8).

Table 4.3.8 Effect of drought on protein, starch, phenol and flavonoid contents

Genotype	Protein (%)		Starch (%)		Phenol (mg GAE/g)		Flavonoid (mg QE/g)	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
IISR Pragati	5.03^{b}	4.35^{ef}	44.57^{a}	36.15^{e}	11.69^{i}	15.5^{c}	5.53^{e}	7.56^{a}
Suguna	4.86^{d}	4.3^{f}	42.66^{b}	33.75^{f}	12.27^{gh}	16.35^{b}	4.86^{g}	6.55^{b}
Suvarna	4.98^{bc}	4.13^{g}	39.85^{c}	31.30^{g}	10.3^{j}	12.92^{f}	4.45^{i}	5.68^{d}
SL 5	5.19^{a}	4.43^{e}	43.34^{b}	34.16^{f}	12.6^{fg}	17.4^{a}	4.71^{h}	6.24^{c}
IISR Kedaram	4.9^{cd}	3.91^{h}	36.34^{e}	25.78^{i}	12.17^{h}	13.78^{e}	3.96^{j}	4.72^{h}
IISRAlleppey Supreme	4.89^{d}	3.8^{i}	38.9^{d}	27.66^{h}	12.41^{gh}	14.53^{d}	4.01^{j}	5.00^{f}
General mean	4.57		36.18		13.5		5.27	
CV (%)	1.21		1.19		1.48		1.47	
CD (P=0.05)	0.094		0.73		0.34		0.13	

*Values represent the mean values of replications. Superscript with different alphabets indicates that values are significantly different between treatments ($P < 0.05$)

According to Parvaiz & Satyavati (2008), plants generate and accumulate a multitude of defensive metabolites in order to fend off abiotic stress situations. Drought stress induces the biosynthesis of phenolics and flavonoids for enhanced plant protection (André, 2009; Rezayian, 2018; Li *et al.,* 2018). Through various biological and structural mechanisms, phenolic substances such as esters, flavonoids, lignin, and tannins serve as antioxidants, safeguarding plant cells from these stressors (Selmar *et al*., 2008).

In our investigation, drought considerably enhanced the total phenol and flavonoid concentration compared to control. This result was in line with studies on the effects of salt stress in turmeric (Bonacina *et al*., 2022) and drought stress in wheat (Kaur & Zhawar, 2015) and ginger (Gatabazi *et al.,* 2022). Secondary metabolites with beneficial antioxidative action, particularly against ROS, include phenolic compounds and flavonoids (Mostajeran *et al.,* 2014; Jungklang *et al.,* 2017). These antioxidants support the equilibrium of electron transport and reduce the free radicals produced in plants under abiotic stress (Nazar *et al.,* 2011; Naghiloo *et al.,* 2012).

Increased level of endogenous secondary metabolites was noticed in various medicinal plants which can increase its therapeutic potential as reported in *Trachyspermum ammi* (Azhar *et al*., 2011). Therefore, both irrigation treatments and genotype need to be considered by breeders during production to produce rhizome with the required levels of polyphenolics for targeted end-use (Wu *et al.,* 2017).

4.3.12 Characterization of curcuminoides fractions of genotypes under drought condition using HPLC

Curcuminoides are a set of polyphenolic compounds present in turmeric rhizomes. These were separated utilizing isocratic flow within the framework of high-performance liquid chromatography (HPLC), a reliable analytical approach. We have used HPLC to characterize different fractions among genotypes under control and drought condition. Curcumin, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) were the major curcuminoides.

In current study the total curcuminoides which constitutes, BDMC, DMC and curcumin were analysed using HPLC with pure standards procured from Sigma Aldrich (Fig. 4.3.14 a-d).

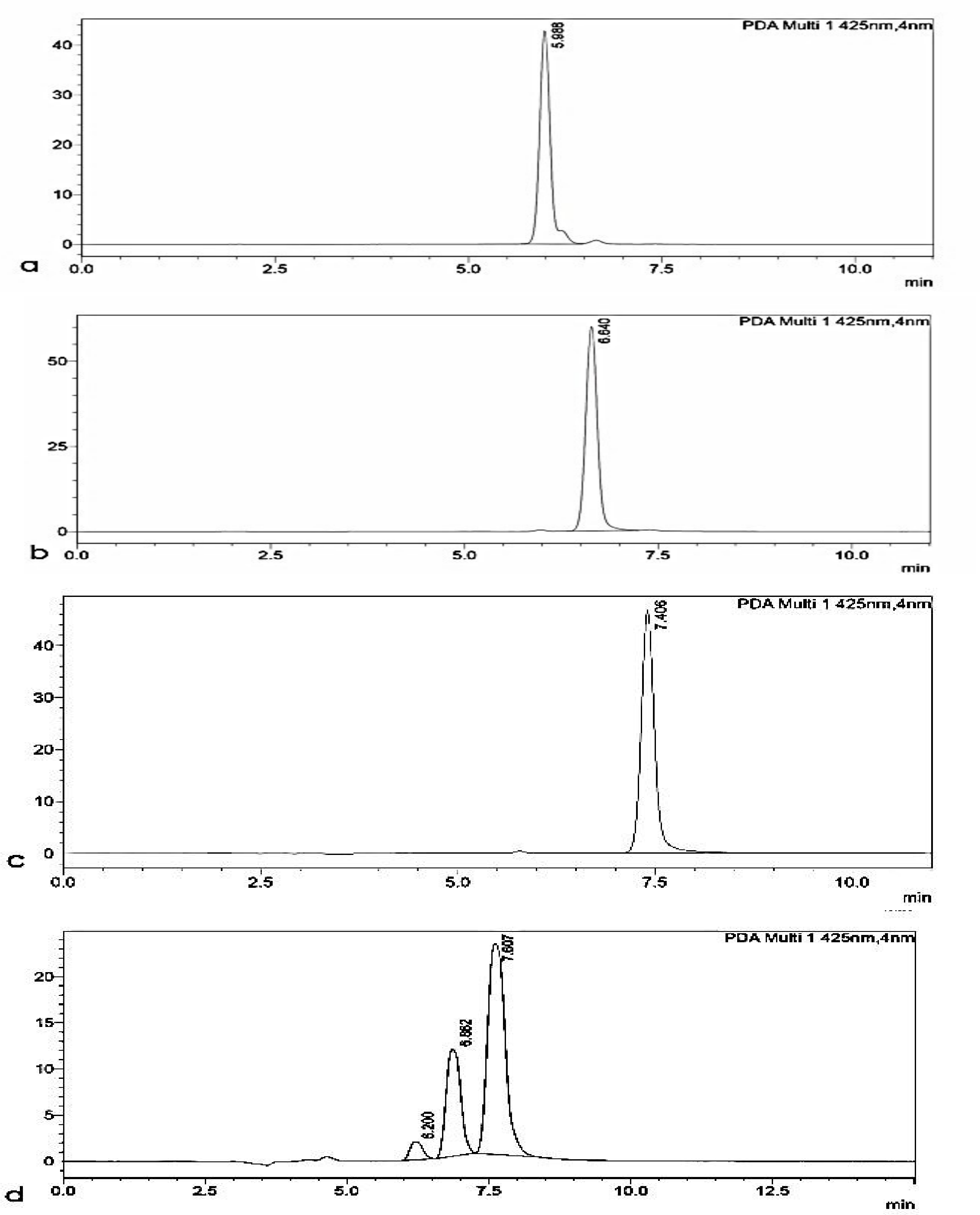


Fig. 4.3.14 Curcuminoid standards using HPLC: a. Bisdemethoxycurcumin; b. Demethoxycurcumin; c. Curcumin; d. curcuminoides mixture.

The HPLC analysis for curcuminoides showed that among the fractions, curcumin was the highest followed by BDMC and DMC. The representative HPLC chromatograms are depicted in the Fig.4.3.15 a-b.

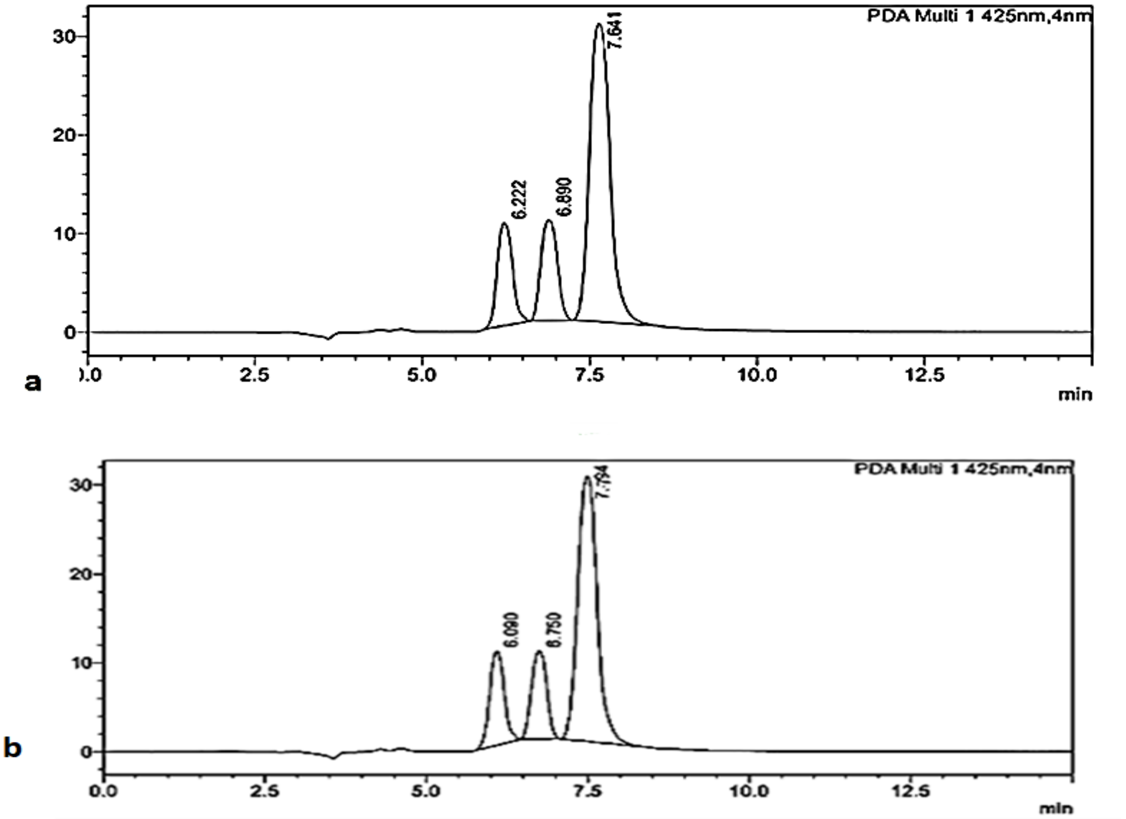


Fig. 4.3.15 Curcuminoides profile of genotype IISR Pragati a. Control): b. Drought treatment.

