Effect of Salicylic Acid on Physiological, Biochemical and Molecular Changes in Drought Stressed Rice (*Oryza sativa* L.) Plants

A Thesis submitted in partial fulfillment for the Degree of

DOCTOR OF PHILOSOPHY

in

School of Biological Sciences & Biotechnology
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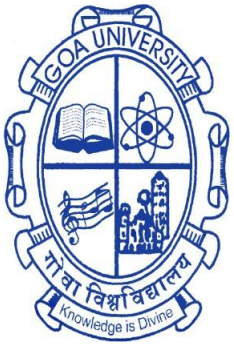

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DECLARATION

I, Shravani Narayan Korgaonker, hereby declare that this thesis represents work which has been carried out by me and that it has not been submitted, either in part or full, to any other university or Institution for the award of any research degree.

Place: Taleigao Plateau.
Date: 16-11-2023 **Shravani Narayan Korgaonker**

CERTIFICATE

I hereby certify that the work was carried out under my supervision and may beplaced for evaluation.

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Ms. Shravani Narayan Korgaonker

Dedicated to

My Almighty,

Loving parents

&

Family

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ABBREVIATIONS

ABA	Abscisic acid
ACR	Acrolein
APX	Ascorbate peroxidase
AQP	Aquaporin
ASA	Acetylsalicylic acid
ATP	Adenosine triphosphate
CAT	Catalase
CE	Carboxylation efficiency
Chl a	Chlorophyll 'a'
Chl b	Chlorophyll 'b'
Chl*	Excited chlorophyll molecule
C_i	Intercellular CO_2
CSA	Cross-section area
DNA	Deoxy ribonucleic acid
DNPH	2,4-Dinitrophenylhydrazine
DREB⁄CBF	Dehydration-responsive element-binding transcription factors
DS	Drought sensitive
DT	Drought tolerant
DW	Dry weight
E	Transpiration rate
EL	Electrolyte leakage
ETC	Electron transport chain
Fv/Fm	Photosynthetic efficiency
FW	Fresh weight
GCL	Glutamate-cysteine ligase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidised glutathione
GTA	Gentisic acid
H_2O_2	Hydrogen peroxide
HKT1	High-affinity potassium transporter gene
HNE	4-hydroxy-2-nonenal
HPLC	High-pressure liquid chromatography
HSPs	Heat shock proteins
IC	Isochorismate
ICS	Isochorismate synthase
IPL	Isochorismate pyruvate lyase
IRRI	International Rice Research Institute
LEA	Late embryogenesis abundant
LPO	Lipid peroxidation
MC	Maximum carboxylation
MDA	Malondialdehyde
MeSA	Methyl salicylate
MGDG	Monogalactosyldiacylglycerol

MSI	Membrane stability index
NIPs	Nodulin 26-like intrinsic proteins
$O_2^{\bullet}$	Superoxide radicals
OAA	Ortho-anisic acid
OH·	Hydroxyl radical
PAL	Phenylalanine ammonia-lyase
PC	Protein carbonyl
PEG	Polyethylene glycol
PIPs	Plasma membrane intrinsic proteins
PM	Plasma membrane
P_N	Photosynthetic rate
PR	Pathogenesis-related proteins
PSI	Photosystem I
PSII	Photosystem II
PTM	Post-translational modification
PUFA	Polyunsaturated fatty acids
qP	Photochemical quenching
RCS	Reactive carbonyl species
RDW	Root dry weight
RES	Reactive electrophilic species
RFW	Root fresh weight
ROS	Reactive oxygen species
RT-PCR	Real-time polymerase chain reaction
RubisCO	Ribulose bisphosphate carboxylase/oxygenase
RWC	Relative water content
SA	Salicylic acid
SAG	Salicylic acid 2-O-β- glucoside
SAR	Systemic acquired resistance
SDW	Shoot dry weight
SEM	Scanning electron microscope
SFA	Saturated fatty acid
SFW	Shoot fresh weight
SGE	Salicyloyl glucose ester
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TGSH	Total glutathione
TIPs	Tonoplast intrinsic proteins
UFA	Unsaturated fatty acid
VB	Vascular bundle
WUE	Water use efficiency
XOD	Xanthine oxidase
ΦPSII	Quantum photosynthetic efficiency
Ψ	Water potential

ABSTRACT

Drought stress is a significant constraint which negatively affects rice productivity (*Oryza sativa* L.). This study was investigated to compare the growth, morphological, physiological, biochemical, and molecular responses of two rice cultivars, drought-tolerant 'Sahbhagi dhan' and drought-sensitive 'Jaya' under the influence of foliar applied salicylic acid by pot culture in controlled growth conditions. Foliar application with salicylic acid (SA) improved all the examined parameters of the selected rice cultivars. The overall results of the present investigation are well evident that 0.25 mM salicylic acid, used in a foliar spray, is more efficient in improving the vegetative growth of both 'Sahbhagi dhan' and 'Jaya' under well-watered and drought-stressed conditions. Increased drought magnitude decreased plant growth and biomass in the 'Jaya' cultivar compared to 'Sahbhagi dhan'. Drought tolerance of the 'Sahbhagi dhan' cultivar depended on their ability to maintain better relative water content than 'Jaya'. The effect of the exogenous application of salicylic acid (SA) on drought-tolerant and drought-sensitive plants showed enhanced growth characteristics, biomass, and relative water status under well-watered and drought-stressed conditions. A significant decline was observed in net photosynthesis rate (P_N), transpiration rate (E), stomatal conductance (g_s), internal CO_2 concentration (Ci), water use efficiency (WUE), photochemical quenching (qP), and lower quantum efficiency of the PSII system (Fv/Fm ratio) in 'Jaya' compared to those of 'Sahbhagi-Dhan' as a response to drought stress.

A significant decline in chlorophyll content was observed in 'Sahbhagi dhan' than in 'Jaya'. The effect of the exogenous application of salicylic acid, particularly at 0.25 mM concentration, on the drought-tolerant and drought-sensitive cultivar showed improved photosynthetic efficiency by enhancing the light and dark reactions and -

- enhancement in the plant pigments under well-watered and drought-stressed conditions. Furthermore, the increased density of cuticular papillae and wax deposition and the reduction in the number and size of stomata due to drought treatment in 'Sahbhagi dhan' indicated the role of morphological adaptation to drought tolerance by preventing water loss. The drought-sensitive leaf and root anatomical characteristics were more severely affected by drought stress than the drought-tolerant cultivar.

Drought-sensitive cultivar exhibited a greater amount of oxidative damage, electrolyte leakage (EC), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH•), lipid peroxidation (MDA), and protein carbonyl (CO) accumulation compared to that of drought-tolerant cultivar under drought stress. GC-MS analysis showed higher levels of saturated fatty acids (palmitic acid and stearic acid) in drought-sensitive than in the drought-tolerant cultivar. In contrast, the drought-tolerant cultivar displayed an enhanced level of unsaturated fatty acid (linoleic acid and linolenic acid), which may suggest an adaptational role probably through maintaining membrane fluidity in making 'Sahbhagi dhan' more tolerant to drought stress.

Furthermore, 'Sahbhagi dhan' showed relatively higher antioxidant capacity (SOD and GR) and lower proline content, i.e., low osmotic stress, compared to the drought-sensitive variety. These results were further substantiated by more significant expression of SOD and APX in 'Sahbhagi dhan' than in 'Jaya,' suggesting better protection against oxidative damage. An increased level of OsAQP expression indicated an increase in plant tolerance to water deficit. Our study concluded that the drought-tolerant cultivar maintained ion and water homeostasis, which was confirmed through high gene expression of the K^+ transporter and a higher threshold level of antioxidant enzymes, which limits ROS-mediated oxidative damage and upregulates the processes associated with the higher photosynthesis that enhanced the biomass,

overcoming drought stress and enabling sustainable growth. The effect of the exogenous application of salicylic acid, particularly at 0.25 mM concentration, on selected rice cultivars showed an enhanced synthesis and activity of the enzymatic and non-enzymatic antioxidants, increasing the plant's tolerance to PEG-induced drought stress. SA also ameliorated the oxidative damage by reducing reactive oxygen species' buildup, thereby maintaining the membrane integrity. These findings indicate that agronomic treatments with exogenous applications of salicylic acid could help reduce drought's effects in the field.

1. Abiotic Stress

Stress is an altered physiological state created by factors interrupting equilibrium. The changes in environmental conditions fluctuate and are predictable over daily cycles due to flexibility in normal metabolism. Thus, variation or divergence of a factor from its equilibrium does not necessarily result in stress. Stress or highly impulsive fluctuations imposed on optimum metabolic patterns lead to injury or abnormal physiology (Jaleel et al., 2009).

In the wake of climate change, worldwide agriculture in the recent decade has experienced a downfall in growth and productivity due to negative consequences of abiotic stress, including drought, heat, cold, nutrient shortage, excess salt, and toxic metals in the soil, as well as biotic stress, which includes pathogen infection and herbivore predation. In unfavourable or stressful circumstances for growth and development, plants must adapt to their ever-changing surroundings, reducing the total yield of major food crops by more than 50% (Fedoroff et al., 2010).

Factors such as the unavailability of timely inputs, differing growing seasons, and outbreaks of pest and abiotic stresses limit the genetic throughput of the crop variety. Abiotic stress for a crop variety differs with the edaphic factor, geographic location, and the growing season and is considered the most detrimental factor concerning growth and productivity (Gao et al., 2007). The most familiar abiotic stresses are fluctuating environmental conditions, soil moisture availability, high evaporation, atmospheric temperatures (heat and freezing), non-availability of nutrients, and heavy metal toxicity (**Fig.1.1)**. The effect of these abiotic stresses becomes more stressful, causing a significant primary global food deficit due to higher food demand and plant-

based by-products (Pareek et al., 2013). Three significant environmental stressors-drought, salinity, and temperature affect the geographic distribution of plants in nature, restrict plant production in agriculture, and jeopardise global food security.

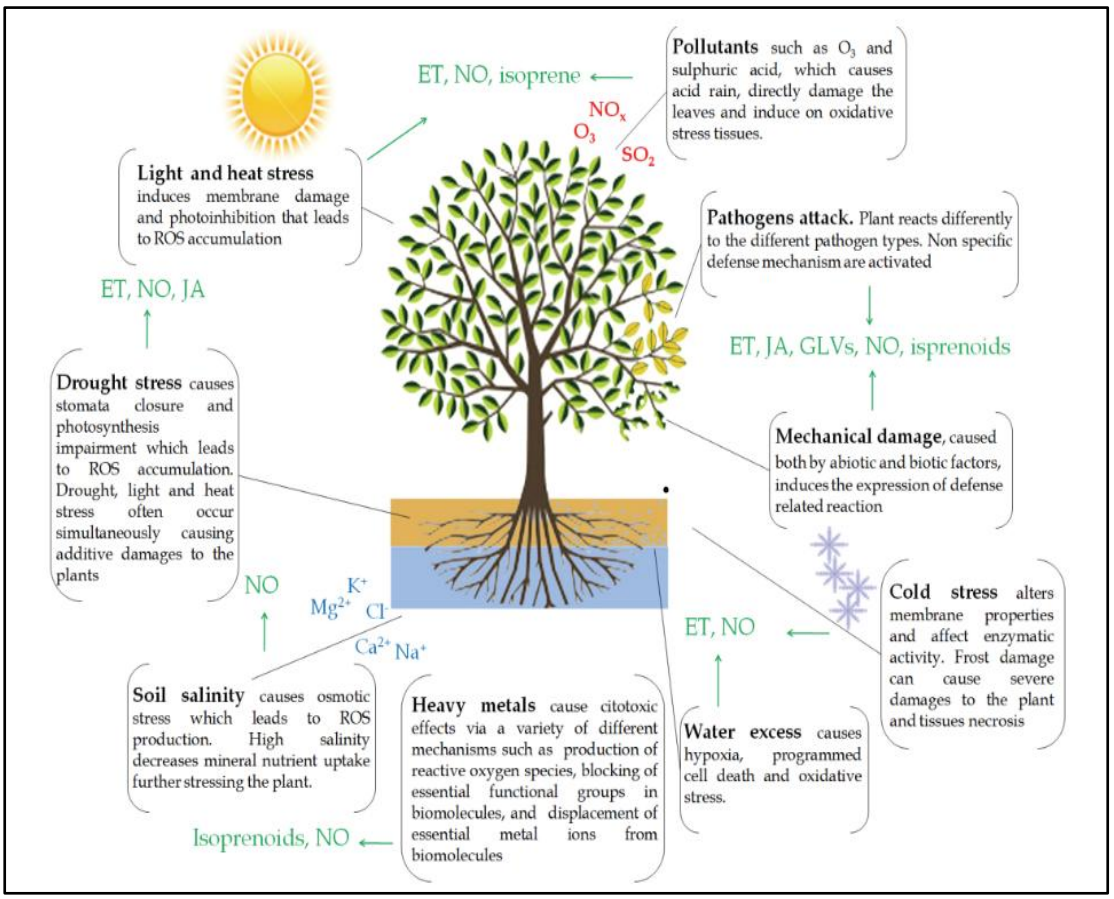

Fig.1.1: Effects of various abiotic stresses causing direct accumulation of ROS hampering plant growth and production (Spinelli et al., 2011).

1.1.Temperature stress

High-temperature stress in plants develops as a result of an array of factors, such as exposing plants to hot ambient temperatures, exposing germinating seeds to the soil that has been warmed by solar infrared radiation, increasing plant transpiration followed by decreased water absorption, decreased transpiration capacity in particular plant organs, causing forest fires or natural gas explosions, etc. Despite extensive

studies of the ultrastructural, molecular, and gene expression levels at various temperature extremes, the perception of temperature and the signalling molecules involved in it are not well understood (Iba, 2002; Rao et al., 2002; Camejo et al., 2005). When an organism is exposed to extreme temperatures, typically 5 to 10 °C above normal growth temperatures, for a short period of up to a few hours, its cells react by synthesising a selected group of proteins known as heat shock proteins (HSPs) (Sri Devi et al., 1999) and these HSPs are an outcome of its recovery. It is crucial to comprehend the processes that lead to the formation of thermotolerant plants in tropical, semiarid, and arid locations worldwide.

1.2. Salinity stress

When rainfall is scarce and inadequate to move salts from the plant root zone, salinity affects agricultural productivity and quality in dry and semiarid countries (Quesada et al., 2000; Tester and Davenport, 2003). Salinity develops when salts, such as magnesium sulphate, sodium chloride, sodium carbonate, or sodium sulphate, are elevated. Ion toxicity, water shortages, nutritional imbalances, and inadequacies are only a few of the negative impacts of salt on plants. The various salinity effects and different genes or sets of genes make salt tolerance and resistance mechanisms extremely complicated (Flowers and Yeo, 1995). In order to tolerate salt, ion homeostasis is necessary (Zhu, 2002). The processes, such as controlled ion absorption, ion compartmentation, and gene products, which include stress proteins, are linked to salt tolerance (Cheeseman, 1988; Winicov, 1998; Zhu, 2001).

1.3. Drought Stress

Variations in temperature and precipitation due to climate change negatively affect crops, resulting in drought stress (Zhao et al., 2017). Drought is among the most severe and common stress factors in many parts of the world, particularly in arid and semiarid regions (Rao, 2009). Drought is a prolonged period of water deficiency in a specific region's ground, surface water, or atmosphere, a significant alarm in modern agriculture. Water scarcity has a global impact on agricultural production and productivity, potentially resulting in substantial yield losses in both quantity and quality (Afridi et al., 2022; Salam et al., 2022; Saleem et al., 2022; Yasmeen et al., 2022). The frequency of drought events in various regions will increase due to climate change. The fluctuations in the climate will show an increasing trend in seasonal and regional droughts, resulting in a decline in average crop yield (Monneveux et al., 2014; Munson et al., 2021). By 2050, under predicted climate change scenarios, the worldwide population will be approximately ten billion people, with about 50% of people facing water shortages for consumption with subsequent water deficits in agriculture (Brown et al., 2019). As a result, two-fold global crop production will be required to meet rising food demand.

Due to the relationship between anthropogenic activities, terrestrial productivity, the hydrological cycle, and global demand for ecosystem services, there has been an increased demand for water (Bernacchi and VanLoocke, 2015). The average rise in drought predicted by climate change scenarios in many parts of the world has emphasised this problem and sparked study into how plants react to water constraints. The World Resources Institute's Aqueduct project has shown that the two European

nations of Moldova and Ukraine have the highest worldwide drought risk. Along with the Middle East, North Africa, Asia, India, China, and Europe are home to more impacted nations facing medium to high drought risk (**Fig.1.2**).

It has been shown that plants respond to various stresses by altering their growth and photosynthesis through various processes (**Fig.1.3**) (Skirycz et al., 2010). Since plants require a certain amount of water to operate normally, its reduced availability due to a lack of precipitation can have negative ecological and economic impacts. Thus, this situation necessitates a greater understanding of crop physiological responses under drought stress to develop new cultivars with enhanced drought tolerance. Water shortage directly impacts plants on a physiological, morphological, and molecular level, affecting the agricultural and ecological output (Xue-Xuan et al., 2010) (**Fig.1.4).**

Lack of rainfall, salt, extremes in temperature, and intense light are a few of the factors that might cause plants to face water deficiency. However, in many situations, there is enough water in the soil, but plants cannot absorb it. This type of water stress is called a pseudo-drought or physiological drought (Lisar et al., 2012). Additionally, it may harm plant development and production in terms of quantity and quality (Jaleel et al., 2009; Nezhadahmadi et al., 2013; Zlatev and Lidon, 2012). Plant responses to a water shortage are influenced by the length and severity of the water deficit and the plant's species, age, and developmental stage (Rao et al., 2006). The systems that help plants tolerate drought follow a basic strategy: preserving cell water homeostasis when drought conditions exist. This is primarily made feasible by restricting water evaporation and enhancing water uptake into the cells, resulting in normal cell

activities. Another typical method of drought resistance in annual plants is drought avoidance and tolerance (Lisar et al., 2012).

According to agricultural and physiological views, drought stress often happens when the amount of water plants can access in the soil at a given time is reduced due to low soil moisture (Dai, 2013). Conversely, water stress in plants happens when the rate of transpiration from leaf surfaces exceeds the rate of water absorption by roots (Lisar et al., 2012). This mismatch between plant water intake and losses occurs mainly when soil moisture potential is lower than plant roots. Many plants may thrive in desert environments without water stress, including water-spending, water-collecting, and water-saving xerophytes. Thus, it is essential to know that drought does not equal plant water deficiency (Trenberth et al., 2014). Therefore, an agricultural drought comes after a meteorological drought (Barriopedro et al., 2012; Dai, 2013).

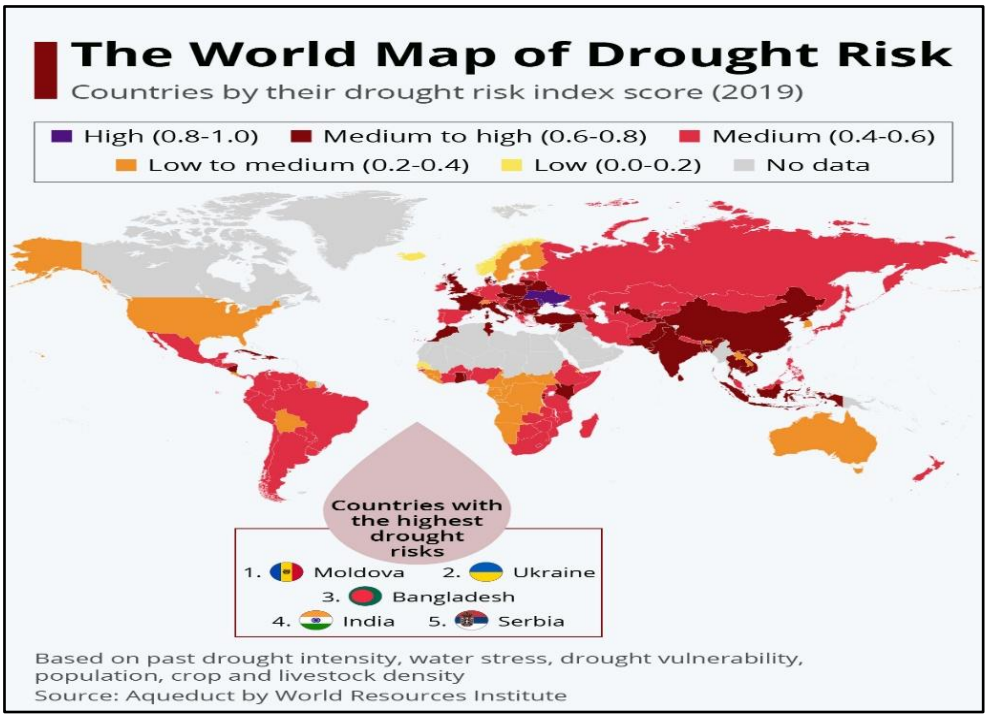

Fig.1.2.: World map indicating drought risk index score (2019).

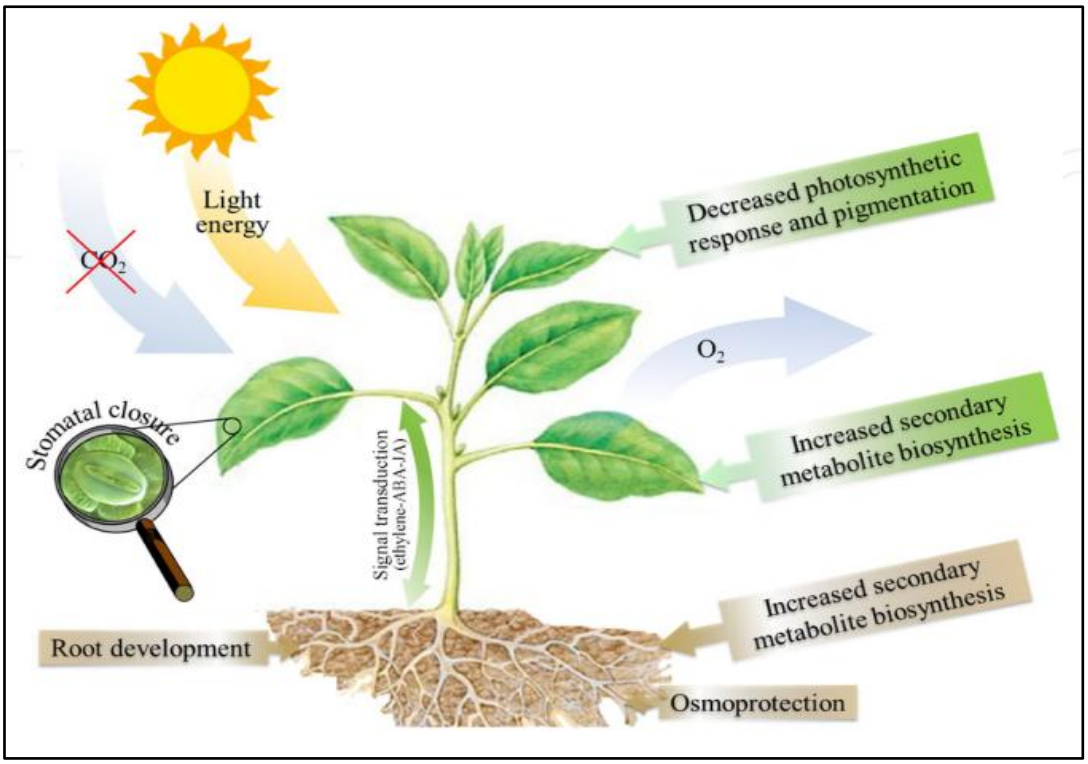

Fig.1.3.: Plant responses to significant abiotic stresses (Zingaretti et al., 2013).

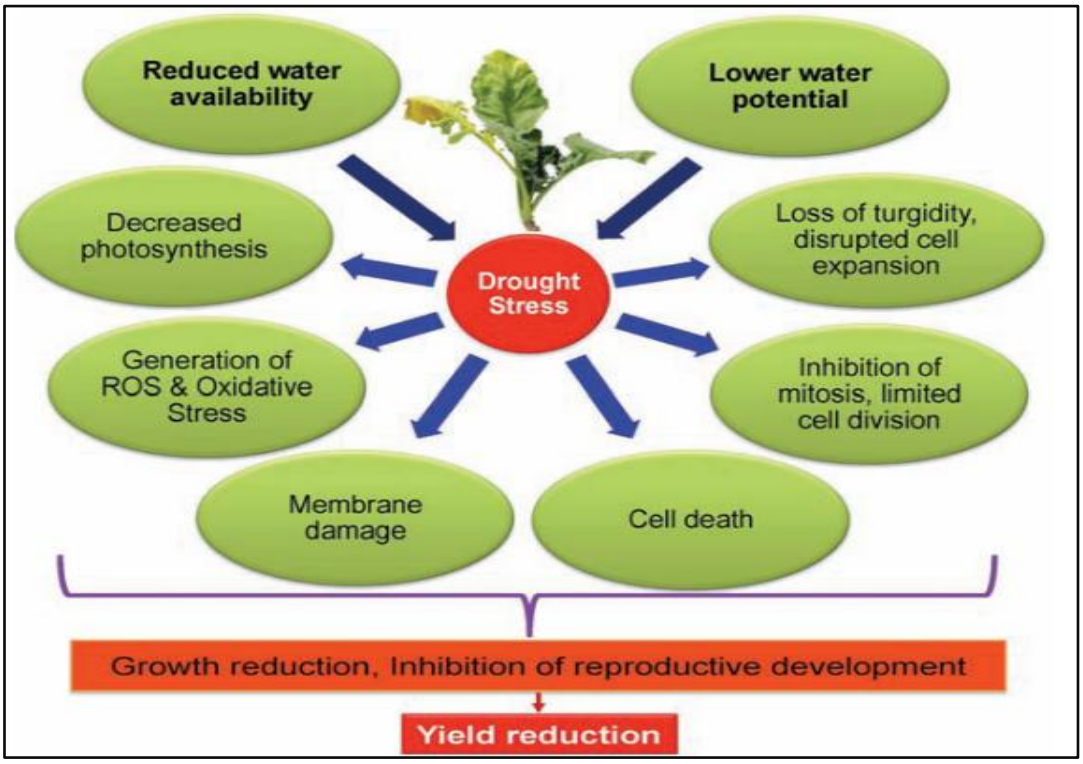

Fig.1.4.: Possible effects of drought stress on plants (Hasanuzzaman et al., 2013).

1.4.Plants under drought stress: morphological impacts

Hyperosmotic stress, frequently referred to as simply "osmotic stress," is the main indication induced by drought in plant cells. Drought, a negative factor, causes multiple impairments overall, including alterations in metabolic activity, membrane stability, and leaf-water relations (Nilsen and Orcutt, 1996). Structural changes are plants' adaptation and resistance mechanism to environmental stresses. The epidermis of plants is the first barrier to environmental stress and acts as a mode of self-preservation in plants. Along with many morphological and anatomical traits susceptible to drought, show a downturn in plant growth and yield. Water stress modifies a leaf's ultrastructure and anatomy (Lisar et al., 2012). The leaf is an assimilative organ and is the largest and most significant part of the plant exposed to the environment. In response to drought, plants undergo a variety of changes such as a reduction in the size of the leaves, a smaller stomatal aperture, a decrease in the number of stomata, thickening of the cell walls, cutinization of the leaf surface, development of the conductive system (an increase in the number of large vessels), submersion of stomata in succulent and xerophyte plants, the development of tube leaves in cereals and early leaf senescence (Chernyad'ev et al., 2005; Jaleel et al., 2009; Keyvan, 2010; Mafakheri et al., 2010; Nezhadahmadi et al., 2013; Shao et al., 2008).

Since water is a vital component, the unavailability of soil moisture content results in an aberrant rate of photosynthetic characteristics in plants (Reddy et al., 2004). Thus, plants need proper gas exchange regulation to function under stressful conditions. This is mediated by partial or complete stomata closure, a significant process during drought stress (Liu et al., 2010), reducing the transpiration rate and curtailing the CO_2

influx and water loss. These drought stress effects can directly affect ATP synthase, which results in a restricted supply of ATP and improper functioning of metabolic processes. Consequently, it results in a decline in the rate of photosynthetic activity accompanied by visible symptoms, including yellowing, etiolation, wilting, and fall (**Fig.1.5)**. Therefore, reduced leaf area, increased stomatal resistance to gas exchange, and increased leaf senescence all contribute to lower net photosynthesis in water-deficient plants, which causes decreased plant size and biomass output (Ding et al., 2013; Mishra and Singh, 2011; Farooq et al., 2009; Shao et al., 2008) (**Fig.1.6)**. Furthermore, reduced chlorophyll levels are a common sign of drought stress and may alter the morphology of plants (Arbona et al., 2013; Kheradmand et al., 2014). Under situations of dehydration, plants expand their roots and create a ramified root structure to enhance water uptake (Akhtar and Nazir, 2013).

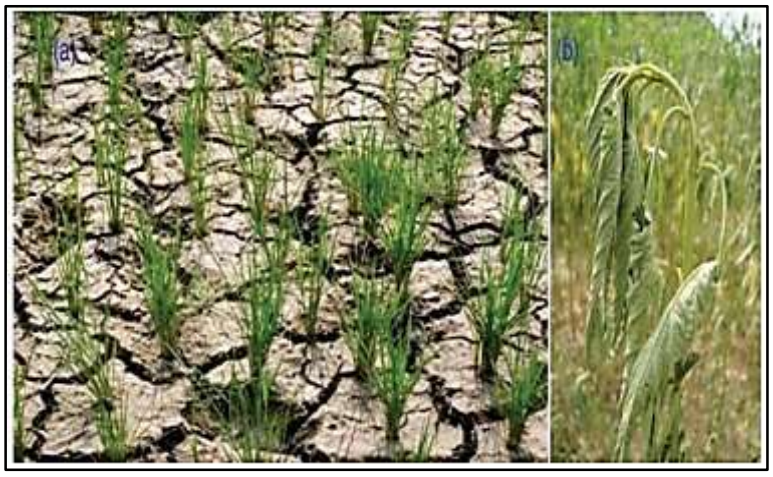

Fig.1.5.: Water deficit induced growth inhibition in rice (a) and wilting in jute (b) plants (Hasanuzzaman et al., 2013).

A larger capacity for water absorption was produced by expanding the plant's root system and allocating more biomass to the roots during drought (Bhargava and Sawant, 2013). As a result, even if the shoot growth is decreased, the root growth is not significantly affected by minor water deficiency. Therefore, in circumstances of dryness, plants' root-to-shoot ratios often rise; nevertheless, their overall biomass significantly decreases (Akhtar and Nazir, 2013). In addition to these effects, water scarcity causes changes in cell volume, solute concentration, turgor, and protein denaturation; ultimately, significant water loss leads to cell dehydration, stunted growth, and development (Bray, 1997).

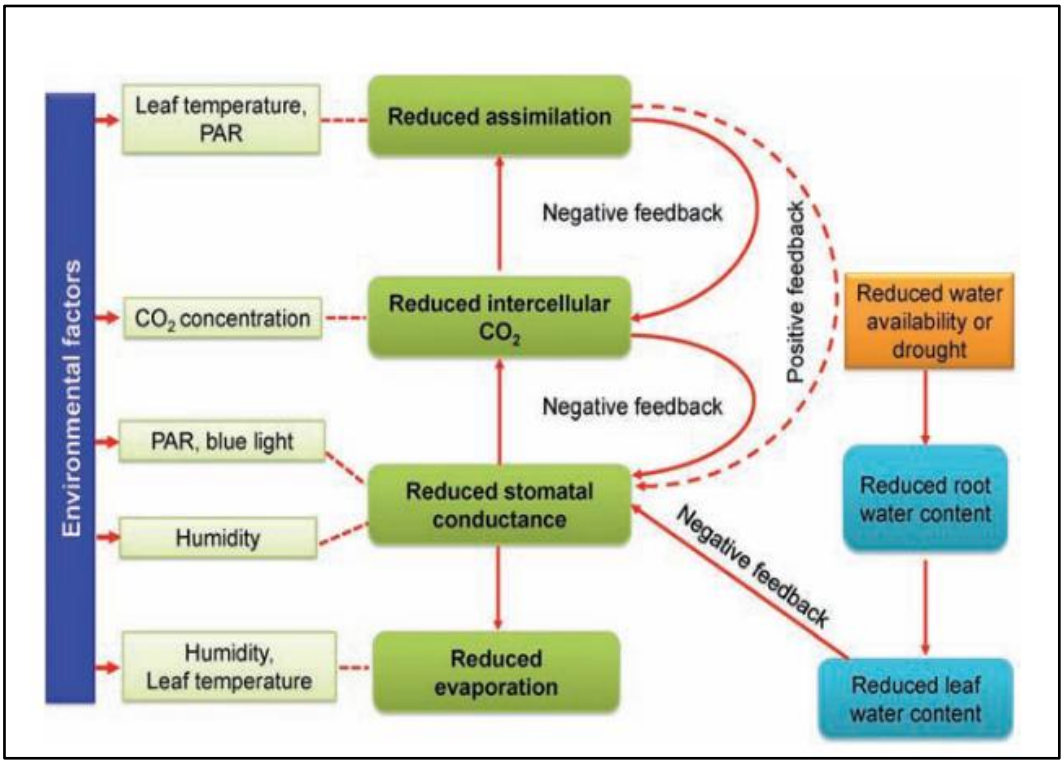

Fig.1.6.: Stomatal adjustment due to water deficit (Sharp and Davies, 2009).

1.5.Plants under drought stress: Physiological impacts

1.5.1. Plant-water Relationships

The relative water content (RWC), leaf water potential, stomatal resistance, transpiration rate, leaf temperature, and canopy temperature are essential factors in plant–water relationships (Bhargava and Sawant, 2013; Farooq et al., 2009; Keyvan, 2010; Shao et al., 2008; Zlatev and Lidon, 2012). A reduction in RWC is the earliest effect of drought on plants (Farooq et al., 2009). A low RWC decreases the leaf water potential and leads to stomatal closer. A higher stomatal resistance due to higher light intensity decreases the transpiration rate and increases the leaf temperature. Therefore, the leaf temperature and stomatal resistance have a positive feedback effect. The high temperature of leaves can lead to the denaturation of proteins, especially enzymes, besides bringing about changes in membrane flexibility that can influence different aspects of metabolism. These alterations disturb cell metabolic functions such as photosynthesis, respiration, ion uptake, mineral nutrition, synthesizing essential macromolecules such as amino acids and proteins, and others (Rana et al., 2013; Sapeta et al., 2013; Zlatev and Lidon, 2012).

1.5.2. Photosynthesis

The physiological responses of plants are subject to several restrictions as a result of changes in the environment. How productive a plant is determined by photosynthesis, which is reliant on a plant's ability to utilize water. Photosynthetic performance has been determined to be an essential indicator of drought stress because of its extreme sensitivity to environmental stresses (Woo et al., 2008). Drought stress affects every stage of photosynthesis because of reduced carbon dioxide diffusion and metabolic restrictions, impacting plant output globally (Ashraf and Harris, 2013). Therefore,

keeping the photosynthetic system operating under drought stress is crucial. Stomatal and non-stomatal restrictions are two ways that water stress can reduce photosynthesis in plants (Grassi and Magnani, 2005).

The severity of drought stress has been linked to several immediate impacts on the photosynthetic apparatus, which suppresses the expression of genes involved in photosynthesis and regulates the level of photosynthetic inhibition in plants. Additionally, transcripts are induced for several glycolysis and pentose phosphate pathway enzymes, suggesting that carbohydrates are used during the drought stress phase. As a result of dryness and heat stress, elevated leaf temperature, faster respiration rate, stomatal closure, and a decrease in photosynthetic rate are readily visible (Rizhsky et al., 2002). When intense drought stress was induced in *Poplus nigra* plants, a significant decrease in light-saturated net photosynthesis was observed, thereby demonstrating the link between drought stress and a decline in photosynthesis (Xu et al., 2010).

The progressive loss of photosynthesis has been studied in several grapevine cultivars that were gradually exposed to drought stress. Therefore, the impact of drought on plants may be precisely assessed. Stomatal conductance values can be utilized to indicate water stress conditions on leaves. Grapevine cultivars that are resilient to drought stress have been shown to have decreased substomatal CO_2 concentration, stomatal conductance, estimated chloroplast CO_2 concentration, and net photosynthetic rate. Besides, mesophyll conductance and photosynthetic electron chain may also be reduced along with increased drought stress magnitude (Flexas et al., 2004).

Reduced inorganic phosphate reserves in the Calvin cycle, which result in the synthesis and buildup of sugars during drought stress, may be due to the drop-in photosynthetic rate. In order to successfully protect photosystem (PS) II from increased production of detrimental reactive oxygen species (ROS), the excitation energy generated as a result of these events must be dissipated and can be expelled out by non-photochemical quenching by the xanthophyll cycle (Farooq et al., 2009). Drought stress can alter carbon partition at both the leaf and whole plant levels by impeding the intake and generation of photo assimilates. Therefore, changes in the carbohydrate pool's size rely on the length of the water deficiency stress and its intensity. However, the starch content reduction is followed by a buildup of soluble carbohydrates under mild drought stress. This change in carbon division may be adaptive and give plants the flexibility to regulate their osmotic environment (Lisar et al., 2012). In a recent study, four Robusta coffee clones representing drought-sensitive and tolerant genotypes were gradually subjected to drought stress. These clones showed a marked decline in stomatal conductance, linked to a striking decline in the internal to atmospheric CO_2 concentration ratio. Despite drought stress, starch levels were significantly decreased (Praxedes et al., 2006).

1.5.3. Mineral Nutrition

Water stress affects plant mineral nutrition and disrupts ion homeostasis in plant cells (Akhtar and Nazir, 2013; Bray, 2007; Kheradmand, 2014). Generally, reduced water availability under water stress conditions limits soil nutrient availability, decreases roots' nutrient uptake, and reduces plant tissue concentrations (Kheradmand, 2014). Changing nutrient uptake by root and their transport to the shoots is an essential effect of water deficit on plants. Drought stress increases N, causes a reduction in the P

concentration, and has no definitive effects on the K^+ concentration in plants (Akhtar and Nazir, 2013; Farooq et al., 2009; Sapeta et al., 2013). A decrease in the Ca content of plants has been reported earlier (Akhtar and Nazir, 2013; Bhargava and Sawant, 2013; Lisar et al., 2012). The cell membrane is one of the earliest targets of many stresses, such as drought. The membrane stability in the roots plays an essential role in the appropriate mineral nutrition of plants. Therefore, preserving membrane stability is a significant factor in plant resistance to drought. Damage of cell membranes under water-deficit conditions leads to disruption of ion homeostasis in plants (Kheradmand, 2014).

1.5.4. Hormonal Balance

Hormones play vital roles in the regulation of plant processes. Some hormones are involved in plant interactions with environmental stresses such as drought (Kheradmand, 2014). Abscisic acid (ABA) is one of the most influential hormones in plant response to drought stress (Bhargava and Sawant, 2013). After plants are exposed to drought, ABA is synthesized in roots and translocated to shoot, especially leaves. Furthermore, water stress induces ABA synthesis in chloroplasts. In addition, the plasma membrane ATPase (PM-ATPase) activity decreases under water-deficit conditions due to a lower ATP supply by photosynthesis and respiration. Low PM-ATPase increases the apoplastic (cell wall) pH and leads to the conversion of ABA to its anionic form (ABA^-). ABA^- cannot cross the leaf cells' plasma membrane and translocate toward the guard cells of stomata by a transpiration stream in the leaf apoplast. ABA translocation to stomata induces stomatal closure and decreases the stomatal conductance capacity. A higher stomatal resistance leads to lower water losses from the leaf surface, which is one of the early plant responses for resistance to

water stress (**Fig.1.7**). However, low CO_2 uptake by stomata leads to a reduction in the photosynthesis rate in leaves (Osakabe et al., 2014; Rangan et al., 2014). ABA also plays a crucial role in regulating aquaporin activity. ABA accumulation under drought conditions reduces ethylene production (Bhargava and Sawant, 2013).

In contrast, auxins act as negative regulators of drought tolerance in plants because indole- 3- acetic acid (IAA) downregulation facilitates late embryogenesis abundant (LEA) mRNA accumulation. LEA proteins are involved in plant adaptation to drought stress (Nezhadahmadi et al., 2013). Plant levels of endogenous cytokinin (zeatin) and gibberellin (GA3) decline rapidly under water stress. Cytokinins have been shown to delay senescence, leading to better plant adaptation by delaying drought-induced senescence (Bhargava and Sawant, 2013). Generally, drought increases plants' brassinosteroid (BR) accumulation, increasing water uptake and cell membrane stability and reducing ion leakage from the membrane under drought-stress conditions (Rahdari and Hoseini, 2012) (**Fig.1.8)**.

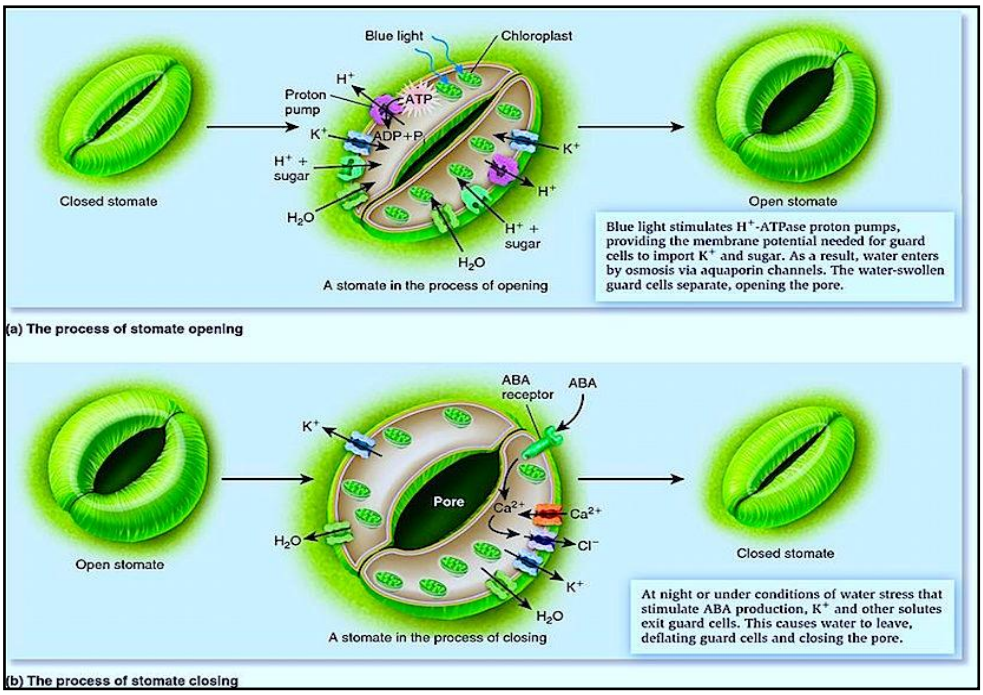

Fig.1.7.: Schematic diagram depicting stomatal conductance due to water deficit. (Illustration: plantstomata.wordpress.com).

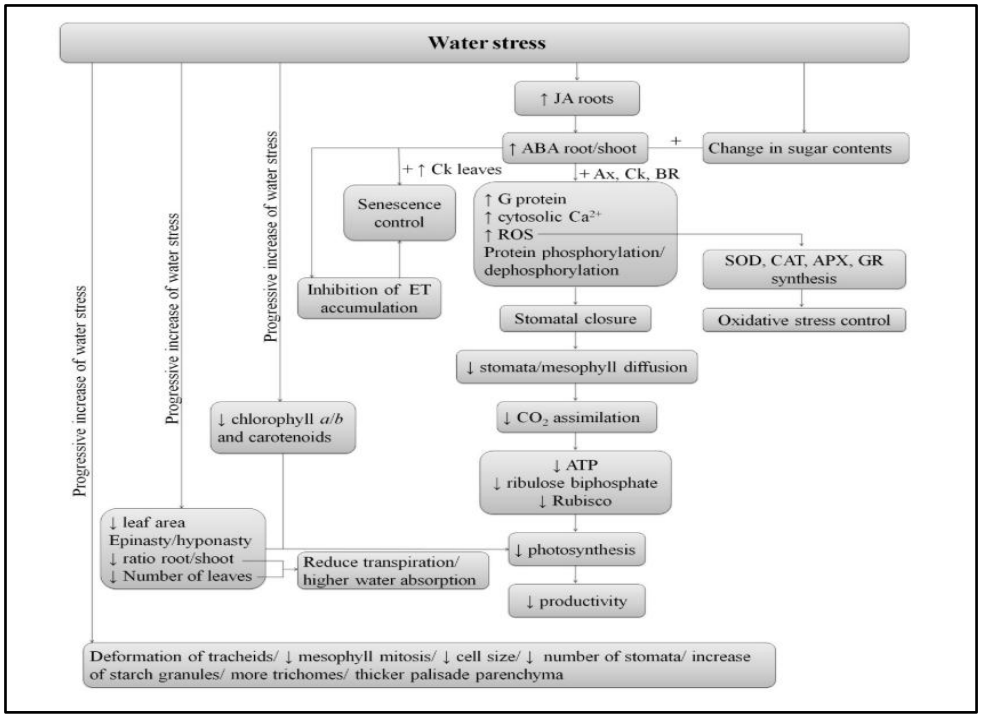

Fig.1.8.: Physiological impact of drought on plants (Zingaretti et al., 2013).

1.6.Plants under drought stress: Biochemical impacts

1.6.1. Reactive Oxygen Radicals

The effects of drought stress might be immediate or delayed. Reduced stomatal and mesophyll conductance causes a reduction in carbon dioxide (CO_2) availability, which is a direct consequence (Flexas et al., 2012; Zivcak et al., 2013). Modifications in photosynthetic metabolism are associated with indirect consequences (Parry et al., 2002). Photorespiration reduces the energy efficiency of photosynthesis in C_3 plants under conditions of constrained CO_2 diffusion. Oxidative stress is another indirect impact contributing to the non-stomatic restriction of photosynthesis (Foyer and Noctor, 2009). The content, structure, and activity of the various parts of the photosynthetic apparatus can change over time as a result of drought (Balaguer et al., 2002; Zivcak et al., 2008, 2014; Kohzuma et al., 2009; Brestic and Zivcak, 2013).

Aerobic organisms require oxygen to continue their biological processes. As a result, when oxygen is not entirely reduced throughout the metabolic process, Reactive oxygen species, an essential signalling molecule, and a group of metabolites and their derivatives with more active chemical characteristics are generated as by-products. According to Foyer and Noctor (2005), the balance between ROS production and scavenging is crucial in determining whether a plant cell will survive or die. Excessive ROS production combined with insufficient or inefficient ROS scavenging is typically considered an indication of oxidative stress (Mittler, 2002). The primary subcellular ROS-producing organelles are peroxisomes, mitochondria, and chloroplasts (**Fig.1.9)**. However, NADPH-dependent oxidases generate a sizeable quantity of O_2^- in the apoplast during different abiotic and biotic stress responses, growth, and developmental process activities (Marino et al., 2012). ROS is thought to

harm cells because it directly oxidises macromolecules, including DNA, proteins, and membrane lipids (Mittler, 2002). Both under normal circumstances and in times of oxidative stress, ROS and antioxidants play significant roles in maintaining cellular homeostasis. However, it appears that all abiotic and biotic stresses, including salt (Miller et al., 2010), drought (Yang et al., 2015), cold (Theocharis et al., 2012), heavy metals (Hossain et al., 2012), pathogen attacks (Scheler et al., 2013), and physical damage will inevitably result in the production of ROS (Suzuki and Mittler, 2012). The reactions generating different ROS derivatives are presented in **Fig.1.10.**

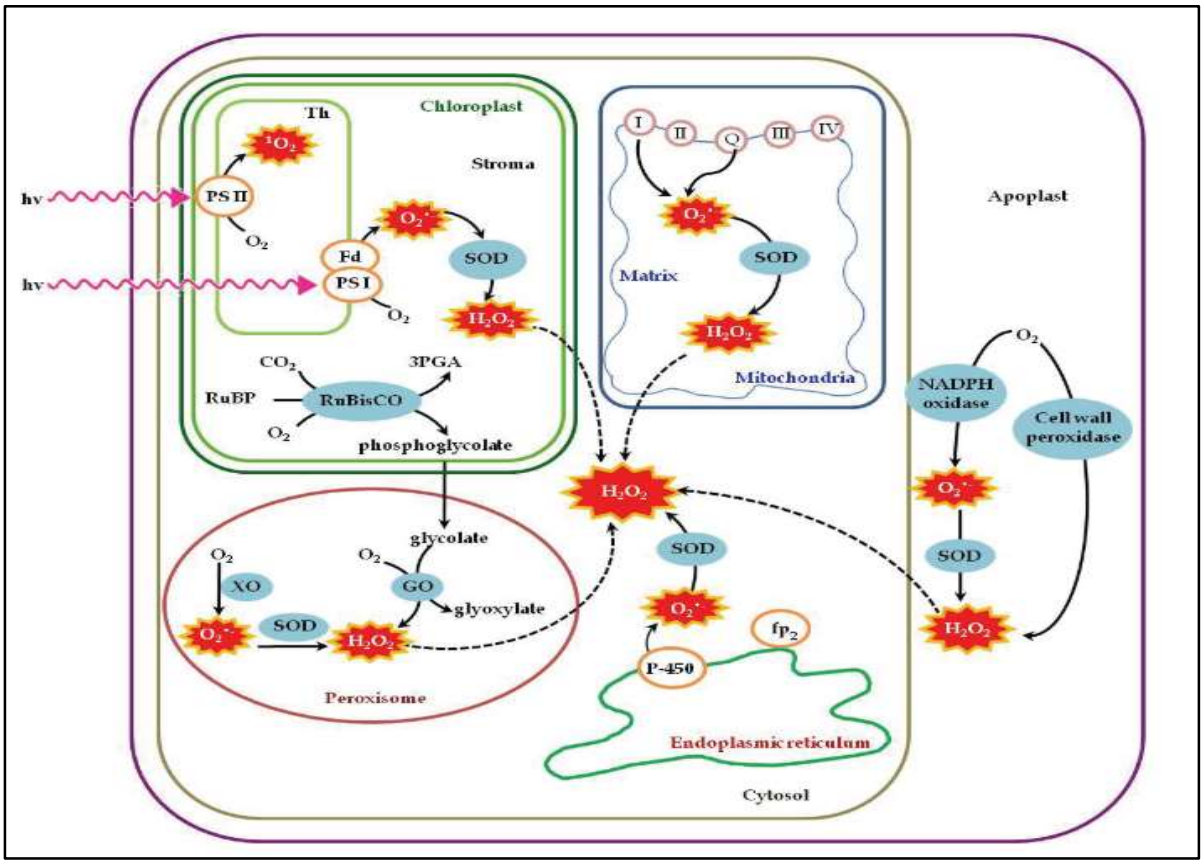

Fig.1.9.: Major sites of ROS production in plants (Hossain et al., 2011).

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scavenge $^1O_2$ with the help of β-carotene, tocopherol, and plastoquinone and can also react with the D1 protein of PS II. Alternatively, singlet oxygen plays a role in upregulating genes responsible for protecting against photooxidative stress (Krieger-Liszkay et al., 2008).

1.6.2.3.Hydrogen peroxide (H_2O_2)

Hydrogen peroxide, a moderately reactive ROS, is formed when $O_2^{\bullet-}$ undergoes univalent reduction and protonation. It can occur non-enzymatically by being dismutated to H_2O_2 under low pH conditions or mainly by a reaction catalyzed by SOD.

$2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$

H_2O_2 is produced in plant cells under normal conditions and by oxidative stress caused by factors such as drought, chilling, intense light, UV radiation, wounding, and pathogen infection (Sharma et al., 2012). Due to stomatal closure, low availability of CO2, and limited fixation, Ribulose1 5-bisphosphate (RuBP) oxygenation is favoured and thus enhances photorespiration. This accounts for more than 70% of the H_2O_2 produced due to drought stress (Noctor et al., 2002). The primary sources of H_2O_2 production in plant cells include the ETC in the chloroplast, mitochondria, ER, cell membrane, β-oxidation of fatty acid and photorespiration. Additional sources include reactions involving NADPH oxidase and xanthine oxidase (XOD) photooxidation. H_2O_2 in plants behaves like a double-edged sword; it is beneficial at low concentrations but damaging at higher concentrations in the cell. At low intracellular concentrations, it acts as a regulatory signal for essential physiological processes like senescence (Peng et al., 2005), photorespiration and photosynthesis

(Noctor et al., 2002), stomatal movement (Bright et al., 2006), cell cycle and growth and development (Tanou et al., 2009). Due to its significantly longer half-life of 1ms, it can traverse longer distances and cross plant cell membranes than other ROS members. It can cross membranes via aquaporins and cover considerable lengths within the cell (Bienert et al., 2007), causing oxidative damage. H_2O_2 at high intracellular concentration oxidizes both cysteine (-SH) and methionine ($-SCH_3$) residues and inactivates Calvin cycle enzymes, Cu/ZnSOD and Fe-SOD by oxidizing their thiol groups (Halliwell, 2006). It causes a 50% loss in activity of different enzymes like fructose1, 6 bisphosphatase, sedoheptulose 1, 7 bisphosphatase and phosphoribulo kinase at concentrations of 10 μM H_2O_2 and is also responsible for programmed cell death at high cellular concentrations (Dat et al., 2000). However, like $O_2^{\bullet-}$, H_2O_2 is moderately reactive; therefore, its damage is realised only when converted into a more reactive species.

1.6.2.4.Hydroxyl radical (OH•)

Hydroxyl radical (OH•) is the most reactive and toxic ROS among its family members. It is generated at neutral pH by the Fenton reaction between H_2O_2 and $O_2^{\bullet-}$ catalyzed by transition metals like Fe (Fe^{2+}, Fe^{3+}).

$Fe^{2+} + H_2O_2 + O_2^{\bullet-} \rightarrow Fe^{3+} + OH^- + O_2 + OH\bullet$

It has the ability to damage different cellular components by lipid peroxidation (LPO), protein damage and membrane destruction. Since there is no existing enzymatic system to scavenge this toxic radical, excess accumulation of OH• causes cellular death (Pinto et al., 2003).

Prolonged and severe drought stress have an impact on the physiological system, causing metabolic alterations, the production of secondary metabolites, a considerable buildup of endogenous ROS, and an increase in toxins (such as methylglyoxal) (Hasanuzzaman et al., 2013). The primary defence mechanism limits CO_2 uptake, increases photorespiration, and shifts the photosynthetic machinery to produce too much singlet oxygen by PS II and H_2O_2 by PS I. The same events also trigger glycolate oxidase, which overproduces H_2O_2 in the peroxisomes (Noctor et al., 2014). Asada (2006) identified PS I and PS II reaction centres as plants' principal ROS generators.

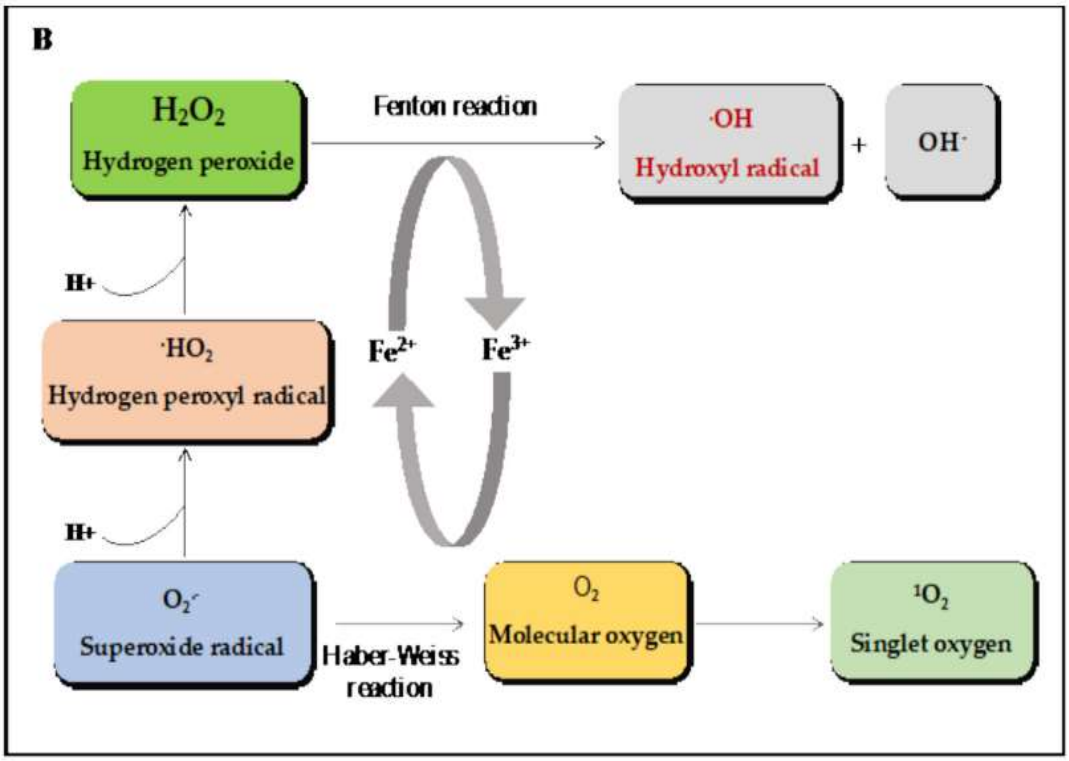


Fig.1.10.: ROS production in plants through Fenton reaction and Haber-Weiss reaction (Halliwell and Gutteridge, 1990).