Under control condition, the curcuminoides fractions among the genotypes ranged widely viz., BDMC (0.57 - 0.65%), DMC (0.26 - 0.59%) and curcumin (0.89 -1.87%). In drought samples, the curcuminoides fractions among the genotypes ranged from 0.60 - 0.77% (BDMC), 0.39 - 0.69% (DMC) and 1.21 -2.74% (curcumin) (Table 4.3.9).

Under control, the BDMC was maximum in SL 5 (0.65%) and IISR Kedaram (0.63 %) while it was lowest in IISR Pragati (0.57%). While under treatment, BDMC was maximum in SL 5 (0.77%) and IISR Kedaram (0.76%) and was lowest in IISR Alleppey Supreme (0.64%).

In control samples, DMC was maximum in IISR Pragati (0.59%) followed by Suguna (0.50%) and lowest in Suvarna (0.26%). While in treatment, it was maximum in IISR Pragati (0.69%) and Suguna (0.63%) and minimum in Suvarna (0.39%).

Curcumin content was maximum in IISR Pragati (1.87%) followed by Suguna (1.77%) and minimum was observed in Suvarna (0.89%) under control. While it was highest in IISR Pragati (2.74%) followed by Suguna (2.61%) and lowest in Suvarna (1.21%) in drought treated samples.

The results revealed considerable variation in the constituents of curcuminoides among the genotypes under both control and drought condition. Curcuminoid fractions increased under drought stress, but there was no drought tolerance related variation among genotypes.

Under drought stress, BDMC increased from 9.8% (Suvarna) to 22.8% (IISR Pragati), DMC increased to about 8.3% (SL 5) to 57.7% (Suvarna) while curcumin increased from 14.8% (IISR Alleppey Supreme) to 47.5% (Suguna).

Table 4.3.9 Effect of drought on BDMC, DMC and Curcumin content

Genotype	**BDMC (%)**		**DMC (%)**		**Curcumin (%)**	
	Control	**Treatment**	**Control**	**Treatment**	**Control**	**Treatment**
IISR Pragati	0.57^{j}	0.70^{c}	0.59^{c}	0.69^{a}	1.87^{d}	2.74^{a}
Suguna	$0.62f^{g}$	0.73^{b}	0.50^{d}	0.63^{b}	1.77^{e}	2.61^{b}
Suvarna	0.61^{hi}	0.67^{d}	0.26^{i}	0.41^{ef}	0.89^{h}	1.21
S L 5	0.65^{e}	0.77^{a}	0.36^{gh}	0.39^{fg}	1.67^{fg}	1.98^{c}
IISR Kedaram	0.63^{gh}	0.76^{ab}	0.41^{ef}	0.52^{d}	1.73^{ef}	2.03^{c}
IISR Alleppey Supreme	0.59^{ij}	0.64^{ef}	0.35^{h}	0.42^{e}	1.62^{g}	1.86^{d}
General mean	0.66		0.46		1.83	
CV (%)	0.03		0.04		0.19	
CD (5%)	3.43		6.19		7.17	

* Values represent the mean values of replications. Superscript with different alphabets indicates that values are significantly different between treatments ($P < 0.05$)

According to Lee *et al*. (2014) terpenoids and curcuminoides are the principal bioactive components of turmeric of which curcuminoides are mainly responsible for the rhizomes' yellow colour and its high therapeutic potential. The major components of curcuminoides are curcumin (77%), demethoxycurcumin (17%), and bisdemethoxycurcumin (3%) (Strimpakos & Sharma, 2008; Goel *et al.,* 2008).

Sandeep *et al.* (2016) and Aarthi *et al.* (2020) reported that the curcumin content was influenced by geographic factors such as soil quality, climate, and environmental conditions. Mostajeran *et al.* (2014) reported increased curcumin content under salt stress. According to Gordon *et al.* (2015), DMC and BDMC are less prone to autoxidation than curcumin and contribute to the enhanced stability of turmeric extract at physiological pH and their oxidative metabolites may contribute to the biological effects of turmeric extract. The composition of curcuminoides fractions can differ based on genetic factors, climate variations, and how genotypes interact with their environments (Elizabeth *et al*., 2011; Niranjan *et al*., 2013; Anandaraj *et al.*, 2014).

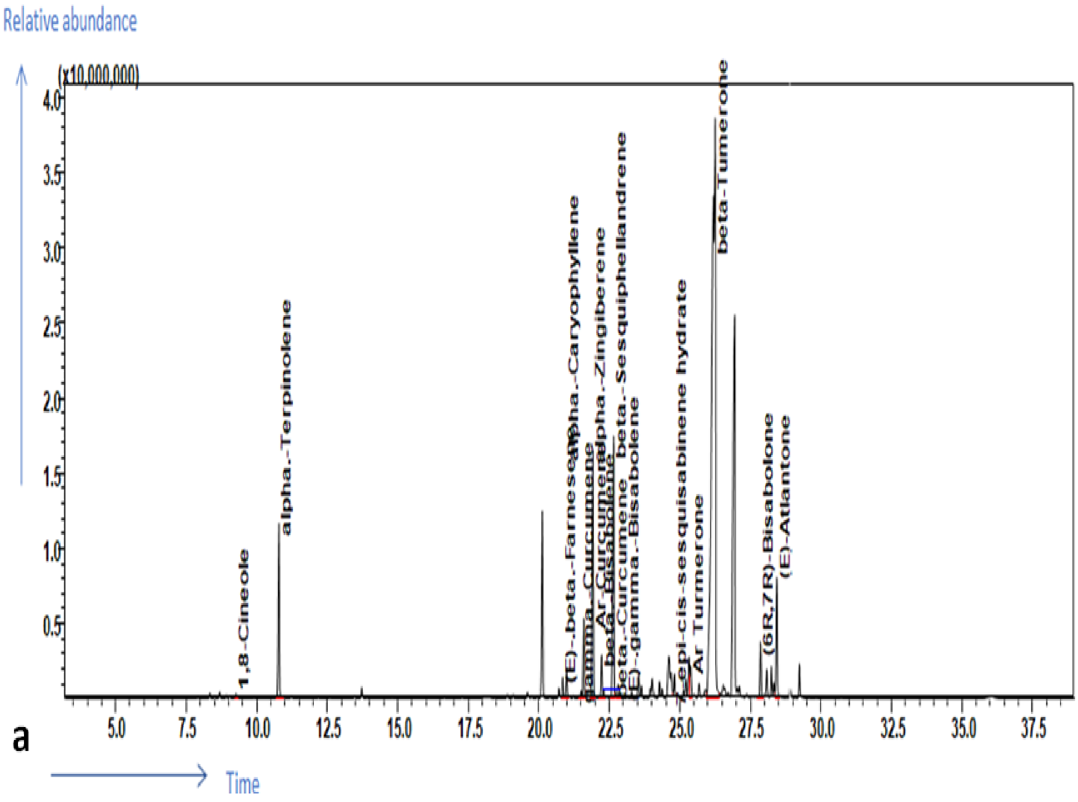
In this research, there was considerable variation in the constituents of curcuminoides under drought condition, while there was no tolerance related variation among genotypes. It indicated that turmeric might have relied on other mechanisms to cope with drought that were more critical for immediate stress response and survival, such as osmoprotectants and antioxidants, under water deficit stress.

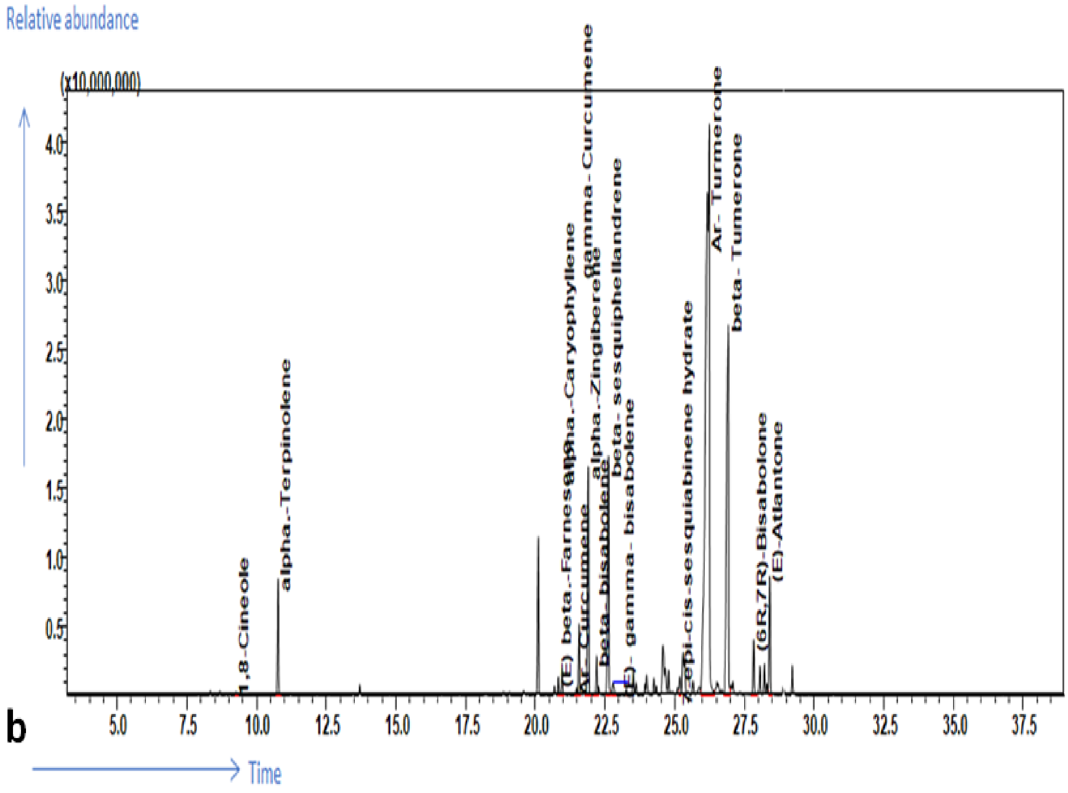
4.3.13 Variation of phytoconstituents of essential oil with drought treatment using gas chromatography-mass spectroscopy

The present study was conducted to analyse the variation of phytoconstituents in essential oil of six different turmeric genotypes grown under irrigation and as well as those subjected to water stress as represented in the table 4.3.10.

Gas chromatography-mass spectrometry (GC-MS) analysis was used to identify the compounds by comparing their retention indices and mass spectra to the data from various sources, including the National Institute of Standards and 151 Technology (NIST 2020), the WILEY library, and compared this with Adams book. Results suggested that the major components of the volatile oil of six turmeric genotypes across control and drought treatments varied for relative abundance of compounds. Mainly sixteen major volatile compounds were selected quantitatively from the genotypes across control and drought treatments. The representative GC-MS chromatograms are depicted in the Fig. 4.3.16. Among these, curlone (β-tuberose), ar-turmerone, beta-sesquiphellandrene and zingiberene were the most abundant compounds found in genotypes across treatments. These findings are in agreement with earlier studies by Leela *et al.* (2002), Raina *et al.* (2002), Chowdhury *et al*. (2008), Naz *et al*. (2010), Akbar *et al*. (2015) and Sandeep *et al*. (2021).

Relative abundance in essential oil constituents of 6 genotypes under control and drought treatment is represented as heat map in Fig. 4.3.17 to visualize easily the differences in eucalyptol (1,8 cineole), α-terpinolene, β-farnesene, α-caryophyllene, ar- curcumene, γ-curcumene, α-zingiberene, β- bisabolene , β-curcumene, β-sesquiphellandrene, (E)-γ-bisabolene, 7-epi-cis-sesquiabinene hydrate, ar-turmerone, curlone (β-tuberose), (6R,7R)-bisabolene and E-atlantone under control and drought treatment.


Relative abundance
(x10,000,000)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1,8-Cineole
alpha.-Terpinolene
(E)-beta.-Farnesene
alpha.-Caryophyllene
gamma.-Curcumene
alpha.-Zingiberene
Ar-Curcumene
beta.-Bisabolene
beta.-Sesquiphellandrene
beta.-Curcumene
(E)-gamma.-Bisabolene
epi-cis-sesquisabinene hydrate
Ar Turmerone
beta-Tumerone
(6R,7R)-Bisabolone
(E)-Atlantone
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
30.0
32.5
35.0
37.5
a
Time


Relative abundance
(x10,000,000)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1,8-Cineole
alpha.-Terpinolene
(E) beta.-Farnesene
alpha.-Caryophyllene
Ar-Curcumene
gamma- Curcumene
alpha.-Zingiberene
beta- bisabolene
beta- sesquiphellandrene
(E)- gamma- bisabolene
epi-cis-sesquiabinene hydrate
Ar- Turmerone
beta- Tumerone
(6R,7R)-Bisabolone
(E)-Atlantone
5.0
7.5
10.0
12.5
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35.0
37.5
b
Time

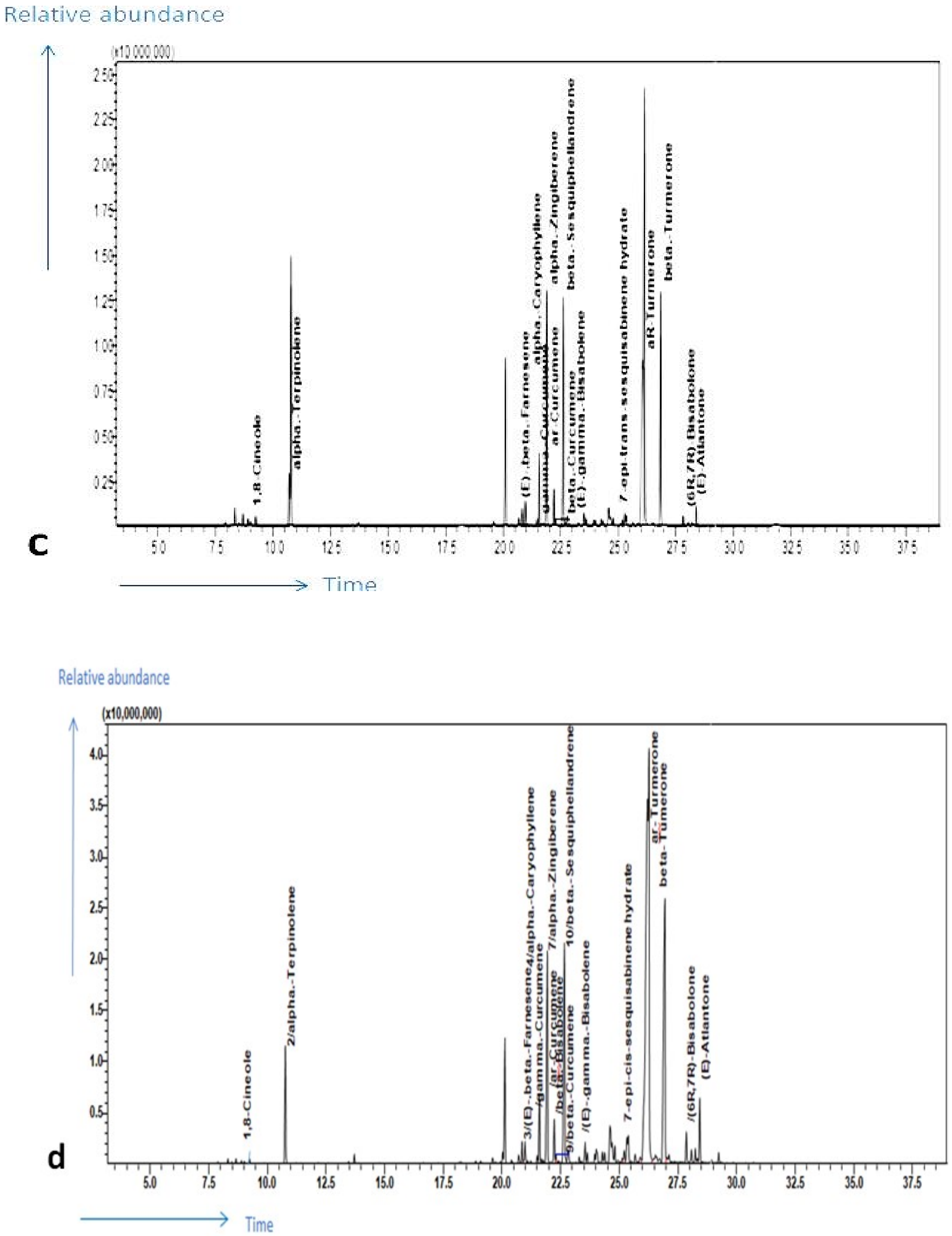


Fig. 4.3.16 Variation in volatile compounds of essential oil under in IISR Pragati ((a) control and (b) drought treatment) and Suguna ((c) control and (d) drought treatment).

1. **1,8 cineole (Eucalyptol)**

In control highest 1,8-cineole (eucalyptol) was recorded in IISR Kedaram (1.00%) followed by SL 5 (0.89%) and minimum in in Suguna (0.08%).

Under drought stress also it was maximum in SL 5 (1.51%) followed by IISR Kedaram (0.89 %) and minimum in Suguna (0.10%) followed by IISR Alleppey Supreme (0.49%). It was increased in tolerant genotypes but decreased in susceptible. Compared to control, the monoterpene, I,8 cineole increased to about 261.24% in IISR Pragati which was maximum followed by SL 5 (70 %).

2. α -terpinolene.

α **-terpinolene** content increased in drought treatment. In control it was maximum in Suguna (2.97%) followed by IISR Pragati (1.39%) while minimum in IISR Alleppey Supreme (0.29%) and SL 5 (0.33%). In drought stress also it was maximum in IISR Pragati (6.02%) and Suguna (4.40%) and minimum in IISR Alleppey Supreme (0.32%). Maximum increase of **α-**terpinolene was noticed in IISR Pragati (331.7%) followed by Suvarna (78.26%) and there was least increase in susceptible ones particularly in IISR Alleppey Supreme (13.1%).

3. α -caryophyllene.

α-caryophyllene increased in tolerant ones but decreased significantly ($P > 0.05$) in susceptible ones. In control, it was maximum in IISR Pragati (0.85%) and Suguna (0.78%) while it was absent in Suvarna and had minimal quantity was detected in IISR Kedaram (0.12%). Under drought treatment, it was maximum in IISR Pragati (1.34%) and SL 5 (0.85%) and minimum in susceptible ones such as IISR Kedaram (0.05%) and IISR Alleppey Supreme (0.06%).

The rise in α-caryophyllene was maximum in SL 5 (83.4%), followed by IISR Pragati (58.25%) but decreased in susceptible ones by more than 50% (IISR Alleppey Supreme: 55% and IISR Kedaram: 56%).

4. β-farnesene

In both control and stress, Suvarna recorded maximum (E)-β-farnesene content. In control, maximum was observed in Suvarna (4.25%) and minimum was in SL 5 (0.19%). While under stress, highest content was observed in Suvarna (3.30%) and lowest in IISR Kedaram (0.27%). (E)-β-Farnesene increase was maximum in IISR Pragati (79.47%) followed by SL 5 (54.83%). Conversely, there was a reduction in Suvarna. But there was no drought tolerance related variation.

5. **Ar-curcumene**

It increased significantly under drought treatment. Ar- curcumene was maximum in Suvarna (2.70%) and IISR Pragati (2.38%) whereas it was minimum in IISR Kedaram (1.16%) followed by IISR Alleppey Supreme (1.69%) under control. Whereas IISR Pragati (5.21%) and Suvarna (2.96%) had the highest content in drought and lowest was in susceptible ones such as IISR Kedaram (1.64%) and IISR Alleppey Supreme (1.72%).

The increase in ar-curcumene was maximum in IISR Pragati (118.7%), followed by SL 5(50.55%) and was lowest in IISR Alleppey Supreme (1.7%). But there was no tolerance related variation with respect to drought.

6. γ-curcumene

There was only a slight increase in γ-curcumene under treatment. Suvarna (0.53%) and IISR Pragati (0.26%) recorded maximum and minimum of 0.12% was recorded in SL 5 under control condition. In treatment IISR Pragati (0.54%) followed by Suvarna (0.35%) had maximum concentration whereas minimum was recorded in IISR Kedaram (0.18%) and Suguna (0.19%).

Maximum increase in γ-curcumene was recorded in IISR Pragati (105.8%) followed by SL 5 (87.47%). It decreased in Suvarna and IISR Alleppey Supreme by 33.87% and 1.53 % respectively.

7. **α-zingiberene**

α-zingiberene increased significantly under drought. α-zingiberene was maximum in Suvarna (13.86%) and IISR Pragati (8.73%) and minimum in IISR Kedaram (2.75%) and SL 5 (3.28%) in control. Under stress, it was highest in tolerant ones such as IISR Pragati (18.00%) and Suvarna (13.92%)

and minimum in susceptible ones such as IISR Kedaram (4.01%) and IISR Alleppey Supreme (4.77%).

Maximum increase of α-zingiberene was observed in IISR Pragati (106%) followed by SL 5 (72.5%). There was no change in its content in Suvarna under drought and. only a slight increase in Suguna (3.5 %).

8. **β-bisabolene**

β -bisabolene showed an increase under drought treatment. In control, Beta bisabolene was recorded highest in Suvarna (3.41%) and IISR Pragati (1.56%) and minimum in SL 5 (0.48%) followed by IISR Kedaram (0.60%). Under stress, it was maximum in Suvarna (6.83%) followed by IISR Pragati (2.79%) and minimum in SL 5 (0.79%) and IISR Kedaram (0.84%).

For β-bisabolene maximum increase was observed in Suvarna (585%) followed by IISR Pragati (81%). Susceptible ones such as IISR Kedaram (40 %) showed least increase.

9. **β-curcumene**

β-curcumene increased under drought. In control, Suvarna (0.68%) and IISR Pragati (0.29%) had the maximum but it was not detected in Suguna and SL 5. Under stress, Suvarna (4.63%) and IISR Pragati (0.52%) had maximum beta curcumene while it was minimum in SL 5 (0.16%) and IISR Kedaram (0.18%). Suguna (0.21%) and SL 5 (0.16%) were detected under drought treatment.