The reduced photosynthetic activity mainly causes a more significant transfer of electrons to oxygen. However, the formation of additional ROS in areas other than the chloroplast, such as the mitochondria or peroxisomes, will result from a chain reaction and are plants' primary sources of ROS generation (Miller et al., 2010). Cruz de Carvalho (2008) proposed that the drought stress response occurs in three successive phases. In plants, the normal ROS steady-state level is disturbed by drought stress, where initially, the enhancement of ROS production enhancement due to stomatal closure shifts the equilibrium upwards, triggering defence signal transduction pathways (**Fig.1.11)**. However, prolonged drought stress exacerbates ROS production that cannot be counterbalanced by the antioxidant system, leading to deleterious oxidative events that ultimately result in cell death (**Fig.1.12)**

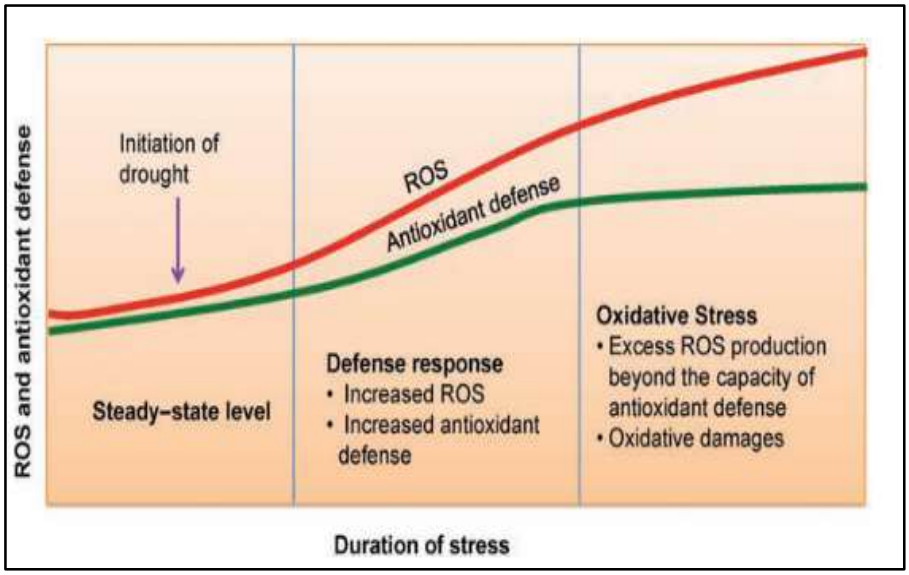


Fig.1.11.: Development of drought stress regarding plants' ROS buildup and defence system (Cruz de Carvalho, 2008).

1.6.3. Oxidative damage

1.6.3.1.Lipid oxidation

Lipids are the most abundant component of cell membranes and play an essential role in the resistance of plant cells to environmental stresses (Bhargava and Sawant, 2013). Malondialdehyde (MDA) is the outcome of lipid peroxidation due to oxidative damage, which is the well-known effect of drought and many other environmental stresses in plants (Mafakheri et al., 2010; Rahdari and Hoseini, 2012). ROS is known to destroy plant biofilm systems (**Fig.1.11)**. For example, ·OH can directly induce the peroxidation decomposition of the unsaturated fatty acid chain in phospholipids, thus destroying membrane structure. However, the peroxides and Nitric Oxide (NO) in ROS are mainly produced by NADPH oxidase, glutathione oxidase, and NO synthase, with low activity, so they cannot directly interact with lipids to induce lipid peroxidation (LPO). They react quickly to produce peroxynitrite, which initiates the LPO reaction (Pacher et al., 2007).

It has been found that the PUFAs (linoleic acid (18:2) and linolenic acid (18:3)) are particularly susceptible to attack to 1O2 and HO•, giving rise to complex mixtures of lipid hydroperoxides. Increased PUFA peroxidation decreases the membrane's fluidity, increases leakiness and causes secondary damage to membrane proteins (Moller et al., 2007). Several aldehydes, such as 4-hydroxy-2-nonenal (HNE) and MDA, as well as hydroxyl and keto fatty acids, are formed due to PUFA peroxidation (**Fig.1.13**). The aldehyde breakdown products can form conjugates with DNA and proteins. Aldehydes formed in the mitochondria may be involved in causing cytoplasmic male sterility in maize because a restorer gene in this species encodes a mitochondrial aldehyde dehydrogenase.

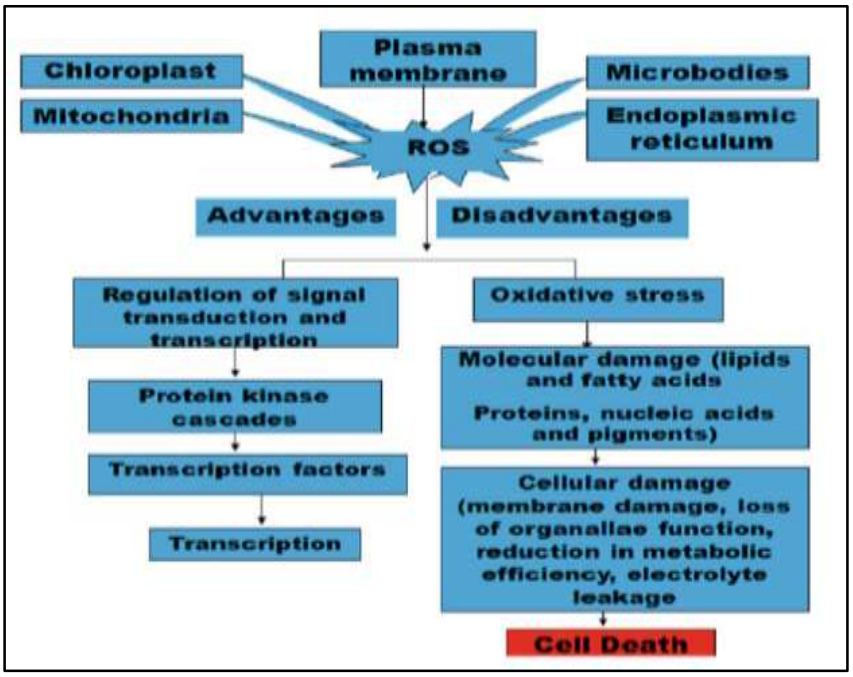


Fig.1.12.: Reactive oxygen species and its biological consequence and physiological dysfunction causing cell death (Ahmad et al., 2008).

The forced destruction of membrane structure will lead to biological dysfunction, damaging lipids via the oxidation of unsaturated fatty acids and leaking solutes through the membrane, proteins, or DNA. This results in the impairment of organelles through mutations and other lethal genetic effects (Møller, 2001; Foyer and Noctor, 2003; Apel and Hirt, 2004; Foyer and Noctor, 2005; Ahmad et al., 2008). Generally, drought stress leads to disturbance in the association between membrane lipids and proteins and decreases the membrane-bound enzyme activity and transport capacity (Farooq et al., 2009; Kheradmand et al., 2014). Monogalactosyldiacylglycerol (MGDG) is a significant glycolipid that decreases after plant exposure to drought.

MGDG is the most crucial component of the chloroplast membrane; its lower content destroys the chloroplast membrane and negatively affects photosynthesis.

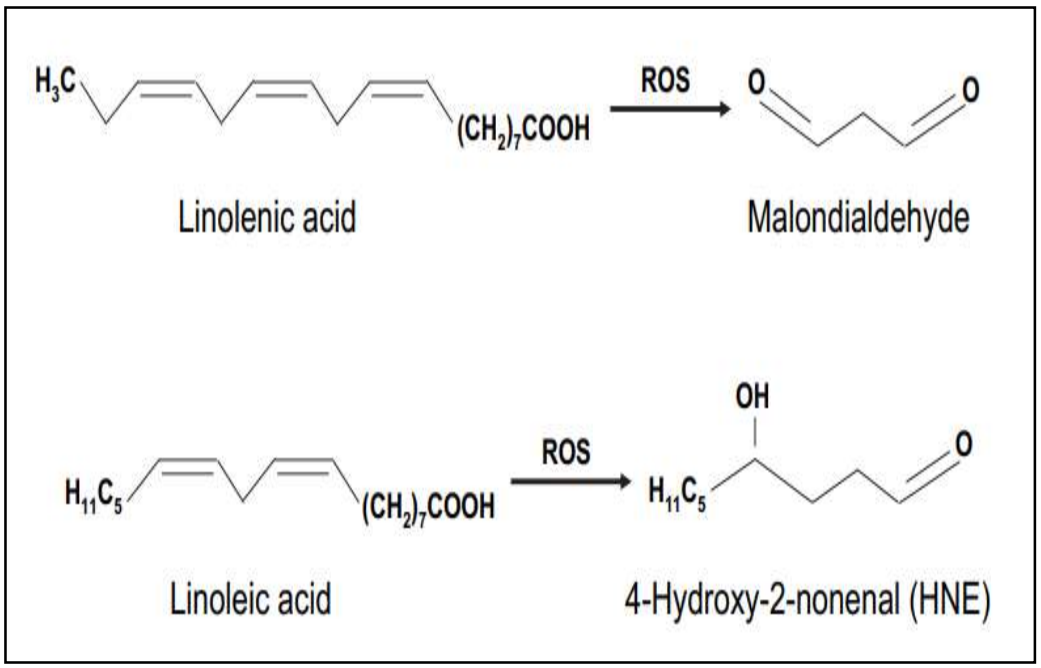


Fig.1.13.: Peroxidation of polyunsaturated fatty acid (PUFA) (Moller et al., 2007).

The preservance of cellular integrity and membrane structure is paramount in plant species to withstand drought stress. Electrolyte leakage, an indicator of cell membrane integrity, is considered one of the finest physiological components of plants tolerance to drought stress (Xu et al., 2008). The three remarkably interrelated parameters, namely membrane stability index (MSI), electrolyte leakage, and MDA content resulting from oxidative stress in drought-stressed plants, suggest that increased MDA content is accompanied by elevated electrolyte leakage that leads to membrane fluidity and higher permeability and is expressed by lower MSI values (Liu et al., 2011).

Drought inhibits all essential biological processes, including photosynthesis and the formation of cellular components, proteosynthesis, lipid metabolism, membrane permeability, and enzyme activity (Liu et al., 2011; Filippou et al., 2014). Drought indirectly increases ROS generation in the mitochondrial electron transport pathways (Noctor et al., 2014). C_4 and CAM plants reduce ROS-caused impairment, photorespiration, and water loss, suggesting reduced photorespiration is insufficient for drought resistance. However, C_4 or CAM plants create excessive ROS when there is a water shortage. As demonstrated in maize, effective antioxidant activity is also required to distinguish between drought-tolerant and susceptible lines (Yang et al., 2015).

1.6.3.2. Protein oxidation

Protein carbonylation is an irreversible post-translational modification (PTM) that involves adding aldehyde and ketone carbonyl groups into the side chains of specific amino acids (Stadtman, 1990). It represents the most frequent and irreversible chemical modification that affects protein structure (Colombo et al., 2016). Primary protein carbonylation involves metal-catalyzed oxidation (MCO) of the side chains of Lys, Pro, and Thr residues, leading to aldehyde or ketone formation (Fedorova, 2017; Biswas et al., 2020). Metal-catalyzed oxidation is one common mechanism of protein carbonylation in a biological cell (Jung et al., 2014). It is triggered by HO• derived from the Fenton reaction between Fe^{2+} (or divalent metal ions) and H_2O_2 in any part of the cell (Valentine and Gralla, 2008). The hydroxyl radical reacts with side chains of Lys, Pro, Arg, Thr, and sometimes Trp to cleave them, forming carbonyl groups. Protein degradation represents a critical cellular process that assures a healthy proteome and helps recycle amino acids during nutrient starvation or stress. The level

of carbonylated proteins rises in stressed cells. Therefore, the increased level of carbonylated proteins under stress coincides with a rise in proteolysis, which provides cells with amino acids for respiration. This suggests that protein carbonylation could be relevant to proteolysis associated with cell growth and maintenance, particularly under stress (Tola et al., 2021) (**Fig.1.14**).

Secondary protein carbonylation involves the addition of reactive carbonyl species (RCS) or reactive electrophilic species (RES) to the side chains of Cys, His, and Lys (**Fig.1.14)**. They are generated by the peroxidation of membrane polyunsaturated fatty acids (PUFAs; linoleic acid, linolenic acid, and arachidonic acid), particularly in the mitochondria and chloroplasts (Mène-Saffrané et al., 2007; Farmer and Mueller, 2013; Møller et al., 2007). The RCS belong to different chemical classes, i.e., α, β-unsaturated aldehydes (4-hydroxynonenal (4-HNE) and acrolein (ACR)), keto-aldehydes (4-oxo-nonenal), isoketals, dia-aldehydes (malondialdehydes (MDA) and glyoxal), and cyclopentanones (Aldini et al., 2007). Protein and nucleic acids are RCS's primary targets, and their interaction with RCS mainly occurs through Michael adduction or Schiff-base formation (Aldini et al., 2007). Examples of RES species frequently involved in protein carbonylation include 2-propenal (acrolein), 4-HNE, and malondialdehydes (MDA) (Perluigi et al., 2012; West and Marnett, 2006; Mano, 2012). A large number of carbonylated proteins have been detected and quantified in plants and non-plant species (Wong and Suzuki, 2009; Mano, 2012; Milic et al., 2015; Rogowska-Wrzesinka et al., 2014; Mano et al., 2010).

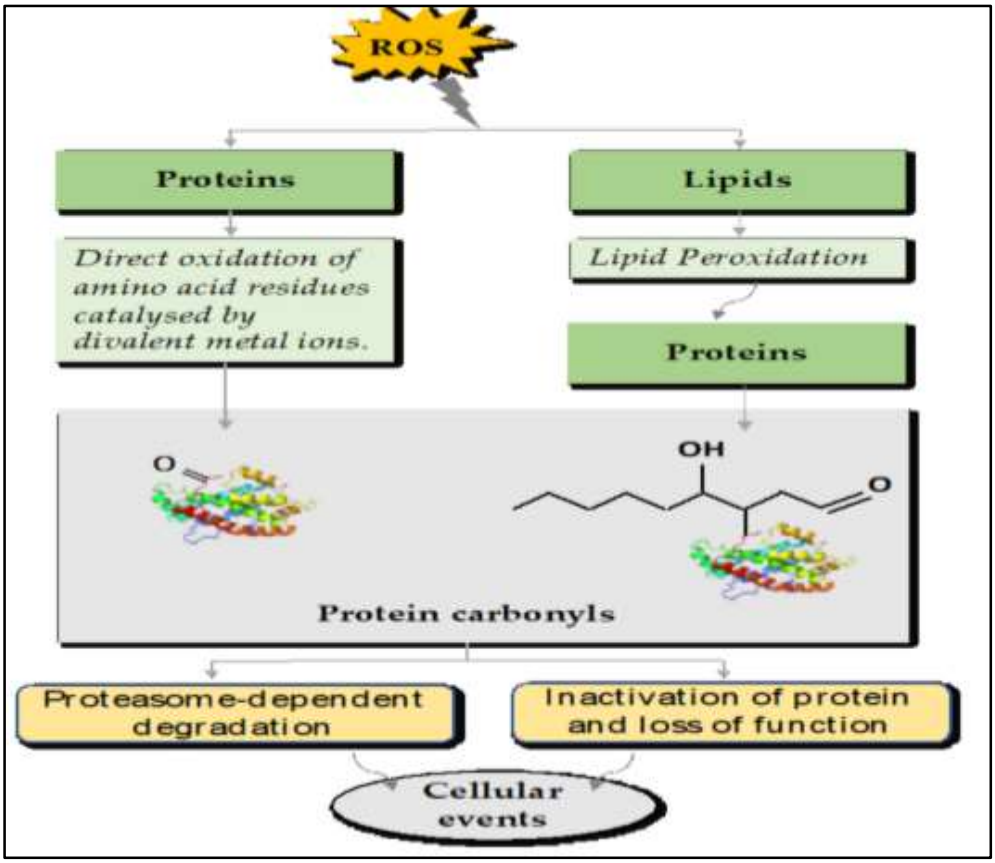


Fig.1.14.: Fate of carbonylated proteins. (Wong and Suzuki, 2009).

1.7.Plant Defence Mechanism

1.7.1. Antioxidants

In response to drought stress, plants adapt by changing their growth rate, morphology, and defence systems (Duan et al., 2007). The term "osmotic adjustment" can be used when new solutes are accumulated and not when the decrease in the osmotic potential is caused by a concentration of existing solutes due to water loss (Babu et al., 1999). The osmotic adjustment (OA), which is a buildup of osmotically active substances (osmolytes), is another crucial adaptive process (Munns, 1988; Zivcak et al., 2009). Although OA is thought to be a key component of plant drought tolerance, it is also a general cellular response to water scarcity (Babu et al., 1999). Plants that can accumulate osmolytes will sustain a greater vitality and have superior survival rates in

habitats where the water supply is constrained (Pintó-Marijuan and Munné-Bosch, 2013).

Most commonly, ROS scavenging mechanisms are distinguished as non-enzymatic and enzymatic antioxidants. Some non-enzymatic antioxidant pairs, such as ascorbate/dehydroascorbate and glutathione/glutathione disulphide, glucose, or tocopherols, or enzymatic antioxidants like superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), or ascorbate peroxidase (APX) (Chaves and Oliveira, 2004; Ahmad et al., 2008; Koyro et al., 2012) define the redox environment to a significant extent and determine the fate of the cell (Kranner et al., 2006; Foyer and Noctor, 2005, 2011; Birtic et al., 2011) (**Fig.1.15** and **Fig.1.16)**.

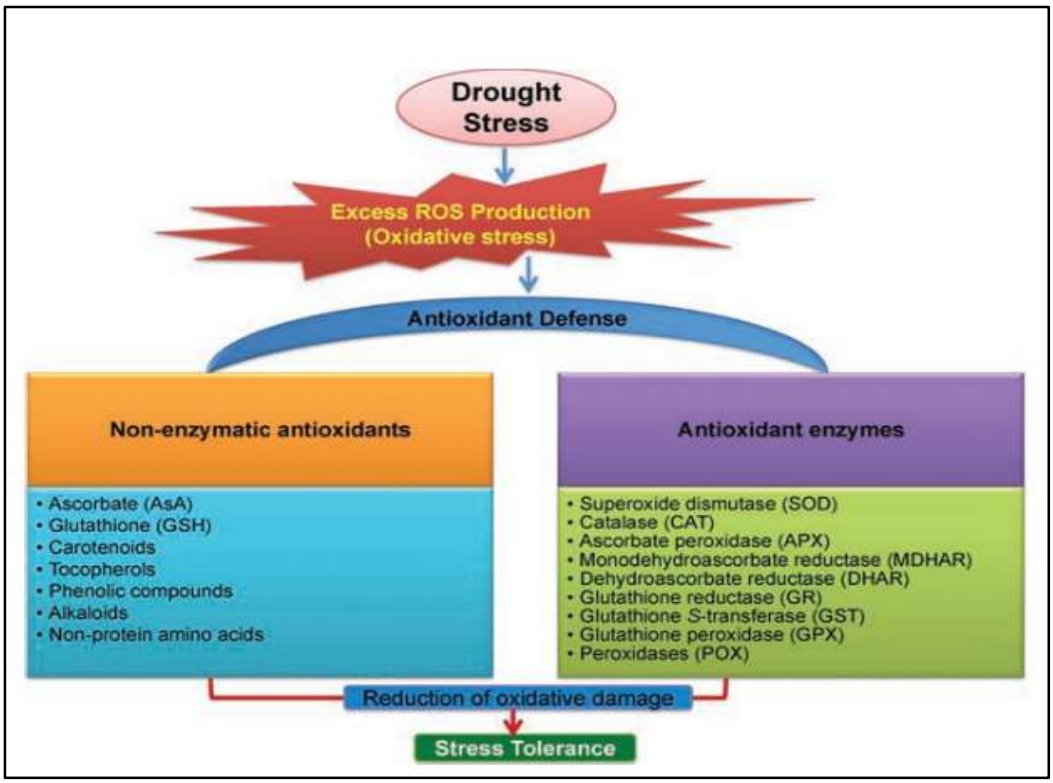


Fig.1.15.: Endogenous plant defence system under drought stress-induced oxidative damage (Gill and Tuteja, 2010).

1.7.1.1.Superoxide dismutase

SOD is one of the most essential metal enzymes having a core role in the antioxidant enzyme system. It sequentially oxidizes and reduces the metals associated with the enzyme and catalyzes the disproportionate reaction of O_2^- to generate O_2 and H_2O_2. Generally, its activity is a crucial index of plant stress resistance, positively correlating with antioxidant capacity (Scandalios, 1993). SOD activity is increased under moderate or short-term drought stress but decreased under severe or long-term water stress. However, it is believed that the change in SOD activity is complex. For instance, with increased stress intensity, SOD activity initially decreases, then increases or remains unchanged. The above alterations may be due to the fact that the response of plants to drought stress is initiated not by the stress itself but by the degree of drought perceived by plants.

Plant SOD can be split into three types according to the metal associated with it, viz., Mn-SOD, Cu/Zn-SOD, and Fe-SOD. Cu/Zn-SOD contains two subunits, each containing a Cu and a Zn, and is the most ample among the three superoxide dismutases. Each subunit of Mn-SOD and Fe-SOD comprises only one metal ion. Mn-SOD and Fe-SOD have similar systems and identical distinguishing domains. Fe-SOD and Mn-SOD dominate lower plants, while Cu/Zn-SOD dominates higher plants. Cu/Zn-SOD is primarily situated in cytoplasm and chloroplasts, Mn-SOD is mainly positioned in mitochondria, and Fe-SOD is in the chloroplasts of some plants. Besides cytoplasm, chloroplast, and mitochondria, SOD also occurs in glyoxylate circulators and peroxisomes (Slooten, 1995).

1.7.1.2.Ascorbate Peroxidase

APX is one of the vital constituents of the AsA-GSH redox pathway in plants. APX is about 30 kDa and generally occurs in monomeric form with a few homodimers that may also appear in some cytosolic APX. It uses ascorbic acid (AsA) as an electron donor to catalyze the reaction between AsA and H_2O_2 to yield monodehydroascorbate acid (MD) and water. Since AsA, as both reactant and reaction product, can be recycled continuously, APX can be catalysed entirely to protect the chloroplast and maintain normal function. Four APX isozymes have been identified: cytoplasmic APX isozyme (cAPX; chloroplasts), soluble (sAPX; chloroplast stroma), and membrane binding form (tAPX; chloroplast thylakoids). tAPX and sAPX exist in similar molar ratios in chloroplasts. Cytoplasmic cAPX and chloroplast APX have different electron donors and internal sequences (Yang et al., 2021).

1.7.1.3.Glutathione Reductase

Glutathione reductase (GR) is a flavoprotein oxidoreductase found in both prokaryotes and eukaryotes (Romero-Puertas et al., 2006). GR is an essential enzyme of the ascorbate–glutathione system and maintains the balance between reduced glutathione (GSH) and the ascorbate pool (Ahmad et al., 2010). GR is primarily found in chloroplasts (70–80%), and small amounts have been found in mitochondria, cytosol, and peroxisomes (Romero-Puertas et al., 2006). GR catalyzes the reduction of glutathione in the cell. GSH is oxidized to GSSG, which is converted back to GSH in normal cells. Rapid recycling of GSH is more critical than the synthesis of GSH. Hence, GR and GSH have been found to play a crucial role in plant stress tolerance by alleviating oxidative stress in plants, as evidenced by increased activities of GR.

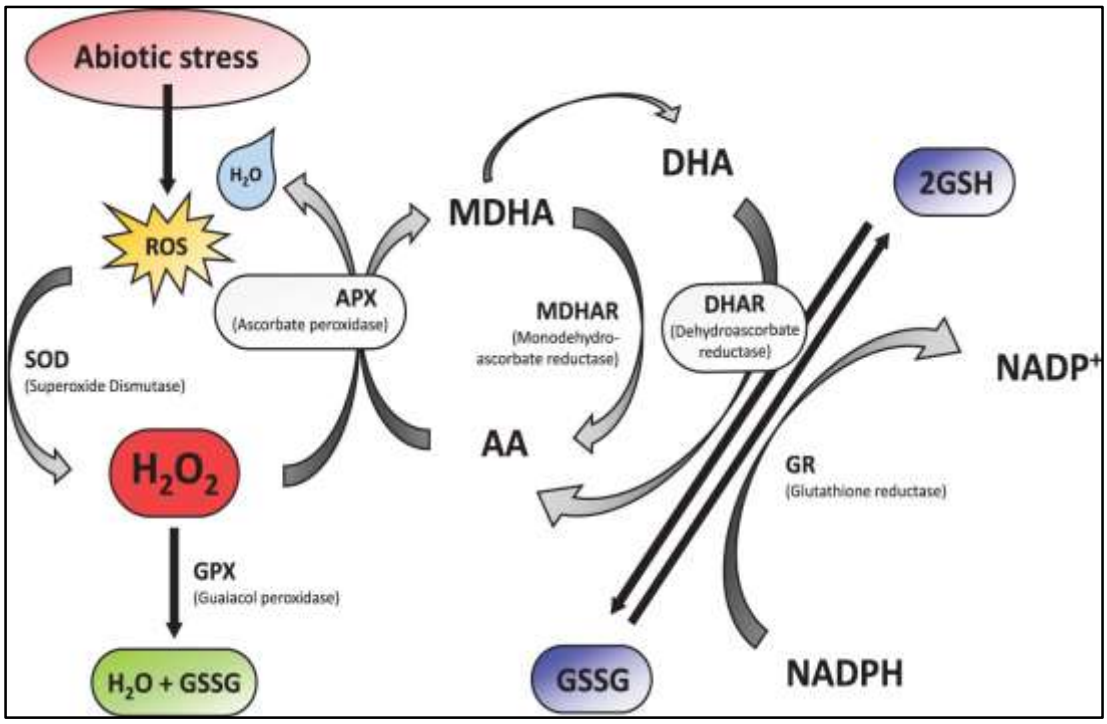


Fig.1.16.: Overall schematic representing ROS and antioxidant defence mechanism (Tuteja, 2007).

1.7.2. Non-enzymatic antioxidant

1.7.2.1.Glutathione

The non-enzymatic ROS scavenging system in plants mainly comprises ascorbate, reduced glutathione (GSH), vitamin E, mannitol, carotenoids, and flavonoids, which can react directly with ROS or act as enzyme substrates in the ROS scavenging mechanism. In addition, as an indispensable part of the antioxidative defence system, such as vitamins also participate in eliminating oxygen free radicals, preventing lipid peroxidation. For instance, some cysteine-rich small molecular proteins, such as metallothionein (MT) (Hassinen et al., 2011) and gibberellin-induced protein (GIP) (Wigoda et al., 2010), can similarly degrade H_2O_2. Overexpression of these antioxidant proteins can significantly reduce the H_2O_2 content in plants on exposure to abiotic stress, thus improving the stress resistance of transgenic plants.

Among the two forms of glutathione are reduced glutathione (GSH) and oxidized glutathione (GSSG). Reduced glutathione (GSH), commonly known as glutathione, can scavenge cells' free radicals. GSH is a mercapto tripeptide formed by the polymerization of glutamic acid, cysteine, and glycine, in which the mercapto group, being the active group, is easy to associate with free radicals and heavy metals to play a detoxification effect. The biosynthesis of GSH is catalyzed by glutamate-cysteine ligase (GCL) and glutathione synthetase (GS) and plays a crucial role in preserving homeostasis and preventing redox damage (Musgrave et al., 2013). For instance, when a minor amount of H_2O_2 is produced inside the cell, GSH reduces H_2O_2 to H_2O under the influence of GPX, and its own is oxidized to GSSG. Under the action of glutathione reductase, GSSG accepts H^+ to reduce to GSH so that the scavenging of free radicals can be carried out uninterruptedly, thus stabilizing the membrane structure.

GSH, one of the most prevalent thiols, is rapidly assuming significance and developing into a molecule of interest because it enhances plant resistance and tolerance to environmental stress conditions. It performs many tasks, including thiol transfer, free radical scavenging, detoxification processes through conjugation reactions, and metabolism of numerous exogenous and endogenous substances. GSH plays a significant role in stress tolerance, antioxidant signalling, and plant stress defence (Foyer et al., 1997; Foyer and Noctor, 2005).

GSH status and its relationship to protein synthesis during water deficit and subsequent rehydration have been examined in the drought-tolerant moss, *Tortula*

ruralis (Dhindsa, 1987). Total GSH was increased due to a water deficit in sunflower seedlings and detached poplar leaves (Morabito and Guerrier, 2000).

1.7.3. Osmotic Adjustment

Osmoregulation and osmoprotection are two separate functions of Osmotic Adjustment (OA). Most osmotically active chemicals provide both osmoprotective and osmoregulatory activities in plant tissues. Osmoregulation, a component of OA, is the intentional reduction of the osmotic potential in plant cells by the buildup of osmotically active substances because it maintains the turgor pressure, which is related to various metabolic and physiological processes. Thus, it is regarded as an efficient and advantageous component of plant drought tolerance (Babu et al., 1999). The osmotic potential decreases when various inorganic ions, such as K^+ and organic solutes, build up. (Yokoi et al., 2002) (**Fig.1.17)**.

However, the accretion differs within the genus as well as plant species. The majority of the organic solutes accumulated are sugars (fructose, glucose, trehalose, and raffinose), sugar alcohols (glycerol and methylated inositols) (Bohnert and Jensen, 1996), quaternary amino compounds (proline, glycine betaine, proline betaine, tertiary amines), and sulfonium compounds (choline O-sulfate, dimethyl sulfonium propionate) (Yokoi et al., 2002). The plant species and environmental events significantly influence individual osmolytes' contributions to overall osmoregulation. For instance, the reduction in osmotic potential caused by a water shortage in cereal crops is mainly caused by inorganic ions like K^+ (Morgan, 1992).

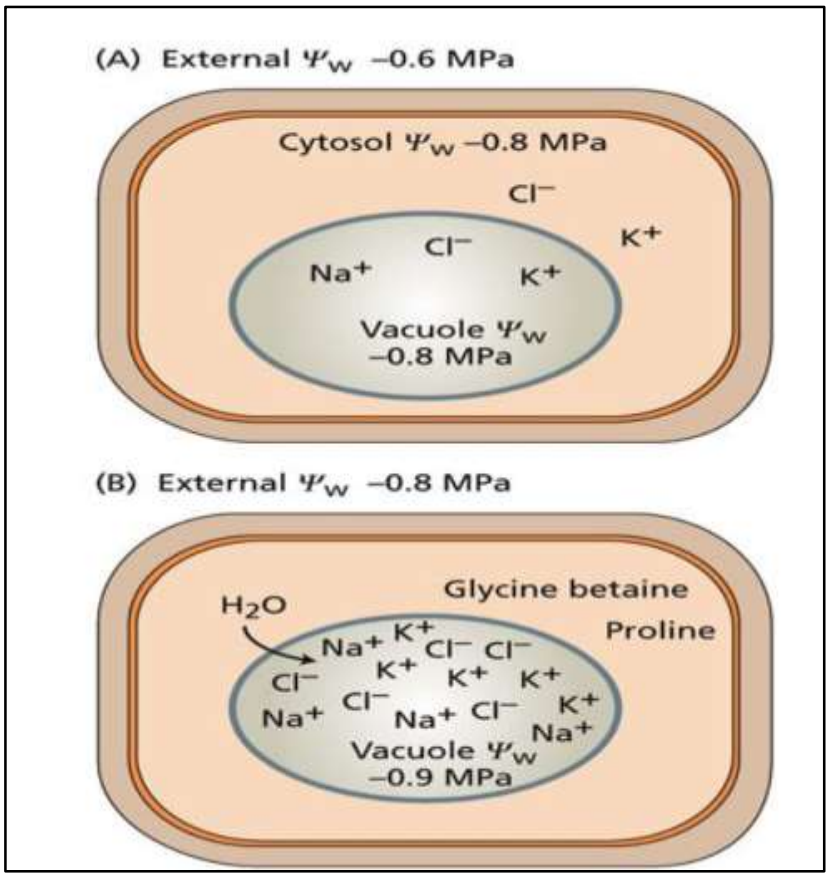


Fig.1.17.: Biosynthesis of osmolytes in response to negative water potential, maintaining ion homeostasis (Taiz and Zeiger, 2002).

The enzymatic and non-enzymatic antioxidants can act alone or in combination to neutralise and detoxify ROS (Apel and Hirt, 2004; Foyer and Noctor, 2005; Ahmad et al., 2008; Koyro et al., 2012). Certain amino acids also have beneficial antioxidant effects when accumulated during drought stress (Smirnoff and Cumbes, 1989; Ashraf and Foolad, 2007; Molinari et al., 2007). One such is proline, a well-known amino acid that builds up in several plant species under drought. When plants, eubacteria, protozoa, and marine invertebrates are subjected to diverse conditions, the buildup of proline is a normal response in these organisms. Salt, drought, low / high temperature, heavy metal stressors, anaerobiosis, UV irradiation, air pollution, nutritional deficiencies, and pathogen infection have all been linked to proline buildup in plants

(Hare and Cress, 1997; Siripornadulsil et al., 2002). Proline accumulation is thus among the most prevalent stress reactions. Although the ability for accumulation varies from species to species, the proline level under stress situations might be 100 times higher than the control plants (Verbruggen and Hermans, 2008).

1.7.3.1.Proline

The accumulation of drought-induced proline has been related to an increase in the activity of the enzyme 1-Pyrroline-5-carboxylate synthetase (P5CS) and 1-Pyrroline-5-carboxylate reductase (P5CR), the two essential enzymes in the biosynthetic pathway for proline synthesis (Nounjan et al., 2012). Parida et al. (2008) reported the upregulation of these essential enzymes in cotton (*Gossypium hirsutum*) under drought stress. Other studies have observed increases in P5CS and proline synthesis in grass species, including barley and cowpea (*Vigna unguiculata*) (Zegaoui et al., 2017; Bandurska et al., 2017). However, proline dehydrogenase activity, which regulates proline breakdown in plants, is hindered under stressful conditions, increasing proline content (Zegaoui et al., 2017). In plants, proline is predicted to have adaptive roles that considerably increase stress tolerance. This amino acid acts as a compatible osmolyte but also represents the means to store nitrogen (N) and carbon (C) (Hare and Cress, 1997). It has been proved that proline is a potent free radical scavenger (Smirnoff and Cumbes, 1989).

Proline has also been suggested as a molecular chaperone helping to buffer the cytosolic pH and stabilising protein structure, both of which are necessary for plant cells to maintain a balanced redox state (Sharma and Dietz, 2006; Hoque et al., 2008; Verbruggen and Hermans, 2008; Filippou et al., 2014). Proteins, membranes, and

enzymes are protected against stress by proline accumulation in the cytosol and chloroplast. It could also help decrease the cytoplasmic pH required to maintain the NADP+/NADPH ratio (Hoque et al., 2008). Genetic modification of osmolytes like proline was reported to increase plant tolerance in agriculture. Compared to wild-type cultivars, soybeans overexpressing the P5CR gene, which generates a high quantity of proline, showed much better drought and heat tolerance (De Ronde et al., 2004). Improved growth and production, greater antioxidant defence, and higher leaf moisture levels have all been linked to increases in endogenous proline concentration (Szabados and Savouré, 2010; Anjum et al., 2012; Bandurska et al., 2017; Zegaoui et al., 2017).

1.7.3.2.Potassium ions

Inorganic ions are hypothesised to have a physiological role in osmoregulation, which lowers the osmotic potential and influences turgor maintenance and the capacity to open stomata. In many plant species, the total osmotic potential is predominately influenced by inorganic ions like K^+, Na^+, and Cl^- during drought. However, in most field crops, K^+ is the most significant ion. Under normal and stressful conditions, plants need K^+, a vital and abundant macronutrient (Agarwal et al., 2009). Potassium plays an active role in a variety of fundamental biochemical and physiological processes, including osmoregulation, enzyme activation, and stomatal movements that reduce the overabsorption of ions like Na and Fe in saline soils (Amtmann et al., 2008; Ahmad et al., 2012; Wang et al., 2013).

Inorganic anions and metabolites are thought to be transported by K^+ and preserve the transmembrane voltage gradients for cytoplasmic pH homeostasis (White and Karley,

2010). Moreover, the K^+ deficit significantly reduces photosynthesis, N metabolism, and leaf area, eventually slowing plant development (Ebelhar and Varsa, 2000). The sink tissues only get a small amount of photoassimilates because the K^+ deficit reduces the amount produced, thus impacting the yield quantity and quality (Jordan-Meille and Pellerin, 2004). The reduced growth rate is the initial sign of a plant potassium deficit and, subsequently, the onset of chlorosis and necrosis in older plants. Loss in turgor, stomatal opening, water relations, and photosynthesis are all disrupted by potassium deprivation (Mengel et al., 2001).

Potassium (K^+) distribution in epidermal cells, guard cells, and leaf apoplast significantly regulates stomata (Shabala et al., 2002). Plants such as wheat (Gupta et al., 1989), maize (Premachandra et al., 1991), and chickpea (Nandwal et al., 1998) grown under water stress can uphold higher leaf water potential, turgor potential, relative water content, and lower osmotic potential with the help of adequate K^+ levels. According to Sangakkara et al. (1996), K^+ and magnesium (Mg^+) improve sucrose transfer to growing roots, which is necessary for their regular growth and development and causes K^+ to stimulate root growth under water stress. Additionally, it improves ion absorption since K^+ is one of the primary components of phloem sap, maintaining osmotica and reducing the detrimental effects of moisture stress on plants by enhancing translocation and preserving water balance in the plants (Jeschke and Hilpert, 1997; Walker et al., 1998). For instance, K^+ accounted for 40–80% of the changes in wheat's osmotic potential brought on by drought (Morgan, 1992). However, individual osmolytes' contributions are influenced by drought stress. Moreover, K^+ supplementation has been reported to increase the synthesis of many organic solutes, like proline, free sugars, and free amino acids, under normal and water stress

conditions, contributing to osmotic adjustment. Under normal and water stress conditions, a K^+-induced increase in proline has been reported in rice (Pandey et al., 2004), wheat (Jatav et al., 2012), and sorghum (Umar et al., 1993) by maintaining higher tissue water content due to osmolyte accumulation and mitigating the ill effects of water stress (Gupta et al., 2000).

Plants exposed to environmental stresses like drought have a more significant internal requirement for K^+, resulting in ROS overproduction (Cakmak, 2005). Potassium has been reported to lessen the detrimental effects of ROS by enhancing photosynthetic electron transport while inhibiting the activity of membrane-bound NAD(P)H oxidases. Plants with appropriate K^+ levels have improved membrane fluidity as K^+ upholds a higher ratio of unsaturated to saturated fatty acids in membranes (Wilkinson et al., 2001). Furthermore, the activity of several enzymes involved in drought resistance is enhanced by supplementing appropriate K^+ and its adequate concentration in the cytoplasm (Kant and Kafkafi, 2002).

1.8. Plants under drought stress: molecular impacts

It is fair to assume that plant cells can perceive numerous environmental signals since plants exhibit a wide array of sophisticated and effective mechanisms by inducing distinct gene expressions in response to varied environmental stress situations that will maintain the cell and membrane architecture (Atkinson and Urwin, 2012). Plant responses to drought stress are mediated by an intricate network of genes, resulting in increased levels of several metabolites and proteins, some of which may be responsible for conferring a certain degree of protection to these stresses. When plants experience drought, several gene expression patterns change (Bernacchia and Furini,

2004). Plant gene expression is regulated at several stages, including the transcriptional, post-transcriptional, translational, and post-translational phases (Bray, 2002; Osakabe et al., 2014). The expression of genes engaged in late responses, such as those involved in water transport, osmotic balance, oxidative stress, and damage-repair processes, has also been modified (Xoconostle-Cazares et al., 2010). Transcriptional and translational levels manage plant response mechanisms to abiotic stressors, such as drought stress (Bhargava and Sawant, 2013; Farooq et al., 2009; Xoconostle-Cazares et al., 2010). Agarwal et al. (2006) focused on the role of dehydration-responsive element binding transcription factors DREB⁄CBF in plants to regulate the expression of genes associated with drought stress tolerance. One of these factors is the DREB transcription factors, which relate to the ERF family consisting of two subclasses, DREB1/CBF and DREB2, induced by cold and dehydration, respectively. The role of DREB transcription factors (TFs), namely, DREB1 and DREB2, which are involved in regulating abiotic stress responses in plants to improve the tolerance of crop plants to low temperature and dehydration as well as their utility in crop improvement programmes through breeding and marker-assisted selection (Yang et al., 2010; Lata and Prasad, 2011). In general, the DREB proteins specifically bind to the DRE sequence, and they are induced by particular signalling molecules such as ABA, which can control the expression of different stress-responsive genes in plants and produce a variety of proteins, especially transcriptional factors in order to avoid adverse effects of water deficit conditions on plant growth. ABA signal transduction and drought signalling are tightly related. ABA is crucial for how plants react to drought and produces genes that are activated by it (Bernacchia and Furini, 2004; Arbona et al., 2013; Bray, 2002; Lisar et al., 2012; Xoconostle-Cazares et al., 2010).

Bioinformatics analyses have identified several transcription factors (TF) induced under drought stress. TFs are classified into several families, including MYB/MYC, zinc-finger protein, and NAC (Bray, 2002; Ding et al., 2013; Nakashima and Yamaguchi-Shinozaki, 2013; Nezhadahmadi et al., 2013; Osakabe et al., 2014; Xoconostle-Cazares et al., 2010). Translational control is another mechanism in plant responses to drought and controls protein production (Xoconostle-Cazares et al., 2010). Molecular biology research has shown that plants respond to stress at the cells' mRNA, post-transcriptional phase, or protein level (Bhargava and Sawant, 2013; Nezhadahmadi et al., 2013) (**Fig.1.18)**. MicroRNAs (miRNAs) are small RNAs recognized as important modulators of gene expression at the post-transcriptional level (Bej and Basak, 2014; Bhargava and Sawant, 2013), which are involved in plant response and resistance to drought (Bej and Basak, 2014; Ding et al., 2013). Studies have shown that these miRNA molecules are involved in responses mediating with ABA, auxin signalling, cell growth, antioxidant defence, osmotic adjustment, photosynthesis, and respiration under drought (Bej and Basak, 2014; Ding et al., 2013; Farooq et al., 2009).

Plants have developed a variety of drought stress adaptation mechanisms, including drought avoidance, drought tolerance, and drought escape mechanisms. Plants that survive drought have quick phenological development, high developmental flexibility, and the ability to finish their life cycle before a physiological water deficit emerges (Blum, 2018). Avoiding drought, creating structures that assist in preserving water, or improving water usage efficiency are all examples of drought adaptation measures (Price et al., 2002). Relative drought tolerance is a plant's ability to grow productively despite low leaf water status. Plants that can withstand droughts have evolved

defences against water loss, antioxidant defences, and systems to stabilise or repair damaged proteins (Hsps, LEAs, AQP).

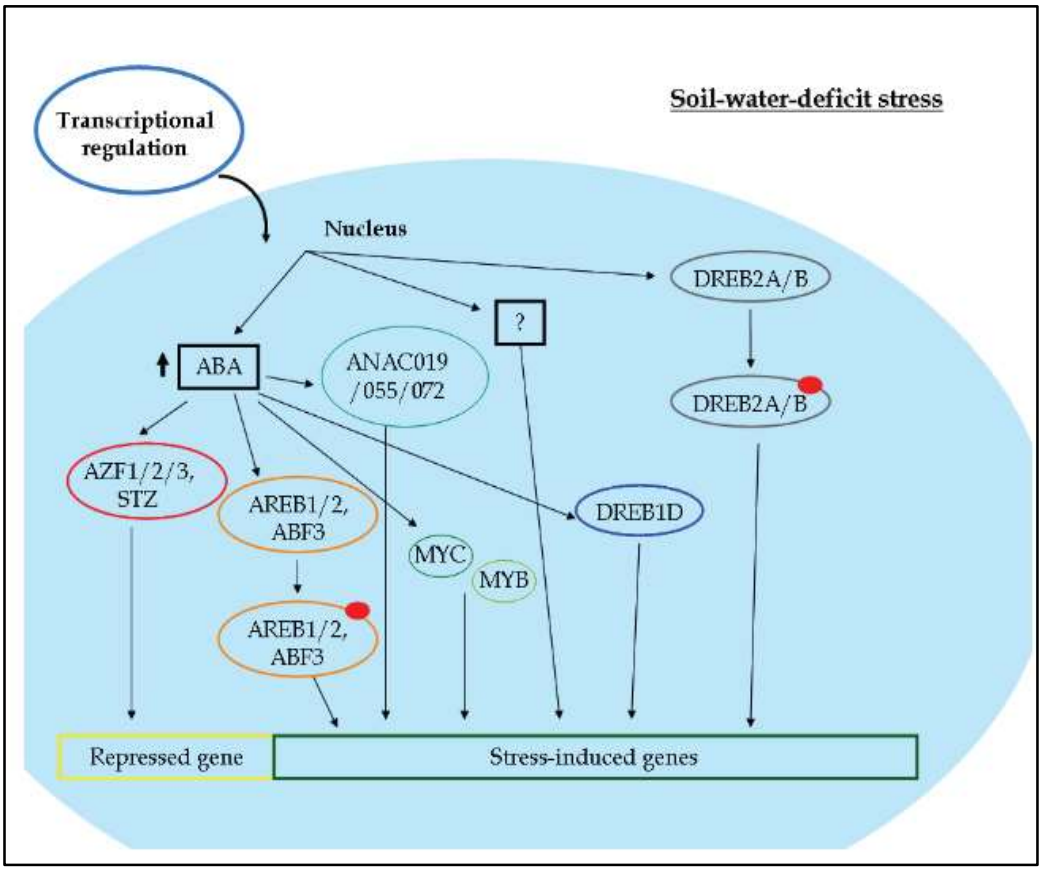


Fig.1.18.: Types of transcription factors involved in induction/repression of regulons under water-deficit conditions (Ciarmiello et al., 2011).

1.8.1. Aquaporins

Plant aquaporins (AQPs) can facilitate and regulate the passive exchange of water across membranes. Aquaporins are a class of membrane channels situated in the plasma membrane and intracellular module, ranging from 26 kD to 30 kD, which can help transport water, small neutral molecules, and gases across biofilm (Kaldenhoff and Fischer, 2006). Aquaporins belong to the MIP family of proteins as ion and glycerol channels that control cellular water movement and maintain water relationships in

plants, especially under drought stress. The structural analysis of aquaporins has revealed the general mechanism of protein-mediated membrane water transport. Nevertheless, it is believed that they can regulate the hydraulic conductivity of membranes and potentiate a ten- to twenty-fold increase in water permeability (Maurel and Chrispeels, 2001).

Based on the homology and structural characteristics of amino acid sequences, the plant AQPs family is classified into four forms: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin 26-like intrinsic proteins (NIPs); small and basic intrinsic proteins (SIPs) (Johanson et al., 2001). Amongst them, plasma membrane intrinsic proteins (PIPs) and tonoplast intrinsic proteins (TIPs) mediate the critical pathways of intracellular water transport, uphold intracellular and intercellular water relations under stress, and are involved in many processes of the drought stress response.

The expression of AQP exhibits strong temporal and spatial specificity. AQP is expressed in tissues that need a lot of water movement, such as the root epidermis, outer cortex, endodermal cells, xylem parenchyma cells, phloem-associated cells, and guard cells. The physiological role of AQP is closely linked to its expression period and location. Its functions cover a sequence of physiological processes such as seed maturation and germination, cell elongation, root growth, leaf extension and movement, petal expansion, pollen and ovule development (Muto et al., 2011; Javot et al., 2003; Heinen et al., 2009; Bots et al., 2005). At the subcellular level, AQP is mainly disseminated in membrane systems such as cells, vacuoles, endoplasmic reticulum, chloroplast, and mitochondrial membranes. It was also found that AQP is

redistributed at the subcellular level in different tissue sites and environments and is mainly responsible for water absorption (Wudick et al., 2009; Maurel et al., 2009). In general, at the cellular level, the plasma membrane intrinsic protein (PIP) is mainly responsible for water absorption and outflow, and the vacuolar membrane intrinsic protein tonoplast intrinsic protein (TIP) is responsible for regulating turgor pressure, thus maintaining the integrity of cells (Fotiadis et al., 2001).

AQP located on the invaginated plasma membrane contributes to water transport amid the protoplast and the vacuole and regulates turgor. The specific distribution of plant AQP specifies that strong water flow across cells occurs in this region. Throughout the transmembrane transport of water in plants, AQP promotes water transport inside and outside of cells along the gradient of water potential by reducing the resistance encountered and accelerating the transmembrane transport rate of water between cells.

Simultaneously, AQP is also the primary way of water in and out of the cell, balancing the water potential inside and outside the cell. For instance, the AQP on the cell membrane of plant root cells can control 70%~90% of the water flowing through the root, which passes through the Casparian strip into the vessels, ensuring that water is transported in large quantities through the plant. In many plants, AQP expression has been found in vascular bundles and adjacent tissues (Kaldenhoff et al., 1995), which suggests that plant AQP can hasten water transport and facilitate water flow in and out of vascular bundles. In addition, plant AQP can preserve the water potential balance between xylem parenchyma cells and transpiration flow (Netting, 2000). In addition to water molecules, aquaporin also transports other physiologically significant neutral small molecules, such as CO_2, H_2O_2, glycerol, NH_3/NH_{4+}, boron,

silicon, and urea, which are involved in a series of critical physiological processes such as photosynthesis, nutrient absorption, cell signal transduction, and stress response.

Zhang et al. (2019) reported that water channel protein RhPIP2;1 can influence plant growth and stress reaction by interacting with rose plants' membrane MYB protein RhPTM. Overexpression of CrPIP2;3 in *Arabidopsis thaliana* (a PIP2 gene from a rose) can promote the survival and recovery of transgenic plants under drought stress by regulating water homeostasis, thus affecting the drought tolerance of plants (Zheng et al., 2021a) (**Fig.1.19**). The seed germination rate, seed yield, seed vigour, and root length of transgenic *Arabidopsis thaliana* lines overexpressing JcPIP2;7 (a plasma membrane intrinsic protein gene) and JcTIP1;3 (a tonoplast intrinsic protein gene) under mannitol condition were significantly higher than those of the control (Khan et al., 2015). Peng et al. (2007) tested the effect of the ginseng PgTIP1 gene by transfer into *Arabidopsis* plants. They showed that it altered root morphology and leaf water channel activity, thereby altering drought tolerance. The overexpression of CsTIP2;1 in *Arabidopsis* plants increased the expansion of mesophyll cells, midrib aquiferous parenchyma abundance, H_2O_2 detoxification, and stomatal conductance, and then significantly improved the water and oxidation state, photosynthetic capacity, transpiration rate, and water use efficiency of leaves under the condition of continuous dry soil (Martins et al., 2017).

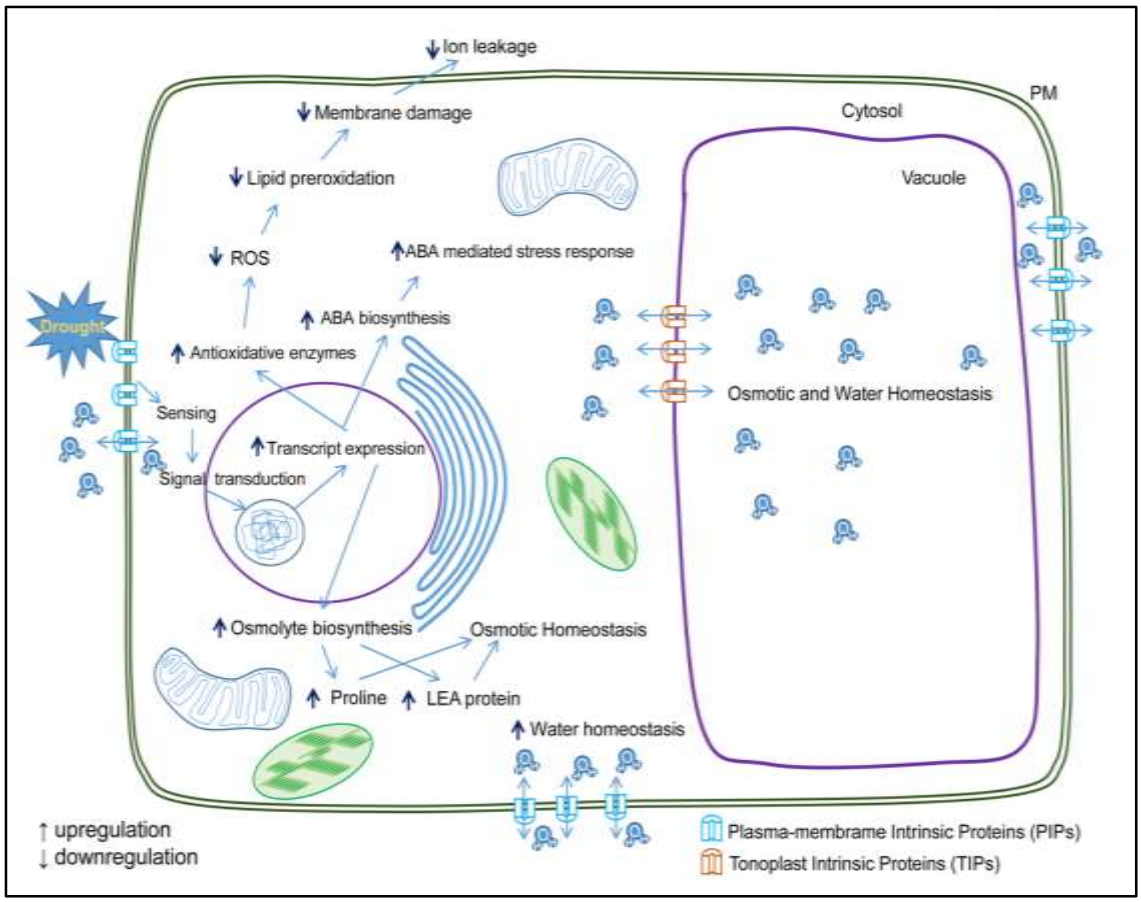


Fig.1.19.: Probable mechanism of aquaporin under drought stress (Zheng et al., 2021b).

1.8.2. Potassium Transporter

Potassium (K^+) is a vital element in plant growth, and its uptake and efflux affect plant productivity and control cell water potential and turgor in osmotic regulation. K^+ affects osmotic pressure in the root xylem (root pressure), which drives long-distance sap flow from roots to shoots (Lebaudy et al., 2007). The high demand for K^+ needs to be met through efficient uptake from the soil solution by roots and further translocation into the aerial parts, distribution within cells into different compartments, and recycling from source to sink organs through various K^+/Na transport systems (Gierth and Mäser, 2007). There are two plant transport systems,

channels, and transporters, for K^+ acquisition and distribution (Ashley et al., 2006; Alemán et al., 2011; Wang and Wu, 2013).

Evidence is emerging from molecular studies that K^+ might regulate plant stress responses (Ashley et al., 2006; Wang et al., 2010). Low K^+ status triggers several signalling cascades, such as the upregulation of K^+ transporters, synthesis of ROS, and phytohormones, including jasmonic acid (JA) and ethylene. In addition to the upregulation of transport proteins, K^+ deficiency also triggers several other responses in roots. All these strategies enable plant species to adapt to changing environmental conditions. It has been reported that the ROS and phytohormone levels increase is accompanied by a transient increase in transcripts coding K^+ transporters and channels, suggesting a possible regulatory role of potassium in plant stress responses (Cheong et al., 2007; Jung et al., 2009). In K^+-deficient plants, drought-induced ABA may trigger Ca^+ flow, which acts as a secondary messenger and initiates the uptake of K^+ by roots and the regulation of stomatal guard cells (Cheong et al., 2007). Ca^+ signalling regulates leaf transpiration and root K^+ uptake and involves membrane-localized Ca^+ sensor-interacting proteins. Jung et al. (2009) reported increased ethylene and ROS production in K^+-deficient plants, and this phytohormone signal is essential for changes in root morphology and plant tolerance to low K^+ supply.

Under drought stress, osmotic stress sensing and signalling are essential to plant water status and lead to rapid changes in gene expression (Yamaguchi-Shinozaki and Shinozaki, 2006; Osakabe et al., 2011) and turgor-dependent stomatal closing, which responds to hydraulic properties in the xylem (Maggio et al., 2006; Hedrich, 2012; Roelfsema et al., 2012). Plant hormones coordinate adaptive changes in cellular

osmotic regulation. Abscisic acid (ABA) regulates several molecular events in response to drought stress and plant growth. Further, under water deficit stress, ABA induces the activation of anion channels, such as SLAC1, which causes depolarization of the plasma membrane in guard cells (Levchenko et al., 2005; Negi et al., 2008; Vahisalu et al., 2008; Geiger et al., 2009). Two types of anion channels, S-type and R-type, control the guard cell movements (Hedrich, 2012). The depolarization of the plasma membrane declines the activity of inward K^+ channels, such as KAT1/KAT2. It stimulates outward K^+ channels, such as the guard cell outward rectifying K^+ channel, and GORK (gated outwardly-rectifying K^+ channel), resulting in K^+ efflux from guard cells. Anion and K^+ efflux from guard cells leads to loss of guard cell turgor and causes stomatal closing (Ache et al., 2000; Hosy et al., 2003; Kim et al., 2010). SLAC1 is directly activated by Snf1-related protein kinase 2 (SRK2E/OST1/SnRK2.6), which is involved in the ABA signalling complex of the ABA receptor PYR family and PP2Cs.

1.9. The Limitations of Endogenous Plant Protection Measures

Plant protection from drought stress is controlled by a group of genes, which include several transcription factors. These genes, however, are not involved in protecting cells but upregulate other genes that produce the regulatory proteins. The transcription factors also regulate stress signal transduction and modulate gene expression. They activate the expression of many target genes responsible for controlling the stress response (Lata and Prasad, 2011). The manipulation of genes involved in cell protection could theoretically increase the survival of plants under severe drought stress. Moreover, the maintenance of metabolic homeostasis of leaf water status under water deficit is achieved by reducing transpiration through controlling the stomatal

aperture and leaf growth, which positively affects plant water status. However, this has a negative effect on photosynthesis and might be regarded as a constraint on plant protection, which contradicts the standard measurement of drought tolerance (Tardieu, 2005).