Maximum increase was observed in Suvarna (585%) compared to control followed by IISR Pragati (81%). It was not present in Suguna and SL 5 under control condition. Susceptible showed less increase with only 3% in IISR Alleppey Supreme and 46% in IISR Kedaram.

10. **β-sesquiphellandrene**

β-sesquiphellandrene increased under drought treatment. Suvarna (12.22%) followed by IISR Pragati (9.71%) showed the highest content while it was lowest in IISR Kedaram (2.71%) and SL 5 (3.34 %) in control Maximum content was noticed in Suvarna (18.68%) and IISR Pragati (18.00%) and minimum in susceptible ones such as IISR Kedaram (3.70 %) and IISR Alleppey Supreme (4.94 %) under drought.

Maximum increase in β-sesquiphellandrene was observed in IISR Pragati (85.4%) followed by SL 5 (62.6%) and the least increase in IISR Alleppey Supreme (26%).

11. **(E)-γ-bisabolene**

In control, Suvarna (2.09 %) and IISR Pragati (0.69 %) had maximum (E)-γ-bisabolene content and minimum was found in IISR Kedaram (0.39) and SL 5(0.40%). Under stress, Suvarna (2.67%) and IISR Pragati (1.15%) had maximum while it was minimum in Suguna (0.52) and it was not detected in IISR Kedaram.

12. **7- epi cis-sesquiabinene hydrate**

7 –epi cis sesquiabinene hydrate, showed significant decrease under stress treatment. In control, Suvarna (1.39%) and SL 5 (1.11%) had maximum 7-epi **cis** -sesquiabinene hydrate content while it was minimum in IISR Kedaram (0.32%) and IISR Alleppey Supreme (0.40%). Under drought, maximum 7 episesquiabinene hydrate was found in SL 5 (0.90%) and Suvarna (0.76%) and was lowest in susceptible ones such as IISR Kedaram (0.30%) and IISR Alleppey Supreme (0.34%). The decrease under stress was maximum in Suvarna (45.6%) and minimum in IISR Kedaram (6.6%). There was no drought tolerance related variation among genotypes with respect to 7 -epi cis sesquiabinene hydrate.

13. Ar-turmerone

Ar-turmerone was the 2nd most abundant compound identified in GC-MS analysis. It increased with drought treatment. In control it was highest in IISR Alleppey Supreme (23.48%) followed by IISR Kedaram (19.20%) and was minimum in Suvarna (11.9%) and SL 5 (14.88%). Under drought, Suguna (50.18%) and IISR Alleppey Supreme (24.49%) had maximum ar-turmerone content and it was minimum in Suvarna (13.79%) and SL 5 (17.28%). Maximum ar tumerone increase was observed in Suguna (167.48%) and least increase in IISR Alleppey Supreme (4.31%).

14. β-tumerone (curlone)

β-tumerone was the most abundant compound and it decreased under treatment. In control it was highest in IISR Kedaram (67.10%) and SL 5 (61.67%) and lowest in IISR Pragati (51.95%) and Suvarna (40.83%). Under stress, IISR Kedaram (61.88%) and IISR Alleppey Supreme (56.50%) had maximum and Suguna (17.48 %) and IISR Pragati (19.69 %) had the minimum concentration. β-tumerone decreased in all genotypes under drought with maximum reduction in Suguna (67.6 %).

15. (6R, 7R)-bisabolone

(6R, 7R)-bisabolone decreased under drought treatment. In control Suguna (1.57%) and Suvarna (1.37%) had maximum while it was minimum in IISR Kedaram (0.99%) and IISR Alleppey Supreme (1.11 %). Under drought t maximum 6R, 7R-Bisabolone was observed in Suguna (1.20%) and Suvarna (1.15%) and lowest in SL 5 (0.27%) and IISR Pragati (0.81%). (6R, 7R)-bisabalone decreased under drought in all genotypes except in IISR Kedaram where 9.5% increase was noticed.

16. (E)-atlantone

E-atlantone content decreased under drought treatment. In control it was maximum in SL 5 (9.81%) followed by Suvarna (4.47%) and minimum in IISR Alleppey Supreme (2.68%) and IISR Pragati (2.23%). While under water stress, it was maximum in SL 5 (9.16%) followed by Suvarna (3.47%) and minimum in IISR Pragati (1.55%). E-atlantone decreased in all genotypes with least decrease in Suguna (6.30%) followed by SL 5 (6.65%) under drought treatment.

Table 4.3.10 Table 4.3.10 Essential oil profile of 6 turmeric genotypes under control and drought stress

Compound	Kovats index	IISR Pragati		Suguna		SL 5		Suvarna		IISRAlleppey Supreme		IISR Kedaram	
		Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
1,8 cineole	1031	0.15	0.52	0.08	0.10	0.89	1.51	0.55	0.63	0.64	0.49	1.00	0.89
α-terpinolene	1088	1.39	6.02	2.97	4.40	0.33	0.52	0.35	0.63	0.29	0.32	0.38	0.54
α-caryophyllene	1419	0.85	1.34	0.78	0.84	0.46	0.85	0.00	0.76	0.13	0.06	0.12	0.05
(E)-β-farnesene	1456	0.71	1.27	0.45	0.50	0.19	0.29	4.25	3.30	0.34	0.50	0.25	0.27
Ar-curcumene	1480	2.38	5.21	1.83	2.10	1.75	2.64	2.70	2.96	1.69	1.72	1.16	1.64
γ-curcumene	1482	0.26	0.54	0.18	0.19	0.12	0.22	0.53	0.35	0.21	0.21	0.12	0.18
α-zingiberene	1493	8.73	18.00	6.58	6.81	3.28	5.67	13.86	13.92	4.08	4.77	2.75	4.01
β-bisabolene	1505	1.56	2.79	1.04	1.81	0.48	0.79	3.41	6.83	0.88	1.35	0.60	0.84
β-curcumene	1515	0.29	0.52	0.00	0.21	0.00	0.16	0.68	4.63	0.21	0.22	0.13	0.18
β-sesquiphellandrene	1522	9.71	18.00	7.28	9.90	3.34	5.43	12.22	18.68	3.92	4.94	2.71	3.70
(E)-γ-bisabolene	1531	0.69	1.15	0.52	0.52	0.40	0.76	2.09	2.67	0.53	0.65	0.39	0.00
7-epi-cis-sesquisabinene hydrate		0.79	0.54	0.59	0.53	1.11	0.90	1.39	0.76	0.40	0.34	0.32	0.30
Ar-turmerone	1583	17.16	22.05	18.76	50.18	14.88	17.28	11.19	13.79	23.48	24.49	19.20	22.14
β-tumerone (curlone)		51.95	19.69	53.94	17.48	61.67	53.56	40.83	25.45	59.40	56.50	67.10	61.88
(6R,7R)-bisabolone	1742	1.14	0.81	1.57	1.20	1.28	0.27	1.37	1.15	1.11	1.04	0.99	1.08
(E)-atlantone	1778	2.23	1.55	3.45	3.23	9.81	9.16	4.57	3.47	2.68	2.39	2.79	2.31

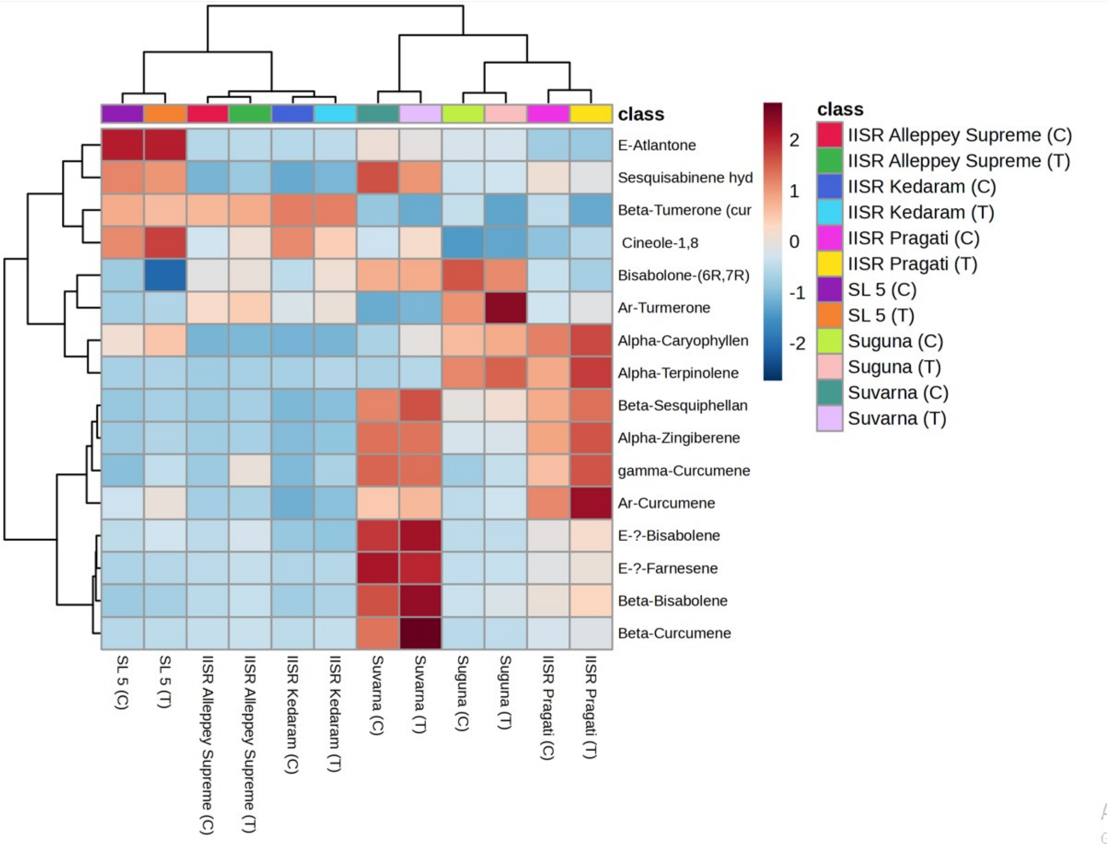


Fig. 4.3.17 Heat map indicating variation of major compounds of essential oil across control and drought treatment in turmeric

On the basis of these results it is concluded that drought had considerable effect on essential oil yield and composition. The chemical composition of the essential oil extracted from turmeric rhizomes has been reported in a number of studies (Mostajeran *et al*., 2014; Zheng *et al*., 2020; Jugreet *et al*., 2020; Bonacina *et al.,* 2022) and these studies indicate that these compounds were present in all turmeric oils, but in different concentrations. The commercial value of turmeric depends on the yield and chemical composition of the essential oil in its rhizome. Major components viz., oxygenated sesquiterpenes are common in all varieties of turmeric which is followed by monoterpene hydrocarbons. Research reports indicate that the production of sesquiterpenes is triggered when plants are exposed to different abiotic stresses (Ibrahim *et al*., 2006).

The results of our study showed relative rise or reduction in abundance of all the compounds which varied significantly among genotypes. Major ones with higher concentration were β-tumerone (most), ar-turmerone (2^{nd} most), α-zingiberene, β-sesquiphellandrene, (E)-atlantone, ar curcumene and β-bisabolene.

IISR Pragati recorded maximum α-caryophyllene, α-terpinolene, α-zingiberene, Ar curcumene and γ-curcumene across control and drought treatment. β-tumerone and (E)-atlantone were highest in SL 5. The evaluation of the composition of essential oil from the turmeric rhizomes showed that plants subjected to drought stress exhibited an increase in the synthesis of 1,8 -cineole, α-terpinolene, (E)-β-farnesene, ar- curcumene, α-zingiberene, β-bisabolene, β-curcumene, β-sesquiphellandrene, (E)-γ-bisabolene and ar-turmerone whereas a decline in the components such as 7-epi-cis-sesquisabinene, β-tumerone (curlone), (6R,7R)-bisabolone and (E)-atlantone. The most abundant compound β-tumerone also got reduced significantly ($P> 0.01$) under drought condition. The increasing content of sesqui and monoterpenes may suggest more isopentenyl pyrophosphate (IPP) flux from the plastid, which is the site of monoterpene biosynthesis (Jin *et al*., 2020). Sesquiterpenes and monoterpenes contribute to drought tolerance through their roles in antioxidant defense, stress signaling, and metabolic regulation (Zhao *et al*., 2019; Peters *et al*., 2019; Chen *et al*., 2017; Barkla *et al*., 2016; Schmidt *et al*., 2017).

The relative abundance of compounds was influenced by genetic constitution of genotype as well as climatic conditions under which they are grown (Dosoky & Setzer, 2018; Kulyal *et al.* 2021; Qiang *et al.* 2021). Abdi *et al.* (2019) and Adichirattle *et al.* (2022) reported that the essential oil components were altered by water stress both positively and negatively in *Mentha piperita* L. plants and ginger respectively under water stress and under salt stress in turmeric (Mostajeran *et al*., 2014) and in different *Curcuma* species (Bonacina *et al*., 2022). Similar results were reported by Singh *et al.* (2022), Hamid *et al.* (2018) and Mandoulakani (2017).

In our study, 1, 8 -cineole, α-terpinolene, α-caryophyllene and β-bisabolene increase was maximum in tolerant genotypes whereas these components decreased or less increased in susceptible ones under drought. Similarly, ar- curcumene, β-sesquiphellandrene and β-bisabolene were maximum in tolerant ones under both control and drought conditions, which points to their possible role in drought tolerance.

4.3.13.1 Principal component analysis

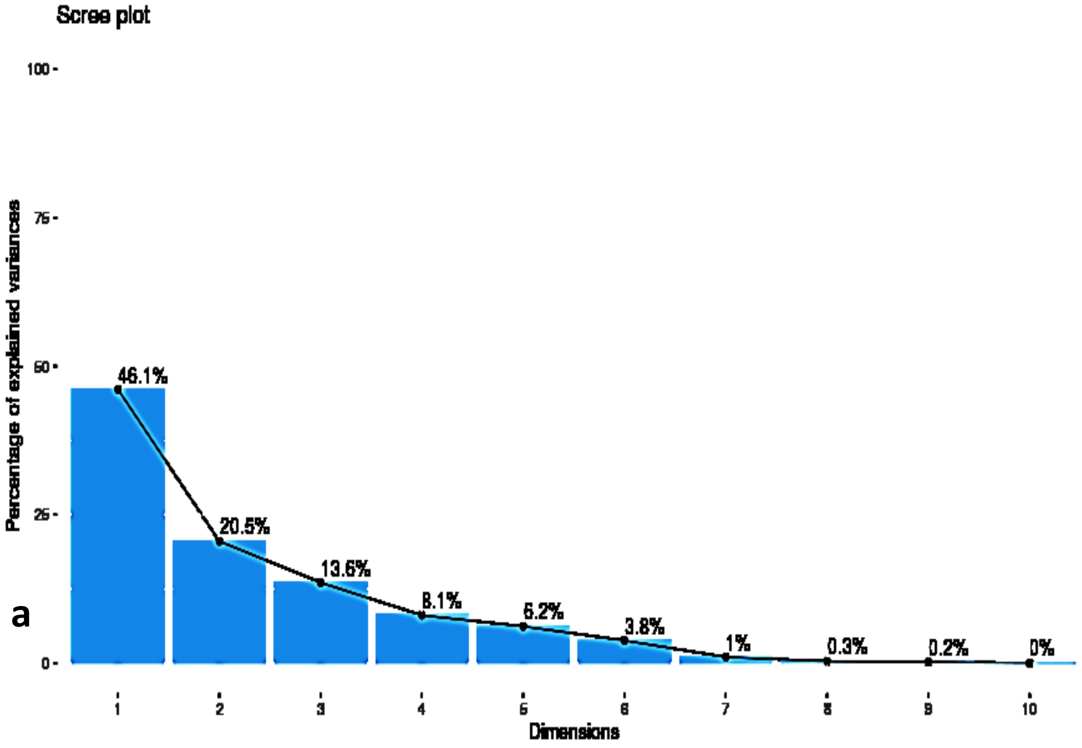
The PCA was performed to evaluate comprehensive pattern of variation and interrelationship among 6 genotypes and essential oil components under drought stress compared to irrigated control. The scree plot illustrated (Fig.4.3.18a) the extent of variance explained by each principal component (PC). The analysis revealed that the first two principal components explained 46.083% and 20.547% of the total variance in the data. Collectively, these components accounted for a cumulative contribution of 66.63 %, considered reasonable for expounding the fundamental connections between genotypes and compounds. The PCA biplot (Fig. 4.3.18b) clearly depicted the distribution of genotypes based on the variation in essential oil components. Percentage contribution of variables to different PCs are given in Table 4.3.11

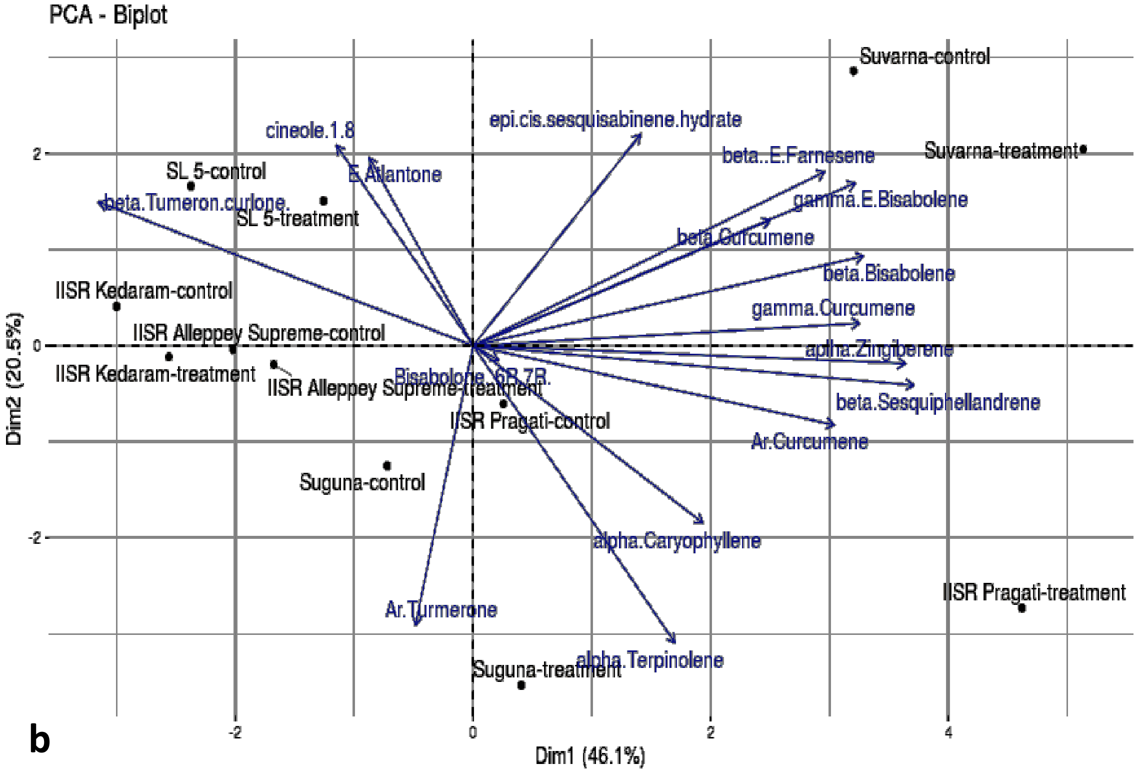
Further analysis of the primary contributors to each principal component more clearly depicted these associations. The volatile compounds β-sesquiphellandrene (13.23%), α-zingiberene (12.77%) and γ-curcumene (10.18%), contributed maximum towards PC1. For PC 2, α-terpinolene (20.6%), ar-turmerone (18.26%) and 7-epi-cis-sesquisabinene hydrate (10.4 %) exerted most considerable influence, followed by 1, 8- cineole (9.37%). Notably, (6R, 7R) - bisabolone, β-curcumene, (E)-atlantone did not contribute significantly to 1st 2 pcs indicating that their concentrations do not vary much with genotype. In the Biplot, IISR Pragati and Suguna were distinct for α-terpinolene and α-caryophyllene and plotted at the lower right quadrant. SL 5 was distinct for 1,8 -cineole and (E)-atlantone. Suvarna was distinct for (E)-β-farnesene, α-zingiberene and (E)-γ-bisabolene which were located towards the upper right quadrant. While IISR Alleppey Supreme and IISR Kedaram (susceptible) which showed negative correlation with tolerant genotypes, were concentrated at the middle portion of left quadrant. From correlation plot (Fig. 4.3.18c) most contributing are α-zingiberene, β-bisabolene, γ-curcumene, Ar-curcumene and β-sesquiphellandrene. Whereas (6R,7R)-bisabolone and (E)-atlantone have neutral response.

Table 4.3.11 Percentage contribution of variables (essential oil components) to different PCs.

Variables	PC1	PC2	PC3	PC4	PC5
1,8 cineole	1.274	9.372	18.502	9.538	0.168
α-terpinolene	2.78	20.634	1.892	2.667	0.012
α-caryophyllene	3.602	7.356	11.76	0.789	6.587
(E)-β-farnesene	8.432	7.064	3.415	0.538	0.505
ar-curcumene	8.892	1.473	9.811	0.045	5.864
γ-curcumene	10.188	0.117	0.448	1.216	18.192
α-zingiberene	12.742	0.074	0.355	0.343	4.503
β-bisabolene	10.39	1.867	2.407	5.777	4.313
β-curcumene	6.014	3.674	2.055	17.119	12.903
β-sesquiphellandrene	13.23	0.369	0.002	0.239	0.143
(E)-γ-bisabolene	9.967	6.168	0.767	0.708	1.052
7-epi-cis-sesquisabinene hydrate	1.916	10.45	1.637	33.06	2.37
ar-turmerone	0.227	18.267	0.408	0.506	14.368
β-tumerone(curlone)	9.566	4.805	0.171	0.007	7.697
(6R,7R)-bisabolone	0.044	0.051	32.081	16.545	0.8
(E)-atlantone	0.736	8.258	14.289	10.901	20.523

Principal component analysis is one of the best tools for multivariate analysis which estimates and reveals the different response of genotypes by the variables (Abdi & Williams, 2010). Our results showed that genotypes vary significantly for most of the essential oil compounds under control and drought treatments. Essential oil components in drought tolerant and the susceptible peanut genotypes was explained by PCA (Srutiben *et al.*, 2020). Essential oil component variation in *Salvia* genotypes (Farzaneh *et al.*, 2022) and *Baccharis trimera* (Moreno-Pizani *et al.,* 2020) under drought stress was depicted through PCA also showed comparable results.