1.10. Plant Protection Measures

Several drought management measures for agricultural areas may help minimize drought impacts on crop plants. Plants commonly resist drought conditions by avoiding tissue dehydration and maintaining tissue water potential at a high level or by tolerating low tissue water potential to maintain metabolic activity. Avoidance is a mechanism associated with various responses against drought, which involves minimizing water loss by the closure of stomata to maximise water retention and by rolling the leaves, which leads to reduced leaf area and, therefore, reduced transpiration (Chaves et al., 2003). The variation in a morpho-physiological mechanism of plant protection on two canola (*Brassica napus* L.) in hydroponic culture indicated that water deficit conditions caused a substantial decrease in leaf area (Kauser et al., 2006). The capacity of plants to alleviate the toxic effects of high ROS is essential for their tolerance to biotic and abiotic stresses. The quenching of this super-ROS by the frontline defence includes the antioxidant enzymes, for instance, superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), against the free radical oxidative damage.

Additionally, increasing plant stress tolerance is essential for agricultural production and environmental sustainability since crops with low-stress tolerance require excessive amounts of water and fertiliser, placing a heavy strain on the environment.

Increasing a plant's tolerance to various abiotic and biotic stimuli may be accomplished using various priming techniques. Because priming techniques frequently leverage the plant's built-in defences and stress memory, they successfully enhance drought resistance. Ding et al. (2012) reported that plants exposed to many periods of drought had higher transcriptional activity associated with drought tolerance during a subsequent stressful phase. After a first stress, a plant can likely better withstand subsequent stresses due to epigenetic modifications or other metabolic responses (Kinoshita and Seki, 2014). Chemical priming may also successfully change a plant's memory and enhance its natural reaction or offer the plant a new resource that it does not usually use to survive.

1.10.1. Gene modifications

The genetic modification approach has become popular as a more rapid and capable mechanism to improve stress tolerance rather than the ordinary reproduction strategy such as plant breeding. Such efficiency can provide the tools for successfully applying stress-tolerant traits and searching for an explanation of the complicated molecular mechanism of plant stress responses. Since drought stress tolerance is a complex trait, it needs a prolonged research time; much research which has studied genetic modification for improved drought tolerance was not agronomic plants but model plants such as tobacco and *Arabidopsis*. These species have a much greater facility to transform, and *Arabidopsis* has a shorter life cycle than many crop plants, making it an ideal model plant for such research. Moreover, many genetic modifications that improve drought stress tolerance have a growth reduction consequence. Only a few

crop plants have been tested for genetic modification of drought tolerance in the field (Moreno et al., 2005).

1.10.2. Plant breeding

The plant breeding process is the selection of two plants with desirable traits crossed to combine their genes and then selected to obtain new genetic arrangements in new individuals. This improvement aims to test individual plants for the expression of favourable characteristics and plant regeneration, which has now been applied to many crop species. Thus, the process has opened the opportunity for crop improvement genetically through a comprehensive collection of "drought-related" genes, and this can be supported by using recombinant DNA technology, which includes familiar tags linked to target genes; these are known as molecular markers, which are based on polymorphisms that occur naturally in the DNA sequence (Xoconostle-Cazares et al., 2010).

1.10.3. Exogenous Application of Plant Growth Regulators

Plant hormones regulate various processes, protecting plants against abiotic stresses. It has been reported that these regulators are synthesized in roots and translocated to leaves, where they cause stomatal closure and inhibit plant growth, consequently enabling the plant to adapt to stress conditions (Bhargava and Sawant, 2013). This is supported by Farooq et al. (2009), who showed that exogenous application of substances such as plant growth regulators at very low concentrations can help maintain physiological activity during long periods of drought. To improve the performance of a particular crop, exogenous application of different chemicals such as auxins, cytokinins, salicylic acid (SA), brassinosteroids (BR), strigolactones (SL),

gibberellins to above-ground plant sections is a widespread method in plant research and agricultural practice (**Fig.1.20)**. In addition, plant growth regulators have been recognized as an essential substance for delaying dehydration damage in water-limited environments by continued maintenance of cell turgor and other physiological processes and giving tolerance against drought injuries by maintaining high tissue water potential.

Depending on the desired outcome of the priming practice, foliar spray priming techniques may be used if seed or root integration techniques are impractical or the substance must come in contact with leaves or stems for the plant to absorb it. Compared to annual crop plants that may be reseeded yearly, perennial crop plants may not benefit from seed priming procedures as much over the long run. It could be challenging to integrate root zone-based priming techniques into perennial crop or no-till annual cropping systems that are already established.

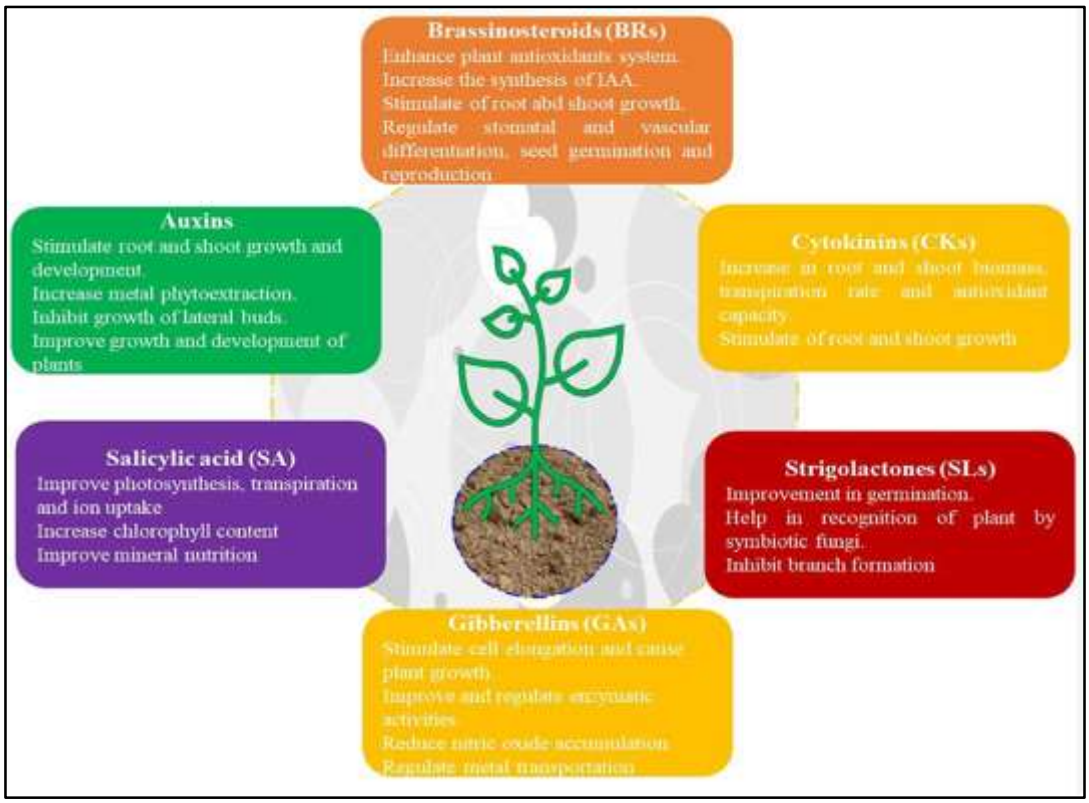


Fig.1.20.: Possible role of plant growth regulators on growth and development (El-Sabagh et al., 2021).

Additionally, annual and perennial crops frequently exhibit drought resistance mechanisms, such as escape and avoidance, that contradict high-yield production. Foliar priming techniques are used to increase annual and perennial crop drought resistance and are crucial for agriculture for the aforementioned reasons. Practices for foliar priming are possible at any point in the plant's life cycle. Different technologies may differ in terms of whether priming with a specific drug should be done on a mature plant compared to a seedling since some priming compounds may have a degree of associated cell toxicity. Hormone metabolism can be significantly impacted by drought stress, and alterations in hormone levels are frequently noticed. For instance, during the early stages of a drought, when the plant starts to wilt, growth promoters

like cytokinins (CK), gibberellins (GA), and auxin may drop while growth inhibitors like ethylene and abscisic acid (ABA) may rise (Nilsen and Orcutt, 1996; Gupta and Kaur, 2005). The effects of drought can be changed through the exogenous administration of hormones or plant growth regulators (PGRs), such as Salicylic acid (SA), which may help increase drought stress resistance.

1.10.4. Salicylic acid

Salicylic acid (SA) or orthohydroxy benzoic acid is a ubiquitously distributed endogenous phenolic plant growth regulator and a signalling molecule that plays a critical role in controlling physiological processes such as plant growth, photosynthesis, and other metabolic functions along with defence against various abiotic and biotic stresses (Raskin, 1992; Idrees et al., 2011; Jini and Joseph, 2017).

Salicylic acid biosynthesis can occur through two distinct pathways, viz. isochorismate (IC) pathway and phenylalanine ammonia-lyase (PAL) pathway, both of which are initiated by chorismic acid, the end product derived from the shikimic acid pathway in plastid (Wildermuth et al., 2001; Uppalapati et al., 2007; Catinot et al., 2008). As reported in several plant species, chorismic acid is converted to IC by isochorismate synthase (ICS) (Dewdney et al., 2000; Garcion et al., 2008; Wan et al., 2012). Isochorismate pyruvate lyase (IPL) is hypothetically known to catalyze the conversion of IC to SA; however, the mechanism is unclear (**Fig.1.21**) (Mercado-Blanco et al., 2001). After the biosynthesis of SA, it is modified into different forms through glycosylation, methylation, and conjugation. Glycosylation of SA results in the formation of salicyloyl glucose ester (SGE) and salicylic acid 2-O-β- glucoside (SAG) stored in the vacuole. The methylation process produces methyl salicylate

(MeSA) and is transported to different plant parts (Song et al., 2008; Chen et al., 2003). Furthermore, the conjugation of SA with amino acid results in the formation of salicyloyl-L-aspartic acid (SA-Asp), and hydroxylation of SA leads to the production of 2,5-dihydroxybenzoate (Gentisic acid). These modifications of SA often render it inactive but are also related to accumulation, function, and mobility. Glucosylation inactivates SA and allows vacuolar storage. Methylation inactivates SA and increases its membrane permeability and volatility, which is essential for the long-distance transport of this defence signal (Baek et al., 2010; Dempsey et al., 2011). In plants, SA induces systemic acquired resistance (SAR) to different pathogens (Métrauxs, 2001). It is well-established that SA may generate various metabolic reactions in plants and influence plant-water relations. Numerous studies indicate that SA significantly regulates how plants react to biotic and abiotic stressors. Under unfavourable environmental conditions, SA was also highly effective in mitigating oxidative stress. Since abiotic stress is one of the most significant global barriers to crop production, it is crucial for plant biologists to find efficient solutions.

Exogenously applied salicylic acid in plants has been reported to enhance the efficiency of several developmental, physiological, and biochemical processes. SA applied exogenously has been demonstrated to increase antioxidant responses and cause stomatal closure (Waseem et al., 2006; Mori et al., 2001). Since pathogens frequently target plants already under drought stress, SA activation of defence proteins known as pathogenesis-related proteins (PR) may improve plant performance. As a result, it may be feasible to indirectly promote tolerance for abiotic stress. Taking SA supplements protects the organism from biotic and abiotic challenges (**Fig.1.22)**.

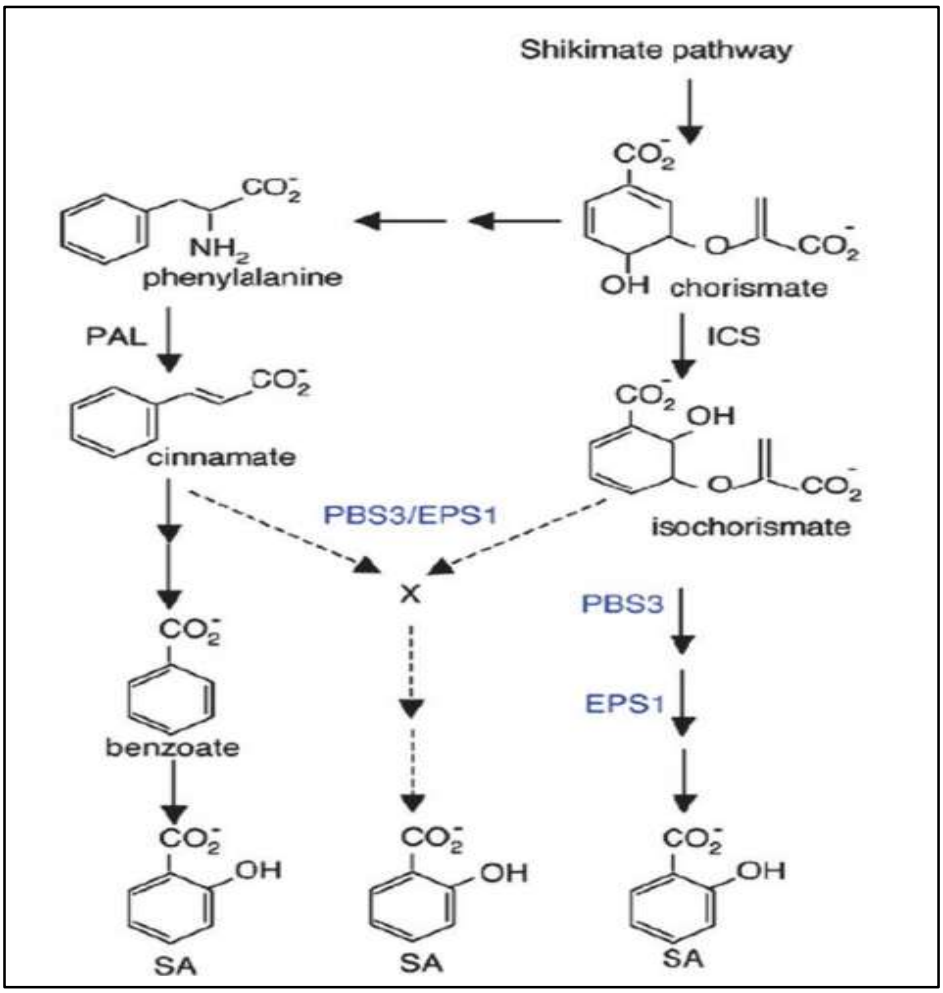


Fig.1.21.: Salicylic acid biosynthetic pathway (Chen et al., 2009).

SA appears to have longer-lasting effects when used as a priming agent (Kadioglu et al., 2011). The role of SA in promoting stress tolerance in plants has been the subject of several investigations. For instance, SA was discovered to produce heat tolerance in mustard (Dat et al., 1998), chilling tolerance in maize, and drought tolerance in wheat (Singh and Usha, 2003). The growth of maize and soybean plants' leaves was increased when SA, acetylsalicylic acid (ASA), gentisic acid (GTA), or other analogues of SA were applied; nevertheless, the plants' height and root length remained unaffected (Khan et al., 2003). Much emphasis has been given to SA's capacity to generate protective effects on stressed plants since it has a significant role

in the ability to withstand abiotic stress (Farooq et al., 2008). SA alters the defence response by enhancing photosynthetic activity and antioxidant metabolism (Horváth et al., 2007a).

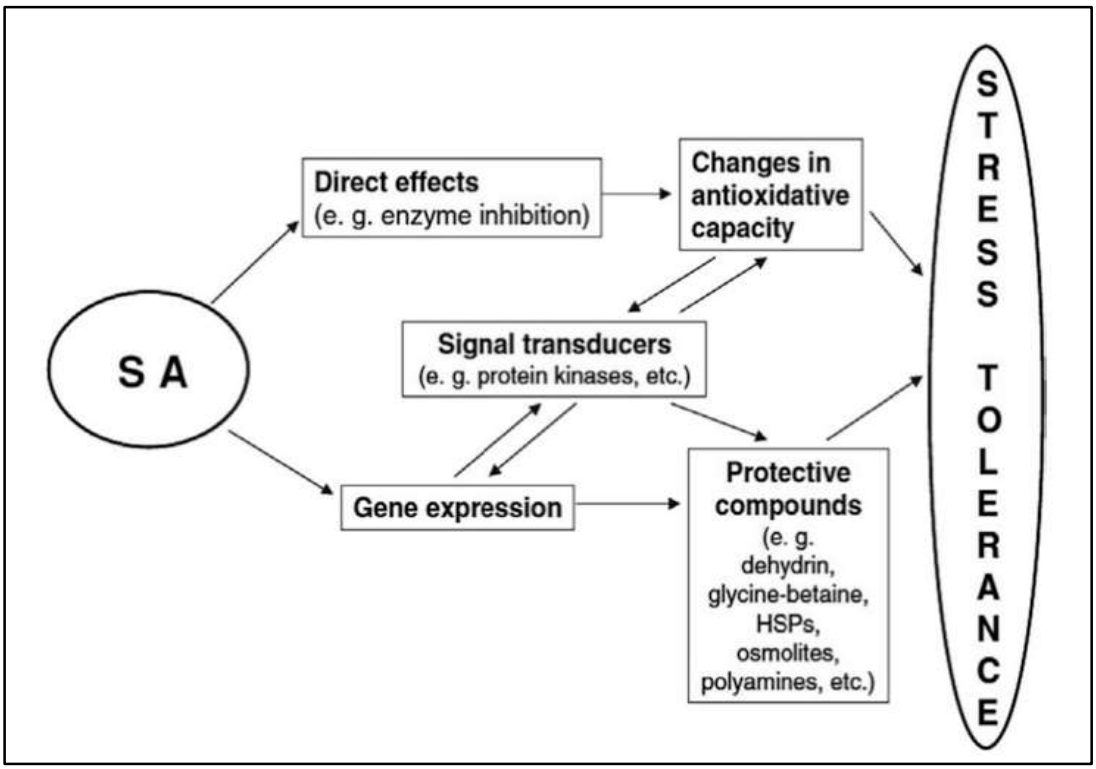


Fig.1.22.: Illustration emphasizing the role of Salicylic acid in stimulating stress tolerance (Horvàth et al., 2007b).

Low SA concentrations protect plants against oxidative damage by enhancing their antioxidant capacity; however, higher SA concentrations may increase the plant's susceptibility to abiotic stress-causing cell death (Hara et al., 2012). Fariduddin et al. (2003) also found that the most significant increase in dry matter accumulation occurred when foliar application of SA at a concentration of $1\ 0^{-5}$ M on leaves of *Brassica juncea*, while concentrations beyond were inhibitory. Fariduddin et al. (2003) further noticed greater water utilisation and carboxylation efficiency in

conjunction with high photosynthetic rates. In addition, wheat seedlings grown from grains soaked in the same concentration of SA produced more leaves and increased fresh and dry mass than in control plants (Hayat et al., 2005).

Salicylic acid boosted the synthesis of carotenoids, xanthophylls, and the rate of de-epoxidation while lowering the quantity of chlorophyll pigments in wheat and moong plants as well as the chlorophyll a/b ratio in wheat seedlings (Moharekar et al., 2003). Additionally, Spirodela-fed SA had lower levels of anthocyanin and chlorophyll pigments (Khurana and Maheshwari, 1980). In *Phaseolus vulgaris*, SA applied to the leaf caused stomatal closure and reduced leaf transpiration rates in *Phaseolus vulgaris* and *Commelina communis* (Larque-Saavedra, 1979). SA boosted rubisco activity in stressed maize plants (Khodary, 2004) and photosynthetic growth rate in mustard plants (Fariduddin et al., 2003). Similarly, SA increased the rate of photosynthetic activity in soybean, barley, and maize (Khodary, 2004; Kumar et al., 2000; Khan et al., 2003). Therefore, increasing plants' ability to withstand abiotic stress requires a knowledge of the physiological role of SA.

1.11. Rice as a model plant

Between 11,000 and 12,000 unique species make up one of the most notable families of flowering plants, the Poaceae (Kellogg, 2016; Soreng et al., 2017). The grass family Poaceae is one major plant species badly influenced by drought stress. Several annual grain species are cultivated as nutritional staples, such as sorghum (*Sorghum* spp.), wheat (*Triticum* spp.), rice (*Oryza* spp.), and maize (*Zea* spp.). (USDA, 2018). *Oryza sativa* (L.) is a typical rice variety (Lu, 1999), adapted to varied environmental conditions, and is planted in dry and wetland regions at high and low elevations.

Around 85 to 90 M hectares of paddy lowland regions, where rice is sown on the same field up to three times a year, generate 75% of the world's rice (IRRI, Africa Rice and CIAT, 2010). About 56% of Asia's rice land is produced using this approach (Swain et al., 2005). Nearby, 20% of the world's rice crop is produced on 40 to 45 million hectares of rainfed lowland systems (IRRI, Africa Rice and CIAT, 2010). In such ecological conditions, rice output in Asia is relatively low due to drought or flooding. Because of erratic rainfall patterns and minimal input, rice is probably more susceptible to drought because of its water-loving nature and is affected at any stage of rice growth and may thus cause reductions in yield than other crops (Filgueiras et al., 2020; Napasintuwong and Pray, 2014). Upland rainfed rice systems frequently produce average yields of just about 2 t ha^{-1} due to irregular rainfall patterns, restricted input consumption, poor weed management, and a high prevalence of infections. Around 20% of the daily caloric requirements of more than 3.5 billion people worldwide are met by rice, making it the leading food for more than half of humankind (IRRI, Africa Rice and CIAT, 2010).

India's main grain crop, *Oryza sativa* L., comes in 5000 different kinds with variable yields, sizes, and textures (Vachhani et al., 1962). In India, rice is grown in various environments, including 24% irrigated, 34% rainfed lowland, 26% flood-prone, and 37% rainfed upland (**Fig.1.23**). Since India is the only nation with various rice ecosystems, Indian rice research is the primary driving force globally. The key motive of the programme is to evaluate genotype x environment interactions in different ecosystems to improvise multidisciplinary rice research (Prasad et al., 2001). . Contrarily, rice suffers from a variety of nature's wrath, including varied biotic stressors, drought, salt, and temperature fluctuations (Al-Zaban et al., 2022; Metayi et

al., 2022). According to the United Nations, the world's population will increase from six to eight billion people between 2000 and 2025 (United States Bureau of Census, 1993). This will necessitate the production of 40% more rice (Fahad et al., 2018). As a result, there is a stern need to multiply rice output to feed the growing population. Hence, rice was selected as a model plant for the present study.

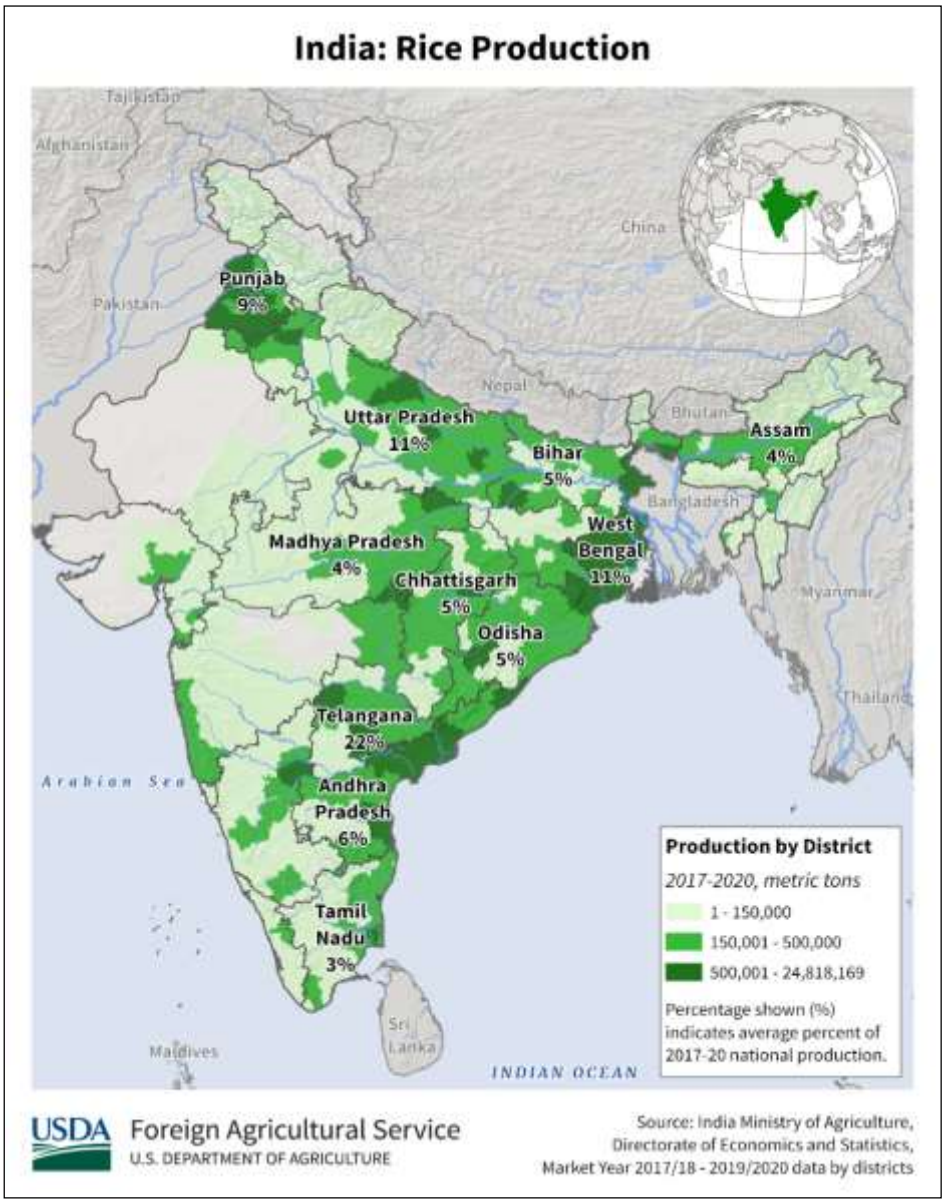


Fig.1.23.: Map depicting rice production in India

1.12. Aims and Objectives

There is a prerequisite for increased agricultural productivity of primary food crops due to rapid reduction in its natural habitat owing to growing exploitation and environmental conditions due to influencing dynamics of population growth. Crop cultivation and agricultural output are restricted due to the potential for future drought stress brought on by climate change. Hence, the present investigation was carried out to assess the effect of SA as a synthetic plant growth regulator during the drought period. This was primarily to overcome drought stress-induced osmotic and oxidative stress and enhance and improve economic production. The study was initiated with an aim to determine the effects of different salicylic acid concentrations on two rice cultivars, viz., Sahbhagi Dhan (drought tolerant) and Jaya (drought sensitive), as a model crop plant, from drought injury that occurred at the two- to three-leaf stage. The following goals were intended to be achieved by the current study.

Objective I: To study the effect of Salicylic acid on the growth, morphology, photosynthesis, and photosynthetic pigments of drought-stressed rice plants

- Analysis of the growth traits (root/shoot length) of plant biomass (Chen et al., 2014). and its relative water content (Barrs and Wheatherley, 1962).
- Determination of the external and internal morphological changes using **a** Scanning Electron Microscope (SEM) and Light Microscope (Butterbach-Bahl et al., 2000).
- Analysis of the photosynthetic efficiency to determine plant productivity (Genty et al., 1989; Murchie and Lawson, 2013).

- Extraction and analysis of the plant pigment using HPLC (Pocock et al., 2004).

Objective II: To study the effect of Salicylic acid on the biochemical parameters of drought-stressed rice plants.

- Determination of the accumulation of Reactive Oxygen Species: Hydrogen peroxide (H_2O_2) (Sagisaka,1976) and Hydroxyl Radical (OH•) (Liu et al., 2009).
- Determination of the level of oxidative damage by estimating the accumulation of TBARS (Sankhalkar and Sharma, 2002) and analysed the membrane integrity (Shi et al., 2006).
- Estimation of the formation of protein carbonyl content (Levine et al., 1990).
- Analysis of fatty acid lipid profile using GC/HRMS (Turnham and Northcote, 1982).

Objective III: To study the effect of Salicylic acid on protective processes of the biochemical and molecular levels of drought-stressed rice plants.

- Analysis of the enzymatic antioxidant assay; Superoxide Dismutase (SOD) (Beauchamp and Fridovich, 1971) and Glutathione Reductase (GR) (Schaedle and Bassham, 1977).
- Estimation of the content of Non-Enzymatic Antioxidant: Total Glutathione (tGSH) according to Griffith (1980).
- Determination of Osmolyte (Proline) Accumulation according to Bates et al. (1973).
- Gene Expression Studies for Enzymatic antioxidants (SOD, APX) and Membrane Transporters (K^+ Transporter, Aquaporin) using RT-PCR, according

to Livak and Schmittgen (2001).

2.1. *Plant material*

Oryza sativa L., cultivar Jaya (IET-723), a short-duration, high-yielding, drought-sensitive and cultivar Sahbhagi Dhan (IR74371-70-1-1), a short-duration, high-yielding, drought-tolerant, were used as the model plants for the present study. The seeds were obtained from the Indian Council of Agricultural Research-Central Coastal Agricultural Research Institute, Goa, and stored in the dark at 4℃.

Drought sensitive: *Oryza sativa* L. cv. Jaya (IET-723)

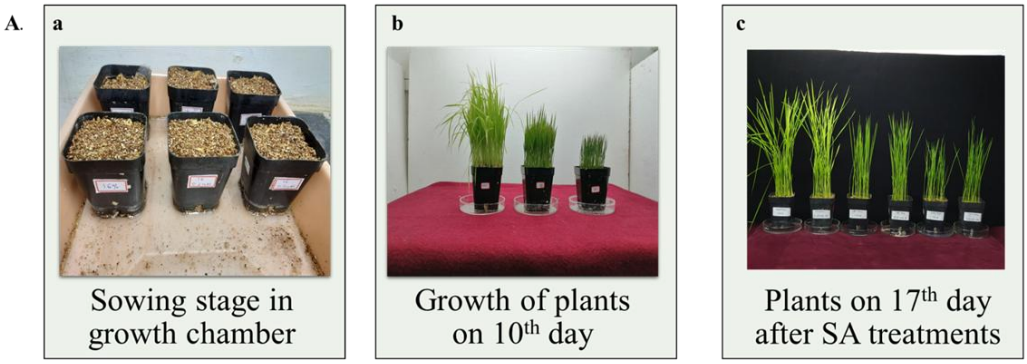
Drought tolerant: *Oryza sativa* L. cv. Sahbhagi Dhan (IR74371-70-1-1)

2.2. *Plant growth condition and PEG_{6000} treatment*

The randomly selected seeds were surface sterilised using 2% sodium hypochlorite (NaClO) for 10 min and washed 3-5 times in distilled water to remove the traces of NaClO. Further, the seeds were soaked in distilled water for 72 h at RT. The soaked seeds were raised in 16.5 x 12.7 x 20.3 cm plastic pots containing vermiculite (20 seeds per pot) in a growth chamber (**Fig.2.1Aa**). The experiment was designed with two gradients of drought stress; 8% (-0.047 bars) and 16% (-0.107 bars) of Polyethylene Glycol 6000 (PEG_{6000}) (Michel and Kaufmann, 1973) in Hoagland's nutrient solution (pH 6.4) (Hoagland and Arnon, 1950) **(Table 2.1).** The optimum watering with Hoagland's nutrient solution served as control. The growth chamber condition of the rice seedlings was 25 ± 2℃ for a 16 /8 h light/dark period with a mean photosynthetic photon flux density (PPFD) of around 200 μmol $m^{-2}s^{-1}$ provided by cool white fluorescent tubes with the average relative humidity of 65-70%. The plants were allowed to grow for ten days (**Fig.2.1Ab**). The water from the trays was changed every third day to avoid fungal growth.

Table 2.1. Composition of Hoagland's Nutrient Solution for Plant Growth (Hoagland and Arnon 1950).

	Component	*Stock solution (g/L)*	*Stock solution (g/100mL)*	*mL/1L*
Macronutrients	2M KNO_3	202g/L	20.2g/dL	2.5
	2M $Ca(NO_3)_2$•$4H_2O$	236g/0.5L	47.2g/dL	2.5
	Iron (sprint BQ Iron chelate)	15g/L	1.5g/dL	1.5
	2M $MgSO_4$•$7H_2O$	493g/L	49.3g/dL	1
	1M NH_4NO_3	50g/L	5g/dL	1
Micronutrients	H_3BO_3	2.86g/L	0.286 g/dL	1
	$MnCl_2$•$4H_2O$	1.81g/L	0.181g/dL	1
	$ZnSO_4$•$7H_2O$	0.22g/L	0.022g/dL	1
	$CuSO_4$	0.051g/L	0.0051g/dL	1
	Na_2MoO_4•$2H_2O$	0.12g/L	0.012g/dL	1
	1M KH_2PO_4	136g/L	13.6g/dL	0.5
	(pH 6.4)			



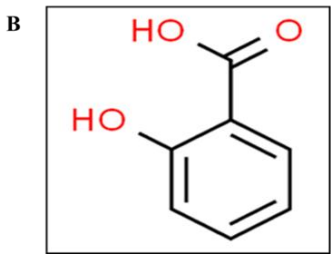


Fig. 2.1: A. Experimental setup with (a) soaked seeds in vermiculite, (b) growth of seedlings on the 10th day and (c) plants on the 17th day after salicylic acid treatment; B. Structure of Salicylic acid (SA).

2.3. *Plant growth regulator treatment*

After ten days of respective treatments viz., control, 8% and 16% PEG_{6000}, the selected rice cultivars were sprayed with 0.1, 0.25, and 0.5 mM Salicylic acid (**Fig. 2.1B**) (2-hydroxybenzoic acid, $C_7H_6O_3$, MW = 138.12 g mol^{-1}, pH 7) dissolved in water at 55°C. Tween-20 of 0.1% (v/v) was added to the spraying solution as a surfactant at the time of spray. Treatments were applied to all the plants on the 11^{th}, 12^{th} and 13^{th} day of the growth stage. A uniform volume (~20 mL/pot) was sprayed on both cultivars with an automiser. Both the rice cultivars were placed separately upon spraying. Three replicates were maintained for each treatment. The effective concentration of SA was preferred for spraying through a series of pilot experiments carried out under 8% and 16% drought stress. The rice seedlings were harvested after three days (17^{th} day) of spraying treatment (**Fig.2.1Ac**), and the following indices were analysed.

2.4. Morphological Assay

2.4.1. Measurement of shoot/root length and biomass

Ten random plants, intact with vermiculite, were gently washed under running tap water to free attached vermiculite from the roots. The extra plant surface water was removed by tapping it on tissue paper.

Biomass analysis: The above and below-ground fresh weight analysis of ten randomly selected plantlets from respective treatments was assessed, and the dry weight was taken by drying the tissue in a hot air oven at 80°C for 48 h (Chen et al., 2014).

Shoot and root measurements for each treatment were taken using a meter scale.

2.4.2. External morphological and anatomical measurements

Morphological analysis of both cultivars under drought stress was carried out to identify the outcomes of stress, which can be reflected on the plant surface or as alterations at the cellular level.

2.4.2.1. External morphology of rice leaves

The external leaf morphology was studied by visualising lyophilised segments of the leaf using a Scanning Electron Microscope (SEM) (ZEISS Evo18) (**Fig.2.2A**) and analysed with INCA viewer software (Oxford Instruments) at Goa University, Goa. The external morphology of the first leaf was used for visualising with SEM. The treated plantlet was placed at -20°C for 24 h in glass beakers. The Cool Safe 110 Freeze Dryer (Fisher Scientific Bio-block) was put on and allowed to reach a temperature of -110°C simultaneously. The vacuum pump was kept on for 30 min with the external valve connecting to the Freeze dryer closed. The samples were then placed on top of the acrylic plate enclosed within an acrylic chamber. The external valve was opened, connecting the freeze dryer to the vacuum pump. It was allowed to undergo sublimation for 4 h.

The external valve was closed, and the vacuum pump was allowed to run for 40 min. The pressure was equalised by opening the release valve at the lid, and the samples were removed, sealed using parafilm and placed in a desiccator until SEM analysis. The leaf's adaxial (upper) and abaxial (lower) sides were carefully placed onto double-sided stick tapes, and the other side was adhered to a specimen mount. This mount was secured to a holder by screws. The stage was set such that the specimen is approx. 50 mm from the bottom of the sputter head.

The leak valve was closed, and the argon pressure was set to 5 psi. The power was switched on, starting the vacuum pump. Once the pressure fell to approximately 600-400 millitorr, the gas leak valve was partially opened to flush the work chamber with argon for about 15 sec. The leak valve was closed, and the work chamber could pump approximately 80 millitorrs. The gas leak valve was opened and adjusted to set the plasma current to 10 mA. A visible discharge was observed in the chamber. The start button was pressed, and gold was sputtered onto the specimen for 110 sec. The plasma was automatically extinguished at the end of the period. The power was switched off, and the air was allowed to enter the chamber using the "vent" valve on the top of the sputter head. The gold-sputtered specimen mount was fixed onto the sample holder in the SEM sample chamber. A vacuum was created, and the SEM was connected to a computer system. The images were obtained at 10 kV and high magnification using the SmartSEM User Interface.

2.4.2.2. Internal morphology of rice leaves and roots

The internal leaf morphology was studied by visualising the leaf and root's transverse section (TS). The freehand TS of leaf and root were prepared from respective plant samples. Cross-sections for the root were taken 20 mm distance above the root tip, whereas the mid-section of the fully expanded first leaf was used. The sections were then stained in saffranine for 1 min. The sections were then submerged in distilled water to remove the excess stain.

Further, the specimens were mounted on a glass slide with a drop of 10% glycerin and delicately placed over the glass cover, ensuring no air bubbles were formed. The slide was wiped off to avoid slide stacking and glycerin seepage with the microscope stage. The examination was completed under an Olympus light microscope and photographed using NIS software (**Fig.2.2B**) (Butterbach-Bahl et al., 2000).

2.5. Physiological assay

2.5.1. Determination of relative water content (RWC)

The standard protocol was used to assess the plant's water status in terms of the physiological consequence of cellular water deficit. Fresh leaves were harvested and weighed randomly, obtaining the fresh weight (FW). Further, the leaves were soaked in distilled water for 24 h at room temperature to get the turgid weight (TW). The leaf samples' dry weight (DW) was attained by placing the samples in the oven at 80 ℃ for 48 h. (Barrs and Weatherley, 1962).

RWC was further calculated using the formula:

$$RWC\ (\%) = \frac{(FW-DW)}{(TW-DW)} \times 100$$

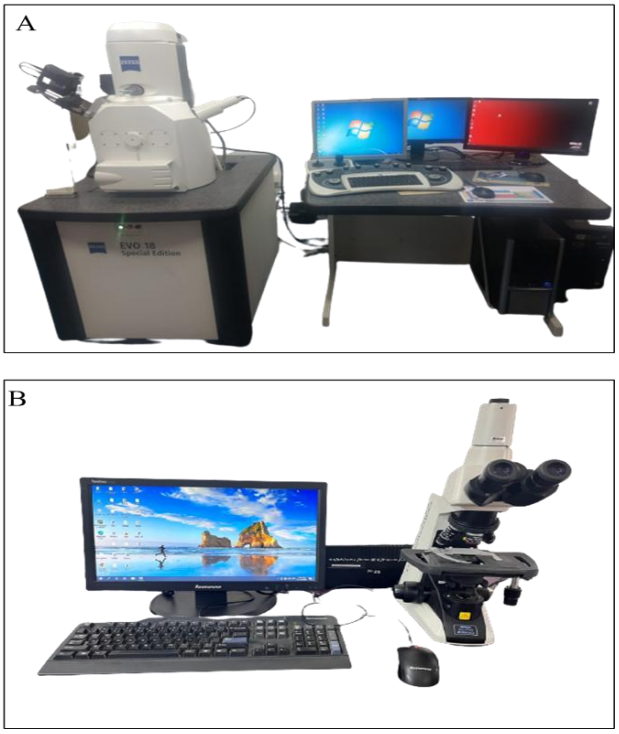


Fig. 2.2: External and Internal morphological study using (A) Scanning Electron Microscope (ZEISS Evo18) with INCA viewer software (Oxford Instruments) and (B) Olympus light microscope with NIS software at Goa University.

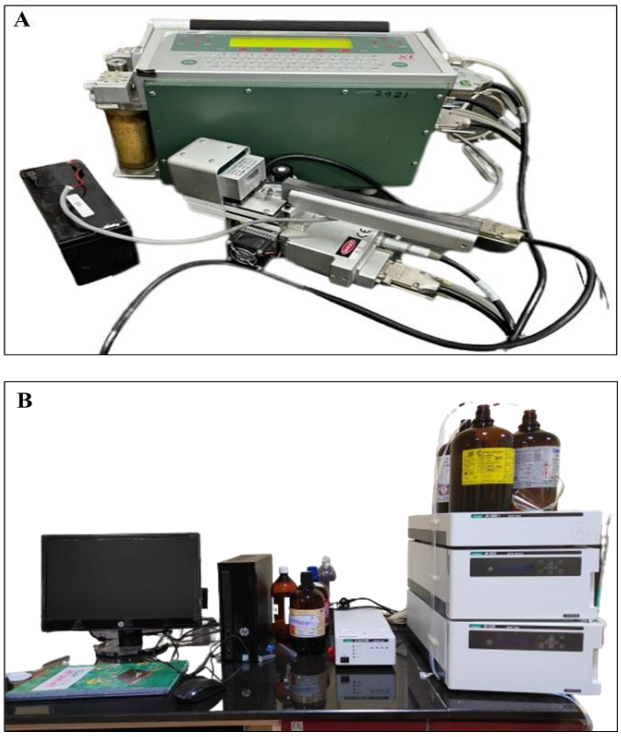


Fig. 2.3: Photosynthetic parameters analysed using (A) Portable Photosynthesis System (Li-6400XT) from ISRO and (B) HPLC system (Jasco HPLC, 4000) at Goa University.

2.5.2. Photosynthetic rate measurements

2.5.2.1. Chlorophyll fluorescence measurements

Photosynthetic efficiency was measured using a chlorophyll fluorometer (Li-6400XT Portable Photosynthesis System) (**Fig.2.3A**). For chlorophyll fluorescence measurements, randomly selected leaves were used. Leaves were adapted to dark for 30 min before measurement. A dark-adapted leaflet was placed in a 2 cm^2 leaf chamber and exposed to the modulated light intensity of 4 µmol $m^{-2}s^{-1}$ to measure the initial chlorophyll fluorescence (Fo). This was followed by exposure to the saturating pulse of the white light of approximately 4000 µmol $m^{-2}s^{-1,}$ which is strong enough to reduce all the PSII reaction centres to provide maximum chlorophyll fluorescence (Fm). The signal was then allowed to stabilise to obtain a steady state of fluorescence (Fs) by exposing the leaf to the actinic light intensity of 800 µmol $m^{-2}s^{-1}$. Another

pulse of saturating light was given to measure secondary maxima of fluorescence (F'm), following a steady state F'o (minimum chlorophyll fluorescence in the light-saturated state), which was measured by exposing the leaflet to far-red radiation of 7 μmol m^{-2} s^{-1}. Variable fluorescence (Fv) was determined by subtracting Fo from Fm, i.e. (Fv = Fm-Fo), and the Fv/Fm ratio, indicative of photosynthetic efficiency, was calculated. The quantum efficiency of PSII open centres in the light-adapted state, ΦPSII, was determined from F'm and Fs values as expressed by Genty et al. (1989).

Maximum quantum efficiency of photosystem II

$$F_v/F_m=(F_m-F_0)/F_m$$

Quantum efficiency of PSII

$$\Phi_{PSII}= (F_m'-F_s)/F_m'$$

Photochemical quenching co-efficient

$$q_P = (Fm'-Fs)/(Fm'-F_0)$$

2.5.2.2. Gas exchange measurements

Gas exchange measurement is the most common technique used to measure photosynthesis. It provides a direct measure of the net rate of photosynthetic carbon assimilation. The photosynthetic gas exchange system consists of a leaf chamber, flow meter and means of generating and controlling airflow over the leaf. The signal from the sample cell is compared to the zero gas reference signals, providing an absolute measurement of CO_2 concentration. A leaf of 2 cm^2 is enclosed in a chamber sealed to prevent gas exchange with the atmosphere, and the rate at which CO_2 and H_2O concentration changes were monitored. Photosynthetic rate (A; μmol m^{-2} s^{-1}), Stomatal conductance ($g_{s;}$ m^2 mol^{-1}), Transpiration rate (E; mol H_2O m^{-2} s^{-1}),

Carboxylation efficiency (MC; µmol m^{-2} s^{-1}), Water use efficiency (WUE; µmol mmol $^{-1}$) and Internal CO_2 concentration (Ci; µmol m^{-1}) were measured (Murchie and Lawson, 2013).

2.5.3. Analysis of photosynthetic pigments using HPLC

2.5.3.1. Extraction of photosynthetic pigments

Fresh leaf tissue (0.2 g) was ground in a pre-cooled mortar pestle using liquid nitrogen. The fine powder was suspended in 100% acetone (Merck, HPLC grade) containing a few crystals of Butylated Hydroxy Toluene (BHT), an antioxidant, making a final volume of 2 mL and incubated overnight at 4°C. The blend was centrifuged (Eppendorf® Centrifuge 5804R) for 10 min at 4,000 *x g* at 4°C. The obtained supernatant was passed through a Nylon filter of 0.45 µm (Whatman Uniflo) (Pocock et al., 2004).

2.5.3.2. Separation of photosynthetic pigments

Filtrate of 10 µL volume was injected in the HPLC system (Jasco HPLC, 4000) (**Fig.2.3B**) using a four-solvent gradient mobile phase composed of solution **A** (Water), solution **B** (Methanol), solution **C** (Ethyl acetate) and solution **D** (Acetonitrile). The complete HPLC solvent system programme is mentioned in **Table 2.2**. The mobile phase flow rate was controlled at 1.5 mL min^{-1} for a run time of 20 min. C18 column was used to separate the pigments (4.6 mml D x 250 mm L x 5 µm), and the peaks were detected at 445 nm. The quantification of separated pigments was carried out using β-carotene as an external standard. Identification of pigments was carried out using retention time against standards and spectral profile of individual peaks using UV detector in the 400-700 nm range.

Table 2.2: HPLC solvent system programme for plant pigments

Time (min)	*Flow rate (mL min^{-1})*	*A (mL) (Water)*	*B(mL) (Methanol)*	*C(mL) (Ethyl acetate)*	*D(mL) (Acetonitrile)*
0.5	1.5	0	12.5	0	87.5
2	1.5	0.4	9.6	20	70
3	1.5	0.2	6	50	43.8
12	2	0.2	6	50	43.8

2.6. Biochemical assay

2.6.1. Quantification of reactive oxygen species accumulation:

2.6.1.1. Determination of hydrogen peroxide (H_2O_2)

The total hydrogen peroxide (H_2O_2) content was estimated according to Sagisaka (1976). Concisely, 0.2 g leaf tissue was suspended in 5% (w/v) Trichloroacetic acid (TCA) followed by centrifugation (Eppendorf® Centrifuge 5804R). The reaction mixture contained supernatant, 2.5 mM potassium thiocyanide and 10 mM ferrous ammonium sulfate; the optical density of the supernatant was considered at 480 nm using an ultraviolet (UV) visible spectrophotometer (UV 2450, Shimadzu). The level of H_2O_2 was expressed as a per cent increase over control.

2.6.1.2. Determination of hydroxyl radical (OH•)

In this method, OH• radical degrade the deoxyribose, forming the **t**hio**b**arbituric **a**cid **r**eactive **s**ubstances (TBA-RS). The formation of the thiobarbituric acid reactive substances (TBA-RS) on reduced deoxyribose by hydroxyl radical (OH•) was spectrophotometrically detected at 532 nm (Liu et al., 2009). Fresh leaf tissue of 0.2 g was homogenised in 1.2 mL of 50 mM sodium phosphate buffer (pH 7.0) and centrifuged at 10000 $\times g$ for 10 min at 4ºC. A reaction mixture

containing 0.5 mL supernatant, 0.5 mL of 50 mM sodium phosphate buffer (pH 7.0) and 1 mL of 25 mM sodium phosphate buffer containing 2.5 mM 2-deoxyribose was incubated at 35°C for 1 h in the dark. After incubation, 1 mL of 1% **t**hio**b**arburtric **a**cid (TBA, Sigma USA) and 1 mL of glacial acetic acid were added and boiled for 10 min. The reaction was cooled immediately on ice for 10 min, and absorbance was recorded. An increase in OH• concentration is directly related to an increase in absorbance, expressed as Abs. Units (Absorbance x 1000).

2.6.2. Oxidative damage caused by ROS

2.6.2.1. Determination of lipid peroxidation

The level of lipid peroxidation was estimated by measuring the malondialdehyde (MDA). The leaf sample (0.5g) was homogenised with 5 mL of 1% trichloroacetic acid (TCA) in an ice bath. The homogenate was centrifuged at 2000 rpm for 10 min, and the supernatant was used for lipid peroxidation analysis. To the 1mL aliquot of the supernatant, 2.5 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA and 2.5 mL of incubation buffer consisting of 150 mM NaCl, 50 mM Tris HCl (Himedia, India) were added. The mixture was incubated for 30 min at 95°C followed by cooling at room temperature. MDA content was detected spectrophotometrically using an ultraviolet (UV) visible spectrophotometer (UV 2450, Shimadzu) at 532 nm and corrected for nonspecific turbidity at 600 nm (Sankhalkar and Sharma, 2002). MDA concentration is expressed as nmol g^{-1} fresh weight of tissue using the extinction coefficient of 155 mM^{-1} cm^{-1}.

2.6.2.2. Determination of electrolyte leakage (EC)

Overall, ion leakage from completely expanded leaves was determined by Shi et al. (2006). Approximately 20 leaf discs were rinsed in glass vials containing 50 mL of distilled water to wash off the leaked electrolytes during excision. The electrical conductivity (EC_1) was measured

by dark incubating the vials for 24 h at room temperature using Water-Proof PCTestr 35 pH/Conductivity. To attain electrical conductivity (EC_2), the vials were heated at 95℃ for 30 min and cooled. The per cent electrolyte leakage was calculated using the formula:

$$(EC_1/EC_2) \text{ X } 100$$

2.6.2.3. Protein oxidation assay

To quantify the protein-bound carbonyls, a biomarker of oxidative stress was measured as described by Levine et al. (1990). 0.5 g of leaf tissue was extracted and homogenised with 30% (w/v) TCA, followed by centrifugation at 3000 rpm for 10 min. 500 µL supernatant was redissolved with an equal volume of 10 mM 2,4-Dinitrophenylhydrazine (DNPH) in 2 M hydrochloric acid (HCl). Further, on 1 h incubation at room temperature, the reagent mixture was given three washes with 500 µL of 1:1 ethanol/ethyl acetate (v/v). The pellets were finally dissolved in 6 M urea in 20 mM potassium phosphate buffer (pH 2.5). The absorbance of the stable yellow-coloured product formed was measured at 280 nm. Carbonyl content for aliphatic hydrazones was calculated using the molar absorption coefficient, 22000, expressed as nmol mL^{-1}.

2.6.2.4. Determination of fatty acid lipid profile using gas chromatography/high-resolution mass spectrometry (GC/HRMS)

2.6.2.4.1. Lipid extraction and FAME preparation

Total lipid extraction was carried out with a slight modification of the protocol described by Turnham and Northcote (1982). Fresh leaf tissue (0.5 g) was boiled over a flame with isopropanol to remove excess chlorophyll, and the tissue was ground to a fine powder using liquid nitrogen. Chloroform and methanol in the ratio of 1:2 (v/v) were used for the extraction.

The residue was again washed with 1:2 v/v chloroform and methanol on decanting the supernatant. The obtained supernatant was centrifuged at 5000 rpm for 5 min.

The reaction mixture was made up to 15 mL for saponification upon adding 4 mL distilled water, 5 mL chloroform and 5 mL KCl solution. Separation was carried out using a separating funnel, and the process was repeated four times. The solvent phase was pooled and dried by flushing it with nitrogen gas, and the residue was dissolved in 1 mL chloroform and stored overnight at –20°C.

For acidification, the sample was again dried and dissolved in 5 mL of MeOH–HCl (hydrochloric acid in methanol) and 10 µL of heptadecanoic acid was added as internal standard, and vials were incubated at 70–80°C in an oven for 2 h. Then distilled H_2O (5 mL) and 2 mL hexane were added, followed by cooling and vortexing for 90 s, and it was allowed to separate; the separated hexane was fractionated in another tube. These steps were repeated three times, and the fractionated hexane layer was pooled together. Then, 5 mL of saturated sodium bicarbonate was added to the hexane and vortexed. The mixture was allowed to settle, and hexane was collected in a fresh tube. Further, 5 mL of distilled water was added to the tube and vortexed for 15 s. Hexane was sequentially collected in a fresh tube. Anhydrous hexane fraction was dried on flushing the nitrogen gas and dissolved in 100–200 µL of hexane before analysis.

2.6.2.4.2. Lipid profile analysis using GC/HRMS

The profiling of resultant FAME samples (1 µL) was analysed using gas chromatography-high resolution mass spectroscopy (Agilent 7890/Joel AccuTOF GCV) equipped with an Elite-5 MS Silica Capillary column (30 m × 0.25 mm) and Flame Ionisation Detector. The sample (1 µL) was injected, and the helium (99.999%) was used as a carrier gas at a 1 mL/min flow rate. The

detection temperature was 255°C, whereas the injection temperature was set at 250°C. The temperature profile of the oven was set as follows: At first, the temperature was set at 70°C for 1 min, then increased to 5°C per min until 100°C; this temperature was held for 2 min, then increased by 10°C per min to 175°C was held for 34 min and again increased by 4°C per min to 225°C and further held at this temperature for 29 min. The line pressure was set at 40 p.s.i. during the first 52.5 min and then increased by 0.5 p.sp.i. per min to 45 p.s.i. and held until the run finished, the MS condition included an EI ion source temperature of 230°C, the ionisation energy of 70 eV, and a mass scan range of 35–800 amu.

2.6.2.4.3. Peak identification

The separated compounds were tentatively identified by comparing their mass spectra with those in the NIST08 MS LIBRARY (National Institute of Standard and Technology, United States) and comparing their retention indices (RIs). Each component was quantified based on relative to the peak area of the internal standard, and the content is expressed as the mg g^{-1} FW.

2.7. Measurement of enzymatic antioxidants

2.7.1. Preparation of enzymatic extract

Fresh leaf tissue (0.5 g) was ground using liquid nitrogen in an ice bath. Extraction of the enzymes was done by placing the leaf powder in a 2 mL phosphate buffer (50 mM, pH 7) containing 1 mM Ethylenediamine Tetra acetic Acid (EDTA) and 1% Polyvinylpyrrolidone (PVP). The homogenised tissue was centrifuged at 15,000 g for 15 min at 4°C. The supernatant was used to assess the antioxidant enzyme activity. All enzyme preparations were estimated for protein content according to Bradford (1976) using bovine serum albumin (BSA, Sigma) as standard.

2.7.2. Determination of SOD antioxidant activity

Superoxide dismutase (EC 1.15.1.1) activity was estimated by assessing its competence to curtail nitroblue tetrazolium (NBT) photo-reduction by forming purple formazone. The 3 mL reaction mixture contained 75 µM NBT, 2 µM riboflavin, 13 mM methionine, 50 mM phosphate buffer (pH 7.8) and 100 µL extract. The addition of the contents mentioned above to the reaction mixture was carried out in dark conditions. Further, the reaction was illuminated for 30 min under a cool white fluorescent lamp. The absorbance of the reaction mixture was read at 560 nm. The reaction mixture without the enzyme extract served as blank. The amount of enzyme essential to cause 50% inhibition of the NBT photoreduction rate is defined as one unit (U) of SOD activity, expressed as U mg^{-1} protein (Beauchamp and Irwin Fridovich, 1971).

2.7.3. Determination of GR antioxidant activity

Glutathione reductase (EC 1.6.4.2) was determined by evaluating the oxidation of NADPH at 340 nm at 30°C as per the method of Schaedle and Bassham (1977). The 1 mL reaction mixture contained 50 mM phosphate buffer containing 2 mM EDTA (pH 7.8), 2 mM Nicotinamide Adenine Dinucleotide Phosphate (NADPH), 20 mM oxidised glutathione (GSSG) and 10 µL enzyme extract. The addition of the reductant, NADPH, initiated the reaction. The enzyme activity was expressed as U mg^{-1} protein.

2.8. Non-enzymatic antioxidants

2.8.1. Determination of GSH content

The antioxidant metabolite, Glutathione content, was spectrophotometrically assessed according to Griffith (1980). 1 mL of supernatant was treated with a precipitating solution for 10 min, followed by filtration. 2 mL phosphate and 250 µL 5,5'-dithio-bis-(2-nitrobenzoic acid)

(DTNB) solution was added to 500 µL filtrate. The sample without the filtrate served as blank. The absorbance was recorded at 412 nm of the yellow colour formed. Total glutathione content (µg mL^{-1}) was obtained through graphical calculation and multiplied with its respective dilution factor.

2.9. Osmolyte accumulation

2.9.1. Proline estimation

The proline content in the fresh leaf tissue was determined by adopting the ninhydrin method of Bates et al. (1973). 0.5 g of leaf tissue was homogenised in 5 mL of 3% aqueous sulfosalicylic acid, followed by centrifugation at 5000 rpm for 5 min. 1 mL of supernatant was mixed with 1 mL of acid ninhydrin (1.25 g Ninhydrin, 30 mL Glacial acetic acid, 20 mL 6 M Phosphoric acid) and 1 mL glacial acetic acid. The mixture was heated at 90°C for an hour, followed by cooling at room temperature. The developed colour was extracted in 5 mL of toluene by mixing vigorously. The mixture was allowed to settle, and the absorbance at 520 nm was measured against toluene using an ultraviolet (UV) visible spectrophotometer (UV 2450, Shimadzu). A standard curve with L-proline was used to quantify the concentrations obtained.