Scree plot
Percentage of explained variances
100
75
50
25
0
46.1%
20.5%
13.6%
8.1%
6.2%
3.8%
1%
0.3%
0.2%
0%
1
2
3
4
5
6
7
8
9
10
Dimensions
a


PCA - Biplot
Suvarna-control
Suvarna-treatment
epi.cis.sesquisabinene.hydrate
cineole.1.8
E.Atlantone
beta..E.Farnesene
gamma.E.Bisabolene
SL 5-control
beta.Tumeron.curlone.
SL 5-treatment
beta.Curcumene
beta.Bisabolene
IISR Kedaram-control
gamma.Curcumene
IISR Alleppey Supreme-control
aplha.Zingiberene
IISR Kedaram-treatment
IISR Alleppey Supreme-treatment
Bisabolone..6R.7R.
beta.Sesquiphellandrene
IISR Pragati-control
Ar.Curcumene
Suguna-control
alpha.Caryophyllene
IISR Pragati-treatment
Ar.Turmerone
Suguna-treatment
alpha.Terpinolene
Dim2 (20.5%)
2
0
-2
-2
0
2
4
Dim1 (46.1%)
b

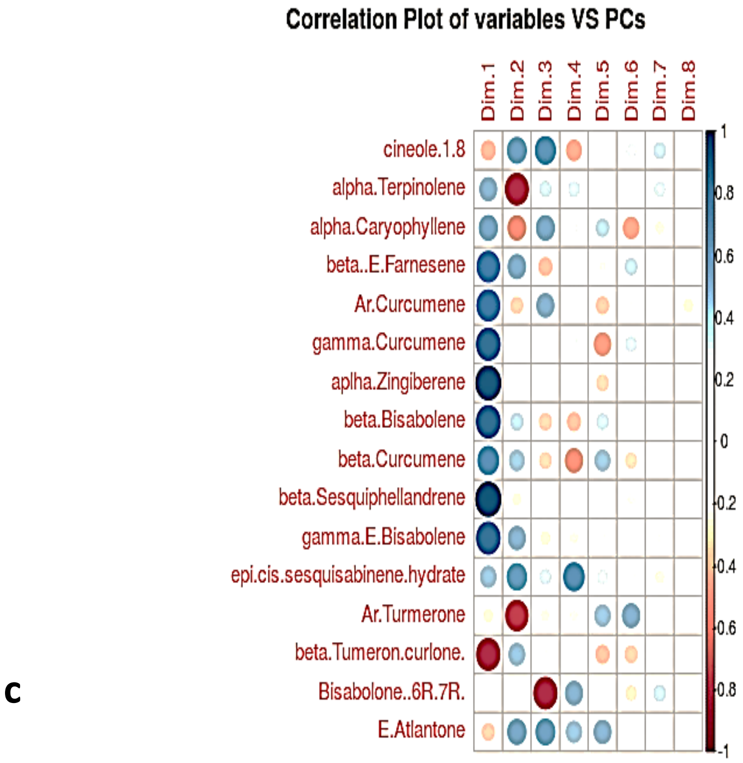


Fig. 4.3.18 Principal component analysis of major essential constituents. (a) Scree plot; (b) PCA biplot (c) Correlation plot of genotypes and essential oil components.

Thus, with respect to yield and quality, a notable variation was identified among different turmeric genotypes under water stress. Yield was notably reduced due to drought stress. However, drought-tolerant varieties like IISR Pragati (215 g plant^{-1}) and Suguna (190 g plant $^{-1}$) demonstrated higher and more sustainable yields. Drought impacts nutrient absorption and the allocation of resources, leading to a marked decline in crop yields. The relative reduction in dry recovery under treatment compared to control was more pronounced in susceptible varieties such as IISR Alleppey Supreme (12.9%) and IISR Kedaram (14.3%).

Under drought conditions, genotypes displayed either a slight increase or decrease in nutrient levels for all elements tested compared to the control. The most significant increase was noted in available concentrations of manganese (Mn), copper (P), and potassium (K). Conversely, concentrations of calcium, zinc, nitrogen, iron, magnesium, and phosphorus were reduced under stress compared to the control. Ca, Cu, Mg, and Fe levels decreased notably across all genotypes, with

Ca and Fe levels particularly low in susceptible varieties. Likewise, Cu and Mn increases were minimal in susceptible varieties, while reductions in Mg, Zn, and N were more pronounced. As a result, drought stress diminished the availability, uptake, transport, metabolism, and redistribution of nutrients (Hu & Schmidhalter, 2005).

Biotic or abiotic stresses can affect the quality characteristics of turmeric. Drought stress led to a decrease in essential oil (%) and oleoresin (%) content, but increased curcumin content, with IISR Alleppey Supreme (4.28) showing the highest curcumin content, followed by IISR Kedaram (4.12%). Prolonged water stress resulted in a decrease in protein content among the turmeric genotypes. Suguna exhibited the least reduction in protein content, with only an 11.6% decrease compared to the control, followed by IISR Pragati with a 13.50% decrease. In susceptible varieties, the reduction in protein content was most pronounced under drought conditions. Similarly, starch content decreased under drought stress, with IISR Pragati showing the smallest decrease of around 18.9% compared to the control, followed closely by Suguna with a reduction of 20.9%.

The total phenol estimation showed an increase in its concentration in drought-treated plant samples compared to the control, with higher increases observed in tolerant genotypes such as SL 5 (38.10%) and Suguna (33.25%). Similarly, the flavonoid estimation also showed a rise in concentration in treated plant samples compared to the control. Furthermore, the chemical composition of essential oils extracted from turmeric rhizomes varied significantly under drought conditions. Plants subjected to drought stress showed higher synthesis levels of 1, 8-cineole, α-terpinolene, α-caryophyllene, and β-bisabolene, particularly in the more tolerant genotypes. ar-curcumene and β-sesquiphellandrene also was higher in tolerant ones. These compounds could serve as key drought stress markers and may be related to specific biochemical pathways and enzymes. Further research is needed to fully understand the tolerance mechanisms involving essential oil components.

CHAPTER V

SUMMARY AND CONCLUSION

Turmeric is an economically important crop. It is widely cultivated for its rhizome as a major spice and for use in medicinal practices. Among abiotic stress drought stress is the most common adverse environmental condition that can seriously reduce crop productivity. Fluctuations in the precipitation and limited availability of irrigation water during dry weather conditions limit the productivity of turmeric. Research on understanding the strategies of plants to cope with drought stress can help us to better use scientific means to improve the adaptability of plants to water shortage environment and increase the yield of crops.

Hence, the present investigation entitled "Morpho-physiological and molecular responses of turmeric genotypes subjected to water stress" was carried out during 2019-2024 at ICAR-Indian Institute of Spices Research, Kozhikode to identify drought tolerant turmeric genotypes suitable for areas with limited water availability. This study was conducted using fifty genotypes collected from National Active Germplasm Site (NAGS) of ICAR-IISR, Kozhikode.

The study was aimed at identification of drought tolerant genotypes with sustainable yield under drought condition, through screening of selected genotypes for drought tolerance from germplasm collection. The other objective was to elucidate the physiological, biochemical and molecular mechanism involved in drought tolerance under different water regimes. Finally, field evaluation of identified genotypes was also intended to identify genotypes with sustainable yield and quality which can be recommended for cultivation under water limited environment.

First objective was morphological and physiological characterization of fifty genotypes to identify a few drought tolerant and also susceptible genotypes for further study through screening of these genotypes for their drought tolerance ability. Fifty turmeric genotypes planted in completely randomized design in polythene bags (15 cm × 30 cm) with three replicates. Plants were subjected to stress treatment from 90 to 120 days after planting (DAP), by suspension of irrigation for 30 days. Observation on morphological characters was recorded after one month of drought treatment whereas physiological characters such as relative water content and membrane permeability were recorded at 10 days interval from 90 to 120 DAP. Genotypes varied significantly ($p < 0.05$) for all the morpho-physiological characters studied. Genotypes with low to medium leaf area, higher relative water content, lower membrane leakage, fewer stomata, and higher epicuticular wax content were considered as drought tolerant. Rhizome yield was very less due to drought treatment during the critical growth period (90 to 120 DAP) which coincides with the rhizome development period but tolerant genotypes showed less yield reduction compared to the susceptible genotypes. Scores and weightages were assigned for each parameter based on tolerance characters in such a way that tolerance category got the highest weighted score. Genotypes were scored based on all the morpho-physiological parameters studied as well as yield.

Weighted score ranged from 39.5 to 16.5 and nine genotypes with higher weighted score (≥33.5) were identified as tolerant ones with sustainable yield. They included SL 10, SL 5, IISR Prabha, IISR Prathibha, IISR Pragati, NDH 1, Suguna, Suvarna and Rajendra Sonia. Genotypes with weighted score of ≤ 25.5 were classified as susceptible genotypes. IISR Alleppey Supreme, IISR Kedaram and Acc 66 which showed higher yield reduction with respect to the highest yielder (Suguna) were selected as susceptible genotypes for further study.

Second objective was to study the mechanism underlying drought tolerance based on physiological and biochemical analysis and molecular characterization through relative gene expression analysis by q RT-PCR. Nine turmeric germplasm accessions, which were selected as most tolerant genotypes from our previous experiment viz. IISR Prabha, IISR Prathibha, IISR Pragati, SL 5, SL 10, Suguna, Suvarna, Rajendra Sonia, NDH 1 and 3 most susceptible genotypes viz. IISR Alleppey Supreme, IISR Kedaram and Acc 66, were used for this study which was conducted during the period 2021-2022. Plants were maintained at soil moisture regimes viz., 100% (control), 50% (moderate stress) and 25% (severe stress) field capacities (soil moisture content -SMC 21.5%, 12.6% and 10.7%), with four replicates. Genotypes were evaluated for their physiological characters (RWC and EL) at 180 DAP, biochemical characters (such as antioxidant enzymes, proline, H_2O_2, MDA) in leaves and rhizomes and photosynthetic pigments, protein, sugar, starch and phenol content in leaves and molecular characterization (relative expression analysis of TFs and AQP gene) for assessing their drought tolerance, at three growth stages *i.e.*, at 120 DAP, 150 DAP and 180 DAP. The differential expressions of the seven TFs (*ABF, BHLH, BZIP, NAC, DREB, WRKY,* and *AP*2) and *AQP* gene were analysed using the selected reference genes EF-1α and UB in leaves and rhizomes.