2.10. Gene expression studies

For the gene expression analysis, the leaf tissue was collected from both cultivars over 72 h post-SA treatment.

2.10.1. RNA extraction and quantification

Total RNA from the fresh leaf tissue (0.1 g) was isolated using 1 mL TRI reagent (RNA-Xpress Reagent, HiMedia); following that, the homogenate was centrifuged at 12,000 *x g* for 15 min

at 4℃ to eliminate the insoluble materials. 500 µL of chloroform was added to the supernatant to ensure complete dissociation of nucleoprotein complexes. The contents were vigorously mixed for 15 s and allowed to stand at RT for 10 min, and then the mixture was centrifuged at 12,000 *x g* for 15 min at 4℃. The colourless upper aqueous phase was transferred to a fresh Eppendorf tube, and an equal volume of isopropanol was added and mixed. Further, the mixture was centrifuged at 12,000 *x g* for 10 min at 4℃. The RNA pellet was washed by adding 1 mL of 75% ethanol and centrifuged at 7,500 *x g* for 5 min at 4℃. The pellet was washed thrice. Furthermore, the pellet was dried at RT and dissolved in 25 µL of DEPC-treated water. The RNA was quantified using a UV-Vis spectrophotometer at 260 and 280 nm. RNA concentration was calculated using the formula:

$$\text{Concentration} = \text{Abs}\,(260) \times 40 \times 100$$

2.10.2. cDNA synthesis

cDNA synthesis was performed in a thermocycler (MiniAmp^{TM}Plus Thermal cycler) using the RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific) following the specified protocol (**Fig.2.4A**). To remove genomic DNA, the obtained RNA was treated with 1 µL DNase I, RNase-free (Thermo Scientific), 1 µL 10X reaction buffer containing $MgCl_2$ and nuclease-free water, making up the volume to 10 µL. The mixture was incubated at 37℃ for 30 min. Further, 1 µL of 50 mM EDTA was added to prevent RNA hydrolysis during heating and incubated at 65℃ for 10 min. In a nuclease-free tube, 1 µg of RNA and 1 µL primer Oligo $(dT)_{18}$ were combined with nuclease-free water, making the volume to 12 µL, mixed gently and incubated at 65℃ for 5 min and immediately snap chilled on ice. Further, a master mix of 8 µL containing- 4 µL 5X reaction buffer, 1 µL of RiboLock RNase Inhibitor (20 U/µL), 2 µL 10 mM dNTP Mix and 1 µL RevertAid M-MuLV RT (200 U/ µL), was added with a gentle mix

and brief spin was followed by incubation at 42℃ for 60 min for cDNA synthesis. The reaction is terminated by heating at 70℃ for 5 min. The reverse transcription product was either directly used for PCR/qPCR with 8X dilution or stored at -20℃ for less than a week.

2.10.3 Primer designing

Gene-specific primers were designed using Primer3 Plus software based on *Oryza sativa* genomic sequences available at the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) and synthesised by Eurofins Genomics. The list of synthesised primers with their Locus ID for the genes of APX, SOD, K^+ Transporter, and Aquaporin from the Rice Genome Annotation Project is mentioned in **Table 2.3**.

2.10.4. Primer stock solution

Tris-EDTA (TE) Buffer was prepared by dissolving 15.8 mg TE buffer powder (Duchefa Biochemie) in 10 mL sterile distilled water, which was used to prepare 100 µM primer stock solutions. The stock solutions were stored at -20ºC until future use. The primers were diluted to 10 µM (5 µL of 100 µM primer stock solution in 45 µL sterile distilled water) to use for RT-PCR and qRT-PCR analysis.

Table 2.3: Primer sequences for real-time polymerase chain reaction analysis

Sr. No.	Gene	Locus ID	Left primer	Right primer
1.	*ACTIN*	***OsActin***	GCCTCAGTCAGCAACACAGG	CGGTGTGATGGTTGGTATGG
2.	*APX1*	***LOC_Os03g17690***	CCGTCTTCCTGATGCTACCA	TAGGAAGCTGAAGAAGGCCC
3.	*Cu,Zn SOD*	***LOC_Os03g11960***	CTCTACCGGGCCCCATTTTA	CTGAGTTCATGACCACCCCT
4.	*AQP TRANSPORTER*	***LOC_Os03g05290***	GCTGGAGATCGTCATGACCT	CCAACCCAGTACACCCACT
5.	*K^+ TRANSPORTER*	***LOC_Os04g32920***	TACGGGATCTGTGTGGTGAC	CATCGTCATCAGCACCATGG

2.10.5. Real-Time analysis

Real-time analysis of gene expression involved in the drought stress (APX, SOD, K^+ Transporter and Aquaporin) and the housekeeping gene (Actin) was performed using CFX96^{TM} Real-Time System with Bio-Rad CFX Maestro software **(Fig. 2.4B)**. All real-time PCR runs were performed in triplicates. Real-time PCR was conducted in a 20 µL reaction volume containing 0.25 µL primers (final concentration µM), 13.5 µL Nuclease-free water, 4 µL q•EvaGreen® (qARTA Bio Inc.) and 2 µL of the diluted synthesised cDNA template (1:8 ratio) was added in a 96-well PCR plate (Applied Biosystems) sealed with optical film. The PCR reaction was performed as follows: Initial denaturation at 95°C for 3 min followed by 39 cycles of denaturation at 95ºC for 15 s, annealing at 55°C for 40 s and extension at 65ºC for 5 s, followed by melting curve analysis: 95ºC for 5 min. Each gene's relative expression levels were calculated using the $2^{-\Delta\Delta C}{}_T$ quantification method (Livak and Schmittgen, 2001).

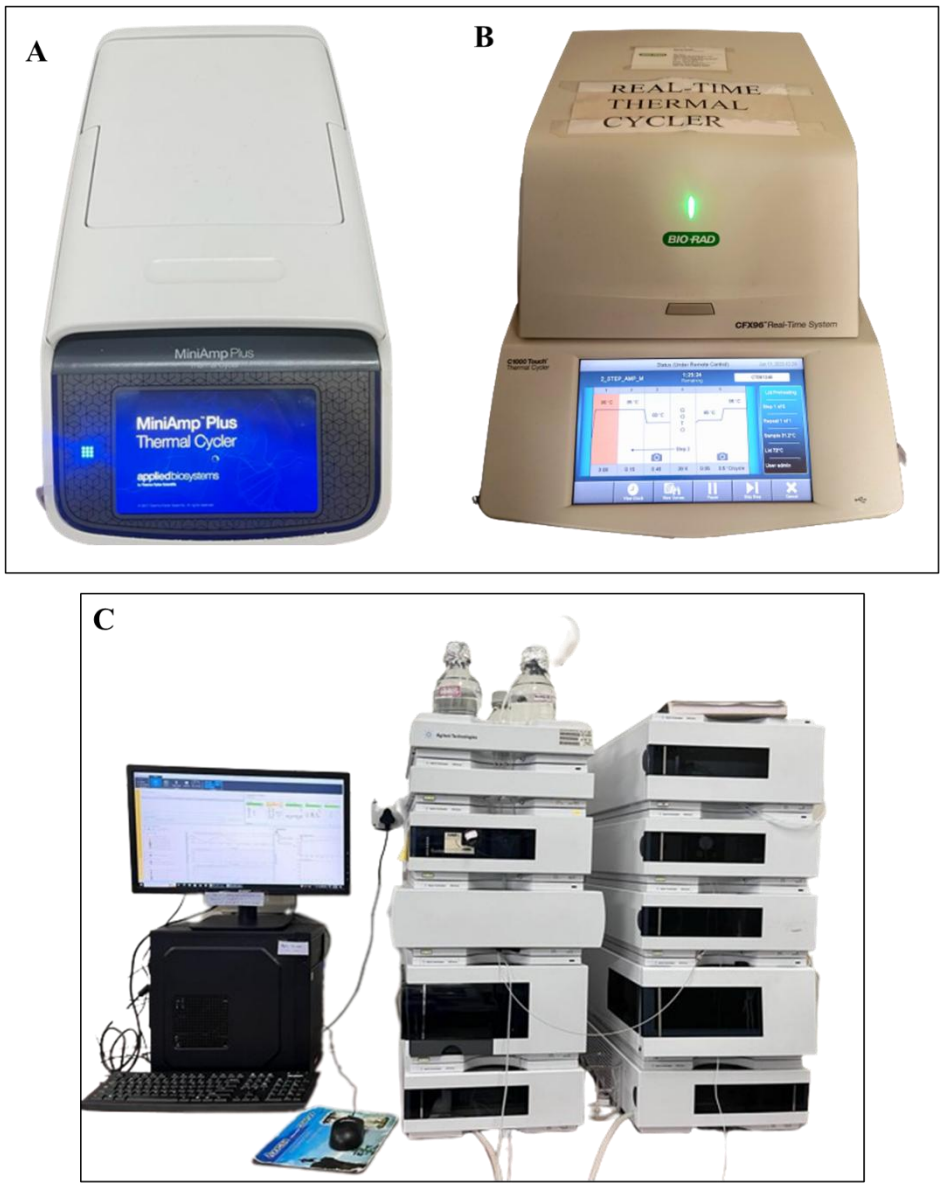


Fig. 2.4: A- MiniAmp^{TM}Plus Thermal cycler used for cDNA synthesis, B- CFX96^{TM} Real-Time System with Bio-Rad CFX Maestro software used for real-time studies at Goa University, and C- Agilent (1200) HPLC system for endogenous salicylic acid estimation at NCPOR, Goa.

2.11. Endogenous Salicylic Acid analysis using HPLC

2.11.1. Extraction of free salicylic acid (SA)

Fresh leaf tissue (0.5g) was ground in a pre-cooled mortar and pestle to a fine powder using liquid nitrogen. The powder was transferred to a 2 mL microcentrifuge tube of 1.6 mL of 70% ethanol (v/v) and 32 µL *o*-anisic acid (OAA; internal standard) vortexed for a minute. On centrifugation at 10,000 *x g* (Eppendorf® Centrifuge 5804R) for 10 min at RT, the supernatant was collected, and the pellet was resuspended in 1.6 mL of 90% methanol (v/v), followed by re-extraction on vortexing for 1 min. The mixture was centrifuged for 10 min for 10,000 *x g* at RT. The obtained supernatant was pooled together and contained the free SA. The pooled supernatant was evaporated to dryness in a vacuum concentrator for ~1.5 h, followed by adding 65 µL of 20% aq. TCA (w/v). To this mixture, 650 µL of ethyl acetate and cyclohexane (1:1 v/v) was added and vortexed for a minute, followed by 2 min centrifugation for 10,000 *x g* at RT for phase separation. The organic (upper) phase was collected, and the aqueous phase was re-extracted with the same amount of ethyl acetate and cyclohexane by centrifugation. The organic phases were pooled together and evaporated the solvents to dryness in a vacuum concentrator for ~8 h. Before loading the sample for HPLC analysis, the dried residue was dissolved in 100 µL of 10% aq. Methanol (v/v) containing 0.1% aq. TFA (v/v) and vortexed (Allasia et al., 2018).

2.11.2. Quantification of SA by HPLC

The 20 µL sample was separated into the HPLC system (Agilent, 1200) with an OpenLAB CDS workstation (**Fig.2.4C**). A linear gradient of aq. Methanol from 10% to 82% containing 0.1% (TriFluoro Acetic acid) TFA on a C18 Column (ZORBAX 300 XDB C18 4.6 x 250 mm, 5 µm) at a flow rate of 1 $mLmin^{-1}$ for 30 min. The column was maintained at 30°C in a set oven. The

eluates passed through the diode array detector followed by the fluorescence detector. The presence of SA and the internal standard validation were carried out using UV spectra. The quantification (ng) of each peak was obtained with fluorimetric detection (excitation: 305 nm; emission: 407 nm) (Allasia et al., 2018).

2.12. Statistical analysis

Based on independent determinations, figures present data as the average value ± standard deviation (SD). Statistical analysis of all data sets was further subjected to the One-way Analysis Of VAriance (ANOVA) and Duncan's Multiple Range Test (DMRT) to confirm the variability of the results between treatment groups at 5% probability to assess the significant value using IBM® SPSS® (SPSS Inc., IBM Corporation, New York, NY, USA) Statistics Version 26 (2019).

In the present chapter, significant observations were made on the overall changes in plant's morphological, physiological, biochemical and molecular processes in the two high-yielding selected rice cultivars, drought-tolerant (Sahbhagi Dhan) and drought-sensitive (Jaya), in response to the interactive effect of PEG_{6000}-induced drought stress (8% PEG_{6000} -moderate drought; 16% PEG_{6000} -severe drought) and varying concentrations (0.1, 0.25 and 0.5 mM) of foliar applied SA at the early vegetative stage. The main goal of this study is to compare the drought-tolerant and drought-sensitive cultivars to understand better and clarify the primary mechanism underlying drought tolerance. It also seeks to understand and identify the relationship between physiological, biochemical, and molecular traits that could be used to develop rice cultivars that can thrive in drought-prone areas.

3.1. Morphological measurements

The interactive influence of PEG_{6000}-induced moderate/severe drought and 0.1, 0.25, and 0.5 mM foliar applied SA on growth, biomass, and external/internal morphological changes of the selected DT and DS rice cultivars were investigated on the 17^{th} day after SA treatment.

3.1.1. Measurement of shoot and root length

A) Shoot length

A significant decrease in shoot length was observed with increased drought magnitude. The data obtained is presented in **Table 3.1; Fig.3.1, 3.2a, b.** The shoot length of plants exposed to moderate stress decreased by 35% in DT and 49% in DS cultivars compared to well-watered control. However, it was observed that the exogenous application of SA

significantly decreased the negative impacts of moderate drought on the shoot length in both cultivars. The maximum increase in shoot length in DT (37%) and DS (58%) cultivars was observed at 0.25 mM concentration of SA. In comparison, 0.1 mM and 0.5 mM concentrations increased by 20% and 28% in the DS cultivar. However, at the same concentrations, the shoot length decreased by 3% and 11% in the DT cultivar compared to its control.

Furthermore, the shoot length of plants exposed to severe drought stress decreased by 52% in DT and 57% in DS cultivars compared to well-watered control. However, the exogenous application of SA significantly decreased the negative impacts of severe drought stress on the shoot length in both cultivars. The maximum increase in shoot length was recorded in DT (49%) and DS (41%) cultivars at 0.25 mM concentration of SA. The SA concentration of 0.1 mM recorded an increase of 6% in DT and 13% in DS, and 0.5 mM showed an increase of 23% in DT and 8% in DS over control.

B) Root length

Results on changes in root length caused by the effects of moderate and severe drought and SA treatments are depicted in **Table 3.1; Fig. 3.1, 3.2a, b**. The results indicate that different treatments significantly influence the development of roots. Exposure to drought caused a significant increase in root length in DT and DS rice plants. The root length of plants exposed to moderate stress increased by 153% in DT and 125% in DS cultivars compared to well-watered control. A further increase of 24% in root length was observed only in the DS cultivar at 0.25 mM concentration of SA. Whereas 0.1 mM and 0.5 mM showed an increase of 14% and 15%, respectively, in DS compared to control. However, no further

increase was recorded in the DT cultivar on the foliar application of SA.

Moreover, the root length of plants exposed to severe drought stress increased by 175% in DT and 109% in DS cultivars compared to well-watered control. The maximum increase in root length was observed at 0.25 mM concentration of SA by 5% in the DS cultivar over control. However, no further increase was seen in the DT cultivar on foliar application of SA. Moreover, 0.1 mM concentration of SA decreased the root length by 32% in DT and 3% in DS cultivars, while 0.5 mM concentration decreased by 26% in DT and 4% in DS cultivars compared to the control. However, the exogenous application of SA showed no significant root growth on exposure to severe drought stress in both cultivars.

Overall, among the varying concentrations of SA used, 0.25 mM markedly increased the shoot and root growth under moderate and severe drought stress in both DT and DS cultivars compared to the control, followed by 0.5 and 0.1 mM concentrations.

Table 3.1: Influence of drought stress (moderate and severe) and exogenously applied salicylic acid (SA) on growth traits (shoot/root length) of drought tolerant (DT) and drought-sensitive (DS) rice cultivars. Data represent mean values ± SD (n=10). Different alphabets among the treatments denote significance at a 5% level.

Treatments		*Growth traits*			
Drought	*SA*	*Drought tolerant*		*Drought sensitive*	
		Shoot length (cm)	*Root length (cm)*	*Shoot length (cm)*	*Root length (cm)*
Well-watered	0 mM	22.45 ± 2.2^{f}	6.50 ± 0.8^{a}	23.65 ± 2.6^{f}	7.25 ± 1.9^{a}
Moderate		15.35 ± 1.1^{cd}	18.35 ± 1.0^{d}	11.50 ± 1.4^{b}	14.6 ± 1.7^{bc}
Severe		11.35 ± 1.8^{a}	17.85 ± 2.6^{d}	9.60 ± 1.2^{a}	15.15 ± 2.4^{c}
Well-watered	0.1 mM	20.70 ± 1.9^{e}	8.55 ± 1.6^{a}	23.40 ± 2.0^{f}	7.17 ± 1.1^{a}
Moderate		14.85 ± 1.9^{c}	16.45 ± 1.8^{cd}	13.75 ± 1.0^{c}	16.7 ± 0.7^{cd}
Severe		12.05 ± 1.6^{ab}	12.20 ± 1.3^{b}	10.80 ± 1.1^{ab}	14.75 ± 2.0^{c}
Well-watered	0.25 mM	28.35 ± 2.1^{g}	8.00 ± 0.9^{a}	28.60 ± 1.6^{g}	8.30 ± 0.9^{ab}
Moderate		21.10 ± 1.7^{e}	17.05 ± 1.4^{cd}	18.15 ± 0.9^{d}	18.15 ± 1.8^{d}
Severe		16.95 ± 1.0^{d}	14.25 ± 2.0^{b}	13.50 ± 0.7^{c}	15.95 ± 2.4^{cd}
Well-watered	0.5 mM	21.50 ± 2.3^{ef}	7.20 ± 1.5^{a}	22.30 ± 2.3^{ef}	10.45 ± 2.0^{b}
Moderate		13.70 ± 1.7^{bc}	14.750 ± 0.6^{c}	14.10 ± 1.2^{c}	16.75 ± 1.8^{cd}
Severe		13.95 ± 1.9^{c}	12.80 ± 1.4^{b}	10.40 ± 0.9^{ab}	14.55 ± 1.7^{c}

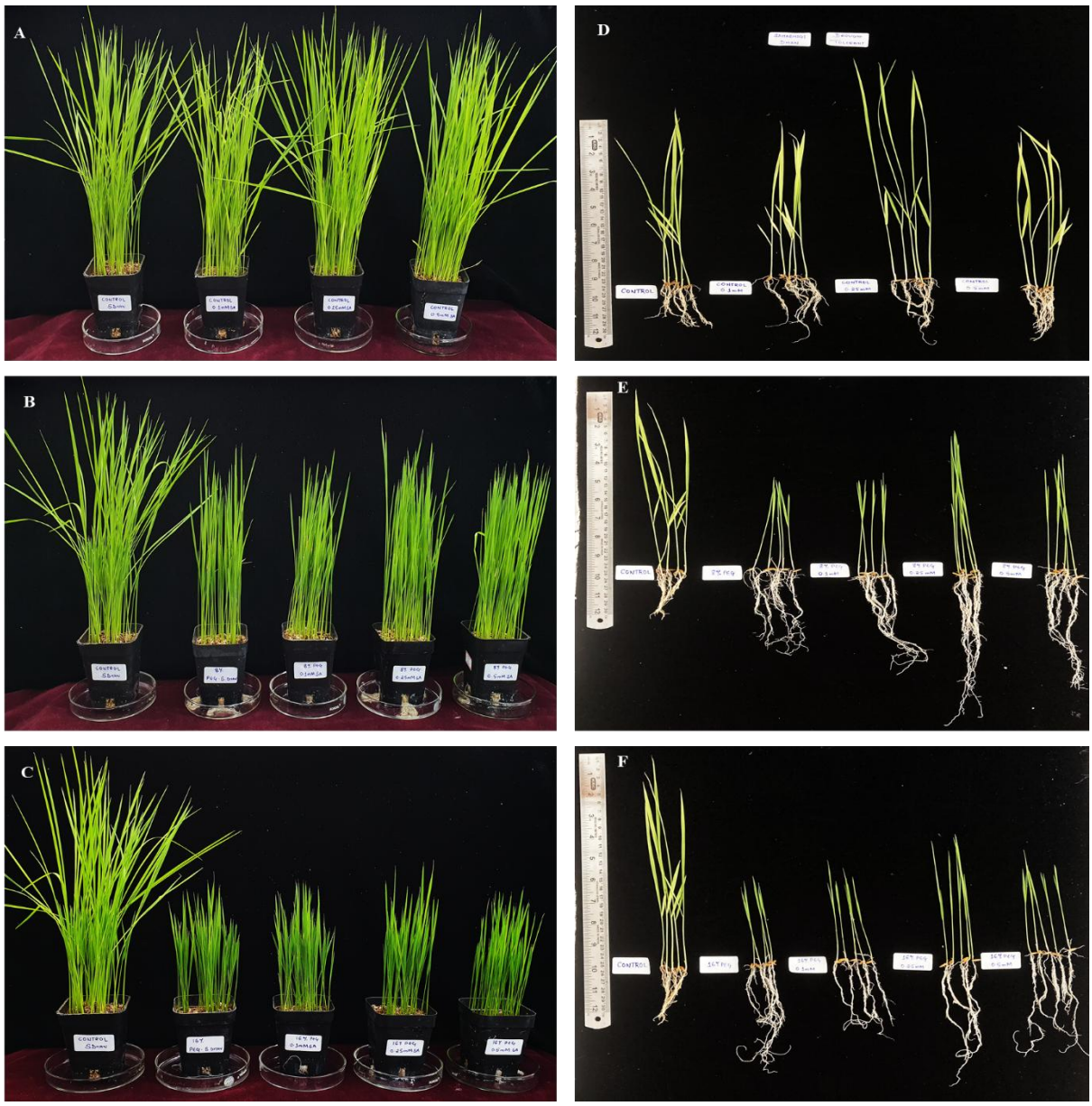


Fig. 3.1a: Photograph showing growth (**A-C**) and shoot and root length (**D-F**) of drought-tolerant (DT) cultivars exposed to well-watered control (**A, D**), moderate stress (**B, E**) and severe stress (**C, F**) and combined effect of drought with varying concentrations of salicylic acid (SA).

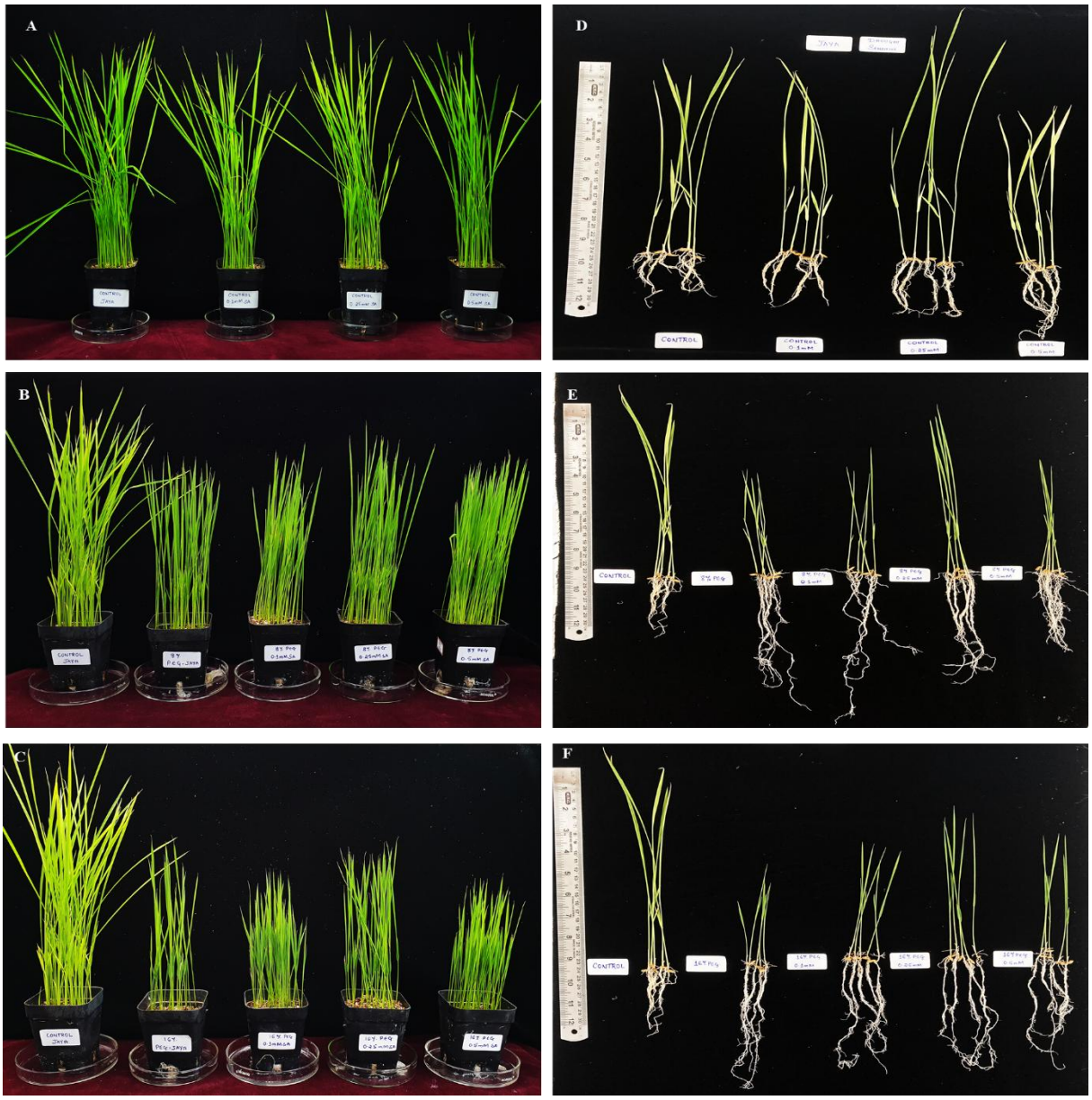


Fig. 3.1b: Photograph showing growth (**A-C**) and shoot/root length (**D-F**) of drought-sensitive (DS) cultivars exposed to well-watered control (**A, D**), moderate stress (**B, E**) and severe stress (**C, F**) and combined effect of drought with varying concentrations of salicylic acid (SA).

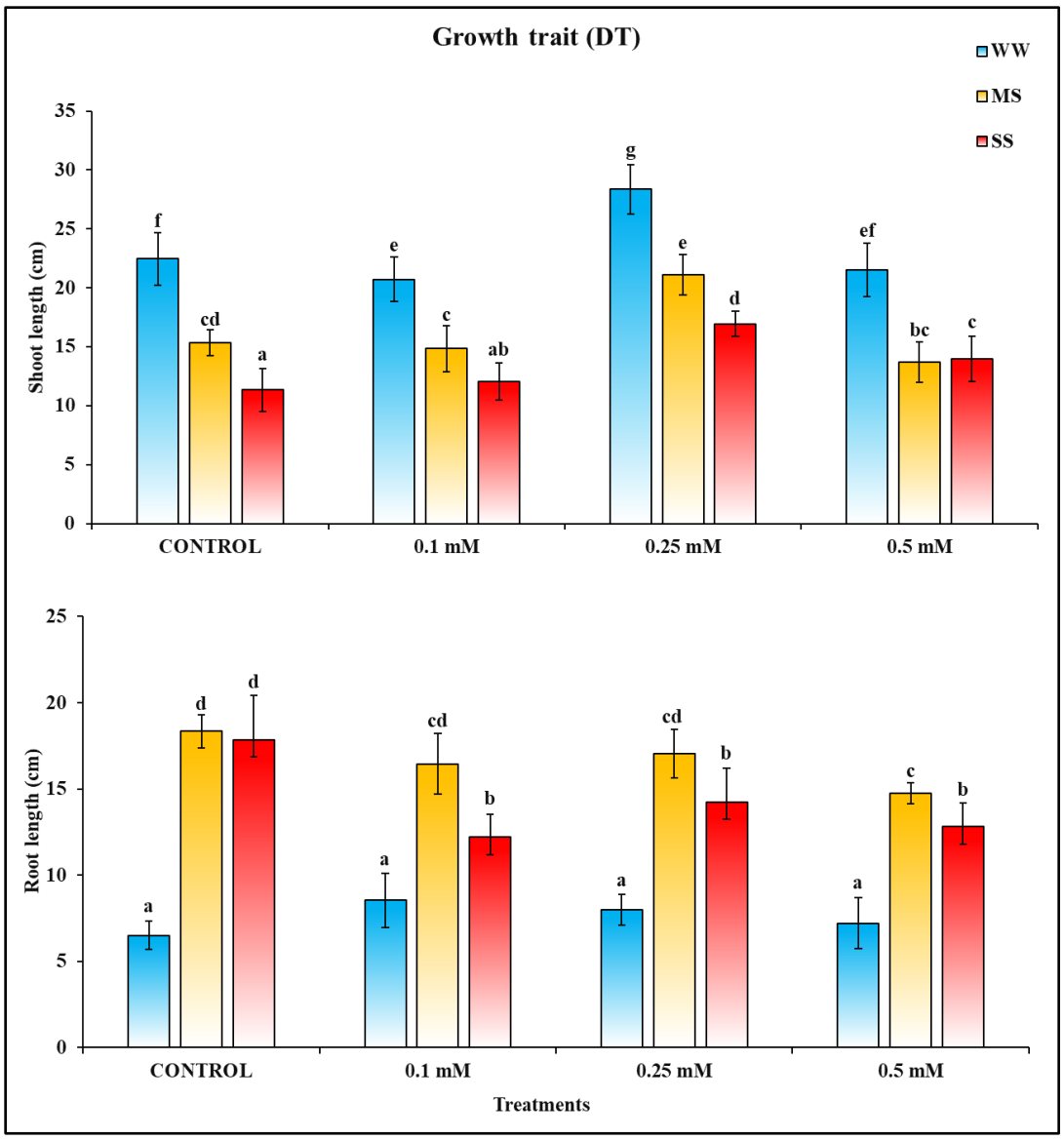


Fig. 3.2a: Growth trait (shoot/root length) in drought tolerant (DT) rice cultivar treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=10). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

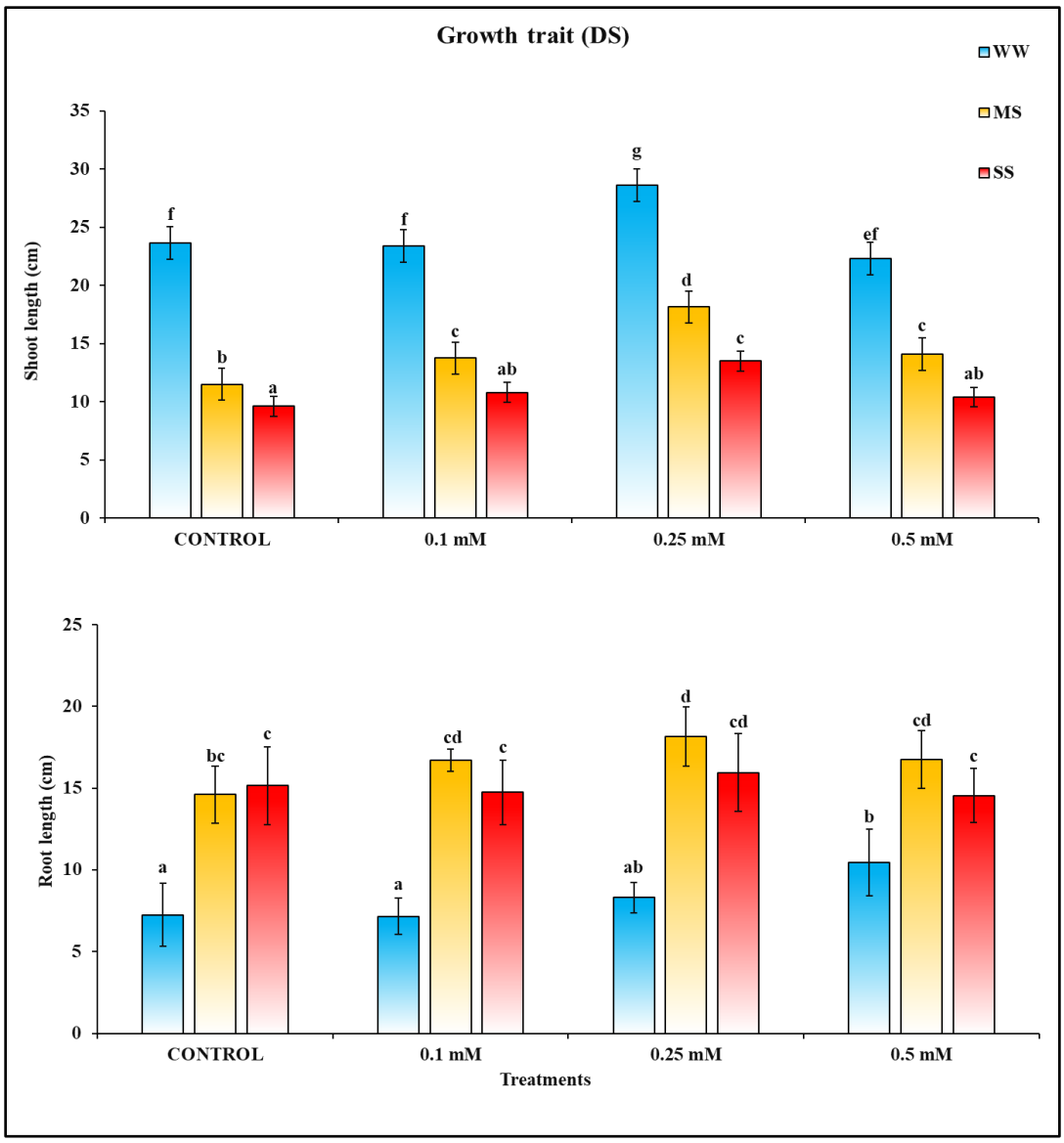


Fig. 3.2b: Growth trait (shoot/root length) in drought-sensitive (DS) rice cultivar treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=10). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

3.1.2. Biomass determination

Results of the shoot/root fresh and dry biomass influenced by PEG-induced moderate/severe drought magnitudes and exogenously applied SA of varying concentrations are presented in **Table 3.2; Fig. 3.2a, b**.

3.1.2.1. Shoot fresh weight (SFW)

Present results showed that moderate and severe drought treatments led to a decline in SFW in both cultivars. Compared to well-watered control, the SFW of plants exposed to moderate stress decreased by 54% in both DT and DS cultivars. The maximum increase in SFW was recorded at 0.25 mM concentration of SA by 10% and 20% in DT and DS cultivars, respectively. It is observed that 0.1 mM concentration recorded an increase of 5% in DT and 9% in DS, while 0.5 mM showed an increase of 14% in DS compared to control. Exposure of plants to severe drought stress further decreased SFW by 70% in DT and 68% in DS cultivars compared to well-watered control. The maximum increase of 10% in DT and 11% in DS in SFW was observed at 0.25 mM concentration of SA. In comparison, 0.1 mM SA concentration recorded a 3% increase in DT and DS cultivars, while 0.5 mM showed an increase of 9% in DT and 7% in DS compared to control.

3.1.2.2. Shoot dry weight (SDW)

The results showed that moderate and severe drought treatment decreased the SDW in both cultivars. SDW of plants exposed to moderate stress decreased by 27% in DT and 39% in DS cultivars compared to well-watered control. Maximum SDW increase of 8% in DT and 22% in DS cultivars was recorded at 0.25 mM concentration of SA. 0.1 mM concentration

of SA resulted in an increase of 6% in DT and 21% in DS cultivars, while 0.5 mM recorded an increase of 8% in DT compared to control. Also, the SDW of plants exposed to severe drought stress recorded a further decrease of 40% in DT and 27% in DS cultivars compared to well-watered control. The maximum SDW increase of 5% in both DT and DS cultivars was observed at 0.25 mM concentration of SA. However, 0.1 mM and 0.5 mM SA concentrations recorded an increase of 2% and 3% in DT over control, while no increase in DS cultivar was observed.

3.1.2.3. Root fresh weight (RFW)

Results of RFW showed that moderate and severe drought treatment led to decreased RFW in both cultivars. RFW of plants exposed to moderate stress decreased 51% in DT and 9% in DS cultivars compared to well-watered control. A maximum increase in RFW of 10% in the DT cultivar was observed at 0.25 mM concentration of SA, followed by 5% increase in 0.5 mM and 0.1 mM concentration. On the other hand, the maximum increase in RFW of 21% in the DS cultivar was observed at 0.5 mM concentration of SA. However, 0.25 mM and 0.5 mM concentrations of SA recorded an increase of 18% and 9% respectively in DS compared to control.

Furthermore, the RFW of plants exposed to severe drought stress showed a further decrease of 68% in DT and 1% in DS cultivars compared to well-watered control. A maximum increase in RFW was observed at 0.25 mM concentration of SA by 10% in the DT cultivar, followed by 0.5 mM and 0.1 mM, showing a 9% and 3% increase in RFW. On the other hand, the RFW recorded a maximum increase of 3% in the DS cultivar at 0.5 mM concentration of SA. While 0.25 mM and 0.1 mM recorded a decrease of 5% and 2%, respectively,

in DS compared to control.

3.1.2.4. Root dry weight (RDW)

The results of the present study revealed that moderate and severe drought treatment increased the RDW in both cultivars. RDW of plants exposed to moderate stress increased by 52% in DT and 82% in DS cultivars compared to well-watered control. The maximum increase in RDW was recorded in the DT cultivar (9%) at 0.25 mM concentration of SA, whereas no increase was observed at 0.1 mM and 0.5 mM concentration. On the other hand, the maximum increase of 12% was recorded in the DS cultivar at 0.5 mM concentration. 0.1 mM and 0.25 mM recorded an increase of 7% and 9% in the DS cultivar compared to control plants.

Furthermore, RDW of plants exposed to severe drought stress increased by 58% in DT and 106% in DS cultivars compared to well-watered control. The maximum increase in RDW (9%) was recorded in the DT cultivar at 0.25 mM concentration of SA. However, the RDW increased by 3% in DT at 0.5 mM and decreased by 18% at 0.1 mM concentration of SA in the same cultivar. Maximum increase of 20% in RDW in the DS cultivar was observed at 0.5 mM concentration of SA, followed by 0.1 mM and 0.25 mM, showing an increase of 16% and 8%, respectively, compared to control.

Overall, it was noted that exogenous application of SA significantly decreased the negative impacts of moderate and severe drought stress in both DT and DS cultivars. However, among the varying concentrations of SA, treatment with 0.25 mM and 0.5 mM markedly alleviated the adverse effects of drought stress in both DT and DS cultivars compared to their respective controls.

Table 3.2: Influence of drought stress and exogenously applied salicylic acid on shoot and root biomass of drought tolerant (DT) and drought-sensitive (DS) rice cultivars. Data represent mean values ± SD (n=6). Different alphabets among the treatments denote significance at a 5% level.

Treatments		*Biomass*							
		Drought tolerant				*Drought sensitive*			
Drought	*SA*	*Fresh weight (g)*		*Dry weight (g)*		*Fresh weight (g)*		*Dry weight (g)*	
		Shoot	*Root*	*Shoot*	*Root*	*Shoot*	*Root*	*Shoot*	*Root*
Well-watered	0 mM	0.727 ± 0.05^{c}	0.680 ± 0.04^{abc}	0.083 ± 0.01^{c}	0.094 ± 0.0^{a}	0.588 ± 0.06^{d}	0.507 ± 0.01^{ab}	0.073 ± 0.01^{b}	0.073 ± 0.0^{a}
Moderate		0.333 ± 0.02^{b}	0.333 ± 0.06^{d}	0.061 ± 0.0^{b}	0.143 ± 0.0^{cd}	0.269 ± 0.02^{b}	0.551 ± 0.07^{abc}	0.044 ± 0.0^{a}	0.133 ± 0.02^{b}
Severe		0.215 ± 0.01^{a}	0.215 ± 0.04^{a}	0.050 ± 0.0^{a}	0.149 ± 0.01^{d}	0.187 ± 0.01^{a}	0.512 ± 0.04^{ab}	0.053 ± 0.0^{a}	0.151 ± 0.01^{bcd}
Well-watered	0.1 mM	0.735 ± 0.05^{c}	0.728 ± 0.04^{bcd}	0.081 ± 0.01^{c}	0.097 ± 0.01^{a}	0.621 ± 0.05^{de}	0.562 ± 0.06^{bc}	0.072 ± 0.01^{b}	0.087 ± 0.01^{a}
Moderate		0.351 ± 0.02^{b}	0.351 ± 0.04^{ab}	0.064 ± 0.01^{b}	0.125 ± 0.01^{bc}	0.295 ± 0.02^{bc}	0.603 ± 0.07^{cd}	0.054 ± 0.0^{a}	0.143 ± 0.01^{b}
Severe		0.220 ± 0.03^{a}	0.220 ± 0.05^{a}	0.051 ± 0.0^{a}	0.122 ± 0.02^{b}	0.194 ± 0.01^{a}	0.500 ± 0.04^{ab}	0.053 ± 0.0^{a}	0.176 ± 0.02^{bcd}
Well-watered	0.25 mM	0.768 ± 0.08^{c}	0.752 ± 0.07^{cd}	0.092 ± 0.01^{d}	0.093 ± 0.0^{a}	0.646 ± 0.07^{e}	0.549 ± 0.04^{abc}	0.090 ± 0.01^{c}	0.100 ± 0.03^{a}
Moderate		0.366 ± 0.02^{b}	0.366 ± 0.03^{d}	0.065 ± 0.0^{b}	0.156 ± 0.01^{d}	0.324 ± 0.03^{c}	0.649 ± 0.05^{d}	0.054 ± 0.01^{a}	0.145 ± 0.02^{bc}
Severe		0.237 ± 0.01^{a}	0.237 ± 0.04^{d}	0.052 ± 0.01^{a}	0.162 ± 0.02^{d}	0.208 ± 0.02^{a}	0.484 ± 0.05^{a}	0.056 ± 0.01^{a}	0.163 ± 0.03^{cd}
Well-watered	0.5 mM	0.762 ± 0.06^{c}	0.736 ± 0.06^{bcd}	0.086 ± 0.01^{cd}	0.099 ± 0.01^{a}	0.637 ± 0.03^{e}	0.602 ± 0.07^{cd}	0.086 ± 0.01^{c}	0.103 ± 0.01^{a}
Moderate		0.351 ± 0.02^{b}	0.351 ± 0.05^{d}	0.065 ± 0.0^{b}	0.127 ± 0.01^{bc}	0.307 ± 0.02^{bc}	0.665 ± 0.05^{d}	0.054 ± 0.0^{a}	0.150 ± 0.02^{bc}
Severe		0.235 ± 0.01^{a}	0.235 ± 0.03^{a}	0.051 ± 0.01^{a}	0.153 ± 0.01^{d}	0.201 ± 0.01^{a}	0.530 ± 0.08^{ab}	0.053 ± 0.0^{a}	0.181 ± 0.03^{d}

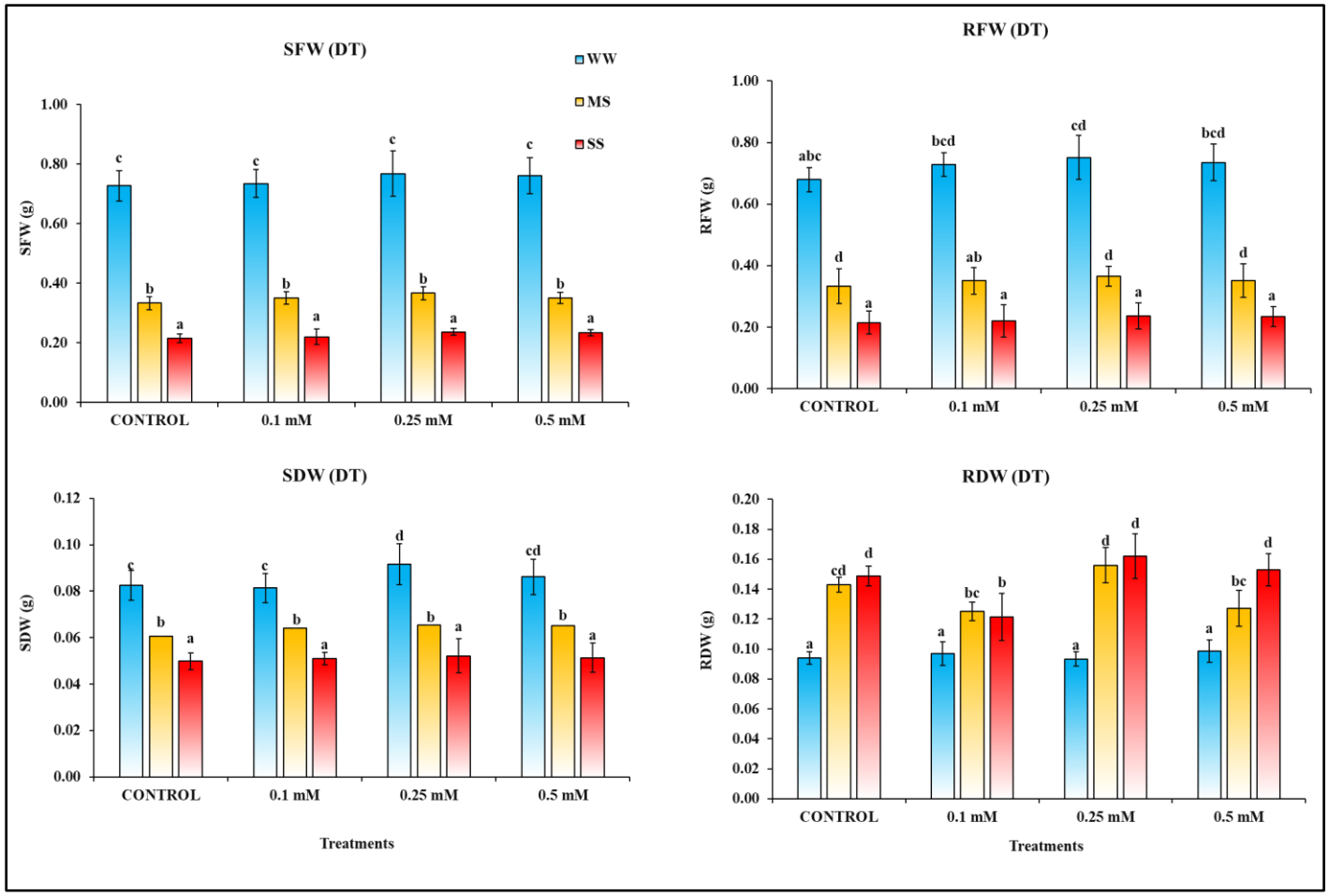


Fig. 3.3a: Interactive effect of drought stress and exogenous salicylic acid treatment on shoot and root biomass of drought tolerant (DT) rice cultivar. Bars represent mean values ± SD (n=6). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

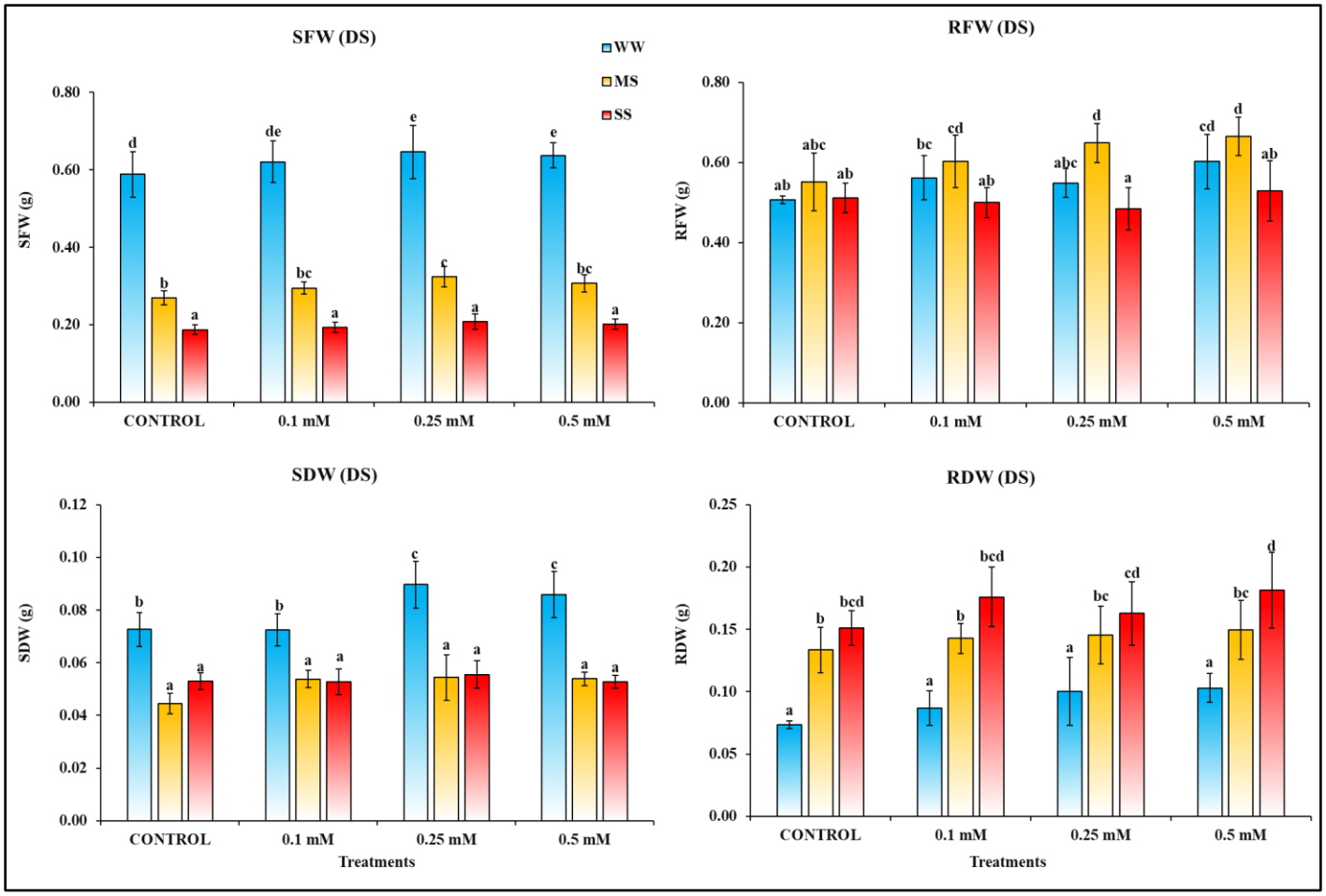


Fig. 3.3b: Interactive effect of drought stress and exogenous salicylic acid treatment on shoot and root biomass of drought-sensitive (DS) rice cultivar. Bars represent mean values ± SD (n=6). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

3.1.3. External morphology of rice leaves

The effect of PEG_{6000}-induced drought stress and ameliorating effects of 0.25 mM SA on the adaxial and abaxial leaf surfaces (trichomes, stomata, wax deposition, and cuticular papillae) on DT and DS cultivars was studied for well-watered, moderate stress and severe stress using a Scanning Electron Microscope (SEM). The present study observed that drought stress and SA concentration significantly affected the stomatal density and morphology (**Fig. 3.4a-d)**. Leaves of DT (**Fig. 3.4a; A, C, E**) and DS plants (**Fig. 3.4b; A, B, C**) exposed to moderate and severe drought stress showed an increase in the number and size of stomata on the adaxial surface whereas a reduction in the number and size of stomata on the abaxial surface was observed in both DT (**Fig. 3.4a; G, I, K**) and DS (**Fig. 3.4b; G, I, K**) cultivars compared to well-watered**.** However, foliar-applied SA showed no significant changes in the stomatal density of both cultivars.

On the other hand, the results also revealed that due to foliar applied SA, a reduction in the opening of the stomata, increased intensity of small cuticular papillae (silica bodies), and deposition of cuticular wax on the leaf surfaces of both DT (**Fig. 3.4c**) and DS (**Fig. 3.4d**) cultivars exposed to moderate and severe drought stress compared to well-watered was observed. Trichomes were observed only on the adaxial surface of the DT (**Fig. 3.4e; A-F**) cultivar, whereas the DS cultivar (**Fig. 3.4c**; **G-L**) showed the trichomes on the abaxial surface. However, the trichome length decreased on drought exposure in both cultivars (**Fig. 3.4e**). Furthermore, exogenous treatment with 0.25 mM SA on moderate and severe drought-stressed plants showed amelioration effects on stomata, wax deposition, and cuticular papillae.

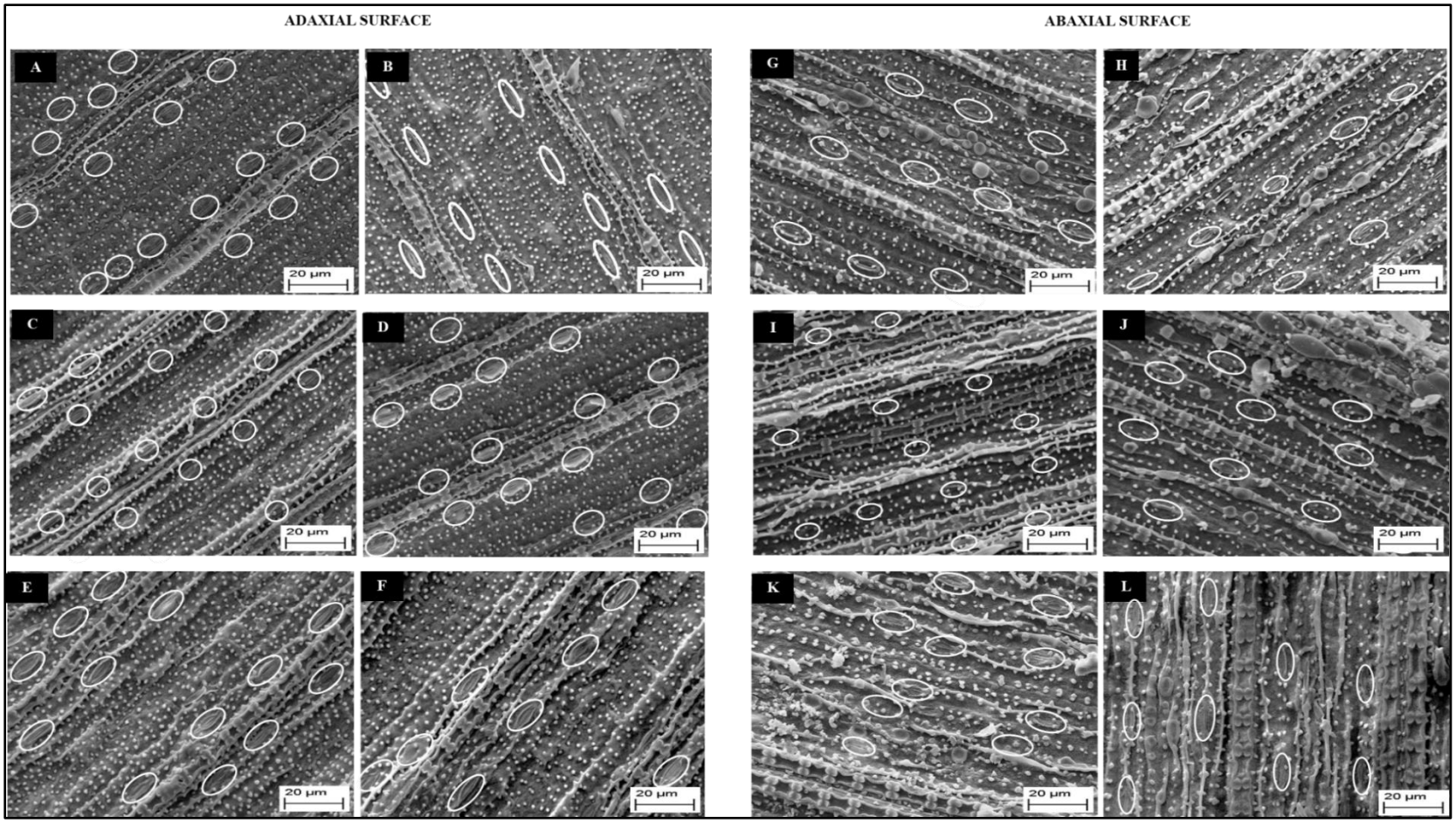


Fig. 3.4a: Scanning Electron Microscope (SEM) images of the leaf (adaxial/abaxial) surface showing the stomatal density of drought-tolerant (DT) plants treated with drought stress and salicylic acid. The circles represent stomata. [Adaxial surface: **A**-well-watered (WW); **B**-WW+0.25 mM SA; **C**- moderates stress (MS); **D**-MS +0.25 mM SA; **E**- severe stress (SS); and **F**- SS +0.25 mM SA; Abaxial surface: **G**- well-watered (WW); **H**-WW+0.25 mM SA; **I**- moderates stress (MS); **J**-MS +0.25 mM SA; **K**- severe stress (SS); and **L**- SS +0.25 mM SA]. Bar=20 µm.

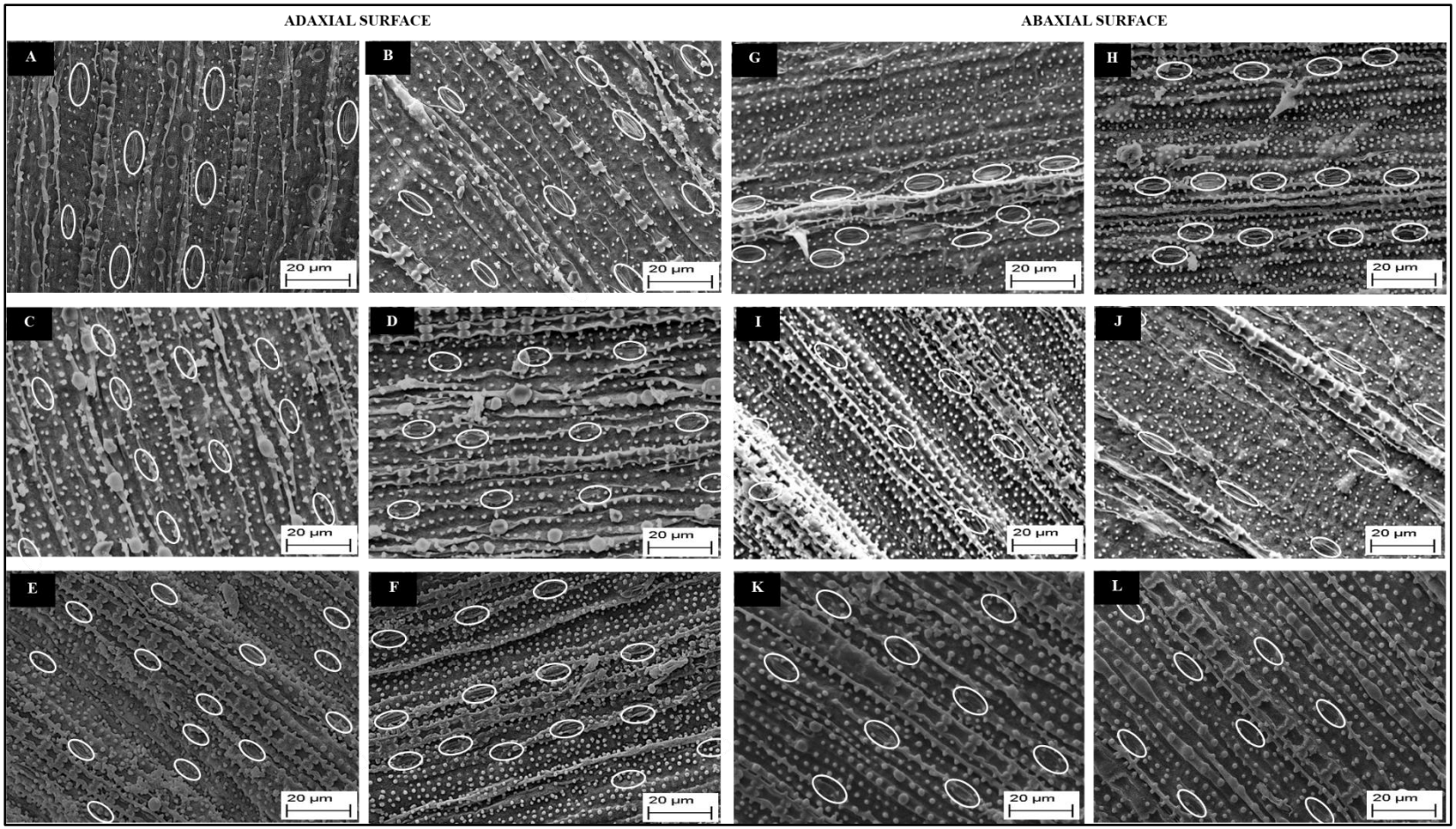


Fig. 3.4b: Scanning Electron Microscope (SEM) images of the leaf (adaxial/abaxial) surface showing the stomatal density of drought-sensitive (DS) plants treated with drought stress and salicylic acid. The circles represent stomata. [Adaxial surface: **A**-well-watered (WW); **B**-WW+0.25 mM SA; **C**- moderates stress (MS); **D**-MS +0.25 mM SA; **E**- severe stress (SS); and **F**- SS +0.25 mM SA; Abaxial surface: **G**- well-watered (WW); **H**-WW+0.25 mM SA; **I**- moderates stress (MS); **J**-MS +0.25 mM SA; **K**- severe stress (SS); and **L**- SS +0.25 mM SA]. Bar=20 µm.

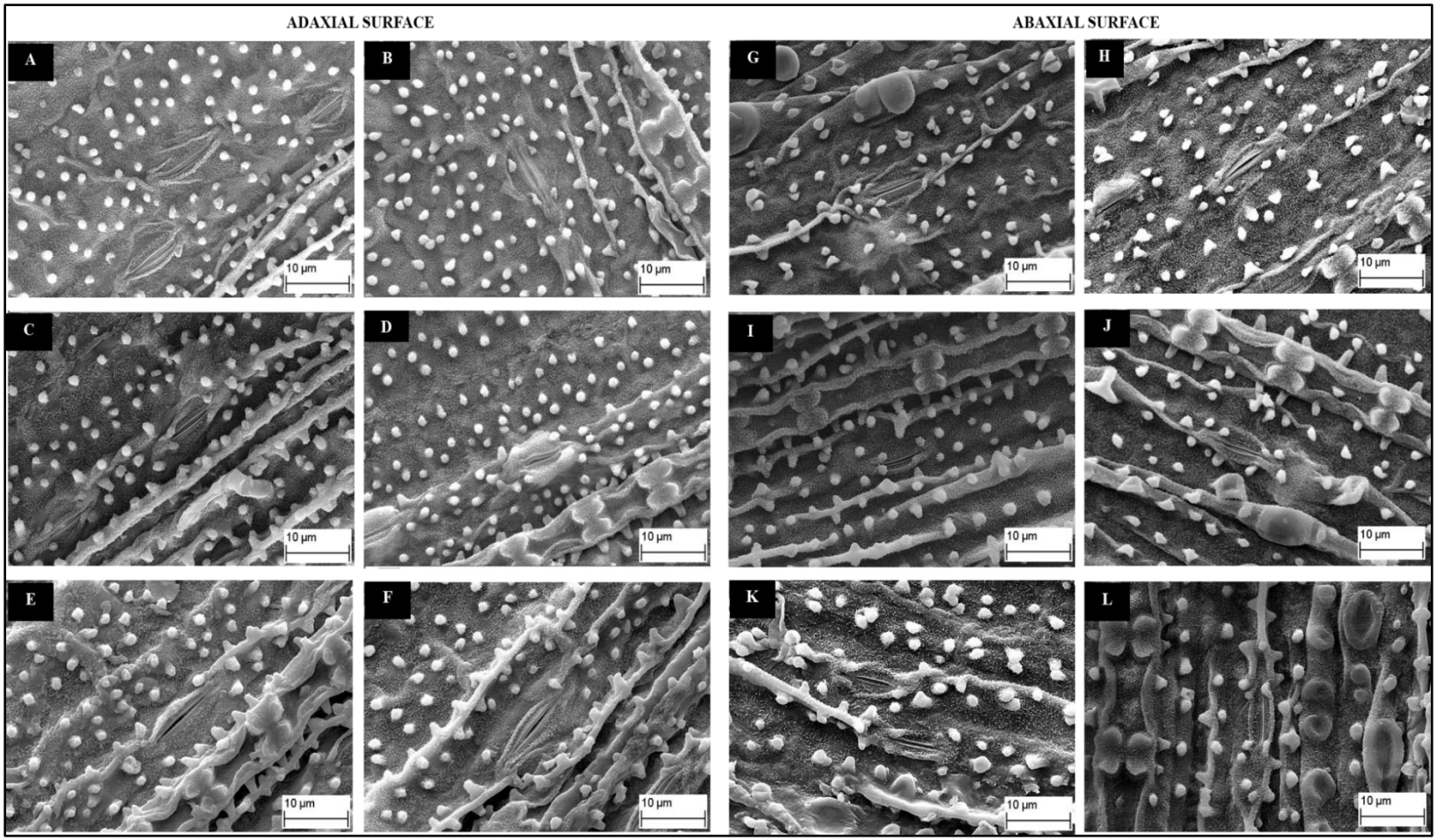


Fig. 3.4c: Scanning Electron Microscope (SEM) images of the leaf (adaxial/abaxial) surface showing the length of stomata and cuticle papillae of drought tolerant (DT) plants treated with drought stress and salicylic acid. [Adaxial surface: **A**- well-watered (WW); **B**-WW+0.25 mM SA; **C**- moderates stress (MS); **D**-MS +0.25 mM SA; **E**- severe stress (SS); and **F**- SS +0.25 mM SA; Abaxial surface: **G**- well-watered (WW); **H**-WW+0.25 mM SA; **I**- moderates stress (MS); **J**-MS +0.25 mM SA; **K**- severe stress (SS); and **L**- SS +0.25 mM SA]. Bar=10 µm.

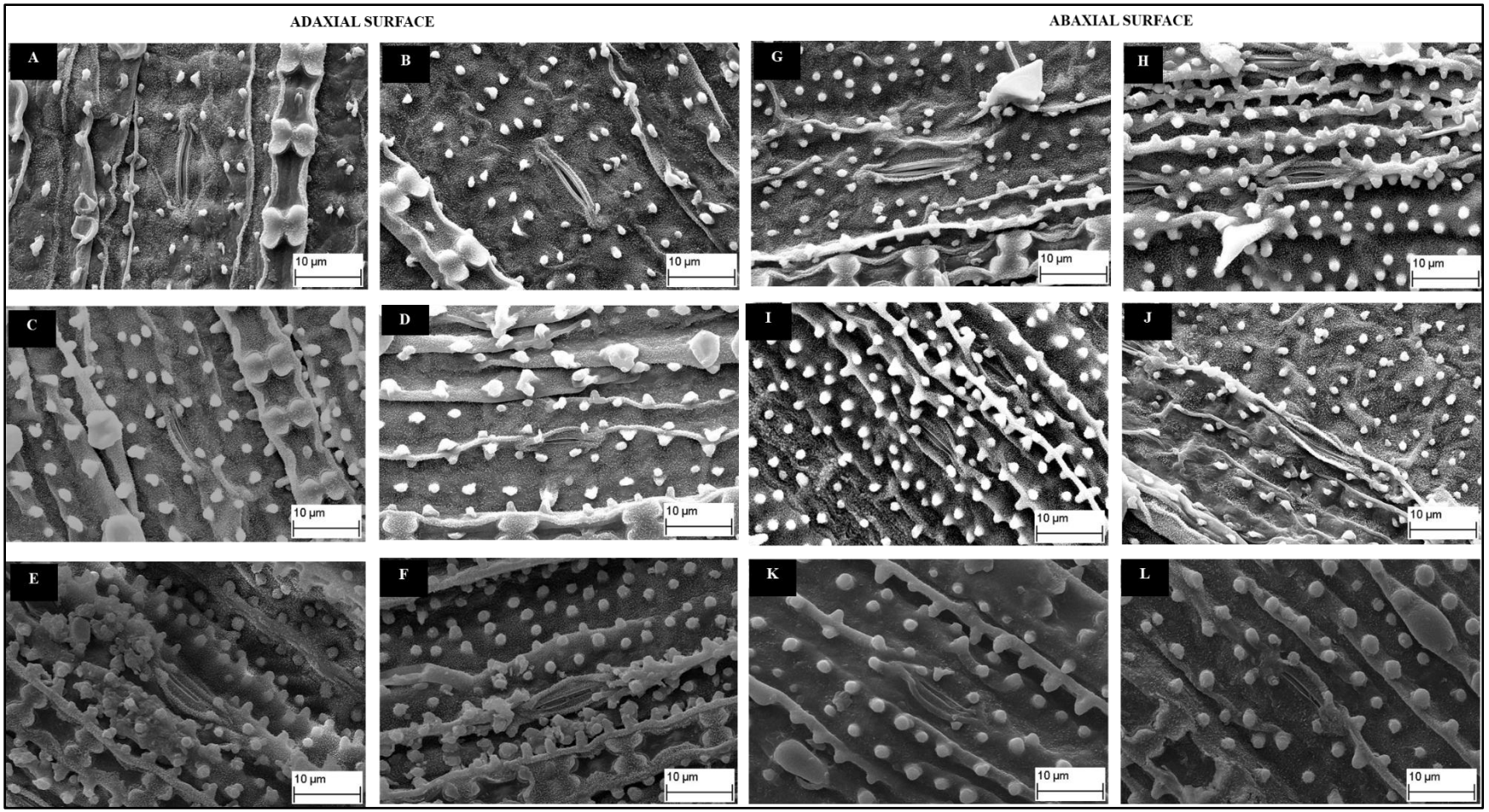


Fig. 3.4d: Scanning Electron Microscope (SEM) images of the leaf (adaxial/abaxial) surface showing the length of stomata and cuticle papillae of drought-sensitive (DS) plants treated with drought stress and salicylic acid. [Adaxial surface: **A**- well-watered (WW); **B**-WW+0.25 mM SA; **C**- moderates stress (MS); **D**-MS +0.25 mM SA; **E**- severe stress (SS); and **F**- SS +0.25 mM SA; Abaxial surface: **G**- well-watered (WW); **H**-WW+0.25 mM SA; **I**- moderates stress (MS); **J**-MS +0.25 mM SA; **K**- severe stress (SS); and **L**- SS +0.25 mM SA]. Bar=10 µm.

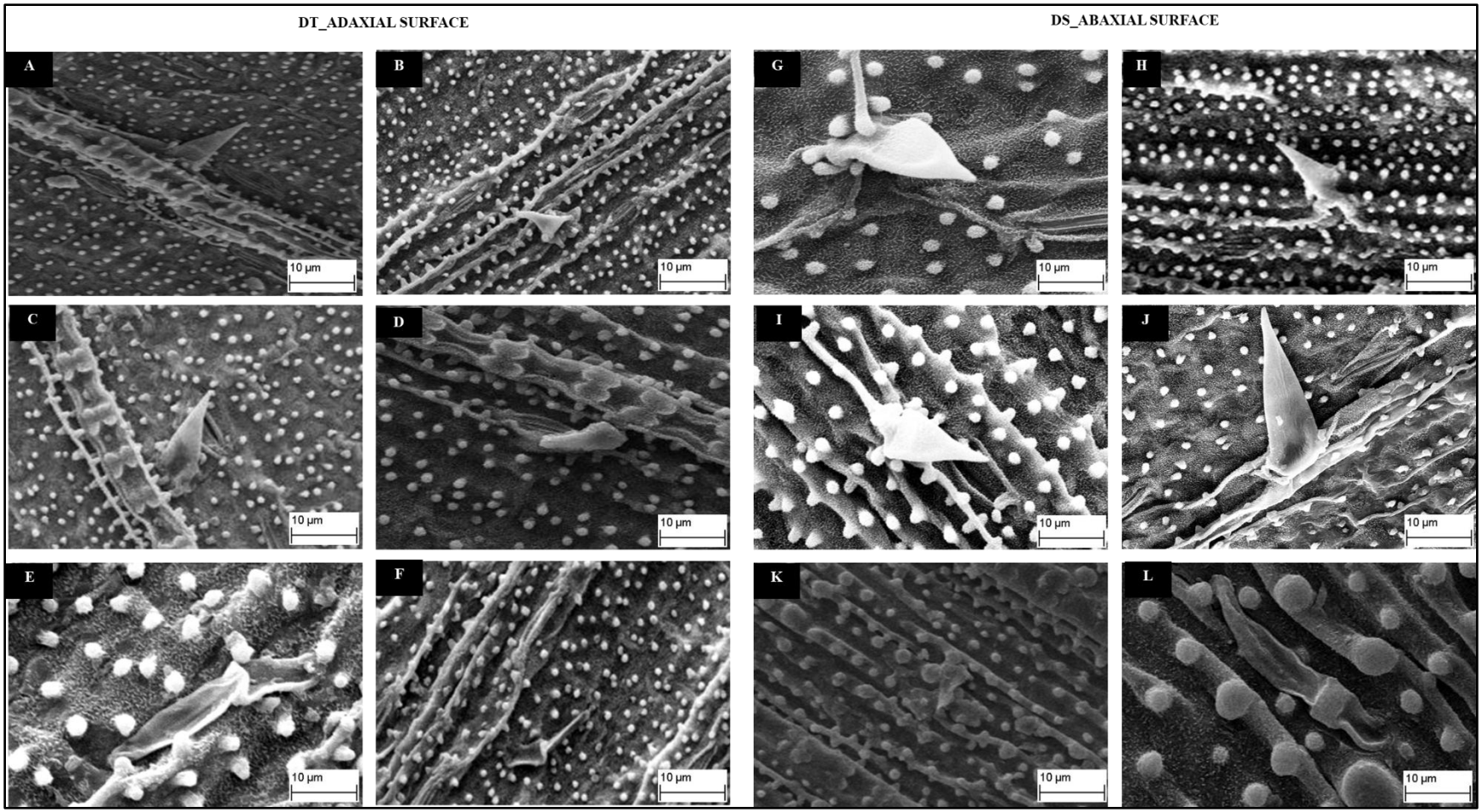


Fig. 3.4e: Scanning Electron Microscope (SEM) images of the leaf surface showing trichome of drought tolerant (DT) on the adaxial surface and drought-sensitive (DS) on the abaxial surface treated with drought stress and salicylic acid. [Adaxial surface: **A**- well-watered (WW); **B**-WW+0.25 mM SA; **C**- moderates stress (MS); **D**-MS +0.25 mM SA; **E**- severe stress (SS); and **F**-SS +0.25 mM SA; Abaxial surface: **G**- well-watered (WW); **H**-WW+0.25 mM SA; **I**- moderates stress (MS); **J**-MS +0.25 mM SA; **K**- severe stress (SS); and **L**- SS +0.25 mM SA]. Bar=10 µm.

3.1.4 Internal Morphological Studies of Rice Leaves and Roots

Light microscopy revealed alterations in the internal leaf and root morphology of DT and DS cultivars treated with drought stress and its interactive effect of 0.25 mM SA (**Tables 3.3 - 3.6**; **Fig. 3.5a-d**).

Leaf thickness decreased in both cultivars with increased drought intensity (**Fig. 3.5a, b**). As drought intensity increased from moderate to severe stress, there was a 5% and 43% leaf reduction in the DT cultivar, respectively. However, this parameter was relatively more adversely affected in the DS cultivar, showing a 34% and 54% decrease in moderate to severe stress, respectively. On foliar application of SA, there was a substantial increase in the leaf thickness in both the cultivars compared to the control. Leaf thickness in the DT cultivar increased by 28% and 63%, whereas in DS, it increased by 7% and 6% under moderate and severe stress conditions, respectively.

It was observed that the adaxial epidermal thickness was reduced by 21% in DT and 44% in DS cultivar under moderate stress, while it was reduced by 36% in DT and 56% in DS cultivar under severe stress conditions (**Fig. 3.5a, b**). However, exogenous SA application increased the adaxial epidermal thickness in DT by 30% and 8%, and an increase of 10% was observed in the DS cultivar as drought intensity increased from moderate to severe. A similar pattern was also seen in the abaxial epidermal thickness with increasing drought levels in both cultivars (**Fig. 3.5a, b**). However, with SA application in drought conditions, the abaxial epidermal thickness significantly increased in both the cultivars compared to well-watered plants.

Drought stress caused a alteration in the bulliform cell thickness and length in both cultivars compared to well-watered plants (**Fig. 3.5a, b**). A considerable decrease in bulliform

length and thickness was observed in DT and DS cultivars as drought intensity increased from moderate to severe. However, in the presence of SA, the DT cultivar showed an increase in bulliform length by 8% and 5% under moderate and severe drought, respectively. A further 4% decrease under moderate and 22% under severe drought in the DS cultivar was recorded.

On the other hand, SA under moderate drought increased the bulliform thickness by 48% in the DT cultivar and 43% in the DS cultivar compared to well-watered plants. Similarly, SA under severe drought increased the bulliform thickness by 2% in the DT cultivar and 49% in the DS cultivar compared to well-watered plants (**Fig. 3.5a, b**).

The cross-section area of the vascular bundle (CSA_VB) showed a significant decrease in both cultivars under moderate and severe drought compared to well-watered plants (**Fig. 3.5a, b**).

A reduction in CSA_VB of 8% in DT and 36% in DS cultivar was observed under moderate drought, while a decline in CSA_VB of 12% in DT and 43% in DS cultivar was observed under severe drought conditions. Plants treated with SA, however, significantly increase CSA_VB by 9% and 14% in DT and 15% and 9% in DS cultivars in similar drought conditions compared to the well-watered plants, respectively.

Overall, increasing drought magnitude caused significant variations in the total leaf cross-sectional area (CSA) in DT and DS cultivars compared to well-watered plants (**Fig. 3.5a, b**). The CSA showed an increase only in DT cultivars by 125% under moderate and 12% under severe drought compared to well-watered plants. However, with SA application, the CSA showed a reduction of only 15% under moderate drought, while an increase of 62% was observed under severe drought conditions compared to well-watered plants. On the

other hand, the DS cultivar showed a significant decrease in CSA due to increasing drought stress. DS cultivars being adversely affected showed a 62% and 76% decrease in CSA under moderate and severe drought, respectively. However, DS cultivars treated with SA under drought conditions increased CSA by 32% and 9% under moderate and severe drought levels compared to the well-watered plants.

The effects of increasing drought markedly changed the root anatomy of DT and DS cultivars (**Table 3.5, 3.6; Fig. 3.5c, d**). Moderate and severe drought stress significantly affected epidermal thickness, aerenchyma area, stele diameter, and the thickness of the root, i.e., the cross-sectional area (CSA).

Under increasing drought magnitude, significant changes were observed in the epidermal thickness in both the cultivars (**Fig. 3.5c, d)**. There was a statistical decrease of epidermal thickness by 3% and 12% in DT and 36% and 45% in DS cultivars under moderate and severe drought, respectively, compared to the well-watered plants. However, with the application of SA, epidermal thickness showed further reduction in both DT and DS cultivars by 2% and 22%, respectively, under moderate stress conditions. On the contrary, it was observed that the epidermal thickness was enhanced by 13% in DT and 3% in DS cultivars exposed to severe stress compared to control plants.

Aerenchyma formation was negatively affected due to water deficit conditions in both rice cultivars (**Table 3.5, 3.6; Fig. 3.5c, d**). The formation of aerenchyma or the aerenchyma thickness was significantly reduced at both drought levels. There was a 17% and 2% decrease in DT and 14% and 19% in DS cultivars on exposure to increasing drought levels, respectively, compared to the aerenchyma of the well-watered leaf. However, under moderate stress, SA enhanced the aerenchyma thickness by 36% in DT and 0.5% in the DS

cultivar. On the other hand, a reduction in the aerenchyma by 22% in DT and 25% in DS cultivar was observed under severe drought conditions compared to well-watered plants. The well-watered condition was beneficial in increasing the diameter of the root stele in both cultivars (**Table 3.5, 3.6; Fig. 3.5c, d**). However, on exposure to increasing drought levels, the stele diameter of both cultivars showed a significant reduction compared to well-watered plants. There was a decrease of 8% and 18% in DT and 32% and 17% in DS cultivar compared to well-watered plants. However, with increasing drought levels, treatment with SA showed a statistical increase of 4% and 15% in DT and 41% and 14% in DS cultivars, respectively.

The effects of increasing drought magnitudes on the root CSA of both cultivars were evident (**Fig. 3.5c, d)** in the study**.** DT cultivars exposed to moderate and severe drought reduced CSA by 33% and 21%, and DS reduced CSA by 30% and 17%, respectively, compared to well-watered plants. The CSA of the cultivar treated with SA was further enhanced under moderate drought stress by 35% and 27% in DT and DS cultivars, respectively, compared to well-watered control. However, a decrease in CSA by 39% in both DT and DS cultivars was observed under severe drought conditions.

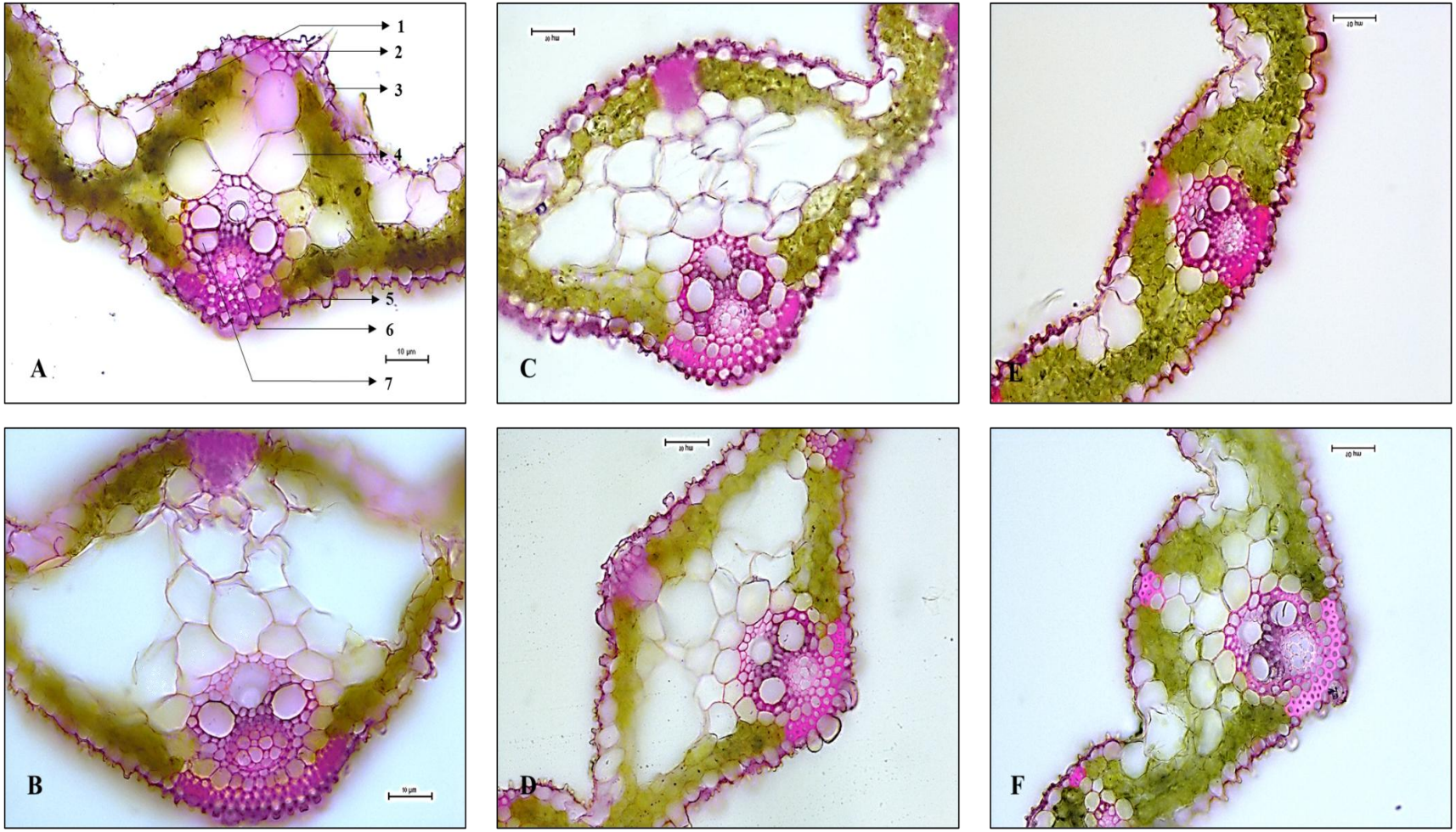


Fig. 3.5a: Leaf anatomical changes in drought tolerant (DT) rice cultivar treated with drought stress and salicylic acid. [**A**- well-watered (WW); **B**-WW+0.25 mM SA; **C**- moderates stress (MS); **D**-MS +0.25 mM SA; **E**- severe stress (SS); and **F**- SS +0.25 mM SA]. Transverse sections showing **1**-bulliform cell**, 2**-sclerenchyma**, 3**-adaxial epidermis**, 4**-bundle sheath**, 5**-abaxial epidermis**, 6**-phloem**,** and **7**-xylem vessel**.** Bar=10µm.

Table 3.3: Influence of drought stress and exogenously applied salicylic acid on leaf anatomy of drought tolerant (DT) rice cultivar. Data represent mean values ± SD (n=3). Different alphabets among the treatments denote significance at a 5% level (Abbreviation: CSA- cross-section area; VB- vascular bundle).

Treatments		*Leaf Anatomy (DT)*						
Drought	*SA*	*Adaxial epidermis (µm)*	*Bulliform length (µm)*	*Bulliform thickness (µm)*	*CSA_VB ($µm^2$)*	*CSA ($µm^2$)*	*Leaf thickness (µm)*	*Abaxial epidermis (µm)*
Well-watered	0 mM	5.20±1.00^{c}	25.01±1.56^{b}	11.28±1.81^{d}	380.97±6.23^{b}	2361.20±31.27^{a}	58.25±1.85^{c}	3.93±0.80^{a}
Moderate		4.12±0.42^{b}	13.05±1.45^{a}	5.05±0.31^{a}	369.76±5.25^{b}	5320.12±96.50^{e}	55.48±1.26^{b}	5.00±0.64^{b}
Severe		3.34±0.23^{a}	14.38±1.35^{a}	9.20±1.69^{c}	336.16±3.28^{a}	2639.53±45.85^{b}	33.29±0.97^{a}	4.59±1.23^{ab}
Well-watered	0.25 mM	4.93±0.36^{c}	30.59±1.28^{c}	9.91±0.64^{d}	453.97±3.84^{c}	5278.94±66.49^{e}	90.25±2.00^{e}	4.39±0.61^{ab}
Moderate		5.37±0.48^{c}	14.06±2.00^{a}	7.47±0.55^{b}	401.45±5.07^{b}	4546.53±68.25^{d}	71.23±3.38^{d}	5.07±0.59^{b}
Severe		3.61±0.62^{ab}	15.10±1.88^{a}	9.40±1.27^{c}	381.95±13.09^{b}	4278.37±42.92^{c}	54.24±0.51^{b}	4.49±0.39ab

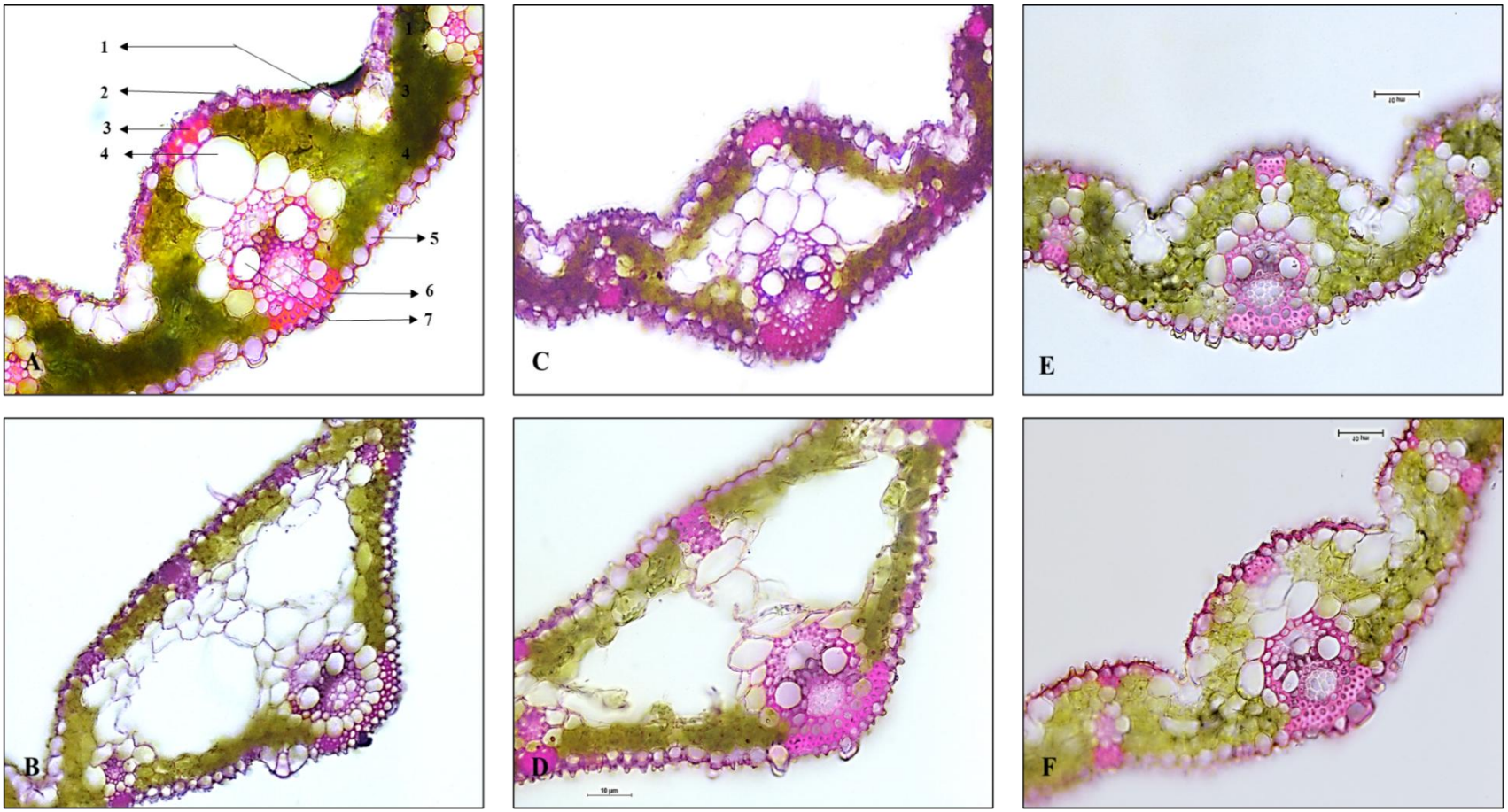


Fig. 3.5b: Leaf anatomical changes in drought-sensitive (DS) rice cultivar treated with drought stress and salicylic acid. [**A**-well-watered (WW); **B**-WW+0.25 mM SA; **C**- moderates stress (MS); **D**-MS +0.25 mM SA; **E**- severe stress (SS); and **F**- SS +0.25 mM SA]. Transverse sections showing **1**-bulliform cell**, 2**-sclerenchyma**, 3**-adaxial epidermis**, 4**-bundle sheath**, 5**-abaxial epidermis**, 6**-phloem**,** and **7**-xylem vessel**.** Bar=10µm.

Table 3.4: Influence of drought stress and exogenously applied salicylic acid on leaf anatomy of drought-sensitive (DS) rice cultivar. Data represent mean values ± SD (n=3). Different alphabets among the treatments denote significance at a 5% level (Abbreviation: CSA- cross-section area; VB- vascular bundle).

Treatments		*Leaf Anatomy (DS)*						
Drought	*SA*	*Adaxial epidermis (µm)*	*Bulliform length (µm)*	*Bulliform thickness (µm)*	*CSA_VB ($µm^2$)*	*CSA ($µm^2$)*	*Leaf thickness (µm)*	*Abaxial epidermis (µm)*
Well-watered	0 mM	7.65 ± 0.30^{c}	30.80 ± 1.04^{c}	15.78 ± 0.82^{e}	572.28 ± 13.85^{e}	13859.73 ± 97.82^{f}	89.79 ± 0.81^{f}	7.33 ± 0.16^{e}
Moderate		4.25 ± 0.39^{b}	31.61 ± 3.41^{c}	7.09 ± 0.97^{b}	366.22 ± 12.37^{b}	5232.70 ± 51.87^{c}	59.11 ± 0.96^{c}	3.74 ± 0.20^{a}
Severe		3.35 ± 0.34^{a}	23.59 ± 1.38^{b}	8.67 ± 0.24^{c}	328.90 ± 11.36^{a}	3361.85 ± 99.89^{a}	41.65 ± 0.71^{a}	4.54 ± 0.07^{c}
Well-watered	0.25 mM	3.35 ± 0.19^{a}	24.87 ± 0.77^{b}	9.52 ± 0.40^{cd}	484.95 ± 11.98^{d}	10758.54 ± 17.31^{e}	87.65 ± 0.85^{e}	4.90 ± 0.21^{d}
Moderate		4.67 ± 0.19^{b}	$19.08\pm^{a}$	10.13 ± 0.23^{d}	419.96 ± 16.70^{c}	6884.92 ± 10.91^{d}	63.37 ± 0.89^{d}	4.23 ± 0.14^{b}
Severe		3.69 ± 0.28^{a}	18.31 ± 1.76^{a}	4.40 ± 0.38^{a}	358.27 ± 22.78^{b}	3658.37 ± 16.63^{b}	44.09 ± 0.50^{b}	3.80 ± 0.12^{a}

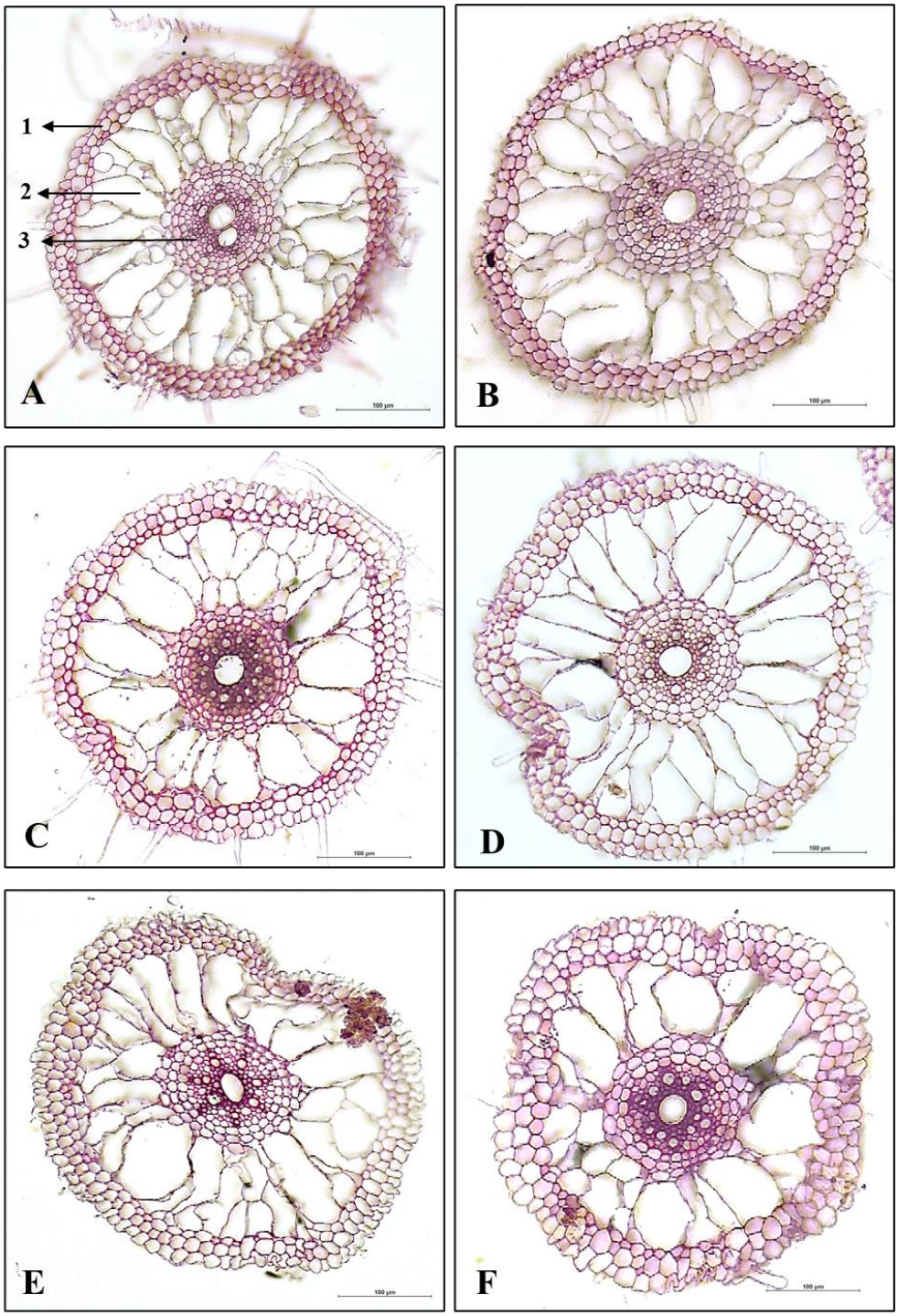


Fig. 3.5c: Root anatomical changes in drought tolerant (DT) rice cultivar treated with drought stress and salicylic acid. [**A**- well-watered (WW); **B**-WW+0.25 mM SA; **C**-moderates stress (MS); **D**-MS +0.25 mM SA; **E**- severe stress (SS); and **F**- SS +0.25 mM SA]. Transverse sections showing **1**-epidermis**, 2**-aerenchyma**, 3**-stele. Bar=100 µm.

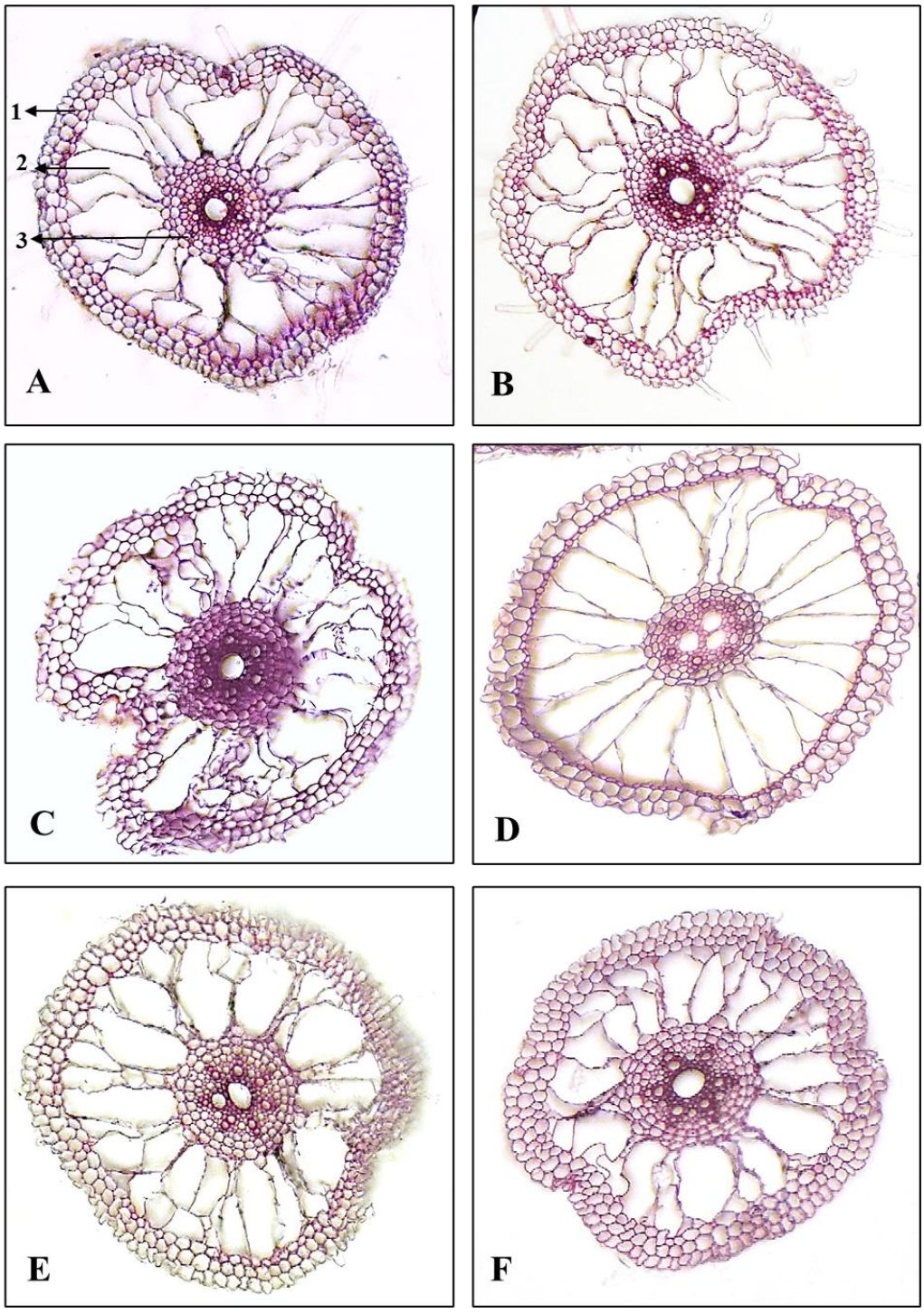


Fig. 3.5d: Root anatomical changes in drought-sensitive (DS) rice cultivar treated with drought stress and salicylic acid. [**A**- well-watered (WW); **B**-WW+0.25 mM SA; **C**-moderates stress (MS); **D**-MS +0.25 mM SA; **E**- severe stress (SS); and **F**- SS +0.25 mM SA]. Transverse sections showing **1**-epidermis**, 2**-aerenchyma**, 3**-stele. Bar=100 µm.

Table 3.5: Influence of drought stress and exogenously applied salicylic acid on root anatomy of drought tolerant (DT) rice cultivar. Data represent mean values ± SD (n=3). Different alphabets among the treatments denote significance at a 5% level (Abbreviation: CSA- cross-section area)

Treatments		*Root anatomy (DT)*			
Drought	*SA*	*Epidermal thickness (µm)*	*Aerenchyma thickness (µm)*	*Stele diameter (µm)*	*CSA ($µm^2$)*
Well-watered	0 mM	15.33±0.58^{b}	43.84±1.79^{b}	36.22±0.93^{c}	16989.74±136.19^{f}
Moderate		14.93±1.31^{ab}	36.55±0.67^{a}	33.20±0.49^{b}	11323.85±177.03^{b}
Severe		17.10±0.12^{c}	43.13±3.32^{b}	29.70±0.80^{a}	13362.06±151.19^{c}
Well-watered	0.25 mM	13.71±0.52^{a}	46.07±0.53^{b}	34.06±1.17^{b}	14862.35±38.49^{d}
Moderate		14.60±0.49^{ab}	49.74±0.15^{c}	34.68±0.85^{b}	15258.63±137.33^{e}
Severe		19.30±1.03^{d}	33.76±2.28^{a}	34.07±0.78^{b}	8096.97±61.09^{a}

Table 3.6: Influence of drought stress and exogenously applied salicylic acid on root anatomy of drought-sensitive (DS) rice cultivar. Data represent mean values ± SD (n=3). Different alphabets among the treatments denote significance at a 5% level (Abbreviation: CSA- cross-section area).

Treatments		*Root anatomy (DS)*			
Drought	*SA*	*Epidermal thickness (µm)*	*Aerenchyma thickness (µm)*	*Stele diameter (µm)*	*CSA ($µm^2$)*
Well-watered	0 mM	22.31±0.98^{e}	42.53±1.81^{c}	53.99±2.28^{c}	14177.81±9.63^{e}
Moderate		14.29±0.83^{c}	36.58±0.17^{b}	36.89±1.70^{a}	9949.14±83.94^{b}
Severe		12.32±0.66^{ab}	34.31±0.6^{b}	44.87±2.86^{b}	11800.12±576.44^{c}
Well-watered	0.25 mM	16.79±0.56^{d}	73.55±1.54^{d}	74.59±1.77^{d}	36506.33±422.04^{f}
Moderate		11.08±0.93^{a}	36.75±1.79^{b}	52.08±1.43^{c}	12603.71±278.92^{d}
Severe		12.64±0.83^{b}	25.85±1.20^{a}	51.06±1.49^{c}	7236.95±104.13^{a}

3.2. Physiological measurements

The interactive influence of PEG_{6000}-induced moderate and severe drought and 0.1, 0.25 and 0.5 mM foliar applied SA concentrations relative to water status, photosynthetic efficiency and photosynthetic pigments of the selected DT and DS rice cultivars were investigated.

3.2.1. Relative Water Content:

The relative water status for each rice cultivar exposed to moderate and severe drought and the interactive effect of drought and salicylic acid of different concentrations *viz.,* 0.1, 0.25, 0.5 mM is presented in **Fig. 3.6**. Data shows that the influence of drought and SA on the leaves of both the rice cultivars markedly affected the relative water content (RWC). It was observed that RWC recorded a more significant decrease in the DS cultivar than in the DT cultivar with increasing drought magnitude. RWC of plants exposed to moderate stress decreased by 4% and 5% in DT and DS cultivars, respectively, compared to well-watered control. However, with the exogenous application of SA, a maximum increase in RWC of 3% in DT and 5% in DS cultivars was observed with a 0.25 mM concentration of SA. While treatment of 0.1 mM and 0.5 mM showed an increase of 1% and 3%, respectively, only in the DS cultivar compared with its control.

Moreover, the RWC of plants exposed to severe drought stress decreased 18% in DT and 20% in DS cultivar compared to well-watered control. The maximum increase in RWC in DT (15%) and DS (7%) cultivars was observed at 0.25 mM concentration of SA. Whereas 0.1 mM and 0.5 mM showed an increase of 2% and 11% in the DT cultivar compared to its control. However, it has been observed that among the varying concentrations of

exogenous application of SA, 0.25 mM SA application significantly decreased the negative impacts of drought stress on the RWC in both DT and DS cultivars compared to the control, followed by 0.5 mM and 0.1 mM concentrations.

3.2.2. Photosynthetic rate measurements

Results of light reactions of photosynthesis measured for both DT and DS cultivars subjected to drought and exogenous SA are presented in **Fig. 3.7a-c**.

3.2.2.1. Light reaction

The maximum photosynthetic efficiency (Fv/Fm) of DT and DS cultivars significantly decreased on exposure to drought stress compared to the control. The Fv/Fm ratio of plants exposed to moderate stress decreased by 5% in DT and 20% in DS cultivars compared to well-watered control. However, it has been observed that exogenous application of 0.25 mM concentration of SA increased the Fv/Fm ratio by 5% in DT and 20% in DS cultivars. Whereas 0.1 mM and 0.5 mM showed an increase of 5% and 2% in DT and 12% and 17% in DS cultivar, respectively, compared to control (**Fig. 3.7a**).

Additionally, compared to well-watered control, the Fv/Fm ratio in plants exposed to severe drought stress decreased by 11% in DT and 33% in DS cultivars. Moreover, a maximum Fv/Fm ratio increase of 4% in DT and 30% in DS cultivars was recorded with the exogenous application of 0.25 mM concentration of SA. Whereas 0.1 mM and 0.5 mM showed an increase of 23% and 24%, respectively, in the DS cultivar compared to its control.

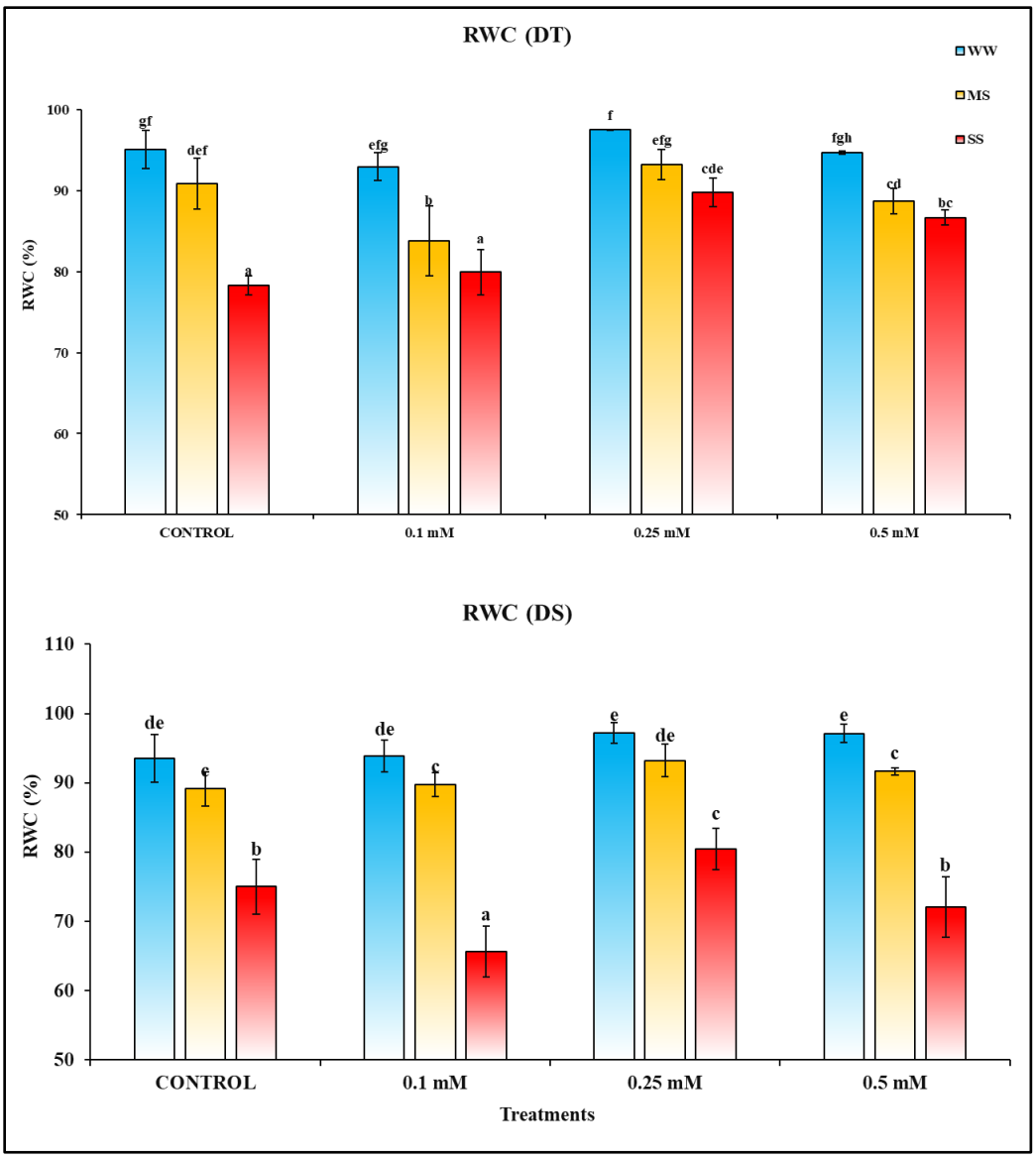


Fig. 3.6: Relative water content (RWC) in drought-tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=8). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

Quantum photosynthetic efficiency (ΦPSII) of DT and DS cultivars showed a linear decline with increasing drought. ΦPSII of plants exposed to moderate stress decreased by 28% in DT and 44% in DS cultivars compared to well-watered control (**Fig. 3.7b**). However, the exogenous application of 0.25 mM concentration of SA recorded a significant increase in ΦPSII by 23% in DT and 38% in DS cultivars. While 0.1 mM and 0.5 mM showed an increase of 10% and 17%, respectively, in the DS cultivar compared to control. However, in the DT cultivar, 0.5 mM showed an increase of 18% in comparison with its control. Likewise, ΦPSII of plants exposed to severe drought stress decreased further in DT (52%) and DS (78%) cultivars compared to well-watered control. The maximum increase in ΦPSII was observed in DT (48%) and DS (113%) cultivars at 0.25 mM concentration of SA. A 26% increase in DT and 37% increase in DS was recorded at 0.1 mM, while a 27% increase in DT and 106% increase in DS was recorded at 0.5 mM concentration of SA compared to control.

The results of light reactions of the photosynthesis, measured in terms of photochemical quenching (qP), are presented in **Fig. 3.7c**. The photochemical quenching (qP) of DT and DS cultivars significantly decreased on exposure to drought stress compared to the control. qP of plants exposed to moderate stress decreased by 19% in DT and 47% in DS cultivars compared to well-watered control. However, it is observed that exogenous application of SA had significantly increased qP at 0.25 mM concentration of SA by 34% in DT and 39% in DS cultivars. Whereas 0.1 mM recorded an increase of 5% while 0.5 mM recorded an increase of 12% in the DS cultivar. However, in the DT cultivar, only an increase of 7% was recorded at 0.5 mM concentration of SA compared to the control.

The study revealed that the qP of plants exposed to severe drought stress decreased by 63%

in DT and 65% in DS cultivars compared to well-watered control. The maximum increase in qP was observed in DT (59%) and DS (68%) cultivars at 0.25 mM concentration of SA. An increase of 5% in DT and 16% in DS was recorded at 0.1 mM, while an increase of 50% in DT and 53% in DS was recorded at 0.5 mM concentration of SA compared to control. In general, it was observed that among the SA concentrations used in the present study, the application with 0.25 mM alleviated the negative impacts of moderate and severe drought stress on light reaction in both DT and DS cultivars compared to control.