Tolerant genotypes showed higher RWC, higher proline accumulation, enzyme activity (SOD, POD, CAT and AsA-GSH pathway enzymes: APX, MDHAR, DHAR and GR), sugar, phenol, protein and lower degradation of chlorophyll, carotenoid, starch as well as lower MDA, H_2O_2 content and membrane leakage compared to susceptible genotypes. Enzyme activity and protein content increased under moderate stress compared to control and decreased under severe stress compared to moderate stress. Chlorophyll, carotenoids and starch decreased with stress intensity. Whereas phenol, proline, H_2O_2 content and sugar content increased with stress intensity. Enzyme activities, starch, chlorophyll, carotenoids, phenol, flavonoid content and protein concentration were maximum at 180 DAP whereas proline, MDA and sugar contents were maximum at 120 DAP.

Tolerant genotypes showed higher fold change for *ABF, bHLH, bZIP, NAC, DREB and WRKY* and relatively more down regulation of *AQP* genes compared to susceptible genotypes while there was no tolerance related variation observed in *AP2* gene. The differential expression of drought responsive TFs and *AQP* gene in tolerant and susceptible genotypes further indicated the drought tolerance ability of the above identified genotypes. There was a reduction in yield with stress intensity compared to control and tolerant genotypes showed lower reduction in yield and lower drought susceptibility index. Similar results were obtained for all analysed parameters at all stages with higher enzyme activity, proline accumulation, H_2O_2 content and lipid peroxidation observed in leaves.

Based on the above results, IISR Pragati, Suguna, Suvarna and SL5 which had higher relative water content, lower-medium electric conductivity, more than average enzymatic activities, more free proline and lower lipid peroxidation , H_2O_2 content and lowest DSI, significantly higher yield than other varieties under lower water regimes (25% and 50% FC) were selected as tolerant ones with sustainable yield. Whereas IISR Alleppey Supreme and IISR Kedaram which had the lowest relative water content, lesser proline accumulation, and reduced enzymatic activities, as well as high levels of electrolyte leakage, malondialdehyde, and hydrogen peroxide, with low yield under low soil moisture regimes were selected as susceptible genotypes for field evaluation.

The final objective was the field evaluation of selected drought tolerant genotypes under water limited environment. It was conducted during 2022-23 at ICAR-Indian Institute of Spices Research, Kozhikode, Kerala. These selected genotypes (four tolerant-IISR Pragati, SL 5, Suguna and Suvarna and two susceptible genotypes -IISR Alleppey Supreme and IISR Kedaram) with contrasting response for drought tolerance were evaluated under field conditions for physiological parameters (RWC and EL) in leaves, biochemical parameters (MDA, proline content, drought-responsive enzymes), and gene expression analysis at three successive growth stages viz., 120, 150 DAP and 180 DAP in both leaves and rhizomes with irrigation (control) and without irrigation (drought treatment which experienced water shortage after 100 DAP till harvest).

Results indicated that RWC reduced and EL increased with drought stress. Tolerant genotypes maintained higher RWC and lower EL and vice versa for susceptible ones. RWC decreased with successive growth stage whereas EL increased. Membrane leakage showed relatively lower increase in tolerant ones but its rise was drastic in susceptible ones under drought condition. Whereas proline accumulation was higher in tolerant ones under drought stress compared to that in susceptible ones and it increased with stress intensity. SOD and POD and AsA-

GSH cycle enzymes activity was higher under drought condition compared to control, and tolerant genotypes showed higher activity. Enzyme activity showed a reduction under severe stress compared to moderate stress. Chlorophyll fluorescence parameters measured at late growth stage revealed significant decrease in F_V/F_M ratio, which represent quantum efficiency of PS II, with less reduction observed in drought tolerant genotypes.

Four TFs (*ABF, bHLH, DREB, WRKY*) that showed high correlation with biochemical parameters from the earlier experiment were selected for field analysis (considering Pearson's correlation study with enzyme activity). Analysis indicated that tolerant genotypes showed maximum expression of TFs compared to susceptible ones under drought conditions.

After harvest, rhizomes of control and treatment were analysed for quality parameters. With respect to yield and quality, a notable variation was identified among different turmeric genotypes under water stress. Yield was notably reduced under drought stress compared to control. However, drought-tolerant varieties like IISR Pragati (215 g plant^{-1}) and Suguna (190 g plant^{-1}) demonstrated higher and sustainable yields. The relative reduction in dry recovery under treatment compared to control was more pronounced in susceptible varieties. Under treatment conditions, samples displayed either a slight increase or decrease in nutrient levels for all elements tested compared to the control. The most significant increase was noted in available concentrations of manganese, copper, and potassium. Conversely, concentrations of calcium, zinc, nitrogen, iron, magnesium, and phosphorus were reduced in treated samples compared to the control.

Drought stress led to a decrease in essential oil (%) and oleoresin (%) content, but increased curcumin content. Tolerant ones showed minimum reduction in essential oil and oleoresin content under drought condition. IISR Kedaram (9.04%) and IISR Pragati (7.4 %) showed higher increase of curcuminoides content compared to control. Curcumin content did not show tolerance related variation. Prolonged water stress resulted in a decrease in starch and protein content in the turmeric genotypes but tolerant genotypes exhibited lower reduction compared to the control under drought conditions.

The total phenol and flavonoid contents showed an increase under drought compared to the control, with higher increases observed in tolerant genotypes such as SL 5 (38.10%) and Suguna (33.25%). Furthermore, the chemical composition of essential oil extracted from turmeric rhizomes varied significantly under drought conditions with higher levels of 1,8-cineole, α-terpinolene, α-caryophyllene, and β-bisabolene, under drought condition, particularly in the tolerant genotypes.

Overall, Among 50 turmeric genotypes screened for drought tolerance based on morpho- physiological traits and yield, nine genotypes (SL 10, SL 5, IISR Prabha, IISR Prathibha, IISR Pragati, NDH 1, Suguna, Suvarna and Rajendra Sonia) with reduced (low to moderate) leaf area, less number of stomata (low to moderate), higher wax content, higher RWC% and lower EL% and higher yield were shortlisted as tolerant ones while IISR Alleppey Supreme, IISR Kedaram and Acc 66 with contrasting characters were selected as susceptible ones.

The tolerant and susceptible genotypes responded differently in terms of physiological, biochemical and molecular characters under different water regimes in greenhouse as well as under field condition. Results revealed that the identified tolerant genotypes, IISR Pragati, Suguna, Suvarna and SL5 had higher RWC, lower-medium membrane permeability, higher enzyme activity, proline accumulation and phenol content whereas lower membrane leakage, H_2O_2 and MDA content and exhibited lower degradation of starch, protein and photosynthetic pigments as well as maintained higher concentration of osmolytes (sugar and proline) and had a lower drought susceptibility index under the drought treatment compared to control condition and maintained a higher yield which indicate their better tolerance with sustainable yield. Elevated accumulation of antioxidant enzymes, proline and sugars helped tolerant plants in lowering oxidative injury under drought stress.

From the result it can be seen that increased lipid peroxidation have a major role under extreme stress which would have resulted in increased cell membrane damage and degradation of macromolecules (including important proteins and enzymes) as well as photosynthetic pigments which ultimately resulted in significant decline in yield under severe drought particularly in susceptible genotypes. Therefore in turmeric also MDA content analysis can be an important drought susceptibility marker. These genotypes showed a relatively higher expression for *ABF, BHLH, BZIP, NAC, DREB and WRKY* and relatively higher down regulation for AQP. Here, lower influence of drought stress on *AQP* is indicating susceptibility to drought stress. Therefore *AQP* can be considered as a molecular marker that indicate susceptibility. IISR Kedaram and IISR Alleppey Supreme which showed just the opposite results were selected as susceptible ones.

Thus drought tolerance in turmeric may be due to combined effect of up regulation or down regulation of many genes and transcription factors which may induce interaction of physiological, biochemical and molecular mechanisms at varying degree which can also bring about morphological changes under water deficit stress. Developmental stage of plant as well as duration and severity of drought have pivotal roles in determining how a plant responds to water deficit. The drought stress affected both yield and quality, however yield was the most affected.Yield was significantly reduced due to drought stress in all the genotypes but drought-tolerant varieties maintained sustainable yields under stress.

The results showed that tolerant genotypes, IISR Pragati, Suguna, Suvarna and SL5, significantly outperformed the susceptible ones in terms of drought tolerance traits as well as yield under limited water availability in field condition as well, which indicate their drought resilience character. These identified genotypes with better tolerance traits under drought conditions with sustainable yield may be suitable for rainfed cultivation and can be further utilised for crop improvement for gene pyramiding incorporating many more useful traits.

CHAPTER VI

RECOMMENDATIONS

Amid apprehension of global climate change, crop plants are inevitably confronted with a myriad of abiotic stress factors during their growth that inflicts a serious threat to their development and overall productivity. The development of climate-resilient crops that withstands reduced water availability is a major focus of the scientific fraternity to ensure crop productivity. So first objective of this study was screening selected germplasm for drought tolerance based on morphological and physiological parameters. Fifty turmeric genotypes were screened for water stress by suspension of irrigation for 30 days. Result of morpho-physiological observation identified, SL 10, SL 5, IISR Prabha, IISR Prathibha, IISR Pragati, NDH 1, Suguna, Suvarna and Rajendra Sonia which manifested with higher relative water content, epicuticular wax content and low to medium leaf area, low to medium stomatal density, lower electrolyte leakage and maintained a higher yield as tolerant ones. Meanwhile IISR Alleppey Supreme, IISR Kedaram and Acc66 with contrasting traits were selected as susceptible genotypes.

Different crops and genotypes vary in their sensitivity; the response to dehydration is generally stage-and trait-specific, which can reveal mechanisms related to tolerance (Bita & Gerats, 2013; Prasad *et al*., 2017). Such variations are often present in adapted genotypes that have evolved under natural selection which we have identified by germplasm screening. Therefore, the identified genotypes are the best source of drought resistance traits (Wang *et al*., 2022). RWC reflects the balance between water supply to the leaf tissue and transpiration rate, exhibited highly positive association with rhizome yield and yield contributing traits. It is possible to use these parameters as rapid indicators for screening turmeric genotypes for drought tolerance in breeding programs (Lugojan & Ciulca, 2011; Robinet *et al.,* 2008). Identification of traits that are directly associated with yield is key to improving drought resistance, which achieves stable yield in drought-prone areas (Bao *et al.,* 2023). Identification of contrasting genotypes with differential response to drought condition facilitate characterization of agronomically important genes and biochemical mechanisms involved in stress response (Nutan *et a*l., 2017; Xu & Bassel, 2020).