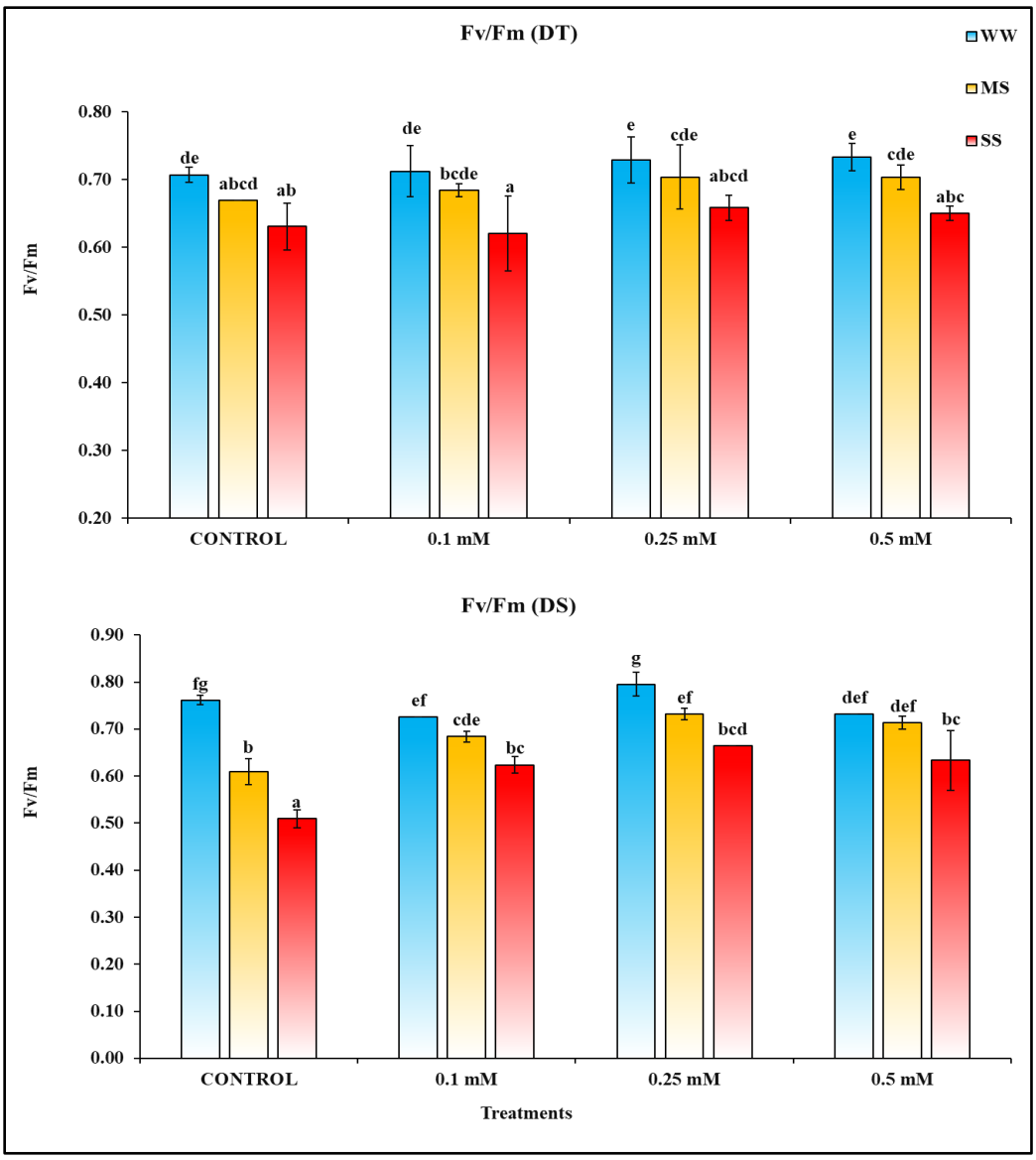


Fig. 3.7a: Chlorophyll fluorescence measurements: **Photosynthetic efficiency (Fv/Fm)** of drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=3). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

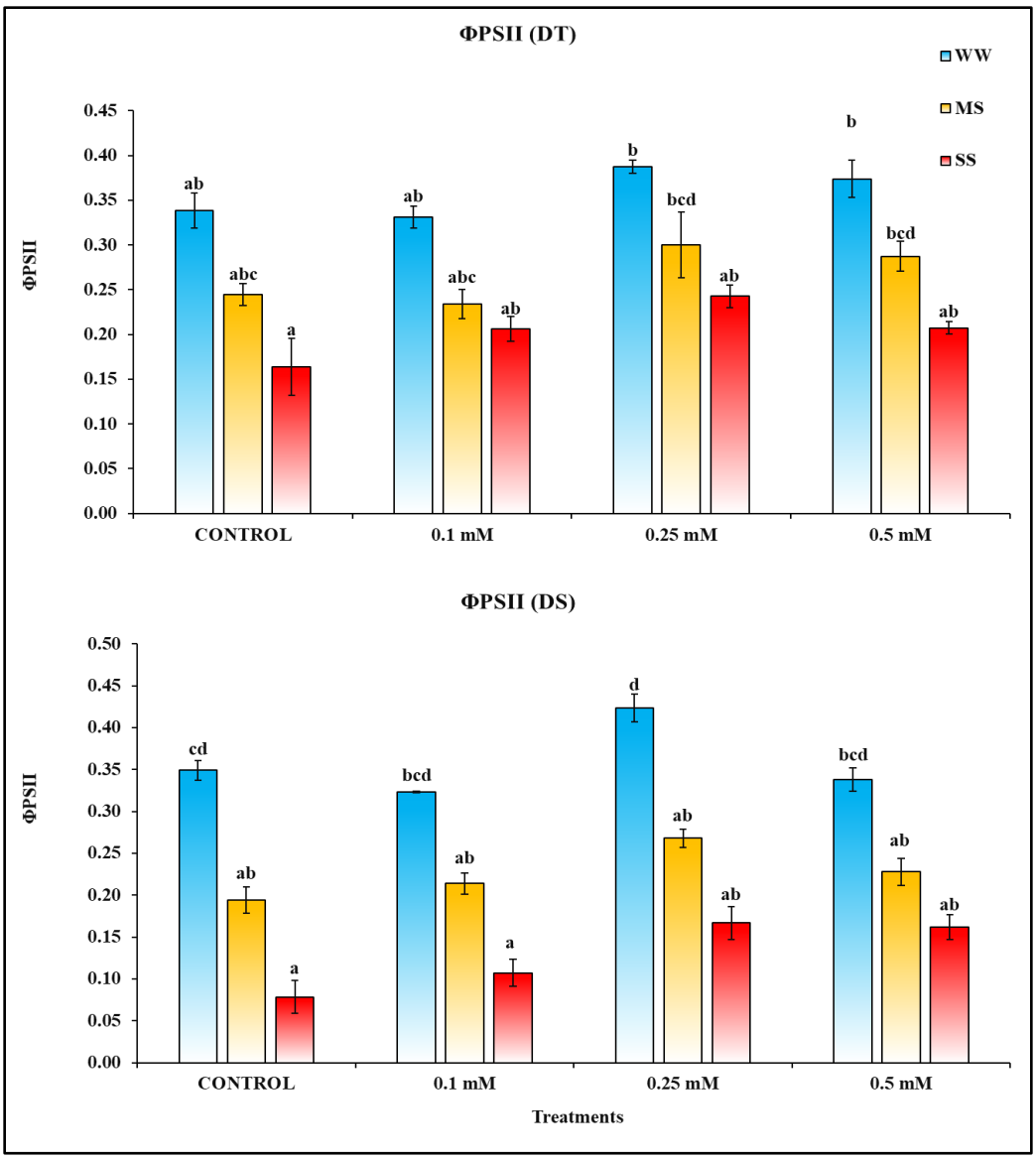


Fig. 3.7b: Chlorophyll fluorescence measurements: **Quantum efficiency of PSII (ΦPSII)** of drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=3). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

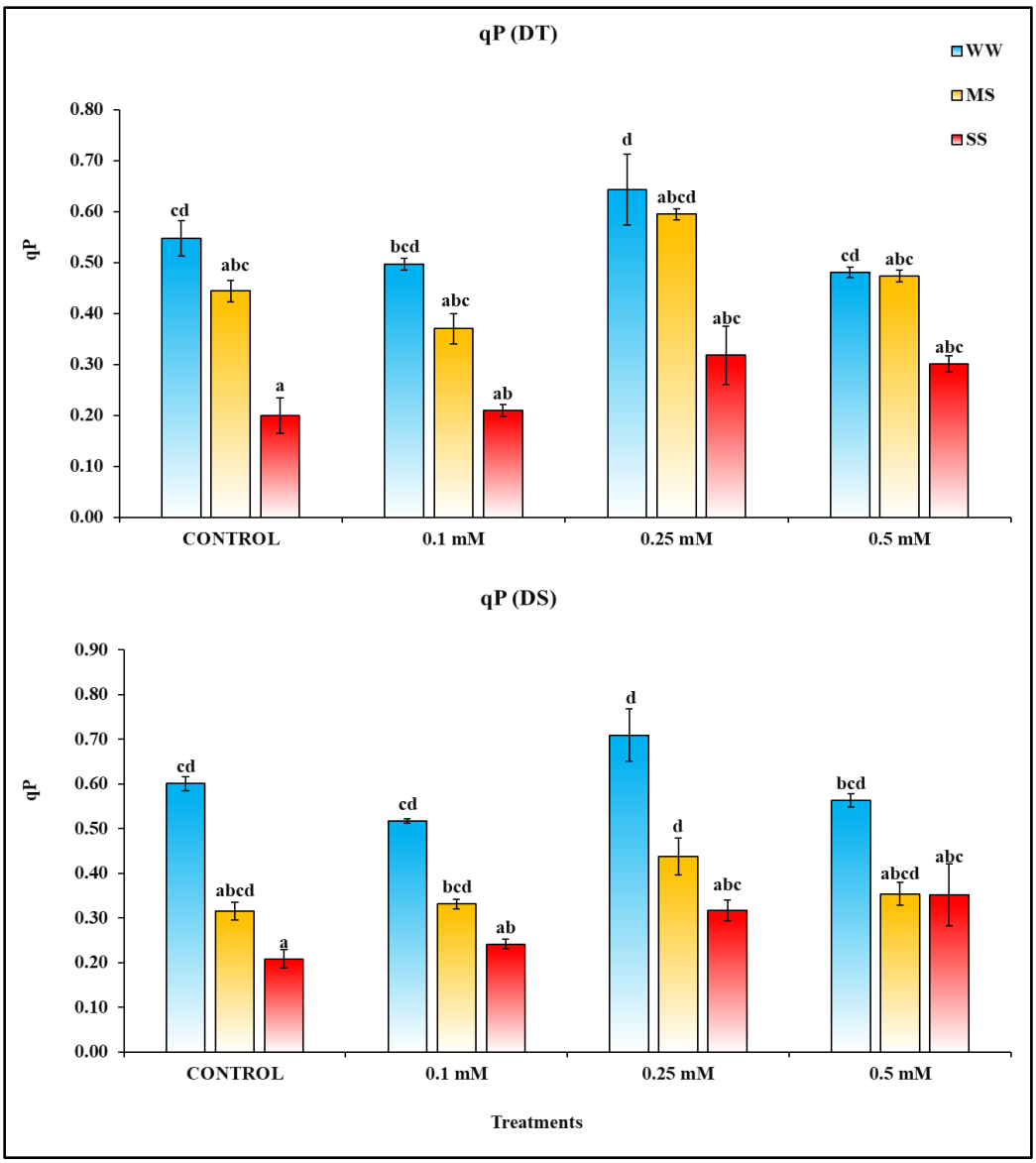


Fig. 3.7c: Chlorophyll fluorescence measurements: **Photochemical quenching (qP)** of drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=3). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

3.2.2.2. Dark reaction

The results of the analysis of CO_2 fixation, i.e., the photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), carboxylation efficiency (CE), water use efficiency (WUE) and intercellular CO_2 (C_i) in DT and DS cultivars exposed to the interactive effect of drought stress and exogenous SA are presented in **Fig. 3.8a-e**. The photosynthetic rate (P_N) of plants exposed to moderate stress decreased by 56% in DT and 62% in DS cultivars compared to well-watered control (**Fig. 3.8a**). Nevertheless, it has been observed that exogenous application of SA significantly increased P_N by 83% in DT and 113% in DS cultivars at 0.25 mM concentration of SA. An increase of 31% in DT and 66% in DS at 0.1 mM and 64% in DT and 104% in DS at 0.5 mM concentration was recorded compared to the control. Furthermore, P_N of plants exposed to severe drought stress reported a further decrease by 88% in DT and 84% in DS cultivars compared to well-watered control. The maximum increase in P_N by 239% in DT and 153% in DS cultivars was recorded at 0.25 mM concentration of SA. However, 0.1 mM recorded an increase of 48% in DT and 102% in DS, while 0.5 mM concentration recorded an increase of 155% in DT and 125% in DS compared to the control.

Moreover, stomatal conductance (g_s) of plants exposed to moderate stress decreased by 48% in DT and 53% in DS cultivars compared to well-watered control (**Fig. 3.8b**). However, it has been observed that exogenous application of SA had significantly increased g_s by 34in DT and 68% in DS cultivars at 0.25 mM concentration of SA. An increase of 56% in DS cultivar at 0.1 mM and 55% in DS at 0.5 mM concentration over control was recorded. While on the other hand, an increase of 14% in DT was recorded at 0.5 mM compared to the control. The g_s of plants exposed to severe drought stress showed a further

decrease by 63% in DT and 73% in DS cultivars compared to well-watered control. The maximum increase in g_s was observed at 0.25 mM of SA, which showed 81% in DT and 162% in DS cultivars. Compared to the control, an increase of 22% in DT and 126% in DS was recorded at 0.1mM concentration, while an increase of 31% in DT and 138% in DS was recorded at 0.5mM concentration.

The transpiration rate (*E*) of plants exposed to moderate stress decreased by 42% in DT and 66% in DS cultivars compared to well-watered control (**Fig. 3.8c**). However, it is observed that the exogenous application of SA significantly increased *E* by 32% in DT and 74% in DS cultivars at 0.25 mM concentration of SA. 0.1 mM concentration increased 23% in DT and 51% in DS, while 0.5 mM concentration increased 31% in DT and 69% in DS compared to control.

Besides, the *E* of plants exposed to severe drought stress showed a further decrease by 74% in DT and 80% in DS cultivars compared to well-watered control. The maximum increase of 47% in both DT and DS cultivars in *E* was observed at 0.25 mM concentration of SA. 0.1 mM concentration decreased by 40% in DT and 6% in DS. Similarly, 0.5 mM concentration decreased 43% in DT and 20% in DS compared to control.

Carboxylation (MC) of plants exposed to moderate stress decreased by 84% in DT and 79% in DS cultivars compared to well-watered control (**Fig. 3.8d**). However, it has been observed that the exogenous application of 0.25 mM concentration of SA significantly increased MC by 112% in DT and 177% in DS cultivars. 0.1 mM concentration increased by 45% in DT and 105% in DS. Similarly, 0.5 mM concentration increased 102% in DT and 174% in DS compared to control.

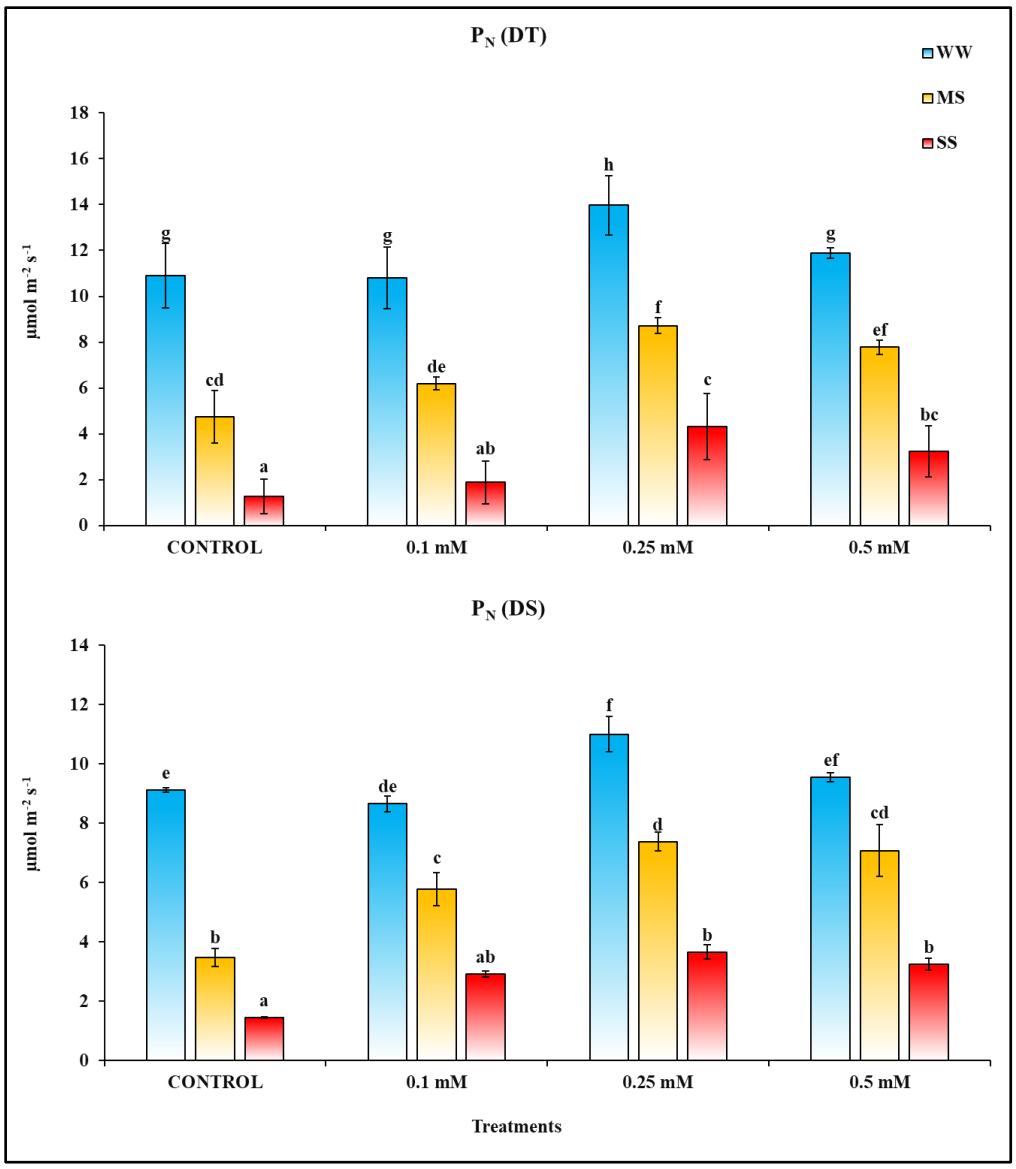


Fig. 3.8a: Leaf gas exchange measurements: Photosynthetic rate (P_N) of drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=3). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

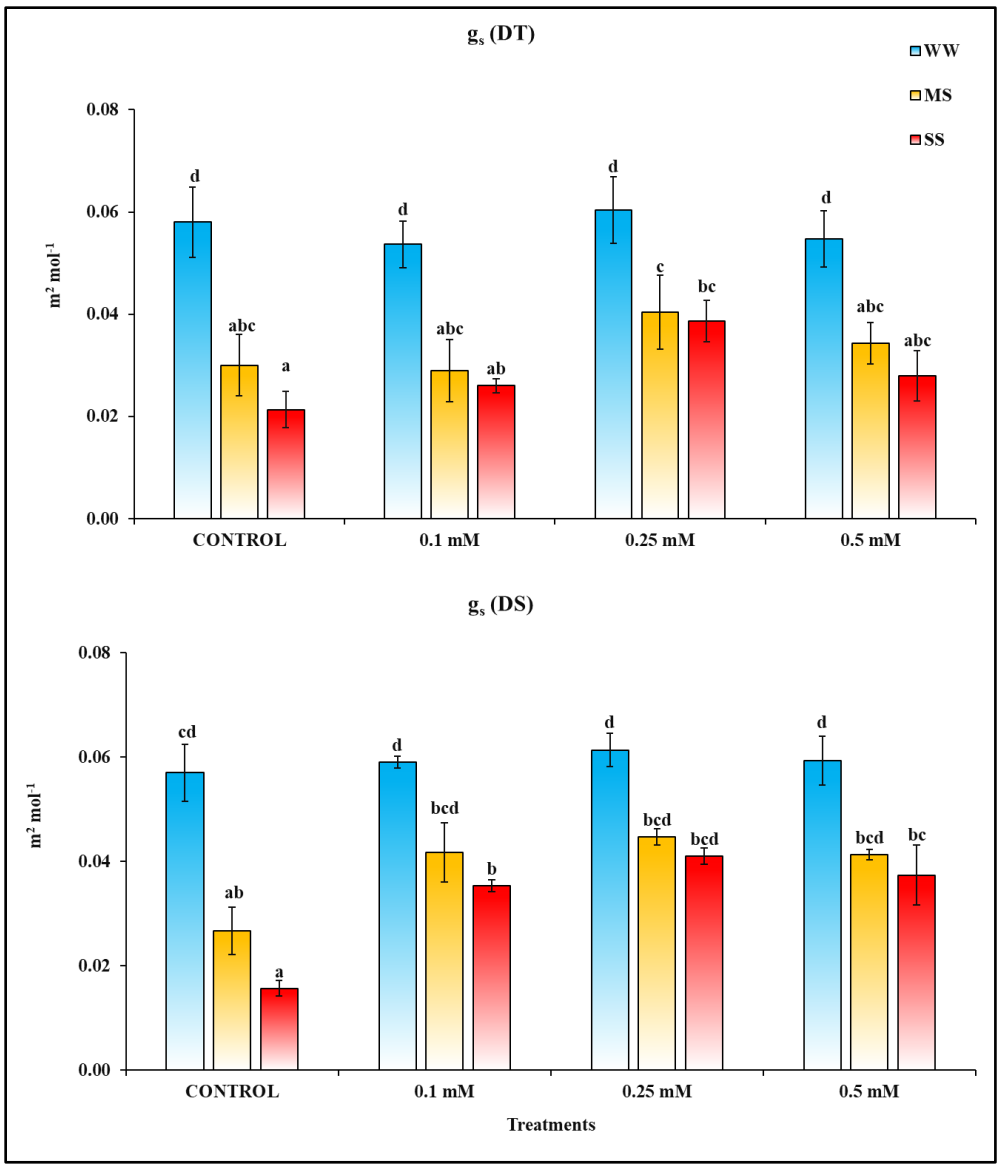


Fig. 3.8b: Leaf gas exchange measurements: **Stomatal conductance (g_s)** of drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=3). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate Stress; **SS**, severe stress.

Besides, the *E* of plants exposed to severe drought stress showed a further decrease by 97% in DT and 93% in DS cultivars compared to well-watered control. The maximum increase in *E* by 345% in DT and 251% in DS cultivars was observed at 0.25 mM concentration of SA. 0.1 mM concentration decreased by 85% in DT and 152% in DS. Similarly, 0.5 mM concentration decreased by 315% in DT and 189% in DS compared to control.

Internal CO_2 (Ci) of plants exposed to moderate stress has increased by 74% in DT and 159% in DS cultivars compared to well-watered control (**Fig. 3.8d**). However, a maximum decrease in Ci by 26% in DT and 17% in DS cultivars was observed at 0.25 mM concentration of SA. 0.1 mM concentration decreased by 22% in DT and 8% in DS. Similarly, 0.5 mM concentration decreased 25% in DT and 13% in DS compared to the control.

Furthermore, Ci of plants exposed to severe drought stress increased by 106% in DT and 221% in DS cultivars compared to well-watered control. The maximum decrease in Ci by 28% in DT and 36% in DS cultivars was observed at 0.25 mM concentration of SA. 0.1 mM concentration decreased by 20% in both DT and DS cultivars. Similarly, 0.5 mM concentration decreased by 22% in DT and 21% in DS cultivars compared to control.

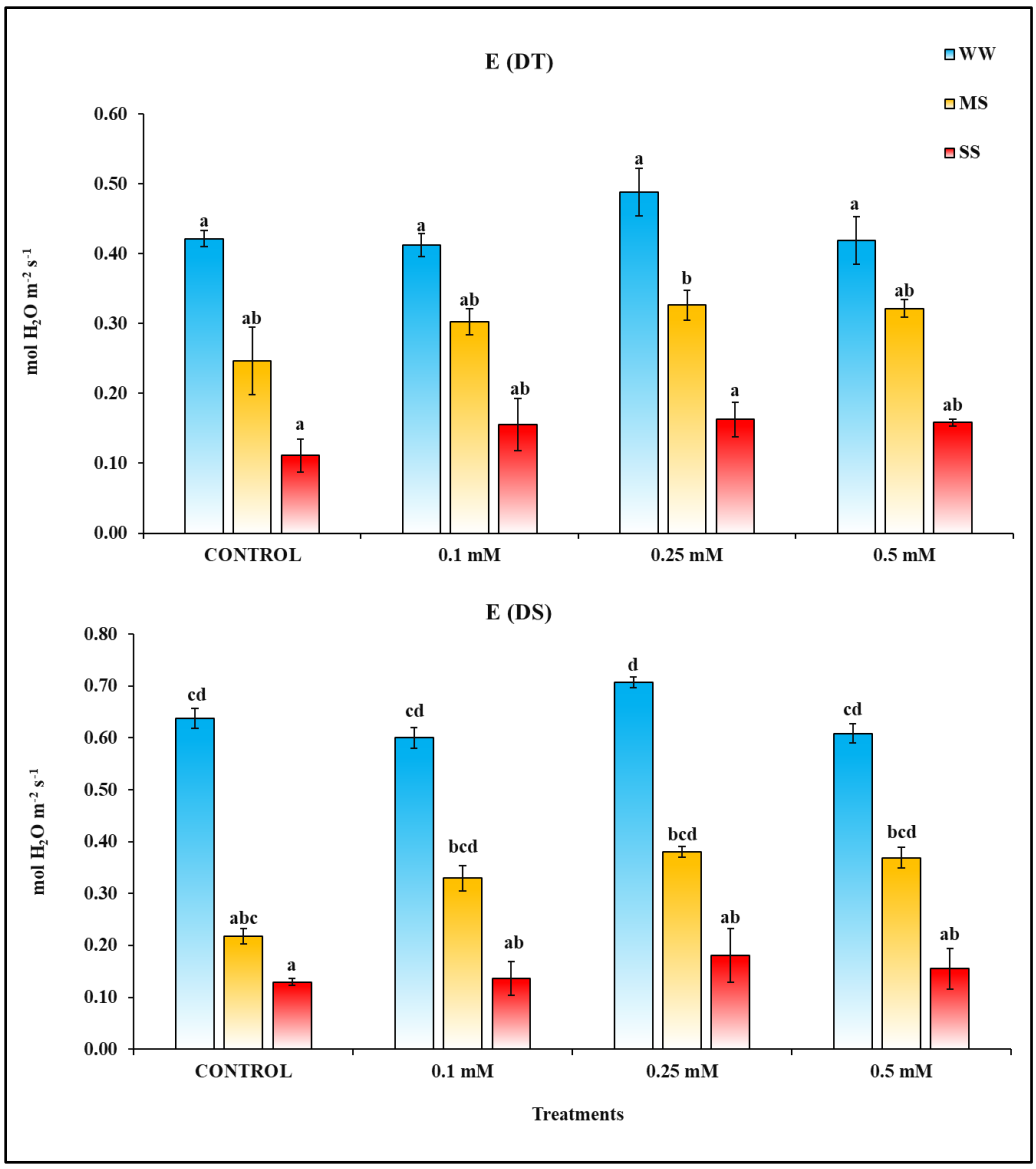


Fig. 3.8c: Leaf gas exchange measurements: **Transpiration rate (*E*)** of drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=3). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

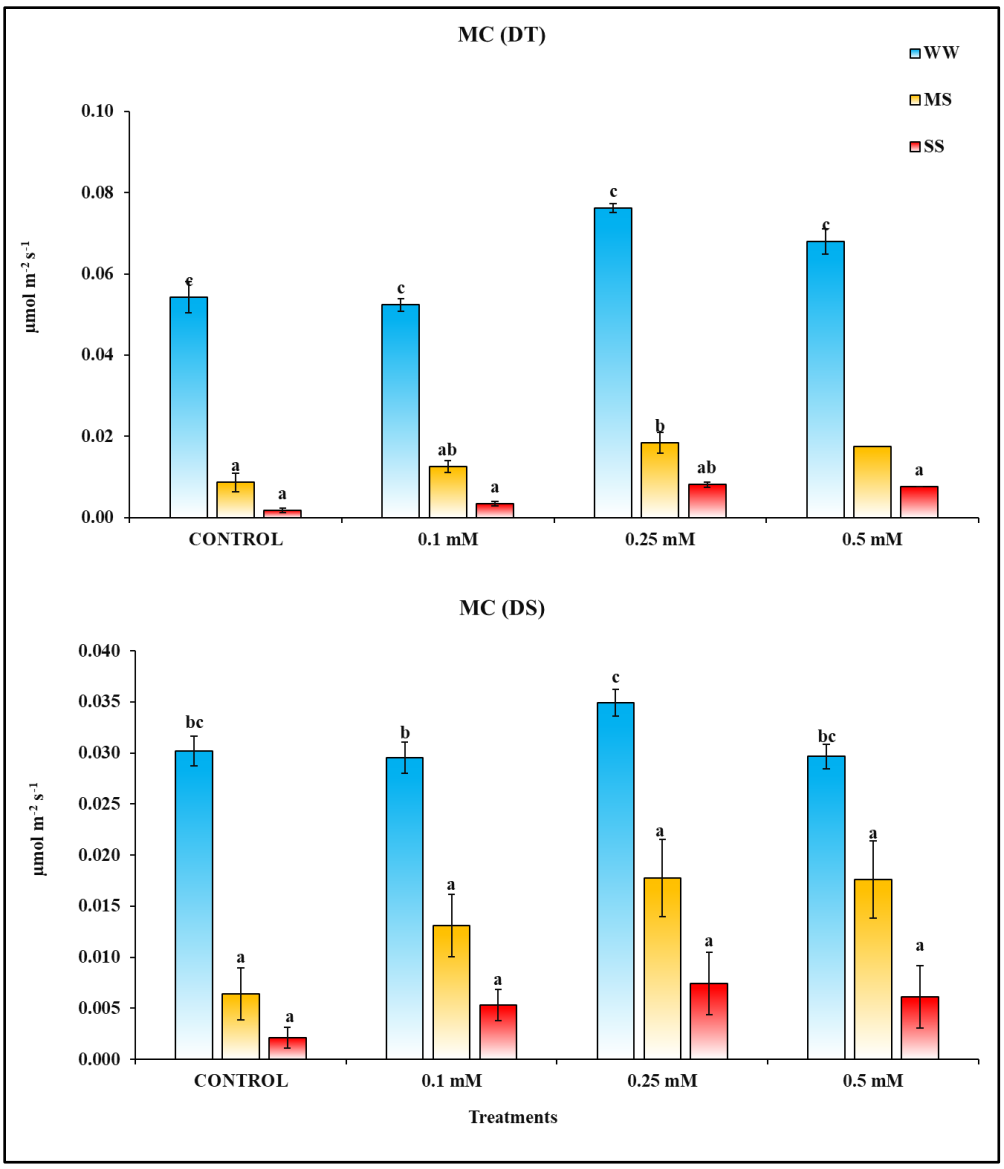


Fig. 3.8d: Leaf gas exchange measurements: Maximum carboxylation (MC) of drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=3). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

Water use efficiency (WUE) of plants exposed to moderate stress has decreased by 7% in DT and 18% in DS cultivars compared to well-watered control (**Fig. 3.8f**). However, applying 0.25 mM concentration of SA increased the WUE by 30% in DT and 31% in DS cultivars. 0.1 mM concentration increased by 24% in DT and 27% in DS. Similarly, 0.5 mM increased the WUE by 25% in DT and 31% in DS compared to control. Furthermore, WUE of plants exposed to severe drought stress has decreased by 66% in DT and 44% in DS cultivars compared to well-watered control. The maximum increase in WUE by 88% in DT and 8% in DS cultivars was observed at 0.25 mM concentration of SA. 0.1 mM concentration and 0.5 mM showed an increase of 16% and 71% in DT. On the other hand, a decrease in WUE was observed by 4% and 3% in DS using the same concentrations compared with its control.

Among the SA concentrations, 0.25 mM SA application markedly alleviated the negative impact of moderate and severe drought stress on dark reaction in both DT and DS compared to control.

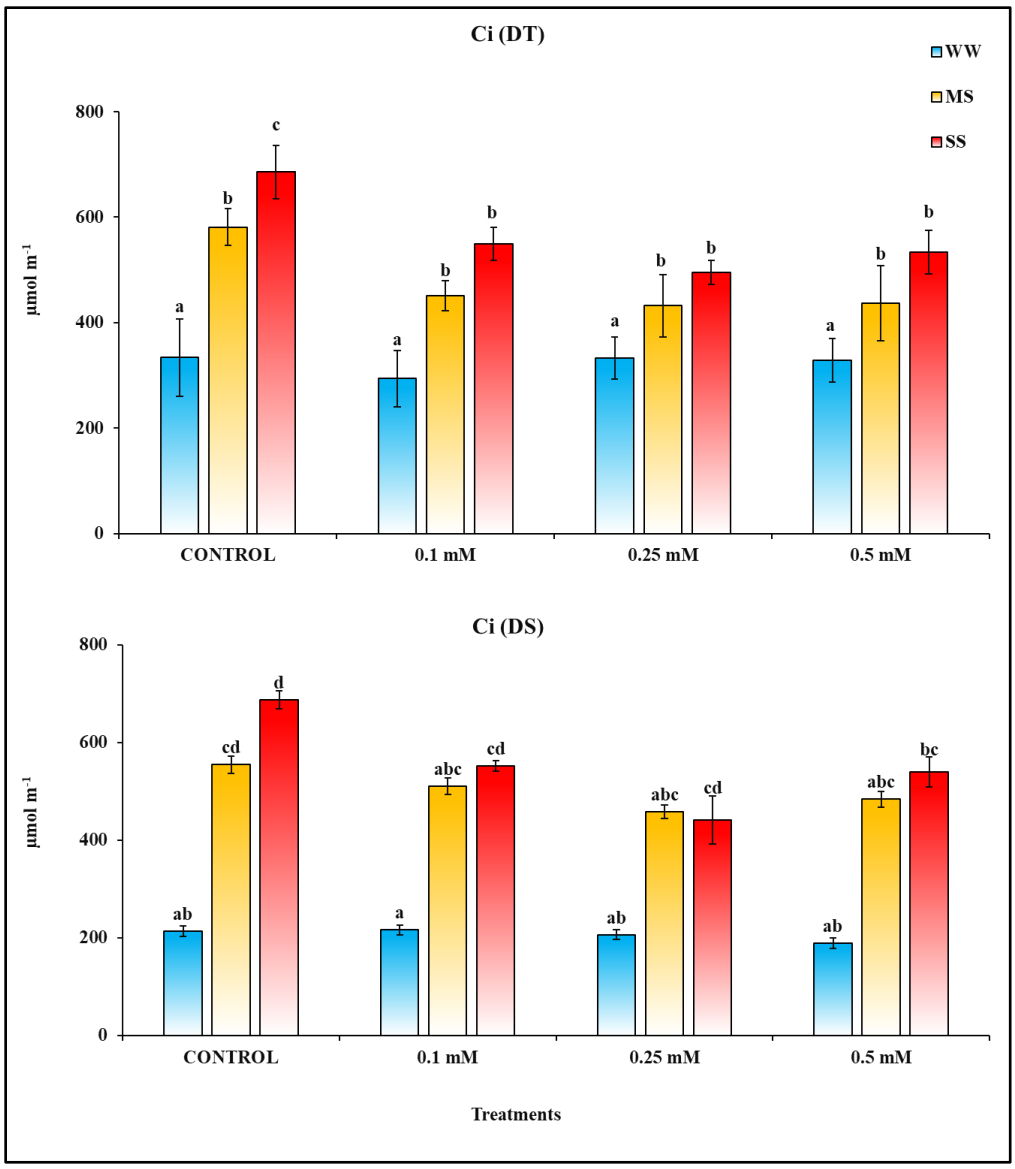


Fig. 3.8e: Leaf gas exchange measurements: **(E)-Internal carbon dioxide (Ci)** of drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=3). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

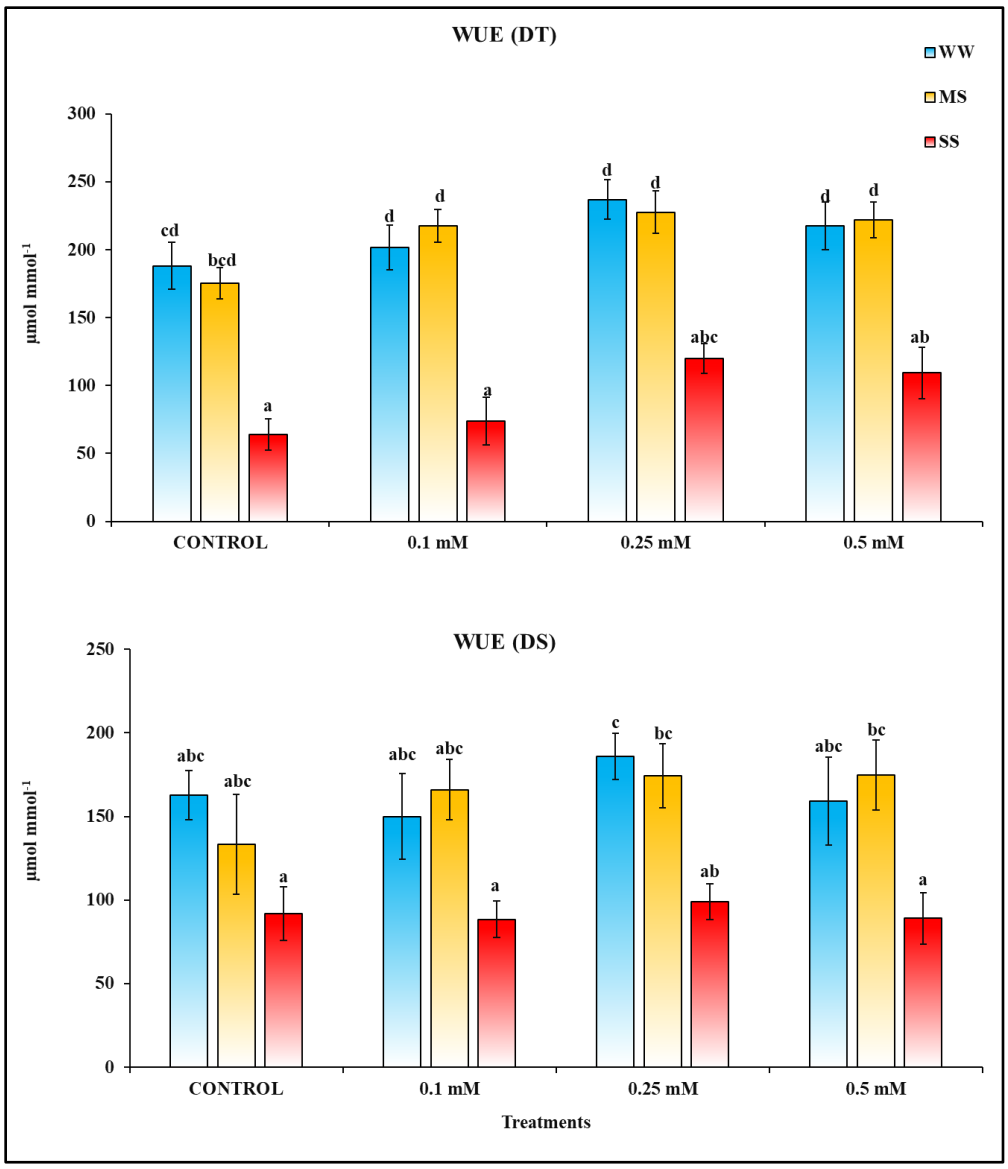


Fig. 3.8f: Leaf gas exchange measurements: **(F)-Water Use Efficiency (WUE)** of drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=3). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

3.2.3. Photosynthetic Pigment measurements

3.2.3.1. Analysis of photosynthetic pigments using HPLC

Qualitative and quantitative profile of photosynthetic pigments in response to drought stress and exogenously applied SA treatment was studied using HPLC. HPLC profile extracted at 445 nm, depicting qualitative changes, is presented in **Fig. 3.9a-b**. It was seen that with the interactive effect of drought stress and SA, there were no qualitative changes in the pigments and showed the presence of lutein, neoxanthin, violaxanthin, and β -carotene as carotenoids and chlorophyll 'a' and chlorophyll 'b' in both the cultivars (**Table 3.7, 3.8**). However, quantitative changes in chlorophyll and carotenoid contents were observed due to the drought stress and SA in both cultivars.

Our results indicate that the total chlorophyll content in the DT cultivar decreased by 19%, with a 20% and 19% decrease in Chl' a' and Chl 'b', respectively, compared to well-watered control on exposure to moderate drought stress. On the other hand, there was a 10% increase in total chlorophyll content in the DS cultivar with a 12% and 7% increase in Chl 'a' and Chl 'b', respectively, compared to well-watered control. Further, lutein, neoxanthin, violaxanthin, and β-carotene also decreased to variable levels in both cultivars. Total carotenoid content dropped by 5% in DT and 22% in the DS cultivar compared to control. The carotenoid to chlorophyll ratio (Car/Chl) also showed a decrease of 15% in DT and 28% in DS cultivars compared to the control. Furthermore, among the SA concentrations, the application of 0.25 mM showed the mitigating effect of drought stress on pigment composition compared to 0.1 and 0.5 mM. The exogenous application of 0.25 mM concentration of SA increased the total chlorophyll content by 22% in DT and 6% in DS cultivars compared to the control. Further, the total carotenoid content was also enhanced by 14%

in DT and 8% in DS cultivars, increasing the Car/Chl ratio by 9% and 2%, respectively, compared to the control (**Table 3.7, 3.8**).

It is observed that on exposure of DT and DS cultivars to severe drought stress, there was a decrease of 42% in the total chlorophyll in the DT cultivar, with 41% and 42% decrease in Chl 'a' and Chl 'b', respectively, compared to well-watered control. At the same drought level, the DS cultivar showed a decline in total chlorophyll by 8%, with a 5% and 11% decrease in Chl 'a' and Chl 'b', respectively, compared to well-watered control. Further, lutein, neoxanthin, violaxanthin, and β-carotene also decreased to variable levels in both cultivars. Total carotenoid content dropped by 26% in DT and 46% in DS cultivar compared to their respective controls. Carotenoid to chlorophyll ratio (Car/Chl) also showed a decline in both DT and DS cultivars by 21% and 42%, respectively.

Furthermore, among the SA concentrations, the application of 0.25 mM showed the mitigating effect of drought stress on pigment composition compared to 0.1 and 0.5 mM. The exogenous application of 0.25 mM concentration of SA increased the total chlorophyll content by 36% in DT and 10% in DS cultivars compared to the control. Further, the total carotenoid content was also enhanced by 25% in DT and 43% in DS cultivars, increasing the Car/Chl ratio by 9% and 32%, respectively, compared to the control (**Table 3.7, 3.8**).

Table 3.7: Influence of drought stress and exogenously applied salicylic acid on drought tolerant (DT) cultivar rice plant pigments. Data represent mean values ± SD (n=3). Different alphabets among the treatments denote significance at a 5% level.

Treatments		*Pigments (μg g^{-1} FW) (DT)*									
Drought	*SA*	*Lutein*	*Neoxanthin*	*Violaxanthin*	*β- carotene*	*Chl a*	*Chl b*	*Chl a/b*	*Total Chl*	*Total carotene*	*Car/Chl*
Well-watered	0 mM	0.017±0.0003^{e}	0.000130±0.00004^{cd}	0.0042±0.00011^{g}	0.0041±0.000088^{d}	0.025±0.001^{e}	0.017±0.00060^{bcd}	1.409±0.017^{a}	0.042±0.0017^{cd}	0.026±0.0003^{e}	1.621±0.056^{bc}
Moderate		0.016±0.0001^{d}	0.00028±0.00002^{fg}	0.0033±0.00005^{d}	0.0052±0.000422^{e}	0.020±0.0007^{c}	0.014±0.00056^{abc}	1.392±0.023^{a}	0.034±0.0013^{b}	0.025±0.0004^{d}	1.377±0.073^{ab}
Severe		0.014±0.0007^{b}	0.00012±0.00002^{bcd}	0.0024±0.00015^{b}	0.0028±0.000095^{a}	0.014±0.0002^{b}	0.010±0.00009^{a}	1.421±0.012^{a}	0.024±0.0003^{a}	0.019±0.0009^{c}	1.279±0.045^{a}
Well-watered	0.1 mM	0.015±0.0001^{cd}	0.000057±0.00002^{ab}	0.0036±0.00005^{e}	0.0040±0.000055^{cd}	0.025±0.0005^{e}	0.018±0.00052^{bcd}	1.406±0.021^{a}	0.042±0.0010^{cd}	0.023±0.0002^{c}	1.839±0.057^{c}
Moderate		0.015±0.0006^{c}	0.00016±0.00003^{de}	0.00278±0.00008^{c}	0.0069±0.000283^{g}	0.025±0.0003^{e}	0.012±0.00927^{ab}	5.701±0.046^{b}	0.037±0.0094^{bc}	0.025±0.0009^{d}	1.520±0.042^{ab}
Severe		0.011±0.0001^{a}	0.00005±0.00001^{a}	0.0026±0.00003^{bc}	0.0036±0.000052^{b}	0.008±0.0003^{a}	0.016±0.00069^{bcd}	0.517±0.009^{a}	0.025±0.0010^{a}	0.017±0.0001^{a}	1.433±0.050^{ab}
Well-watered	0.25 mM	0.018±0.0005^{f}	0.000091±0.00001^{abc}	0.0044±0.00005^{g}	0.0102±0.000427^{h}	0.030±0.0007^{g}	0.021±0.00054^{d}	1.398±0.034^{a}	0.051±0.0010^{e}	0.033±0.0010^{h}	1.549±0.046^{b}
Moderate		0.018±0.0004^{f}	0.00014±0.00001^{cd}	0.0039±0.00008^{f}	0.0059±0.000132^{f}	0.024±0.0004^{e}	0.017±0.00044^{bcd}	1.391±0.033^{a}	0.041±0.0007^{cd}	0.028±0.0006^{f}	1.477±0.031^{ab}
Severe		0.017±0.0006^{e}	0.00024±0.00011^{fg}	0.0039±0.00046^{f}	0.0027±0.000049^{a}	0.030±0.0002^{c}	0.014±0.00019^{abc}	1.418±0.006^{a}	0.033±0.0004^{b}	0.024±0.0010^{cd}	1.396±0.042^{abc}
Well-watered	0.5 mM	0.021±0.0004^{g}	0.000301±0.00001^{g}	0.0049±0.00015^{h}	0.0034±0.000218^{b}	0.026±0.0006^{f}	0.019±0.00050^{cd}	1.360±0.005^{a}	0.045±0.0011^{d}	0.029±0.0005^{g}	1.516±0.049^{ab}
Moderate		0.019±0.0003^{f}	0.00022±0.00002^{ef}	0.0042±0.00007^{g}	0.0037±0.000095^{bc}	0.022±0.0003^{d}	0.015±0.00042^{bc}	1.450±0.052^{a}	0.037±0.0003^{bc}	0.027±0.0004^{e}	1.396±0.030^{ab}
Severe		0.013±0.0002^{b}	0.00026±0.00002^{fg}	0.0021±0.00004^{a}	0.0026±0.000038^{a}	0.009±0.0001^{a}	0.017±0.00027^{bcd}	0.521±0.002^{a}	0.025±0.0004^{a}	0.018±0.0002^{b}	1.396±0.008^{ab}

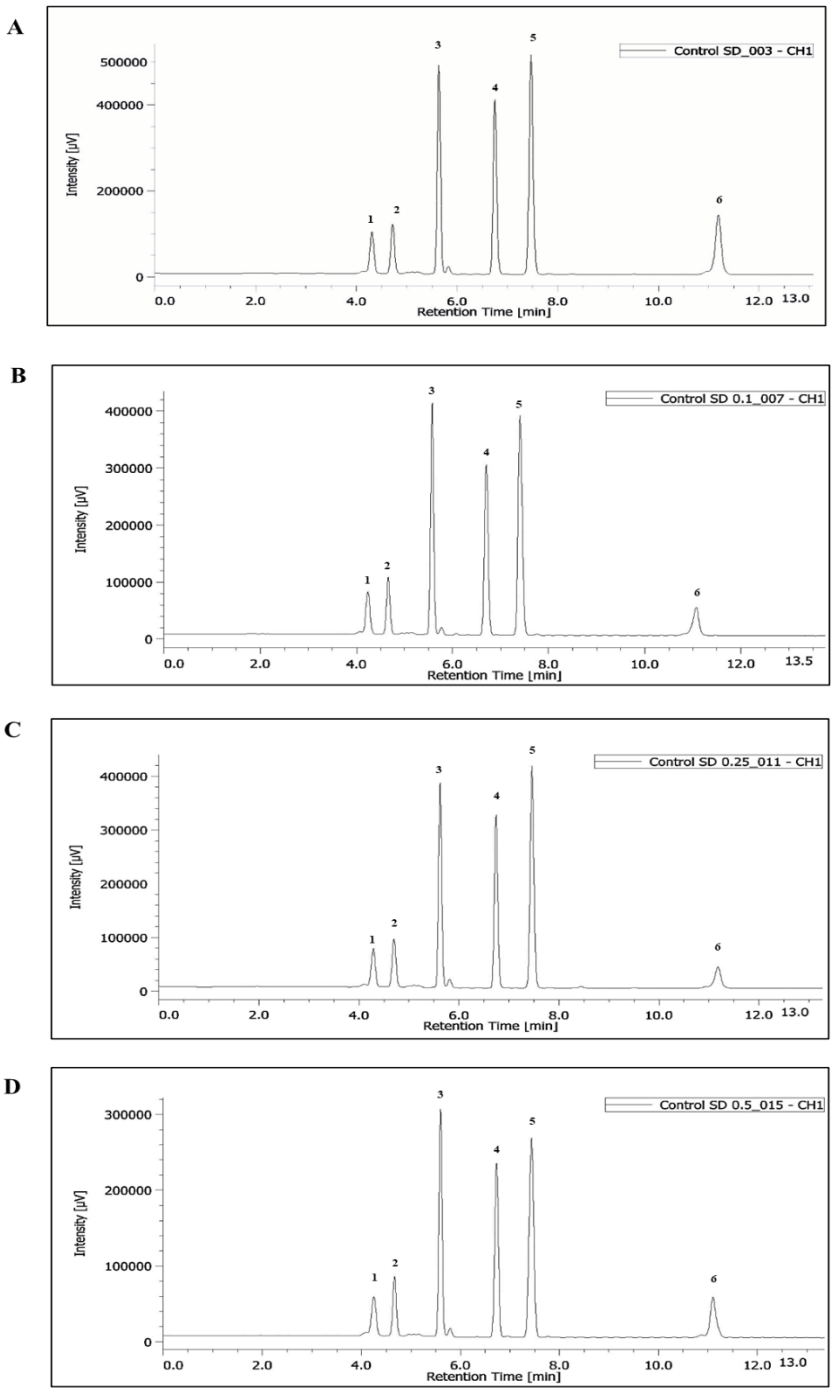


Fig. 3.9a: HPLC profile of photosynthetic pigments of drought tolerant (DT) rice cultivars under well-watered condition and treated with salicylic acid at 445 nm. **A-D**: Well-watered (WW); **A**-WW; **B**-WW+0.1 mM SA; **C**-WW+0.25 mM SA; **D**-WW+ 0.5 mM SA. **1-** Lutein; **2-** Neoxanthin; **3**-Violaxanthin; **4-** Chl a; **5-** Chl b; **6-** βcarotene.

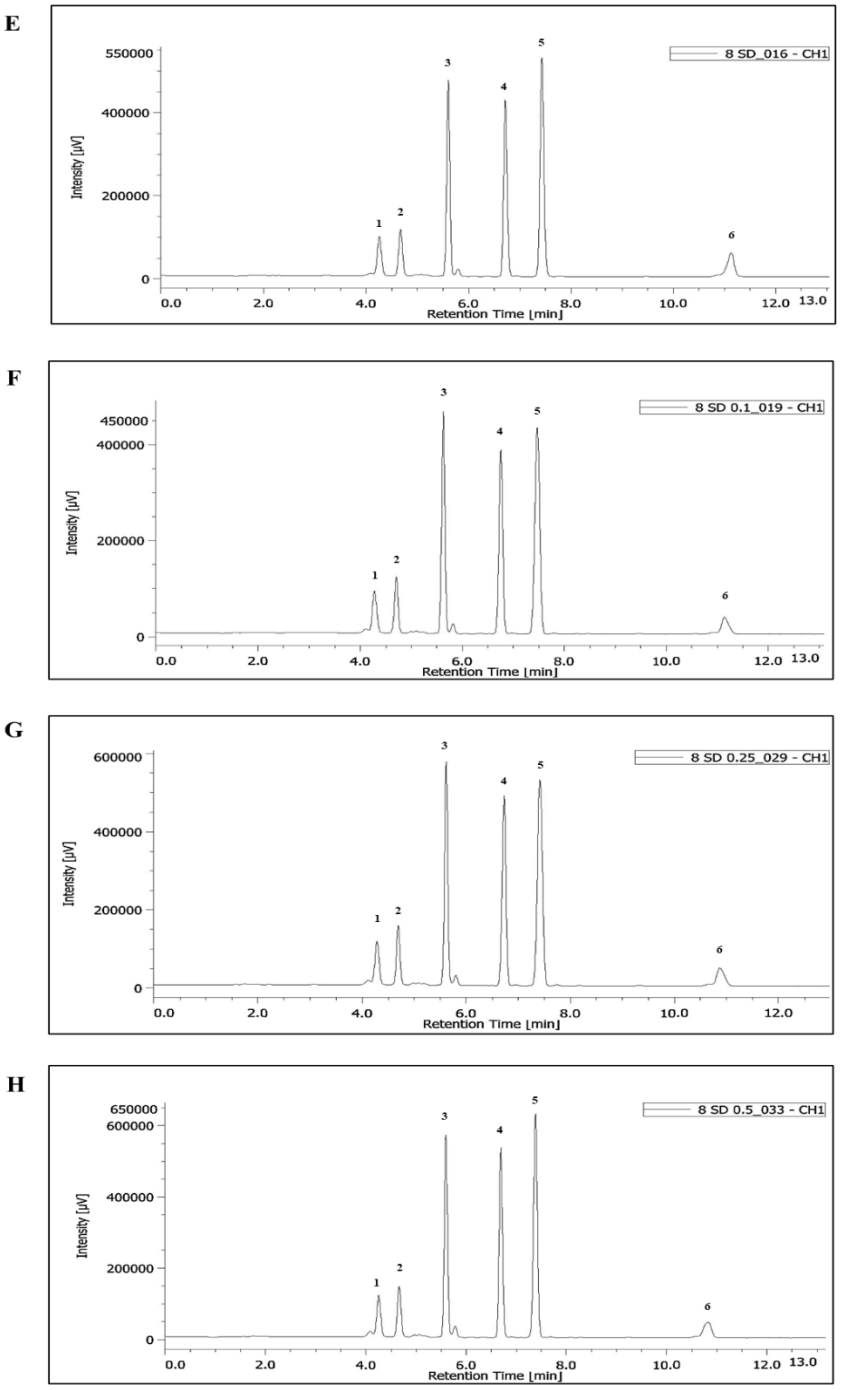


Fig. 3.9b: HPLC profile of photosynthetic pigments of drought tolerant (DT) rice cultivars treated with moderate drought stress and salicylic acid at 445 nm. **E-H**: Moderates stress (MS); **E**- MS; **F**-MS+0.1 mM SA; **G**- MS+0.25 mM SA; **H**-MS+0.5 mM SA. **1-** Lutein; **2-** Neoxanthin; **3-**Violaxanthin; **4-** Chl a; **5-** Chl b; **6-** βcarotene.

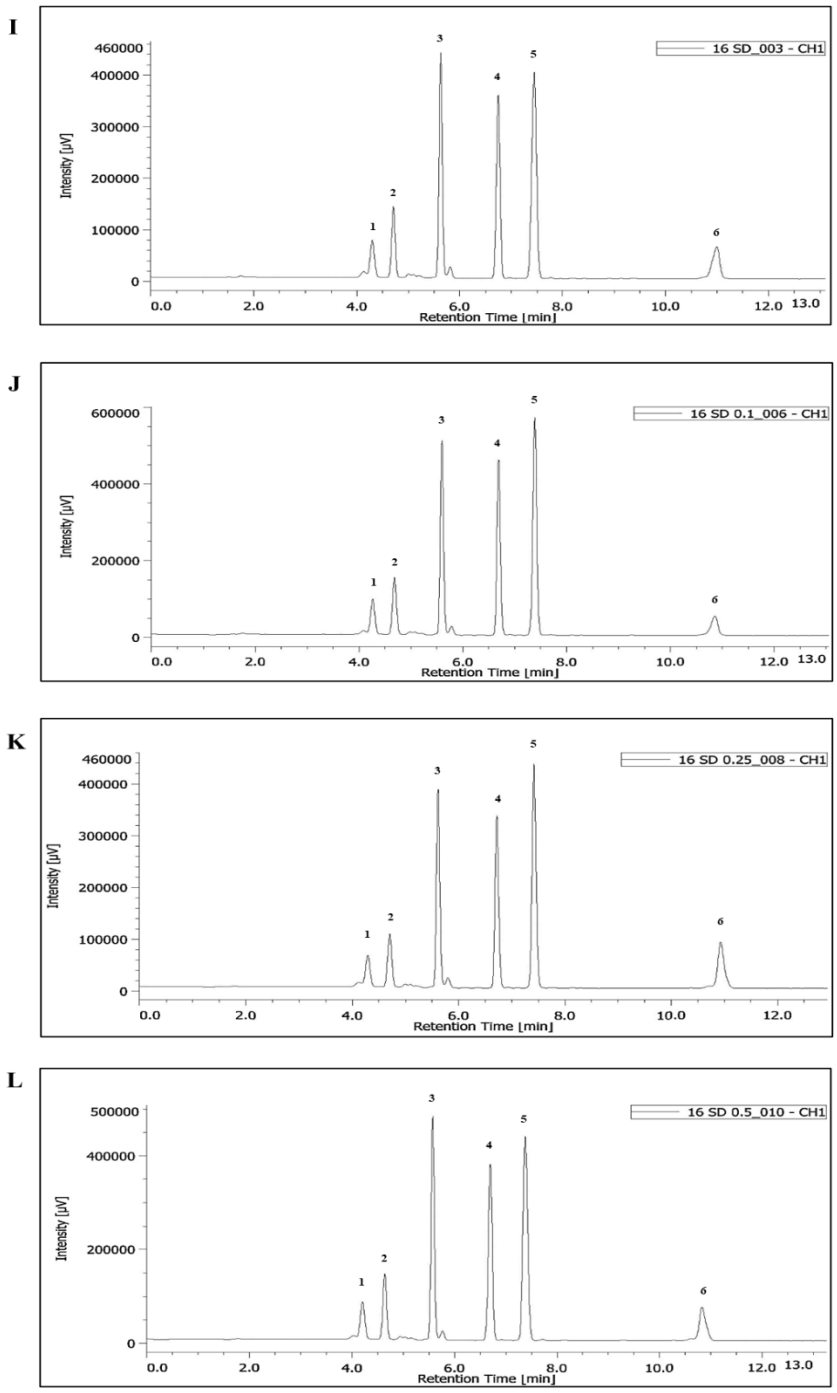


Fig. 3.9c: HPLC profile of photosynthetic pigments of drought tolerant (DT) rice cultivars treated with severe drought stress and salicylic acid at 445 nm. **I-L**: Severe stress (SS). **I**- SS; **J**- SS+0.1 mM SA; **K**- SS+0.25 mM SA; **L**- SS+0.5 mM SA. **1**- Lutein; **2**- Neoxanthin; **3**-Violaxanthin; **4**- Chl a; **5**- Chl b; **6**- βcarotene.

Table 3.8: Influence of drought stress and exogenously applied salicylic acid on drought-sensitive (DS) cultivar rice plant pigments. Data represent mean values ± SD (n=3). Different alphabets among the treatments denote significance at a 5% level.

Treatments		Pigments (μg g^{-1} FW) (DS)									
Drought	SA	Lutein	Neoxanthin	Violaxanthin	Chl a	Chl b	Chl a/b	β- carotene	Total Chl	Total carotene	Car/Chl
Well-watered	0 mM	0.017±0.0017^{cd}	0.00030±0.000007^{a}	0.0034±0.00049^{ab}	0.023±0.0008^{bcd}	0.018±0.0006^{bc}	1.331±0.006^{a}	0.050±0.0056^{c}	0.041±0.0014^{bc}	0.071±0.008^{cd}	1.740±0.13^{def}
Moderate		0.019±0.0003^{de}	0.00051±0.00013^{bc}	0.0039±0.00024^{bc}	0.026±0.0011^{ef}	0.019±0.0007^{cd}	1.396±0.011^{bcd}	0.033±0.0021^{b}	0.045±0.002^{de}	0.056±0.002^{bc}	1.247±0.09^{ab}
Severe		0.018±0.0005^{de}	0.00062±0.00009^{c}	0.0038±0.00021^{abc}	0.022±0.0007^{abc}	0.016±0.0005^{a}	1.420±0.014^{d}	0.015±0.0008^{a}	0.038±0.0012^{ab}	0.038±0.002^{a}	1.014±0.01^{a}
Well-watered	0.1 mM	0.015±0.0015^{ab}	0.00028±0.00016^{a}	0.0033±0.00064^{a}	0.024±0.0023^{f}	0.017±0.0013^{ab}	1.463±0.029^{e}	0.049±0.0261c	0.041±0.0035^{bc}	0.068±0.028^{bcd}	1.638±0.56^{cdef}
Moderate		0.021±0.0002^{f}	0.00055±0.00010^{bc}	0.0042±0.00011^{c}	0.023±0.0011^{bcd}	0.017±0.0006^{ab}	1.393±0.016^{bcd}	0.031±0.0005^{b}	0.040±0.002^{bc}	0.056±0.000^{bc}	1.402±0.05^{bc}
Severe		0.0170\±0.0019^{cd}	0.00024±0.00011^{a}	0.0040±0.00051^{bc}	0.021±0.0011^{a}	0.015±0.0010^{a}	1.371±0.026^{b}	0.032±0.0021^{b}	0.036±0.0021^{a}	0.053±0.01^{ab}	1.473±0.05^{bcde}
Well-watered	0.25 mM	0.016±0.0004^{abc}	0.00039±0.00005^{ab}	0.0032±0.00007^{a}	0.028±0.0003^{cde}	0.017±0.0003^{de}	1.369±0.016^{b}	0.072±0.0005^{d}	0.048±0.0005^{e}	0.092±0.001^{e}	1.894±0.02^{f}
Moderate		0.018±0.0002^{de}	0.00053±0.00006^{bc}	0.0032±0.00010^{a}	0.028±0.0004^{f}	0.020±0.0002^{de}	1.419±0.025^{d}	0.039±0.0008^{c}	0.048±0.000^{e}	0.061±0.001^{bcd}	1.269±0.03^{ab}
Severe		0.016±0.0003^{bc}	0.00037±0.00012^{ab}	0.0033±0.00016^{a}	0.024±0.0028^{cde}	0.017±0.0021^{bc}	1.378±0.012^{bc}	0.035±0.0012^{b}	0.041±0.0048^{bcd}	0.055±0.002^{b}	1.336±0.19^{abc}
Well-watered	0.5 mM	0.019±0.0003^{e}	0.00054±0.00012^{bc}	0.0038±0.00023^{abc}	0.024±0.0004^{de}	0.017±0.0002^{bc}	1.413±0.030^{cd}	0.051±0.0020^{c}	0.042±0.0005^{cd}	0.075±0.002^{c}	1.787±0.05^{ef}
Moderate		0.018±0.0004^{be}	0.00038±0.00012^{ab}	0.0040±0.00041^{bc}	0.027±0.0008^{f}	0.019±0.0003^{de}	1.379±0.025^{bc}	0.037±0.0021^{bc}	0.046±0.001^{e}	0.060±0.003^{bcd}	1.305±0.09^{abc}
Severe		0.015±0.0004^{a}	0.00026±0.00008^{a}	0.0033±0.00023^{a}	0.021±0.0003^{ab}	0.015±0.0002^{a}	1.386±0.003^{bcd}	0.034±0.0002^{b}	0.036±0.0005^{a}	0.052±0.001^{ab}	1.429±0.02^{bcd}

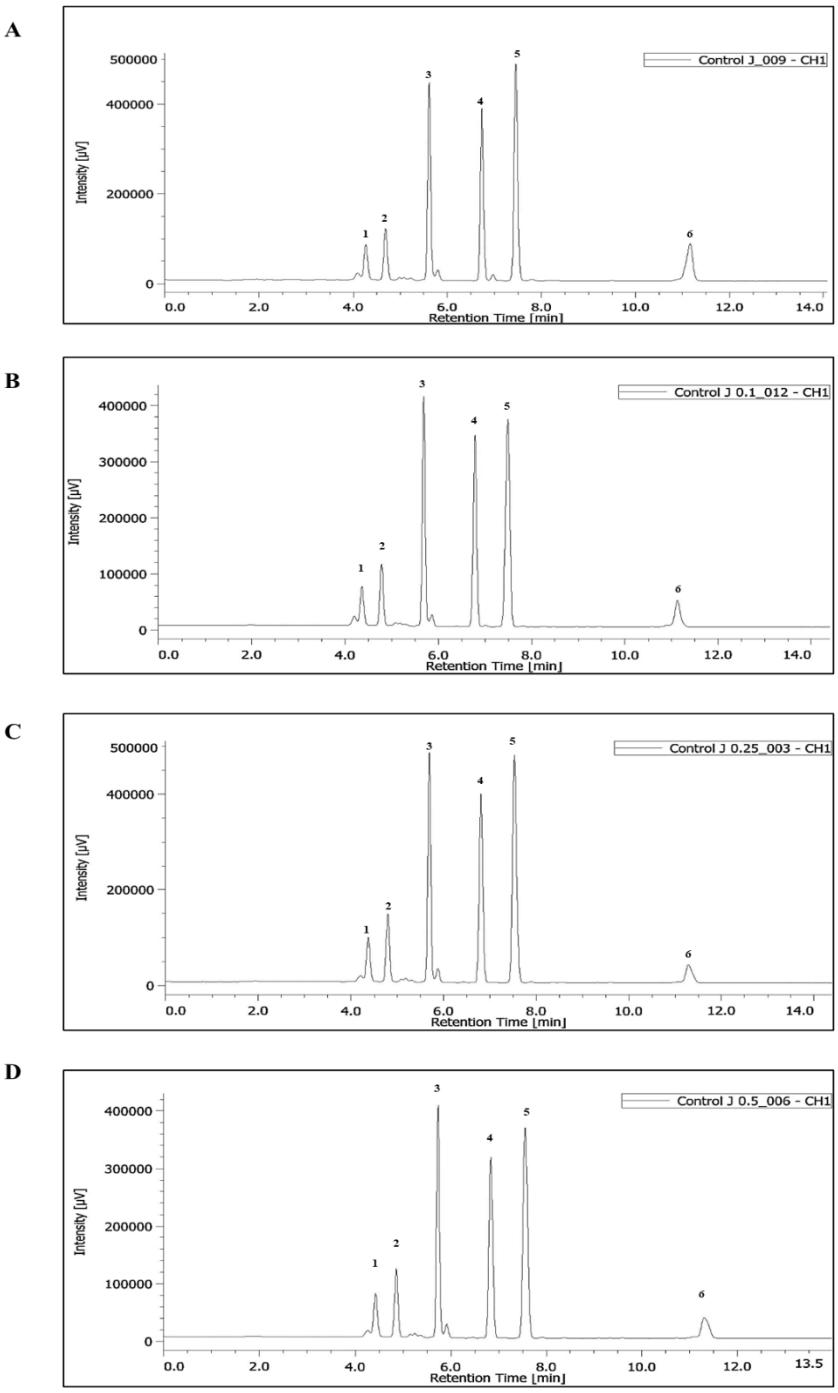


Fig. 3.9d: HPLC profile of photosynthetic pigments of drought-sensitive (DS) rice cultivars under well-watered condition and treated with salicylic acid at 445 nm. **A-D**: Well-watered (WW); **A**-WW; **B**-WW+0.1 mM SA; **C**-WW+0.25 mM SA; **D**-WW+ 0.5 mM SA. **1-** Lutein; **2-** Neoxanthin; **3**-Violaxanthin; **4-** Chl a; **5-** Chl b; **6-** βcarotene.

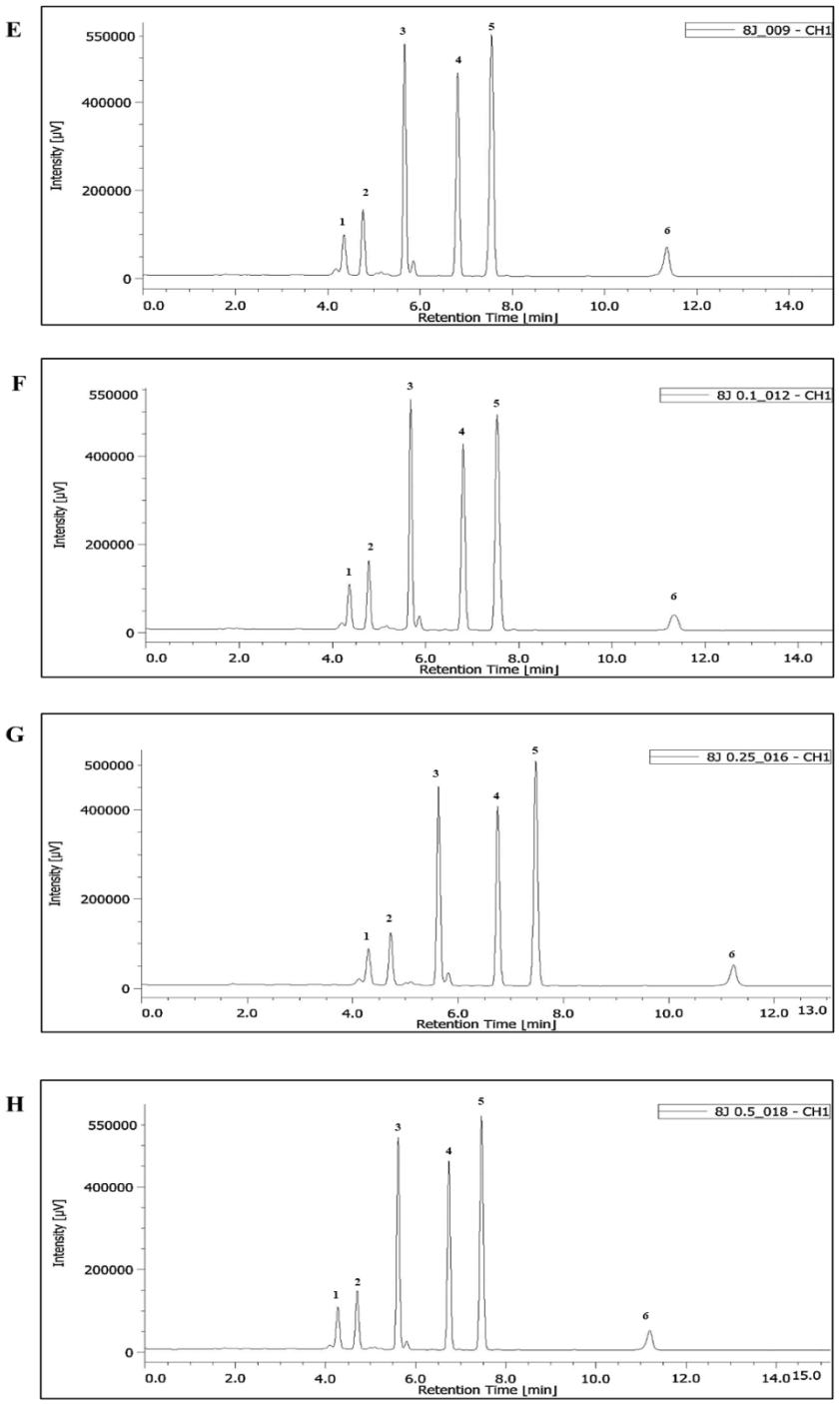


Fig. 3.9e: HPLC profile of photosynthetic pigments of drought-sensitive (DS) rice cultivars treated with moderate drought stress and salicylic acid at 445 nm. **E-H**: Moderates stress (MS); **E**- MS; **F**-MS+0.1 mM SA; **G**- MS+0.25 mM SA; **H**-MS+0.5 mM SA. **1-** Lutein; **2-** Neoxanthin; **3-**Violaxanthin; **4-** Chl a; **5-** Chl b; **6-** βcarotene.

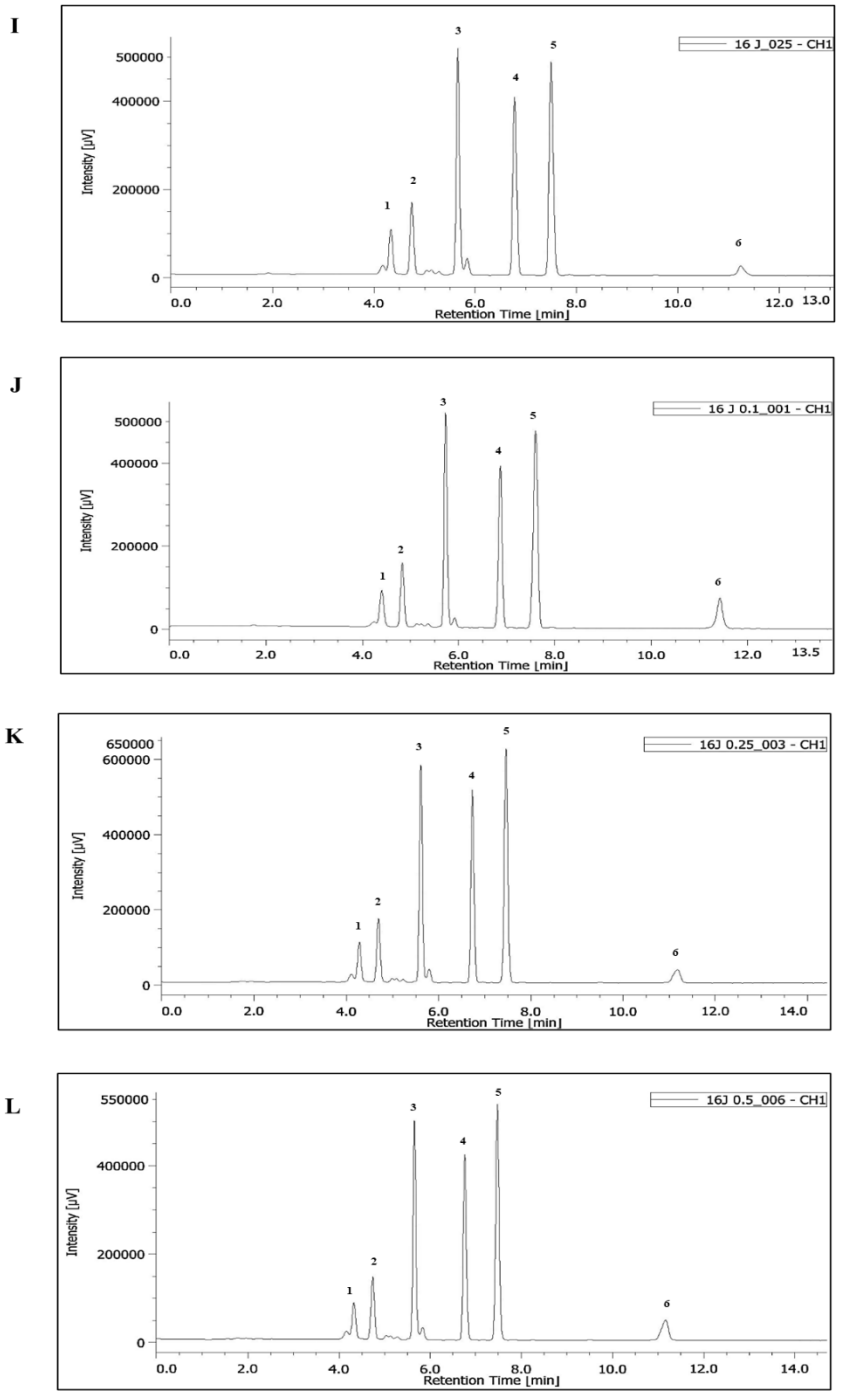


Fig. 3.9f: HPLC profile of photosynthetic pigments of drought-sensitive (DS) rice cultivars treated with severe drought stress and salicylic acid at 445 nm. **I-L**: Severe stress (SS). **I**- SS; **J**- SS+0.1 mM SA; **K**- SS+0.25 mM SA; **L**- SS+0.5 mM SA. **1**- Lutein; **2**- Neoxanthin; **3**-Violaxanthin; **4**- Chl a; **5**- Chl b; **6**- βcarotene.

3.3. Biochemical Assays

The results of the investigation on the interactive influence of PEG_{6000}-induced moderate and severe drought and the various concentrations of foliar applied SA viz., 0.1, 0.25 and 0.5 mM on the accumulation of ROS and oxidative damage analysed as lipid peroxidation, electrolyte leakage, protein oxidation of the selected DT and DS rice cultivars are presented in **Table 3.9**.

3.3.1. Quantification of Reactive Oxygen Species Accumulation:

3.3.3.1. Determination of Hydrogen Peroxide (H_2O_2)

It was observed that both cultivars grown under well-watered conditions showed low-level of H_2O_2 and OH• compared to plants grown under moderate and severe drought conditions. The H_2O_2 content significantly increased in DT and DS cultivars under moderate and severe drought stress compared to control plants (**Table 3.9; Fig. 3.10a**).

The H_2O_2 content of plants exposed to moderate stress increased by 78% in DT and 43% in DS cultivars compared to well-watered control (**Fig. 3.10a)**. The maximum decrease in H_2O_2 content by 24% in DT and 20% in DS cultivar was recorded at 0.25 mM concentration of SA. 0.1 mM concentration decreased by 10% in DT and 9% in DS cultivar. Similarly, 0.5 mM concentration decreased 14% in DT and 15% in DS cultivars compared to control**.** Furthermore, the H_2O_2 content of plants exposed to severe drought stress increased by 115% in DT and 75% in DS cultivar compared to well-watered control. The maximum decrease in H_2O_2 content by 8% in DT and 12% in DS cultivar was recorded at 0.25 mM concentration of SA. 0.1 mM concentration increased by 4% in DT and 8% in DS cultivar. Similarly, 0.5 mM concentration increased 7% in DT and 9% in DS cultivars compared to

control.

3.3.3.2. Determination of Hydroxyl Radical (•OH)

OH• content of plants exposed to moderate stress increased by 31% in DT and 42% in DS cultivars compared to well-watered control (**Fig. 3.10b**). However, it was observed that exogenous application of SA had significantly decreased OH• content by 23% in DT and 22% in DS cultivars at 0.25 mM concentration of SA. 0.1 mM concentration increased by 13% in DT and 8% in the DS cultivar. Similarly, 0.5 mM concentration increased 18% in DT and 22% in DS cultivars compared to control.

In addition, the OH• content of plants exposed to severe drought stress increased by 64% in DT and 67% in DS cultivars compared to well-watered control. The maximum decrease in OH• content by 17% in DT and 34% in DS cultivars was observed at 0.25 mM concentration of SA. 0.1 mM concentration increased by 7% in DT and 20% in the DS cultivar. Similarly, 0.5 mM concentration increased 14% in DT and 32% in DS cultivars compared to control. It may be noted that the exogenous application of SA significantly decreased the negative impacts of severe drought stress on the OH• content of both cultivars. Among the SA concentrations, treatment with the 0.25 mM SA resulted in a marked decrease in the ROS (H_2O_2; OH•) content under moderate and severe drought stress in both DT and DS compared to control.

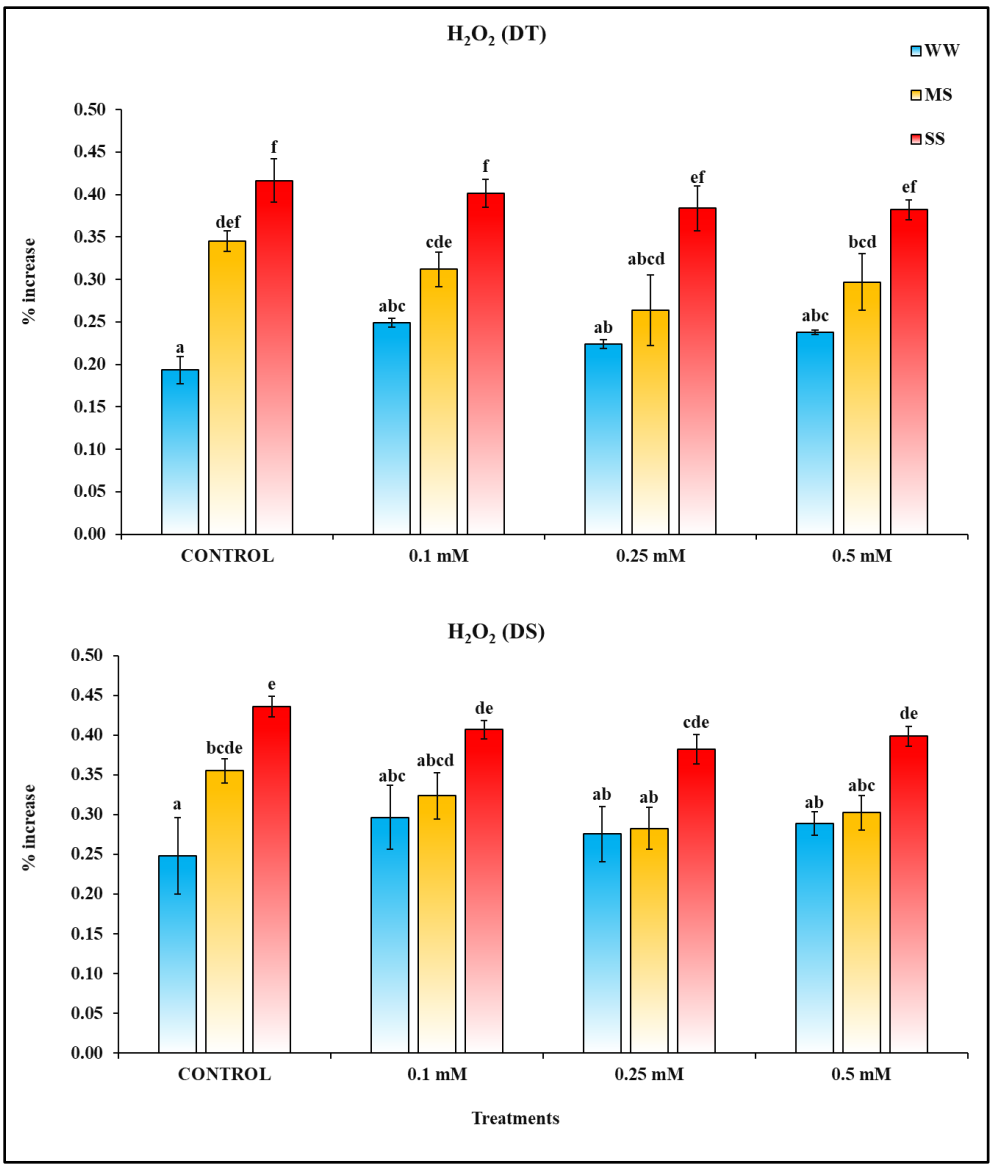


Fig. 3.10a: Reactive Oxygen Species (ROS) (hydrogen peroxide- H_2O_2) content in drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=9). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

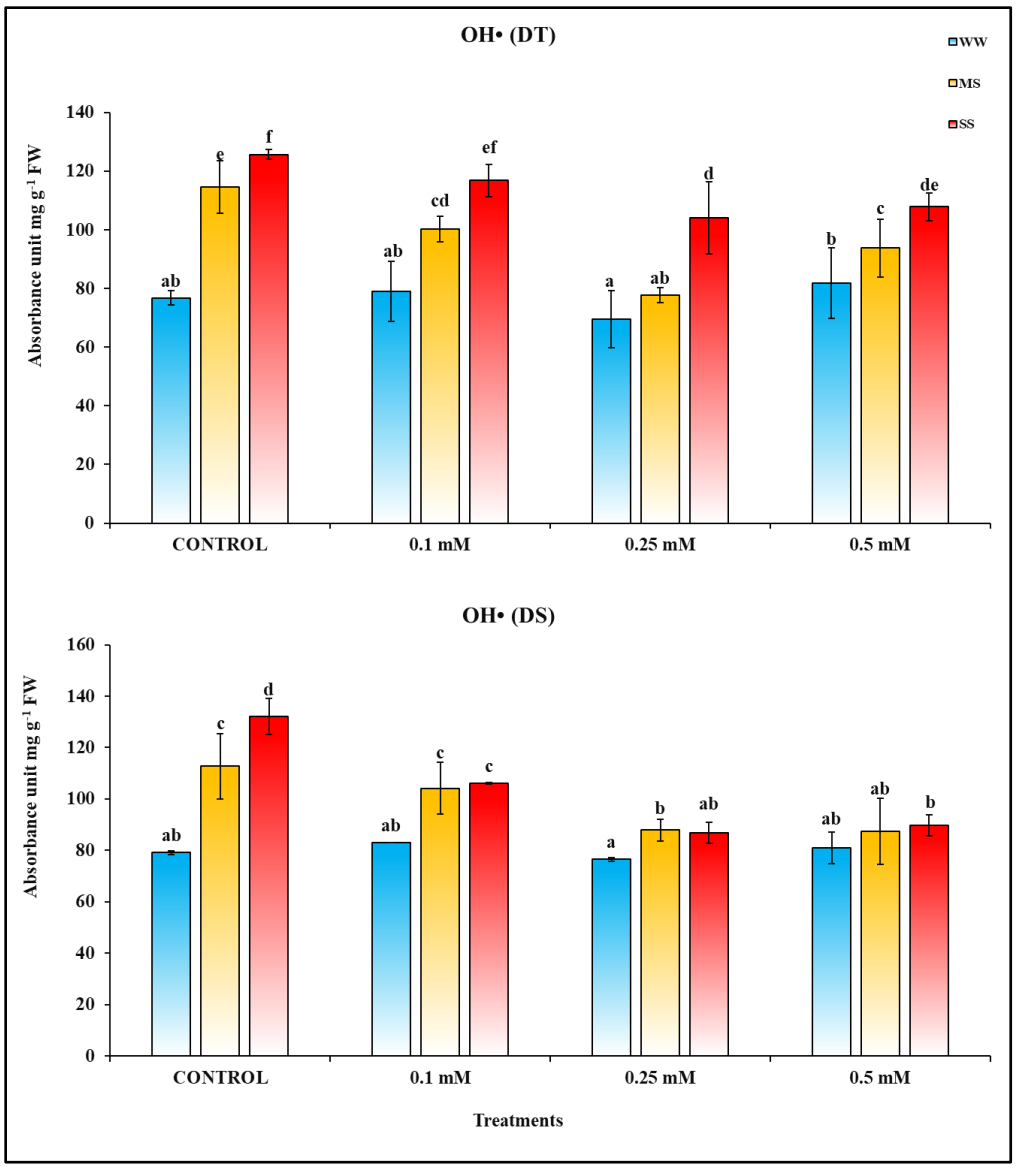


Fig. 3.10b: Reactive Oxygen Species (ROS) (hydroxyl radical- OH•) content in drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=9). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

3.3.2. Oxidative damage caused by ROS/ Effect of salicylic acid and drought stress on oxidative damage:

3.3.2.1. Determination of lipid peroxidation

The malondialdehyde (MDA) content level, an indicator of membrane damage imposed due to oxidative stress, was analysed in rice plants. Under drought stress, both cultivars showed significant MDA accumulation compared to control plants (**Table 3.9; Fig. 3.11)**. Compared to well-watered control, the MDA content of plants exposed to moderate stress increased by 55% in DT and 94% in DS cultivars. The exogenous application of SA significantly decreased MDA content by 26% in DT and 10% in DS cultivars at 0.25 mM concentration of SA. 0.1 mM concentration decreased by 5% in DT and 2% in DS cultivar. Similarly, 0.5 mM concentration decreased 25% in DT and 7% in DS cultivars compared to control.

The MDA content of plants exposed to severe drought stress has increased by 143% in DT and 566% in DS cultivars compared to well-watered control. The exogenous application of SA significantly decreased MDA content by 24% in DT and 27% in DS cultivar at 0.25 mM concentration of SA. 0.1 mM concentration increased by 20% in DT and 7% in DS cultivar. Similarly, 0.5 mM concentration increased 22% in DT and 12% in DS cultivars compared to control.

There was a marked decrease in MDA content under moderate and severe drought stress in both DT and DS with the 0.25 mM SA application compared to its control, followed by 0.5 mM and 0.1 mM.

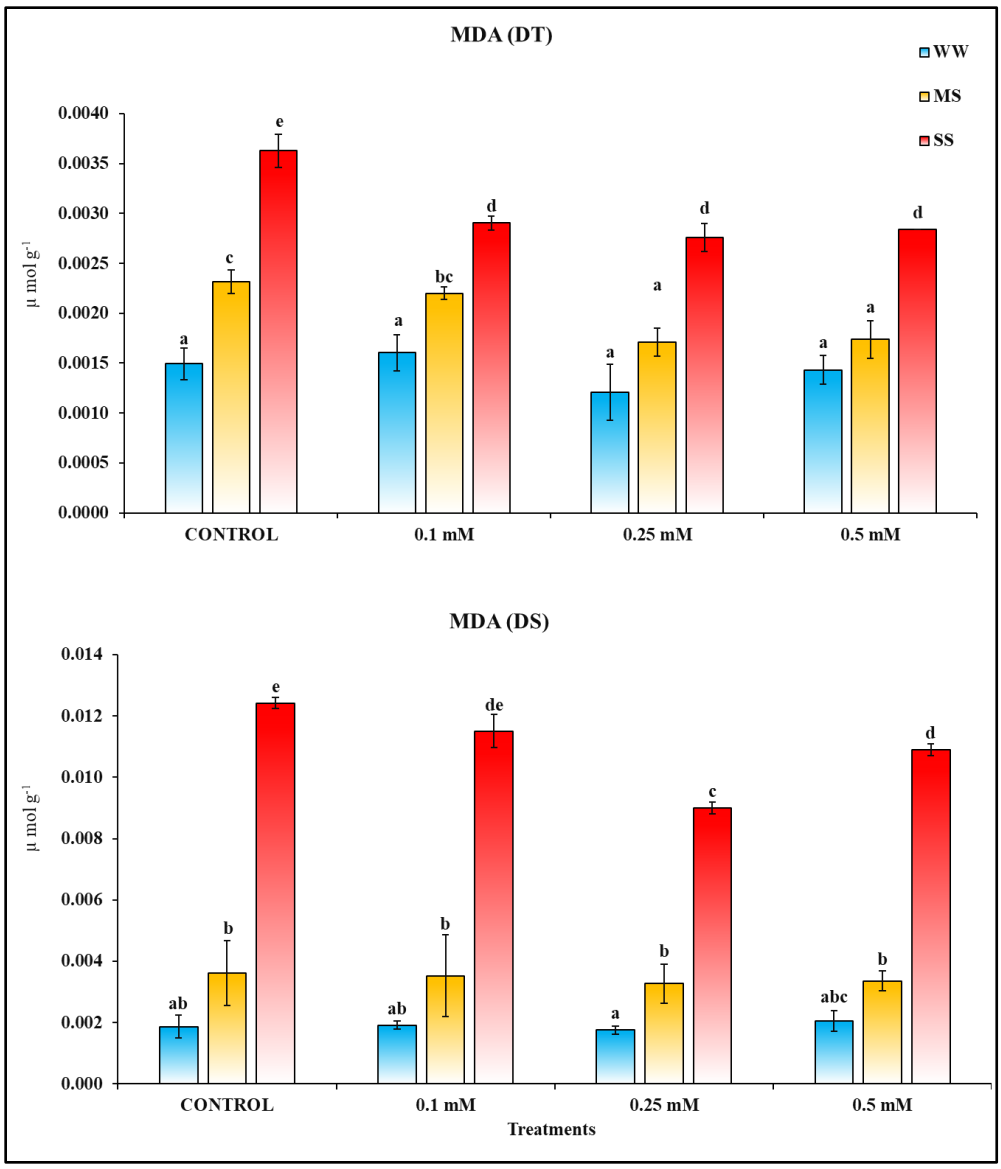


Fig. 3.11: Malondialdehyde (MDA) accumulation in drought-tolerant (DT), drought-sensitive (DS), and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=9). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

3.3.2.2. Electrolyte leakage (EL)

The results indicate drought stress significantly increased EL in both rice cultivars compared to well-watered control (**Fig. 3.11**). Electrolyte leakage of plants exposed to moderate stress increased by 72% in DT and 79% in DS cultivars compared to well-watered control. On the other hand, it has been observed that exogenous application of SA significantly decreased EL by 30% in DT and 19% in DS cultivars with 0.25 mM concentration of SA. 0.1 mM concentration decreased by 23% in DT and 8% in the DS cultivar. In comparison, 0.5 mM concentration decreased by 8% in the DS cultivar compared to the control.

The EL of plants exposed to severe drought stress increased by 87% in DT and 96% in DS cultivars compared to well-watered control. Further, the exogenous application of 0.25 mM concentration of SA significantly decreased the negative impacts of severe drought stress on the EL of both cultivars by 41% in DT and 22% in DS cultivars. 0.1 mM concentration decreased by 11% in DT and 27% in the DS cultivar. In comparison, 0.5 mM concentration decreased by 21% in the DT cultivar and 15% in the DS cultivar compared to control.

The study revealed that among the concentrations of SA used, treatment with 0.25 mM resulted in a marked decrease in EL under moderate and severe drought stress in both DT and DS cultivars compared to control.

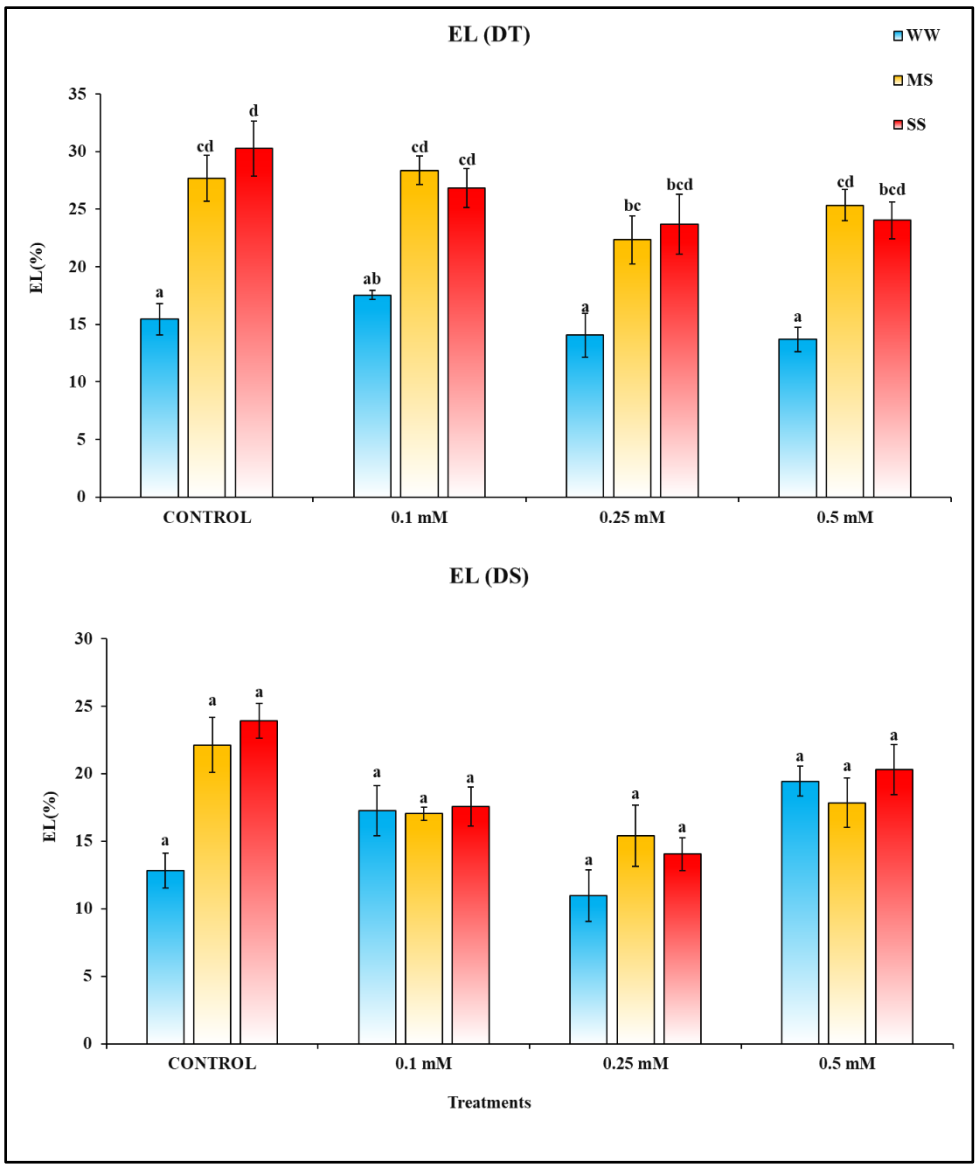


Fig. 3.12: Electrolyte leakage (EL) in drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=9). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

3.3.2.3. Protein oxidation assay

Changes in protein carbonyl content levels were measured in DT and DS cultivars exposed to drought stress and drought combined with SA (**Table 3.9; Fig. 3.13**). The results of the study revealed that protein carbonyl content significantly increased in DT and DS cultivars subjected to drought compared to well-watered control.

The protein carbonyl content of plants exposed to moderate stress increased by 131% in DT and 16% in DS cultivars compared to well-watered control. Nevertheless, it was observed that the exogenous application of 0.25 mM SA had significantly decreased the negative impacts of protein carbonyl content by 39% in DT and 12% in DS cultivars. 0.1 mM concentration recorded a decrease of 8% in DT and 1% in DS cultivar. In comparison, 0.5 mM concentration decreased 33% in the DT cultivar and 5% in the DS cultivar compared to the control. Moreover, the protein carbonyl content of plants exposed to severe drought stress recorded an increase of 165% in DT and 41% in DS cultivars compared to well-watered control. However, it was observed that the exogenous application of 0.25 mM SA significantly decreased the negative impacts of protein carbonyl content by 19% in DT and 16% in DS cultivars. 0.1 mM concentration recorded a decrease of 2% in DT and 3% in DS cultivar. In comparison, 0.5 mM concentration recorded a decrease of 13% in the DT cultivar and 6% in the DS cultivar compared to control (**Fig. 3.13)**. Among the SA concentrations, treatment with 0.25 mM SA markedly decreased the protein carbonyl content under moderate and severe drought stress in both DT and DS as compared to control.

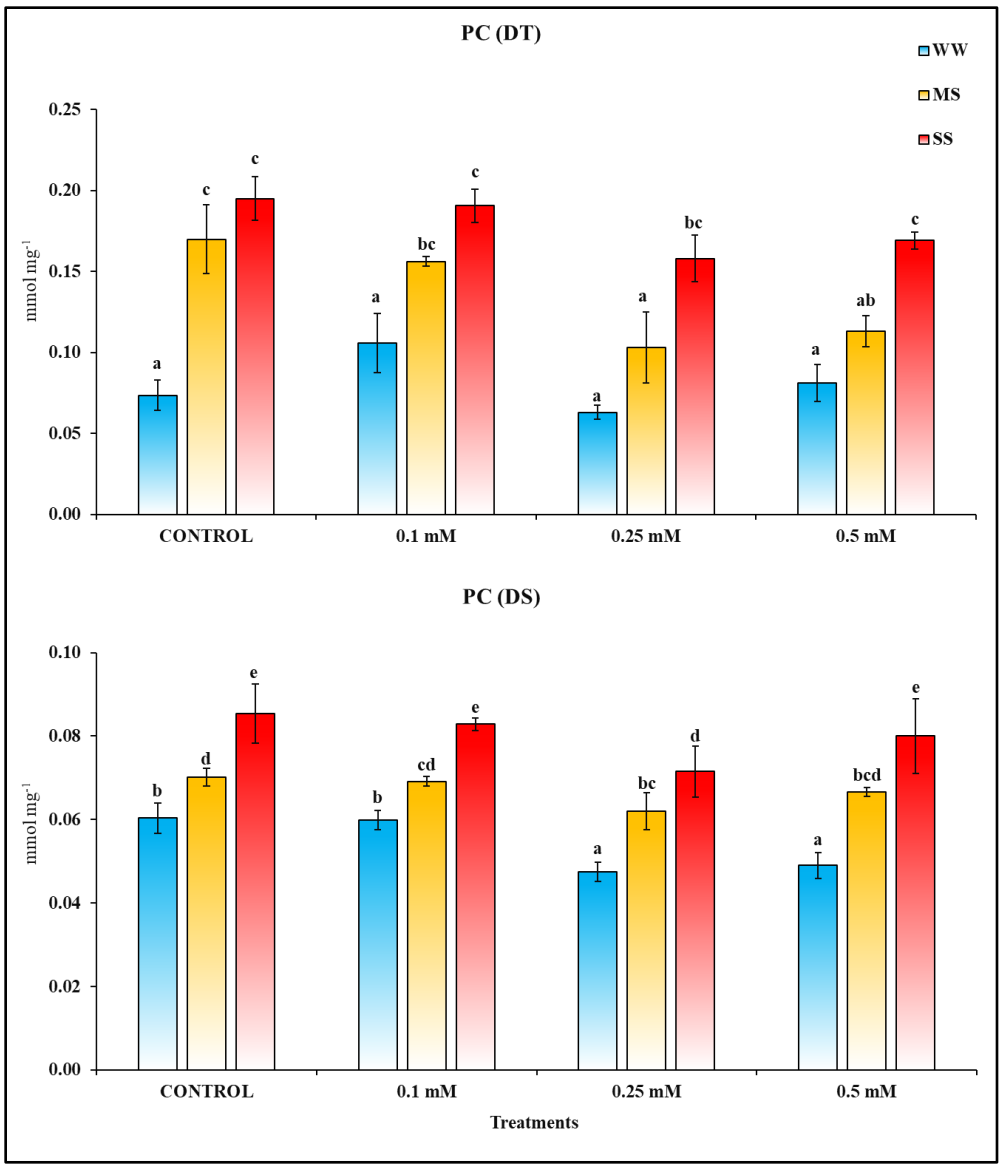


Fig. 3.13: Protein carbonyl (PC) content in drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=9). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

Table 3.9: Influence of drought stress and exogenously applied salicylic acid on ROS accumulation (H_2O_2, OH•), lipid peroxidation (MDA), and protein oxidation (carbonyl content) in drought tolerant (DT) and drought-sensitive (DS) cultivars. Data represent mean values ± SD (n=9). Different alphabets among the treatments denote significance at a 5% level.

Treatments		*Drought Tolerant*				*Drought Sensitive*			
Drought	*SA*	*Reactive Oxygen species*		*Lipid Peroxidation*	*Protein oxidation*	*Reactive Oxygen species*		*Lipid Peroxidation*	*Protein oxidation*
		H_2O_2 (% increase)	*OH• (absorbance units mg g^{-1}FW)*	*MDA (µmol g^{-1})*	*PC (mmol g^{-1} FW)*	*H_2O_2 (% increase)*	*OH• (absorbance units mg g^{-1}FW)*	*MDA (µmol g^{-1})*	*PC (mmol g^{-1} FW)*
Well-watered	0 mM	0.193±0.02^{a}	76.75±2.43^{ab}	0.00148±0.00016^{a}	0.074±0.0094^{a}	0.248±0.05^{a}	79.13±0.83^{ab}	0.00186±0.00037^{ab}	0.060±0.0036^{b}
Moderate		0.345±0.01^{def}	100.25±4.30^{cd}	0.00231±0.00012^{c}	0.170±0.0212^{c}	0.355±0.02^{def}	112.75±22.76^{c}	0.00361±0.00106^{b}	0.070±0.0021^{d}
Severe		0.416±0.03^{f}	125.75±1.58^{f}	0.00363±0.00067^{e}	0.195±0.0134^{c}	0.436±0.01^{f}	132.13±16.97^{d}	0.01242±0.00182^{e}	0.085±0.0071^{e}
Well-watered	0.1 mM	0.249±0.01^{abc}	79.00±10.16^{ab}	0.00143±0.00014^{a}	0.106±0.0183^{a}	0.297±0.04^{abc}	83.00±0.00^{ab}	0.0019±0.00013^{ab}	0.060±0.0023^{b}
Moderate		0.312±0.02^{cde}	93.75±9.90^{c}	0.0022±0.00006^{bc}	0.156±0.0029^{bc}	0.324±0.03^{cde}	104.13±10.05^{c}	0.00353±0.00133^{b}	0.069±0.0012^{cd}
Severe		0.401±0.02^{f}	107.88±4.70^{de}	0.0029±0.00007^{d}	0.191±0.0103^{c}	0.407±0.01^{f}	106.13±0.35^{c}	0.01151±0.00254^{de}	0.083±0.0156^{e}
Well-watered	0.25 mM	0.224±0.01^{ab}	69.50±9.71^{a}	0.00120±0.00028^{a}	0.063±0.0043^{a}	0.275±0.03^{ab}	76.50±0.76^{a}	0.00176±0.00013^{a}	0.048±0.0023^{a}
Moderate		0.263±0.04^{abcd}	77.63±2.56^{ab}	0.00171±0.00043^{a}	0.103±0.0220^{a}	0.283±0.03^{abcd}	87.88±4.19^{b}	0.00327±0.00064^{b}	0.062±0.0044^{bc}
Severe		0.384±0.03^{ef}	104.00±12.31^{d}	0.00276±0.00043^{d}	0.158±0.0142^{bc}	0.382±0.02^{ef}	89.75±4.13^{b}	0.009±0.00194^{c}	0.072±0.0062^{d}
Well-watered	0.5 mM	0.238±0.00^{abc}	81.75±12.06^{b}	0.00161±0.00018^{a}	0.081±0.0114^{a}	0.289±0.01^{abc}	81.00±6.16^{ab}	0.00206±0.00034^{abc}	0.049±0.0031^{a}
Moderate		0.297±0.03^{bcd}	114.63±8.90^{e}	0.00174±0.00019^{a}	0.113±0.0096^{ab}	0.302±0.02^{bcd}	87.50±12.85^{ab}	0.00336±0.00032^{b}	0.067±0.0010^{bcd}
Severe		0.382±0.01^{ef}	116.88±5.49^{ef}	0.00284±0.00^{d}	0.169±0.0051^{c}	0.398±0.01^{ef}	86.88±4.16^{ab}	0.0109±0.00188^{d}	0.080±0.0062^{e}

3.3.3. Determination of Fatty acid lipid profile using GC/MS

From the GC-MS data, a total of 5 fatty acids, two saturated viz., palmitic acid and stearic acid, and three unsaturated viz., pentadecanoic acid, linoleic acid, and linolenic acid, were identified from both the cultivars and drought treatments did not cause any qualitative changes in the fatty acid composition in either of rice cultivar (**Table 3.10; Fig. 3.14a, b**). Under moderate stress, quantitative changes in fatty acid composition were observed. In DT, total saturated fatty acid (SFA) content decreased by 3%, while an increase of 22% in total unsaturated fatty acid (UFA) content was observed. In the DS cultivar, a decrease in total SFA content by 41% and total UFA content by 42% was observed compared to well-watered plants.

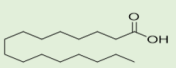
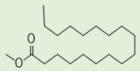
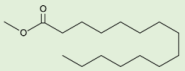
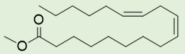
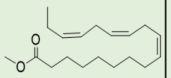
Application of 0.25 SA, plants exposed to moderate drought stress recorded a decrease in total SFA content by 17% and total UFA content by 19% in the DT cultivar. However, in the DS cultivar with foliar application of SA, the total SFA content increased by 91%, and the total UFA content decreased by 1% compared to the control (**Table 3.10**).

Further, quantitative changes in fatty acid composition were observed under severe drought stress conditions. In DT, an increase in total SFA by 72% and total UFA by 88% was recorded. Also, in the DS cultivar, an increase of 42% in total SFA and 57% in total UFA was observed.

The application of 0.25 SA on plants exposed to severe drought stress recorded a decrease in total SFA by 54% and total UFA by 88% in the DT cultivar. In the DS cultivar, a decrease in total SFA by 6% and total UFA by 15% was observed compared to SA untreated control. However, the UFA/SFA ratio increased as the magnitude of drought increased. At severe drought stress treatment, the ratio of UFA/SFA increased by 9% in DT and 11% in

DS cultivar compared to the control. Furthermore, foliar application of SA also showed a decrease in the UFA/SFA ratio under severe drought stress in both DT (75%) and DS (6%) cultivars, compared to SA untreated control (**Table 3.10**).

Table 3.10: Influence of drought stress and exogenously applied salicylic acid on fatty acid profile in the leaves of drought-tolerant (DT) and drought-sensitive (DS) rice cultivars.

Compound		Hexadecenoic acid	Octadecanoic acid	(Z)-pentadecenoic acid	9,12 (Z,Z)-Octadecadienoic acid	9,12, 15(Z,Z,Z)-Octadecatrienoic acid	Saturated fatty acid (SFA)	Unsaturated fatty acid (UFA)	UFA/SFA	Total fatty acid (TFA)
Synonym		(Palmitic acid)	(Stearic acid)	(Pentadecenoic acid)	(Linoleic acid)	(Linolenic acid)				
Mol.Wt. (g mol^{-1})		270.45	298.5	240	280.44	278.4				
Structure										
Drought	*SA*	DT (mg g^{-1}FW)								
Well-watered	0 mM	11.97	5.66	0.89	5.95	16.15	17.63	22.99	1.30	41.92
Moderate		14.23	2.94	0.89	6.73	20.42	17.17	28.04	1.63	46.84
Severe		25.55	4.79	Nd	9.61	33.66	30.34	43.27	1.43	75.04
Well-watered	0.25 mM	14.29	6.78	1.14	6.3	21.99	21.07	29.43	1.40	51.90
Moderate		11.87	2.321	Nd	6.71	16.06	14.191	22.77	1.60	38.57
Severe		11.28	2.69	Nd	5.06	Nd	13.97	5.06	0.36	19.39
		DS (mg g^{-1}FW)								
Well-watered	0 mM	16.62	2.99	0.91	7.4	23.95	19.61	32.26	1.65	53.52
Moderate		9.92	1.56	0.59	4.09	14.07	11.48	18.75	1.63	31.86
Severe		24.59	3.18	2.07	14.25	34.4	27.77	50.72	1.83	80.32
Well-watered	0.25 mM	13.89	3.04	1.22	7.74	22.84	16.93	31.8	1.88	50.61
Moderate		15.57	6.32	1.5	5.85	24.18	21.89	31.53	1.44	54.86
Severe		19.29	6.74	1.93	13.09	27.88	26.03	42.9	1.65	70.58

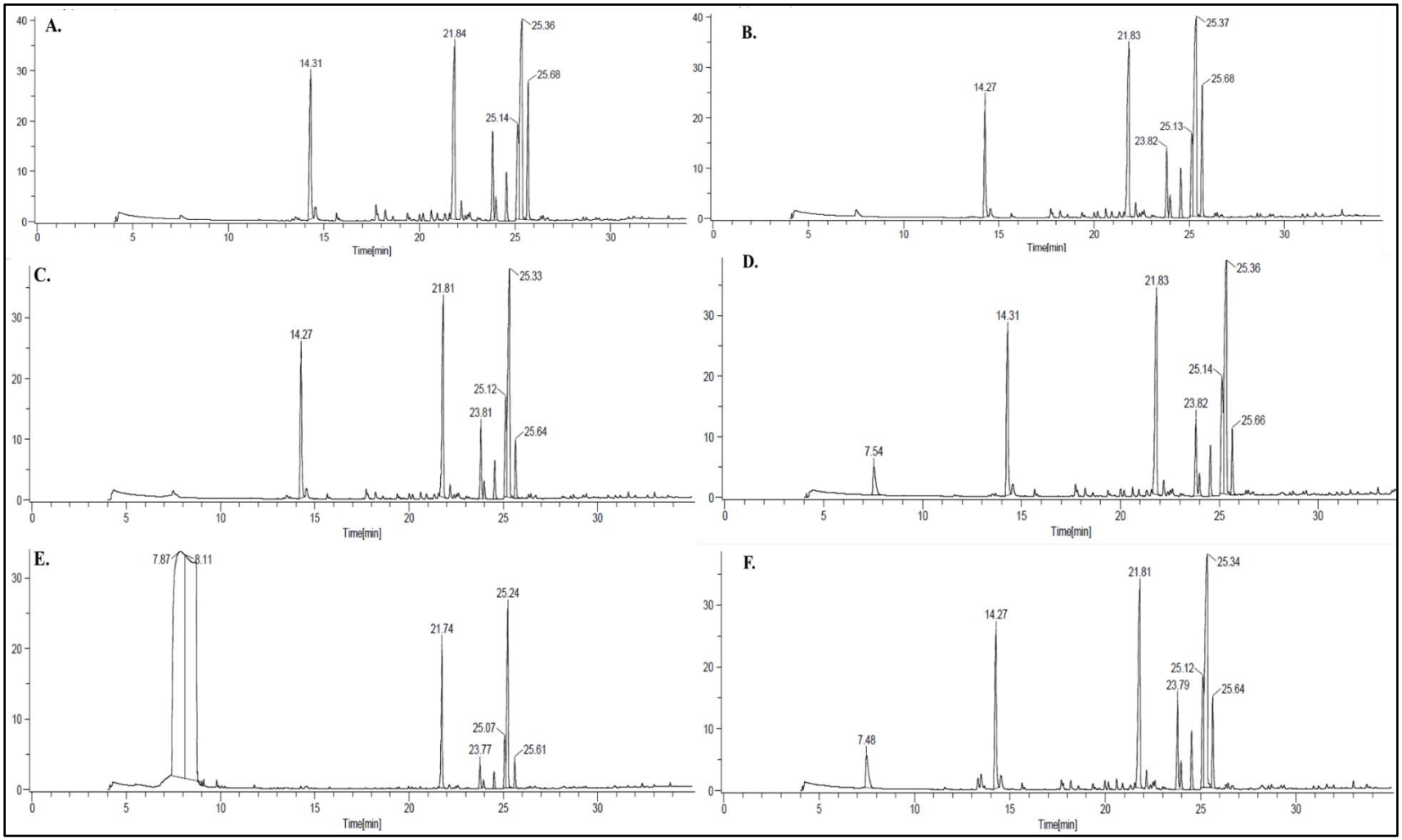


Fig. 3.14a.: GC-MS fatty acid profile of drought tolerant (DT) rice cultivars treated with drought stress and 0.25 mM salicylic acid. [A-WW (well-watered); B- WW +0.25 mM SA; C- MS (moderate stress); D- MS+0.25 mM SA; E- SS (severe stress); F- SS+0.25 mM SA]

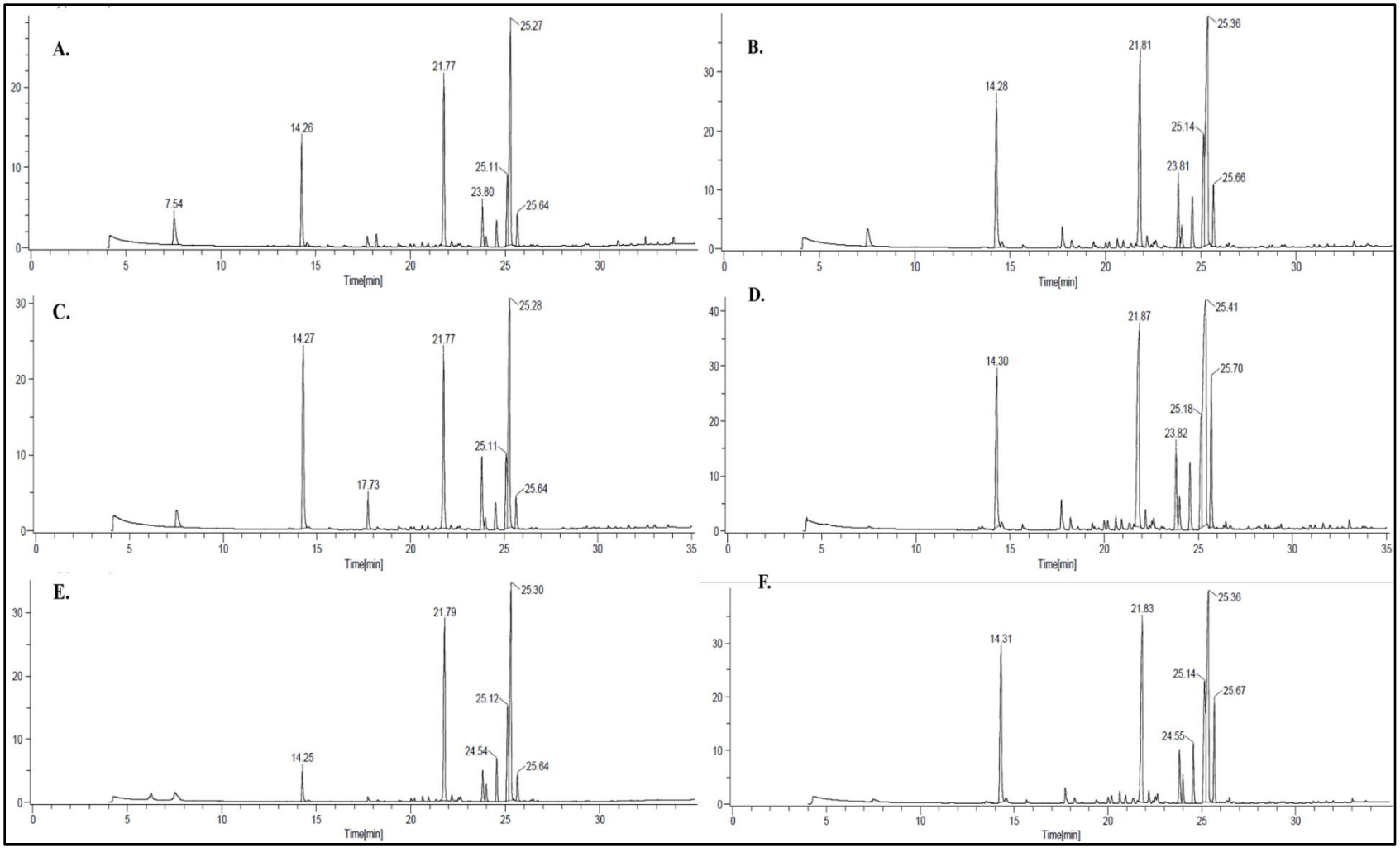


Fig. 3.14b.: GC-MS fatty acid profile of drought-sensitive (DS) rice cultivars treated with drought stress and 0.25 mM salicylic acid. [A-WW (well-watered); B- WW +0.25 mM SA; C- MS (moderate stress); D- MS+0.25 mM SA; E- SS (severe stress); F- SS+0.25 mM SA]

3.3.4. Measurement of enzymatic antioxidants

3.3.4.1. Determination of Superoxide dismutase (SOD) antioxidant activity

The results showing the effects of moderate and severe drought conditions on the SOD activity in the two cultivars are depicted in **Table 3.11 and Fig. 3.15**. The SOD activity in both cultivars exposed to moderate stress decreased by 47% in the DT cultivar and 20% in DS cultivar-compared to well-watered control. The study indicated that the exogenous application of 0.25 mM concentration of SA significantly increased SOD activity by 49% in DT and 42% in DS cultivars. 0.1 mM concentration recorded an increase of 40% in DT and 28% in DS cultivar. In comparison, 0.5 mM concentration recorded an increase of 47% in the DT cultivar and 38% in the DS cultivar compared to the control.