Second objective was to study mechanism underlying drought tolerance using tolerant and susceptible genotypes identified from previous study under different water of 100% (control), 50% (moderate stress) and 25% (severe stress) field capacities. These genotypes were evaluated for their physiological parameters in leaves, biochemical (enzymes, sugar, proline, H_2O_2, MDA) and molecular characters (differential expressions of the transcription factors) for assessing their drought tolerance at three growth stages *i.e*., at 120 DAP, 150 DAP and 180 DAP in leaves and rhizomes. Starch, phenol, protein content and photosynthetic pigments in leaves also were analysed to understand their variation under drought condition. Result indicated that tolerant genotypes showed higher RWC, enzyme activity, proline and phenol content whereas a lower membrane leakage, H_2O_2, MDA content and also exhibited lower degradation of starch, protein and chlorophyll while maintaining a higher sugar and carotenoid content which resulted in sustainable yield.

PCA biplot revealed close positive correlation between higher yield and proline accumulation as well as enzyme activity. Enzyme activity was highest at late stage particularly under moderate stress. Thus impacts of drought condition on yield of crops depend on their severity and the stage of plant growth during which they occur. A positive correlation between proline accumulation and plants' tolerance to abiotic stress has been pointed out in numerous reviews (Spormann *et al.,* 2023; ozturk, 2021). Exogenous application of proline can be done to improve crop productivity. It will increase content of sugars, soluble proteins and phenol, while decrease membrane permeability by acting as ROS scavenger, molecular chaperons and as nitrogen source (Gadallah, 1999; Rai & Kumari, 1983; Santos *et al.,* 1996; Matysik *et al*., 2002; Smirnoff & Cumbes, 1989; Hussein *et al.,* 2019). Activation or overexpression of genes encoding enzymes for glutamate and ornithine-based proline biosynthesis pathways escalate the accumulation of proline that provide tolerance to the plant, which in turn also activates downstream regulation of drought-responsive genes (Singha *et al*., 2020; Sharma & Verslues, 2010). Likewise, overexpression of genes encoding antioxidant enzymes can improve drought tolerance (Hu *et al.,* 2023; Wang *et al*., 2022; Yu *et al*., 2022).

Gene expression analysis revealed that the relative expression of TFs (*bZIP, ABF, bHLH, NAC, WRKY and DREB*) increased with drought stress and were higher in tolerant genotype. Understanding of genomic responses of drought is of utmost importance as it determines the morphological and physiological changes due to the stress, emphasis should be given to the target genes of TFs identified, which may correspond to multiple metabolic pathways and are considered as promising targets for crop improvement. Understanding the role and regulation of the transcription factors will facilitate the crop improvement strategies intending to develop agronomically-superior crops (Manna *et al.,* 2020). Characterization of TFs and promoter regions as well as SNP identification of drought responsive genes aids in further elucidation of molecular mechanisms (He *et al*., 2023; Nagore *et al.,* 2013). Transgenic plants overexpressing specific TFs and their target genes can enhance tolerance to abiotic stresses (Hoang *et al.,* 2017; Lu *et al.,* 2012; Fujita *et al*., 2005). Comparative transcriptomics using RNA-seq analysis, aid in identification of genotype- and developmental-stage specific differentially expressed genes (DEGs) in response to drought stress (Parmar *et al.,* 2019; Bhaskarla *et al.,* 2020).

Based on the result on drought mechanism study, IISR Pragati, Suguna, Suvarna and SL 5 were identified as tolerant ones with sustainable yield and were selected for field analysis along with two susceptible ones (IISR Kedaram and IISR Alleppey Supreme) with contrasting characters for comparative analysis. Physiological, biochemical and molecular characters were recorded at different growth stages 120, 150 and 180 DAP in field condition under irrigated and drought condition. Yield and quality parameters were studied after the harvest. Under field condition also, tolerant genotypes exhibited higher enzyme activity, proline accumulation whereas a lower lipid peroxidation and membrane leakage.

Drought-tolerant varieties demonstrated less reduction in yield and dry recovery. Therefore, these genotypes could be recommended for better rhizome yields. Yield and dry matter accumulation can be considered as primary indicators for drought screening (Sun *et al*., 2021; Nakhforoosh et al, 2016). The drought treatment revealed marked variation in the quality traits of turmeric as compared to the untreated control. Tolerant varieties exhibited less reduction in essential oil and oleoresin under drought condition. Drought increased curcumin content significantly. Here tolerant genotypes including IISR Pragati and Suguna had higher curcumin content under both drought and control treated samples. Phenol and flavonoids were also increased under stress and were higher in tolerant ones. Development of genotypes specially adapted for drought conditions with enhanced secondary metabolite content especially curcumin, will be an innovative approach for turmeric cultivation (Yadav *et al.,* 2021).

Regarding mineral nutrients tolerant genotypes had shown higher concentration of Mn, Cu, Ca, Mg, Zn and N under drought stress compared to susceptible genotypes. Mineral nutrients are involved in signalling, cell wall stability, interaction with osmolytes, used as cofactors for enzymes, and had a central role in photosynthesis (Cakmak & Kirkby, 2008; Herman *et al*., 2013; Dodd *et al.,* 2010; Molassiotis *et al.,* 2016; Taiz & Zeiger, 2015). Supplying these mineral nutrients by exogenous application can improve drought resistance and can effectively alleviate the negative effects of drought stress on growth and production (Zhang *et al.,* 2022; Ashraf *et al.,* 2012; Karim *et al.,* 2012).

The chemical composition of essential oils extracted from turmeric rhizomes varied significantly under drought conditions. Plants subjected to drought stress showed higher synthesis levels of 1, 8-cineole, α-terpinolene, α-caryophyllene, and β-bisabolene. Ar-curcumene and β-sesquiphellandrene was particularly more in the tolerant genotypes. In general, monoterpenes and sesquiterpenes were the main components of essential oils. Terpene biosynthesis is induced by drought and seasonal changes (Griesser *et al.,* 2015). They trigger local and systemic defence signalling and provide drought tolerance by inducing the ROS scavenging system

(Liu *et al.,* 2014). Hence, manipulating selective metabolites and overexpression of biosynthesis pathway-related genes play crucial roles under stress could allow for crop yield improvement under stress conditions (Yang *et al*., 2018; Yadav *et al.,* 2021). Similarly, the application of biostimulant, organic products that influence critical signalling molecules, is a sustainable approach to inducing phenolic compounds in plants to aid against abiotic stresses (Riya *et al*., 2024). In addition, antimicrobial peptides (AMPs), like defensins which are small amino acid sequences, constitute an integral part of plant immunity, play an important role in maintaining the homeostasis of plant cells, The expression of AMP genes in transgenic plants may increase abiotic stress tolerance (Olga *et al*., 2020).

Many preclinical studies indicated high antioxidant, antimicrobial, anticancerous, anti-inflammatory properties of these terpenoides (Parveen *et al*., 2013; Shariffi *et al*., 2017; Patra *et al*., 2016; Russo *et al*., 2015). Essential oil is known for its relaxant effect by regulation of hormonal levels which ultimately affect both mood and emotion (Fung *et al.,* 2021). They also functions in allelopathy, attraction of pollinators and seed dispersers (Fürstenberg *et al.,* 2013). In medicinal and spice plants, drought-mediated induction of aromatic products such as isoprenoids, phenols, and alkaloids can be utilized as a novel strategy to enhance the yield of these valuable compounds (Kleinwachter & Selmar, 2017).

The drought response depends on plant-genotype-specific characteristics, stress intensity and duration. Conserving secondary metabolites especially curcumin content and maintaining proper yields are crucial in turmeric under dehydration stress. Tolerant genotypes can maintain their quality under drought condition. Both irrigation treatments and genotype need to be considered by turmeric breeders and farmers during its production to produce grain with the required levels of poly phenolics and antioxidant activity for targeted end-use. Taken together, strategies to improve drought tolerance in crops need to target several metabolic pathways at the same time. Performance of different turmeric genotypes varied significantly in terms of morpho physiological and molecular traits, yield, and quality parameters. These findings suggest that the selection of superior turmeric genotypes could potentially improve yield and quality and leads to more profitable turmeric cultivation. The identified genotypes (IISR Pragati, SL 5, Suguna, and Suvarna) with better tolerance traits, sustainable yield and quality under drought conditions can be cultivated under rainfed cultivation or areas with limited water availability to obtain better yield with assured quality.

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Scoring of fifty turmeric genotypes based on ideal genotype concept.

SL No.	Genotype	Score
1.	IISR Prabha	39.5
2.	IISR Pragati	39.5
3.	IISR Prathibha	39
4.	SL-P389/ 1	38.5
5.	Rasmi	38
6.	Rajendra Sonali	37.5
7.	SL 5	37.5
8.	Rajendra Sonia	36.5
9.	SL 10	36.5
10.	Suguna	36
11.	SL 2	36
12.	BSR 2	35.5
13.	BSR I	35.5
14.	Kanthi	35
15.	Salem Erigoor	34.5
16.	Ranga	34
17.	SL 7	34
18.	Suvarna	33.5
19.	NDH 1	33.5
20.	SL 1	33.5
21.	Megha Turmeric	32.5
22.	SL 6	32.5
23.	Roma	32
24.	CO- 2	31.5
25.	NDH 98	31.5
26.	Amballur	31
27.	BSR 2 White	31
28.	CO- 1	31
29.	SL 8	31
30.	NTC 189	30.5
31.	PH 2	30.5
32.	Acc 849	30
33.	Duggirala Red	30
34.	NTC 188	30
35.	SL 11	30
36.	Sobha	29.5
37.	Suranjana	29.5
38.	Panth Peetab	29

39.	PH 1	28.5
40.	SL 3	28.5
41.	Sudarsana	27.5
42.	Varna	27.5
43.	SC 61	26.5
44.	Suroma	26
45.	IISR Kedaram	25.5
46.	SL 4	25.5
47.	Sugantham	25
48.	Acc 8	24
49.	IISR Alleppey Supreme	22
50.	Acc 66	16.5

PUBLICATIONS AND PRESENTATIONS

- **Banu, C** & Krishnamurthy, K. (2023). Identification of drought tolerant turmeric (*Curcuma longa* L.) genotypes with sustainable yield. *Journal of Spices and Aromatic Crops*. 129-147. 10.25081/josac.2023.v32.i2.8883.

Oral presentation

- **C V Nazmin Banu** and K S Krishnamurthy (2022). Physiological response of turmeric (*Curcuma longa* L.) genotypes to limited soil moisture regimes. International conference on physiological and molecular mechanisms of abiotic stress tolerance in plants. 26 -28 October 2022, University of Calicut, Calicut, Kerala.

Poster presentation

- **C V Nazmin Banu** and K S Krishnamurthy. 2023. Physiological changes in turmeric genotypes subjected to water stress-international symposium on spices and aromatic crops. SYMSAC X 2021, 09-12 February 2021, International Symposium on Spices as Flavours, Fragrances & Functional Foods, Indian Society for Spices, Kozhikode, Kerala, India.
- **C V Nazmin Banu** and K S Krishnamurthy. 2021. Identification of water stress tolerant turmeric genotypes based on relative water content, electrical conductivity and yield response. IPPVS-2021, 11-12 March 2021, International Plant Physiology Virtual Symposium, ICAR-Sugarcane Breeding Institute, Coimbatore, India.