Consequently, SOD activity in plants exposed to severe drought stress recorded a further decrease by -59% in DT and 34% in DS cultivars compared to the control. A maximum of 26% in DT and 37% in DS cultivars in SOD activity was observed at 0.25 mM concentration of SA. 0.1 mM concentration increased by 1% in DT and 9% in DS cultivar. In comparison, 0.5 mM concentration recorded an increase of 5% in the DT cultivar and 36% in the DS cultivar compared to control.

3.3.4.2. Determination of Glutathione Reductase (GR) antioxidant activity

It was observed that the GR activity in both cultivars exposed to moderate stress increased by 107% in DT and 86% in DS cultivars compared to the control (**Fig. 3.16)**. The study revealed that the exogenous application of 0.25 mM concentration of SA significantly increased GR activity by 33% in DT and 61% in DS cultivars. 0.1 mM concentration recorded an increase of 6% in DT and 7% in DS cultivar. In comparison, 0.5 mM

concentration recorded an increase of 25% in the DT cultivar and 31% in the DS cultivar compared to control. The GR activity in plants exposed to severe drought stress increased by 182% in DT and 107% in DS cultivars compared to control plants. The maximum increase in GR activity by 28% in DT and 18% in DS cultivar was recorded at 0.25 mM concentration of SA. 0.1 mM concentration recorded an increase of 2% in DT and 3% in DS cultivar. In comparison, 0.5 mM concentration recorded an increase of 3% in the DT cultivar and 14% in the DS cultivar compared to control.

The study also revealed that among the SA concentrations, 0.25 mM markedly increased the antioxidant activity (SOD and GR) under moderate and severe drought stress in both DT and DS compared to control plants.

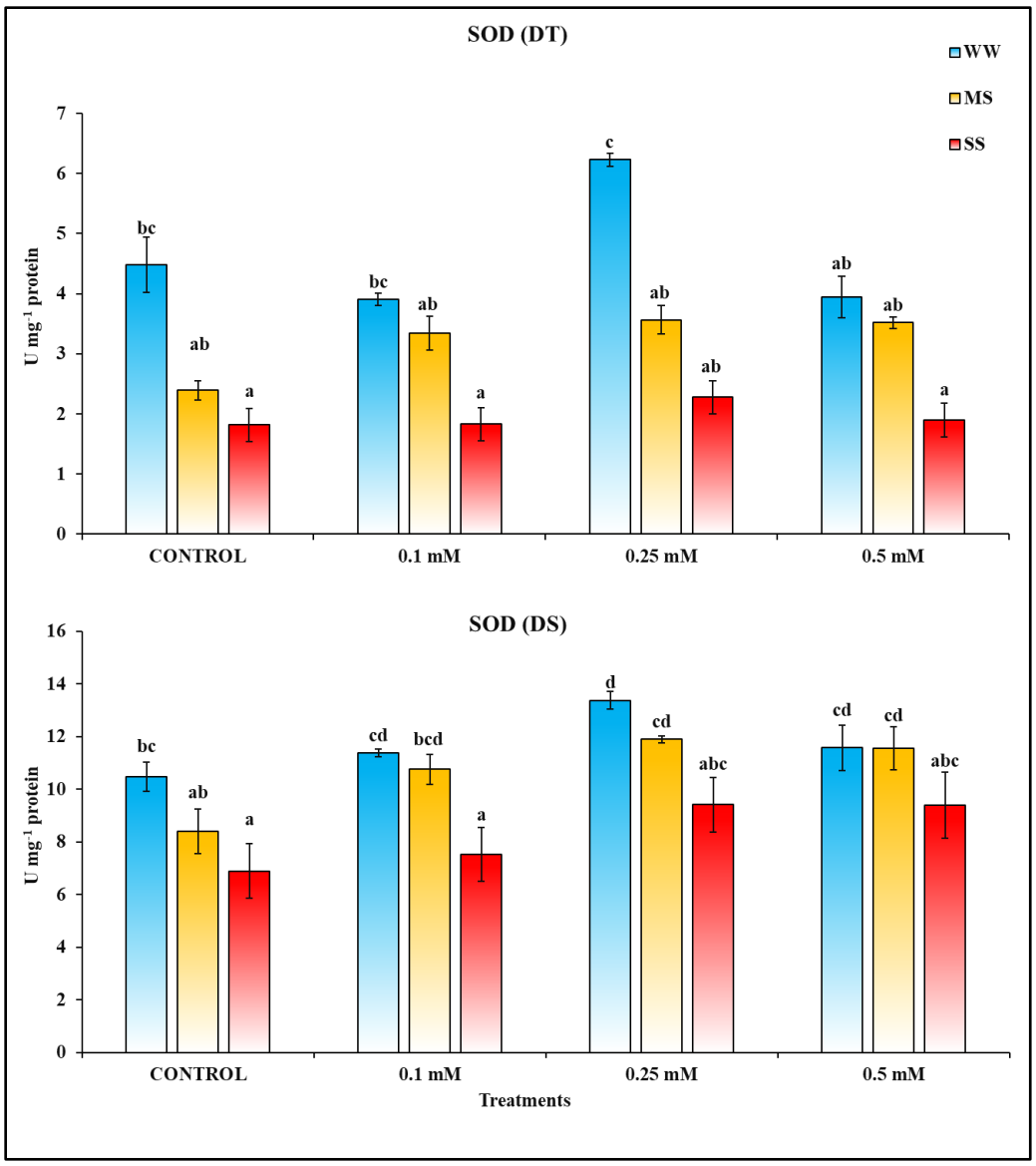


Fig. 3.15: Superoxide dismutase (SOD) in drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=6). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

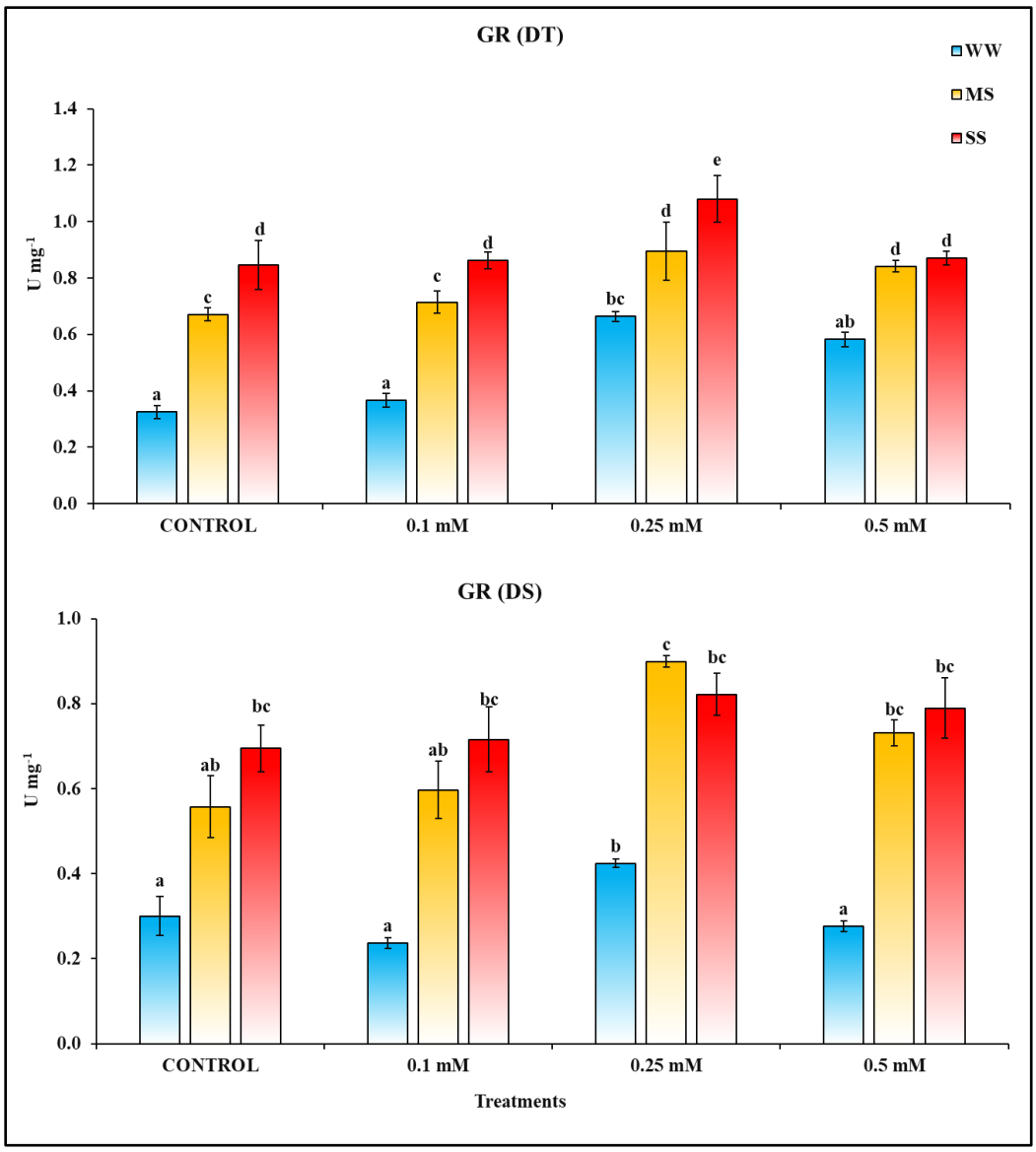


Fig. 3.16: Glutathione reductase (GR) assay in drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=6). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

3.3.5. Measurement of Non-enzymatic antioxidant

3.3.5.1. Determination of GSH activity

The results of total Glutathione content in both the cultivars under moderate and severe drought stress and on the application of SA are presented in **Table 3.11** and **Fig. 3.17**. The tGSH content of both cultivars exposed to moderate stress increased by 335% in DT and 115% in DS cultivars compared to well-watered control. The maximum increase in tGSH content by 13% in DT and 24% in DS cultivars was recorded at 0.25 mM concentration of SA. 0.1 mM concentration recorded an increase of 3% in DT and 0.1% in DS cultivar. In comparison, 0.5 mM concentration recorded an increase of 10% in the DT cultivar and 19% in the DS cultivar compared to control. The tGSH content in plants exposed to severe drought stress increased by 744% in DT and 639% in DS cultivars compared to well-watered control. The maximum increase in tGSH content by 32% in DT and 3% in DS cultivars was observed at 0.25 mM concentration of SA. However, 0.1 mM and 0.5 mM SA concentrations recorded an 18% and 23% increase in the DT cultivar compared to the control.

Among the SA concentrations, treatment with 0.25 mM markedly increased the tGSH content under moderate and severe drought stress in both DT and DS compared to its control.

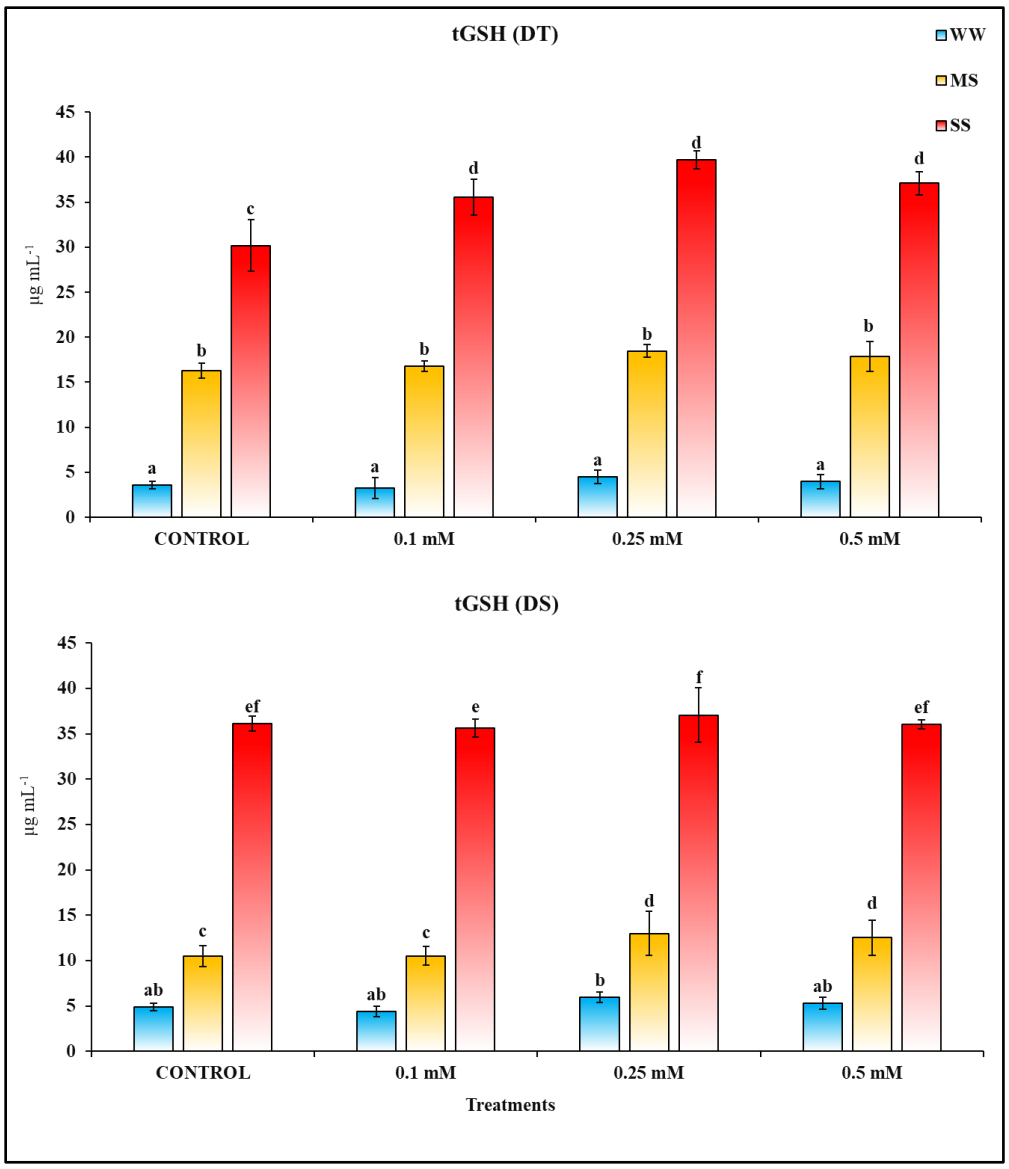


Fig. 3.17: Total Glutathione (tGSH) content in drought-tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=6). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

3.3.6. Osmolyte accumulation

3.3.6.1. Proline estimation

There was a significant increase in leaf proline content, indicating osmotic stress in both cultivars exposed to drought compared to well-watered control (**Table 3.11**; **Fig. 3.18**). The proline content in both the cultivars exposed to moderate stress was recorded as 1.3 folds in the DT cultivar, while a decrease of 13% in the DS cultivar was recorded compared to the well-watered control. The maximum increase in proline content by 63% in DT and 185% in DS cultivars was recorded at 0.25 mM concentration of SA. However, an increase of 36% and 178% over control in SA treatments of 0.1 mM and 0.5 mM, respectively, was recorded only in the DS cultivar. In comparison, only 0.5 mM SA treatment showed an increase of 34% in the DT cultivar compared to the control. The proline content of plants exposed to severe drought stress showed a further increase by 11.8 folds in DT and 2.4 folds in DS cultivars compared to well-watered control. The maximum increase in proline content by 13% in DT and 127% in DS cultivar was observed at 0.25 mM concentration of SA. However, only the DS cultivar recorded an increase of 63% and 114% on treatment with 0.1 mM and 0.5 mM of SA compared to control. At the same time, only 0.5 mM treatment recorded an increase of 12% in the DT cultivar compared with its control.

Among the SA concentrations, the application of 0.25 mM markedly increased the proline content under moderate and severe drought stress in both DT and DS compared to the control.

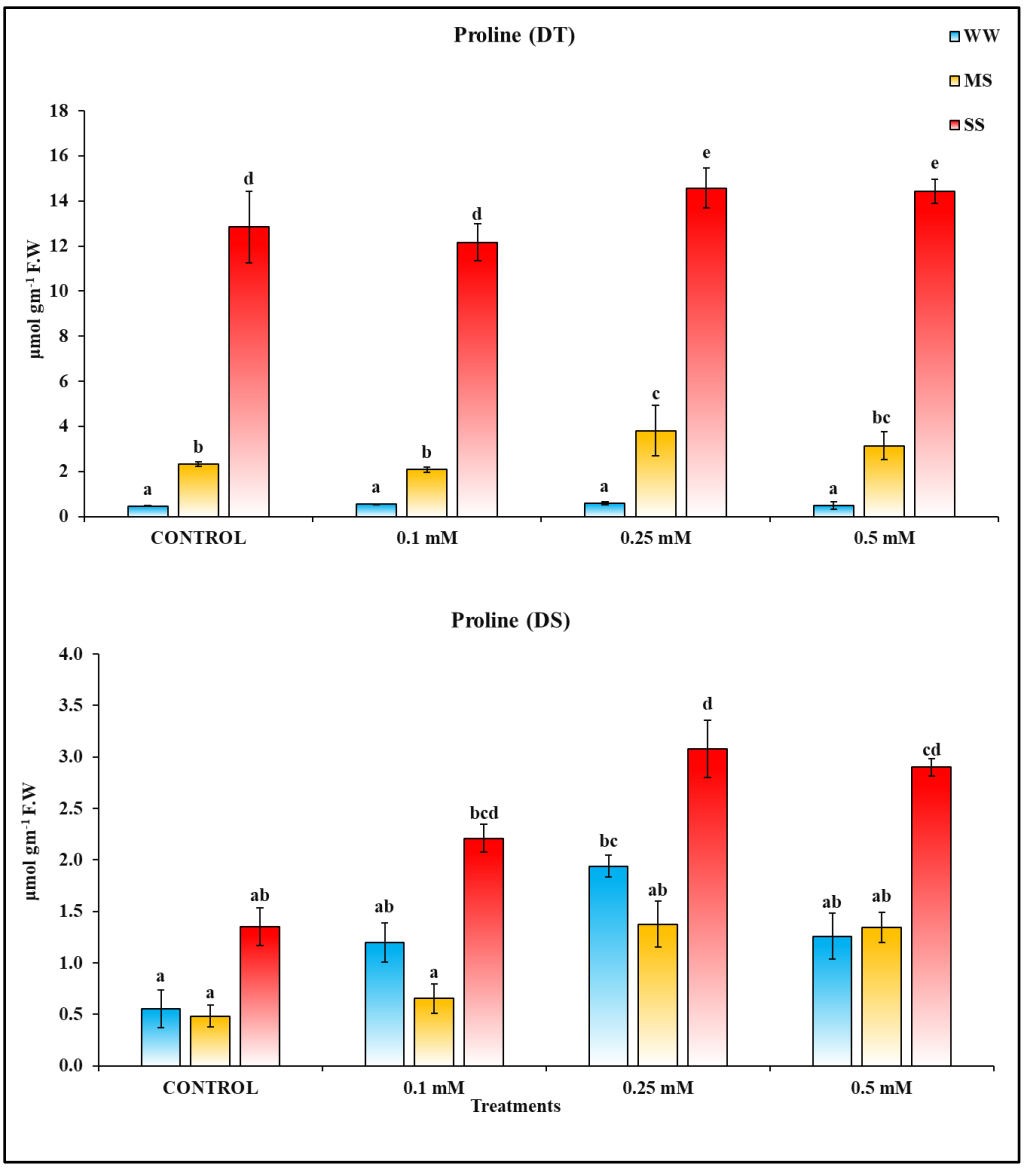


Fig. 3.18: Proline content in drought-tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=6). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **MS**, moderate stress; **SS**, severe stress.

Table 3.11: Influence of drought stress and exogenously applied salicylic acid on activity of antioxidant enzymes (SOD, GR), accumulation of antioxidant metabolite (GSH) and proline in drought tolerant (DT) and drought-sensitive (DS) cultivars. Data represent mean values ± SD (n=6). Different alphabets among the treatments denote significance at a 5% level.

Treatments		*Drought Tolerant*				*Drought Sensitive*			
Drought	*SA*	*Antioxidant enzymes*		*Antioxidant metabolite*	*Osmolyte*	*Antioxidant enzymes*		*Antioxidant metabolite*	*Osmolyte*
		SOD (U mg^{-1} protein)	*GR Umg^{-1}*	*tGSH (µg mL^{-1})*	*Proline (µmol gm^{-1} F.W)*	*SOD (U mg^{-1} protein)*	*GR Umg^{-1}*	*tGSH (µg mL^{-1})*	*Proline (µmol gm^{-1} F.W)*
Well-watered	0 mM	4.480±0.46^{bc}	0.325±0.02^{a}	3.579±0.4^{a}	0.460±0.02^{a}	10.477±0.55^{bc}	0.300±0.05^{a}	4.885±0.4^{ab}	0.555±0.38^{a}
Moderate		2.393±0.16^{ab}	0.671±0.02^{c}	16.272±0.9^{b}	2.335±0.10^{b}	8.390±0.85^{ab}	0.557±0.07^{ab}	10.499±1.2^{c}	0.484±0.11^{a}
Severe		1.817±0.27 ^{a}	0.846±0.09^{d}	30.192±2.8^{c}	12.844±1.58^{d}	6.883±1.03^{a}	0.695±0.05^{bc}	36.113±0.8^{ef}	1.354±0.18^{ab}
Well-watered	0.1 mM	3.910±0.10^{bc}	0.367±0.02^{a}	3.232±1.2^{a}	0.552±0.01^{a}	11.373±0.16^{cd}	0.237±0.01^{a}	4.365±0.6^{ab}	1.198 ±0.39^{ab}
Moderate		3.343±0.28^{ab}	0.714±0.04^{c}	16.802±0.6^{b}	2.075±0.12^{b}	10.753±0.56^{bcd}	0.597±0.07^{ab}	10.512±1.0^{c}	0.656±0.14^{a}
Severe		1.830±0.28 ^{a}	0.863±0.03^{d}	35.525±2.0^{d}	12.160±0.82^{d}	7.523±1.01^{a}	0.716±0.08^{bc}	35.632±1.0^{e}	2.210±0.13^{bcd}
Well-watered	0.25 mM	6.233±0.11^{c}	0.664±0.02^{bc}	4.485±0.8^{a}	0.582±0.06^{a}	13.377±0.33^{d}	0.424±0.01^{b}	5.979±0.6^{b}	1.939±0.31^{bc}
Moderate		3.567±0.24^{ab}	0.895±0.10^{d}	18.459±0.7^{b}	3.811±1.13^{c}	11.900±0.13^{cd}	0.899±0.09^{c}	12.992±2.4^{d}	1.377±0.22^{ab}
Severe		2.280±0.28 ^{ab}	1.081±0.08^{e}	39.712±1.0^{d}	14.573±0.89^{e}	9.407±1.04^{abc}	0.822±0.05^{bc}	37.055±3.0^{f}	3.076±0.28^{d}
Well-watered	0.5 mM	3.943±0.35^{ab}	0.583±0.03^{ab}	3.952±0.8^{a}	0.484±0.16^{a}	11.570±0.86^{cd}	0.277±0.13^{a}	5.272±0.7^{ab}	1.259±0.22^{ab}
Moderate		3.517±0.10 ^{ab}	0.842±0.02^{d}	17.845±1.7^{b}	3.134±0.62^{bc}	11.547±0.82^{cd}	0.731±0.03^{bc}	12.512±1.9^{d}	1.344±0.14^{ab}
Severe		1.900±0.28 ^{a}	0.871±0.03^{d}	37.125±1.3^{d}	14.428±1.54^{e}	9.393±1.25^{abc}	0.789±0.07^{bc}	36.032±0.5^{ef}	2.900±0.08^{cd}

3.4. Gene expression studies of antioxidant enzymes (SOD, APX), aquaporin (AQP) channel and potassium (K^+) transporter by real time-PCR

Gene expression of enzymatic antioxidants such as superoxide dismutase (SOD), ascorbate peroxidase (APX), aquaporin (AQP) channel and potassium (K^+) transporter was investigated in plants exposed to drought and in combination with drought and SA (**Fig. 3.19a-d**).

There was an increase in the relative gene expression of APX upon drought treatment in both DT and DS by 2 fold and 3 fold, respectively, compared to control. However, foliar application of SA on both cultivars showed a further enhancement in the expression of APX by 34 fold and 1 fold in DT and DS cultivars, respectively, compared to the control (**Fig. 3.19a**). An increase in the relative gene expression of SOD upon drought treatment in both DT and DS by 2 fold and 4 fold, respectively, compared to control. However, after 72 hours of SA application, both cultivars showed a further enhancement in the expression of APX by 32 fold and 2 fold in DT and DS cultivars, respectively, compared to the control(**Fig. 3.19b**).

Further, aquaporin's relative gene expression level was increased by 13 fold in DT and a decrease by 45 fold in DS cultivar was observed upon drought treatment compared to the control. However, after 72 hours of SA application, both cultivars showed a further increase by 6 fold in DT and a decrease by 1 fold was observed in the DS cultivar compared to the control (**Fig. 3.19c).** The relative gene expression of the K^+ transporter, upon drought treatment, recorded an increase by 8 fold in DT and 0.98 fold in DS cultivar compared to the control. However, foliar application of SA on both the cultivars showed a further enhancement in the expression of K^+ transporter by 7 fold in DT and 1.06 fold in DS

compared to the control (**Fig. 3.19d**).

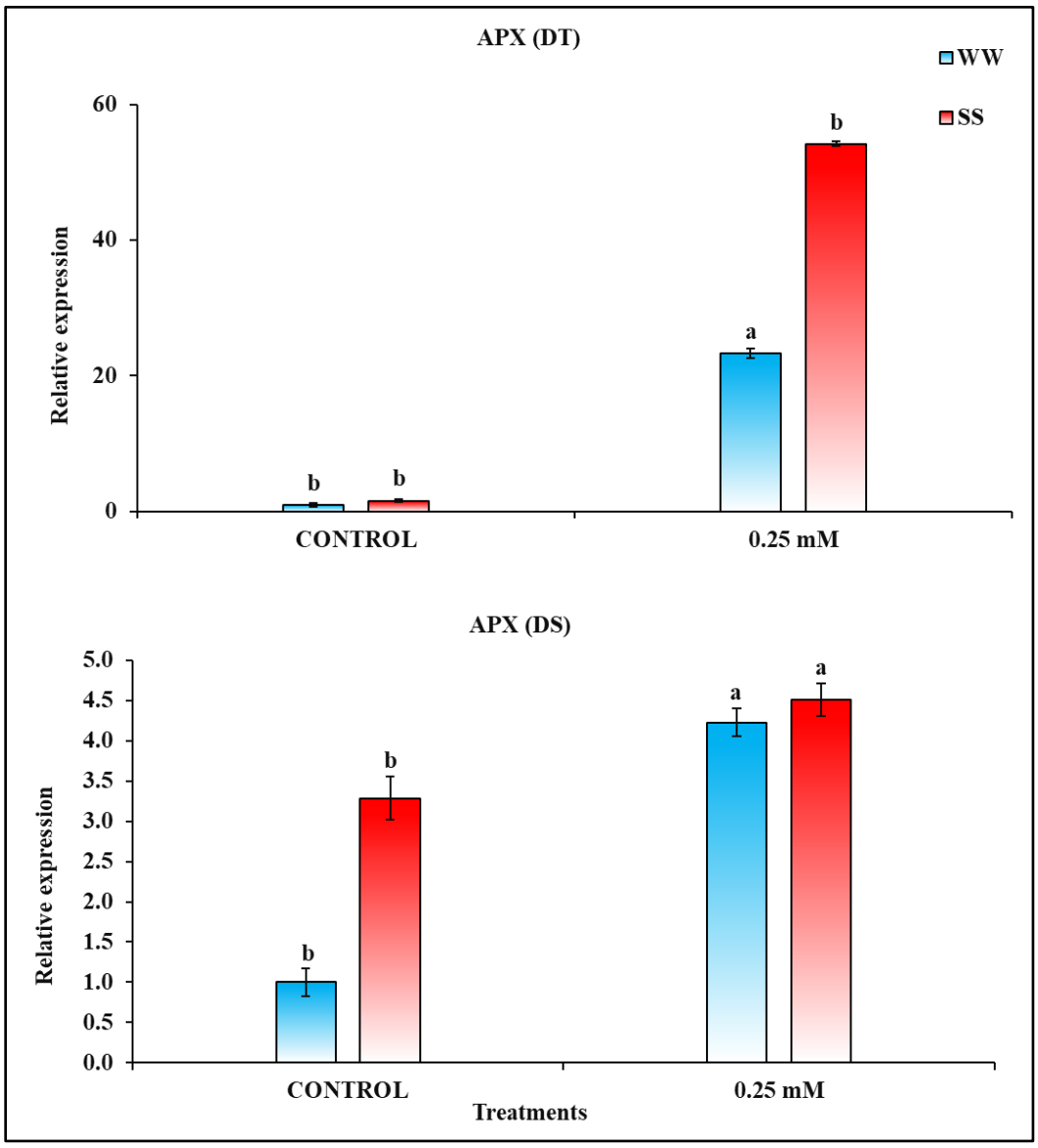


Fig. 3.19a: Gene expression studies: Ascorbate peroxidase (APX) in drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=3). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **SS**, severe stress.

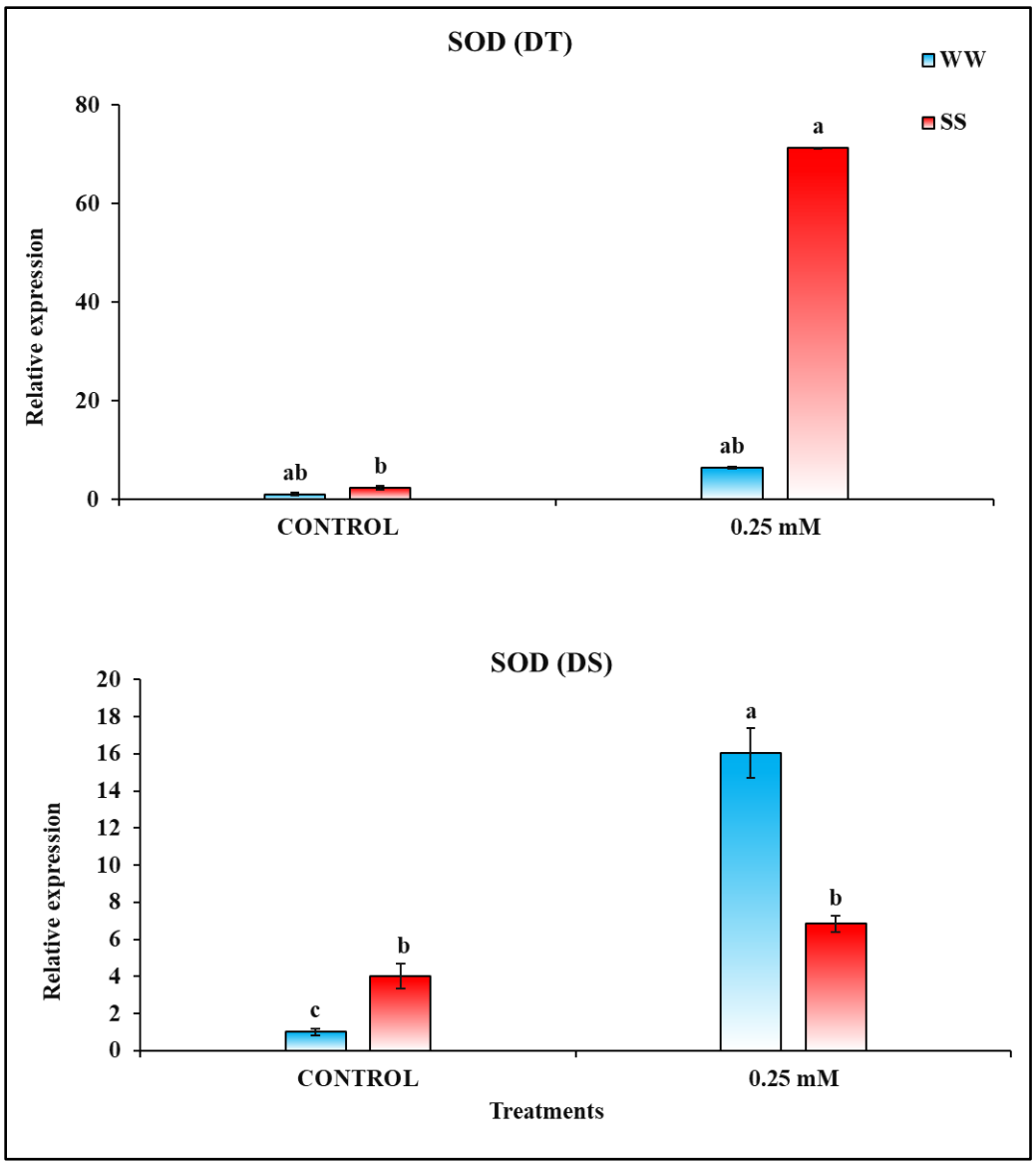


Fig. 3.19b: Gene expression studies: Superoxide Dismutase (SOD) in drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=3). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **SS**, severe stress.

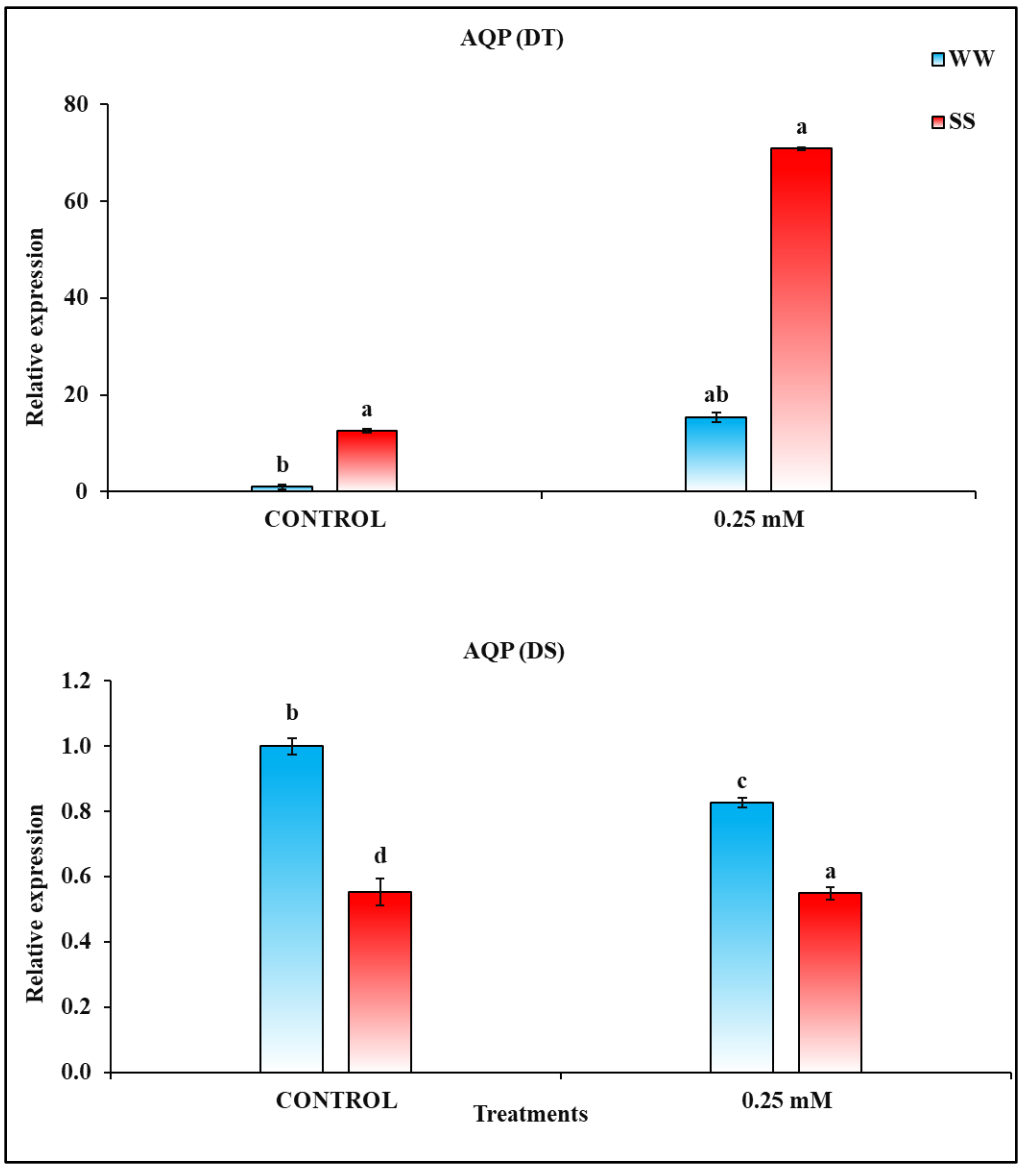


Fig. 3.19c: Gene expression studies: Aquaporin (AQP) in drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=3). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **SS**, severe stress.

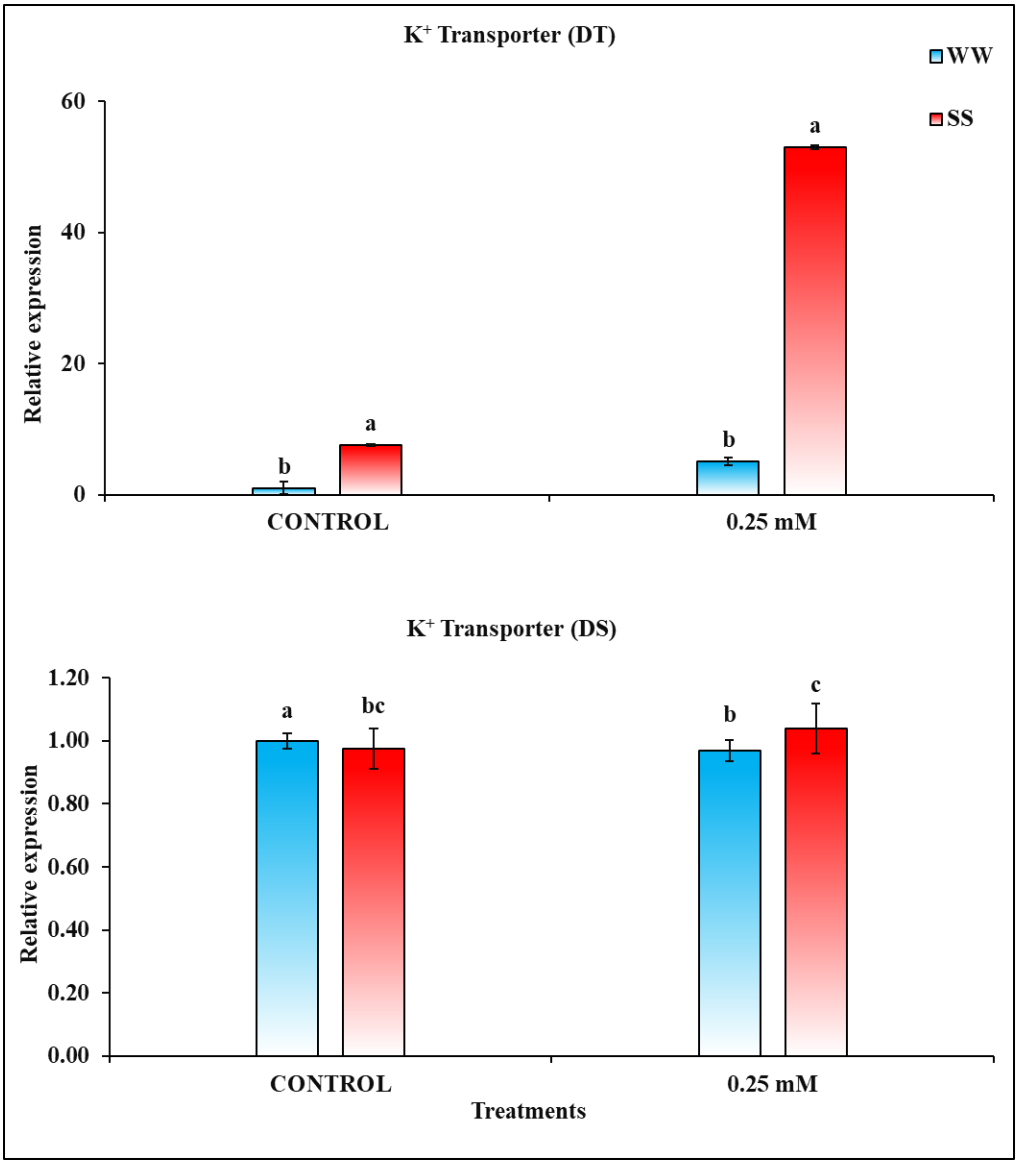


Fig. 3.19d: Gene expression studies: Potassium (K^+) transporter in drought tolerant (DT) and drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. Bars represent mean values ± SD (n=3). Bars denoted by different alphabets among the treatments denote significance at a 5% level using the Duncan Multiple Range Test. Abbreviation: **WW**, well-watered control; **SS**, severe stress.

3.5. Endogenous Salicylic Acid analysis using HPLC

Results of the HPLC analysis of the endogenous salicylic acid levels in the leaves of well-watered, moderate and severe drought-stressed and combined with drought and 0.25 mM SA grown DT and DS cultivars are presented in **Table 3.12** and **Fig**. **3.20b, c.** Ortho Anisic acid (OAA) was used as the internal standard, and synthetic SA was used for developing the calibration curve. The chromatogram of standard SA is presented in **Fig. 3.20a**.

On exposure of the DT cultivar to moderate drought stress, the concentration of endogenous SA increased, along with an increased concentration of the unknown compound compared to well-watered plants. Furthermore, with the application of 0.25 mM SA, the concentration of endogenous SA further increased with a decrease in the concentration of the unknown compound, compared to SA untreated control in the DT cultivar (**Table 3.12**). On the other hand, exposure of the DS cultivar to moderate drought showed a similar pattern as observed in the DT cultivar. However, the exogenous application of SA decreased the endogenous SA concentration with an increase in the concentration of the unknown compound compared to SA untreated control.

Moreover, exposure of the DT cultivar to severe drought stress decreased the endogenous SA concentration with an increase in the concentration of the unknown compound compared to well-watered plants. On the other hand, the application of exogenous SA on the DT cultivar increased the concentration of endogenous SA with a decrease in the unknown compound compared to the control. Similarly, exposure of the DS cultivar to severe drought showed a similar pattern as in the DT cultivar. However, the exogenous application of SA decreased the endogenous SA concentration with an increase in the concentration of the unknown compound compared to SA untreated control (**Table 3.12**).

Table 3.12: Influence of drought stress and exogenously applied salicylic acid on endogenous salicylic acid content in the leaves of drought tolerant (DT) and drought-sensitive (DS) cultivars.

Treatments		*Endogenous salicylic acid (SA)*			
		Drought tolerant (DT)		*Drought sensitive (DS)*	
Drought	*SA*	*Endo. SA ($\mu g\ mL^{-1}$)*	*Unknown ($\mu g\ mL^{-1}$)*	*Endo. SA ($\mu g\ mL^{-1}$)*	*Unknown ($\mu g\ mL^{-1}$)*
Well-watered	*0 mM*	6.75	5.00	62.87	115.75
Moderate		49.03	29.60	25.68	45.55
Severe		37.64	27.42	20.28	46.17
Well-watered	*0.25 mM*	22.99	5.07	8.80	46.29
Moderate		58.13	24.45	20.17	46.99
Severe		50.01	15.52	17.08	49.67

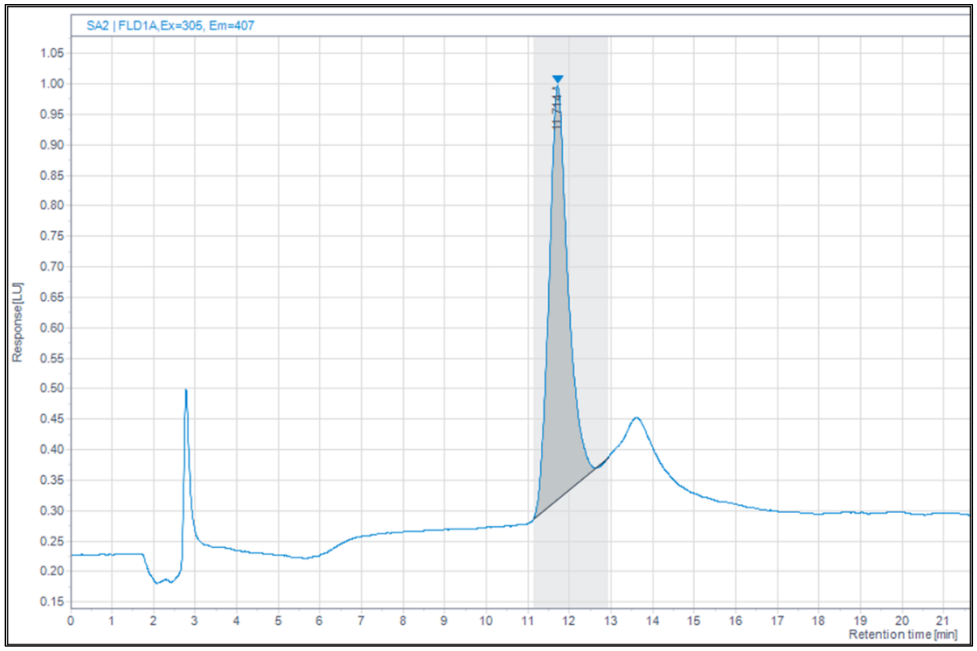


Fig. 3.20a: HPLC profile of standard salicylic acid.

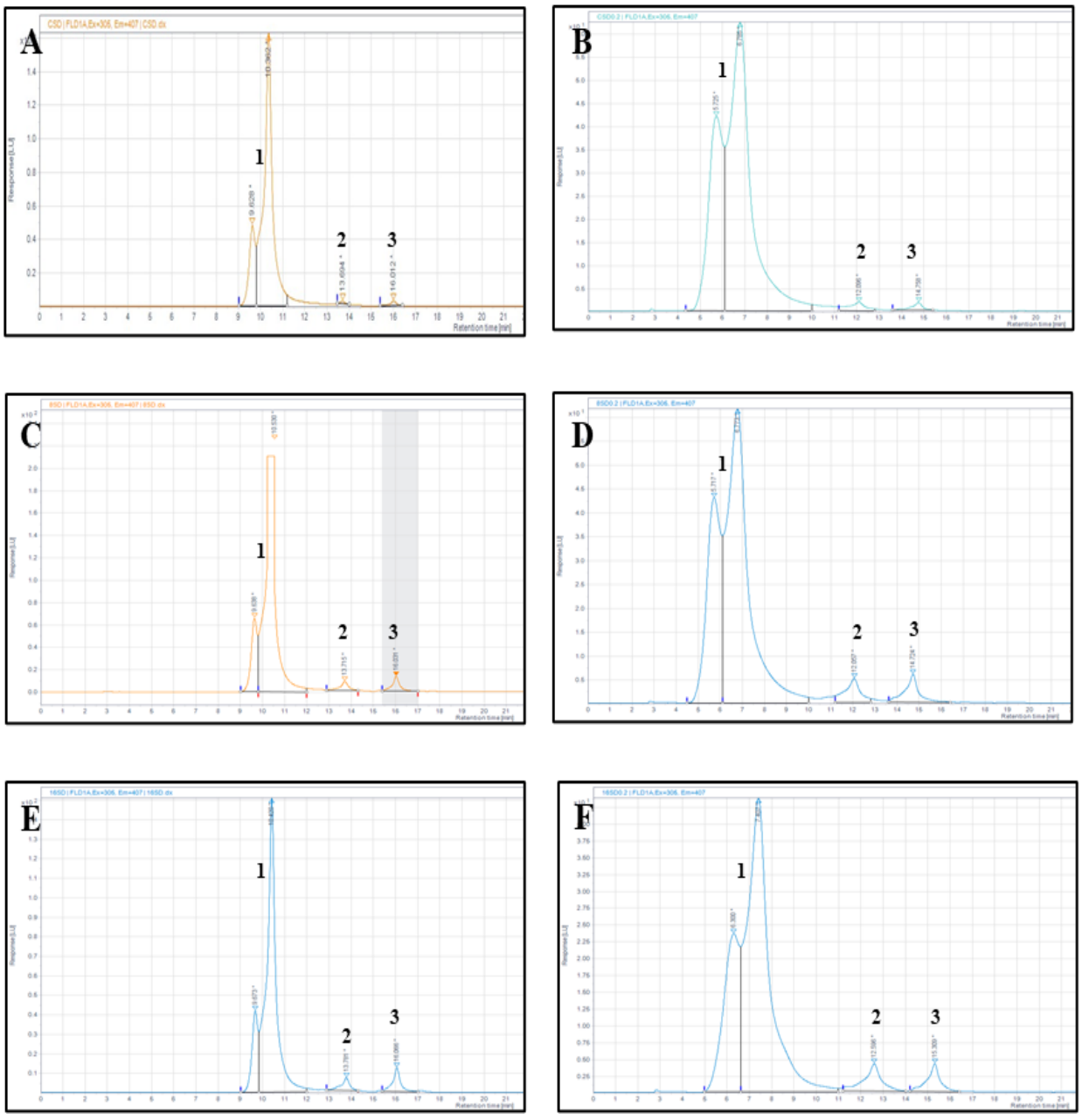


Fig. 3.20b: HPLC profile of endogenous salicylic acid in drought tolerant (DT) rice cultivars treated with drought stress and salicylic acid. [**A-D**: well-watered (ww); **E-H**: moderates stress (ms); **I-L**: severe stress (SS). **A**-WW; **B**- WW+0.25 mM SA; **C**- MS; **D**-MS+0.25 mM SA; **E**- SS; **F**-SS+0.25 mM SA.

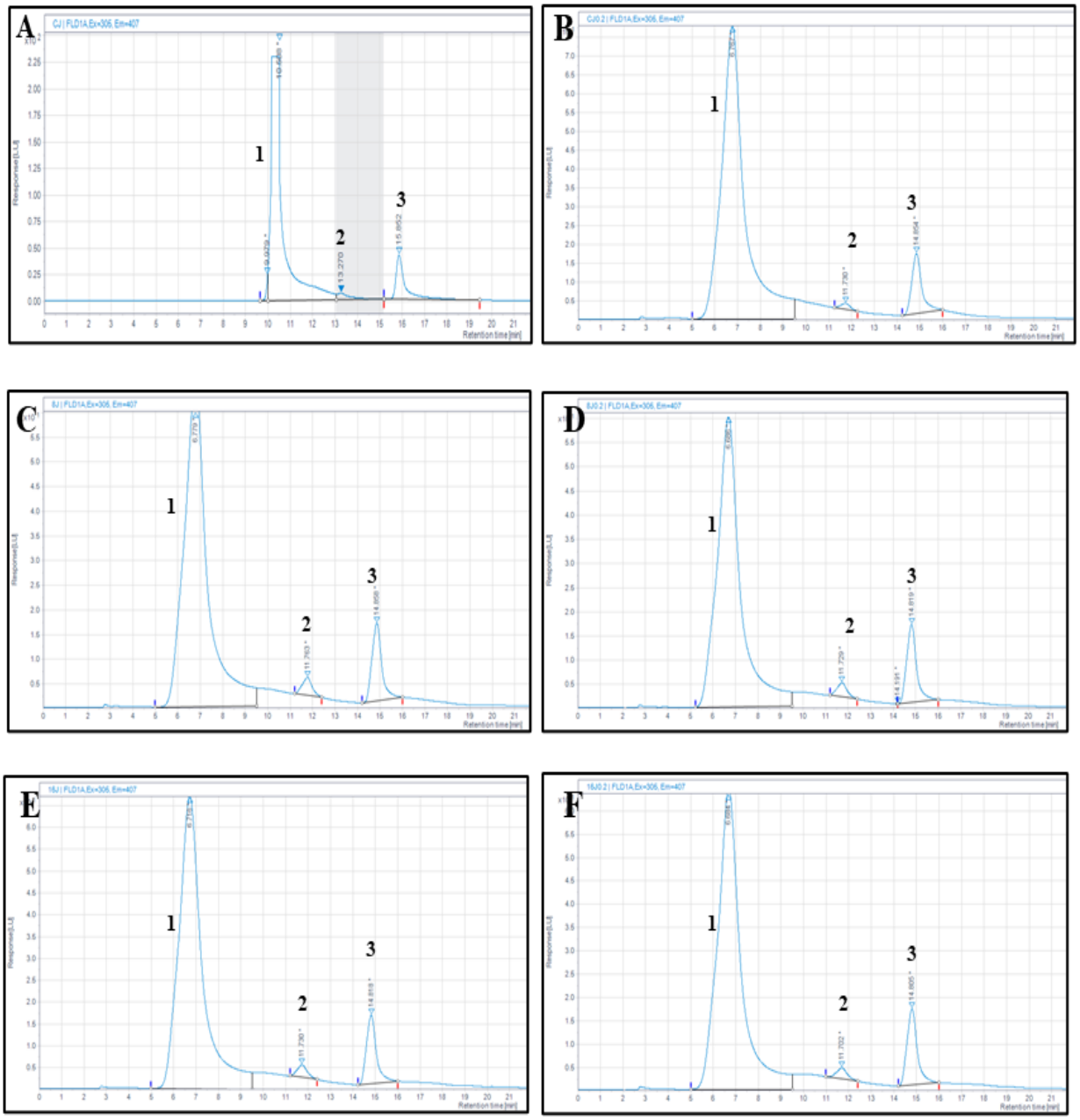


Fig. 3.20c: HPLC profile of endogenous salicylic acid in drought-sensitive (DS) rice cultivars treated with drought stress and salicylic acid. [**A-D**: well-watered (ww); **E-H**: moderates stress (ms); **I-L**: severe stress (SS). **A**-WW; **B**- WW+0.25 mM SA; **C**- MS; **D**-MS+0.25 mM SA; **E**- SS; **F**-SS+0.25 mM SA.

A considerable amount of literature has dealt with the effects of salicylic acid (SA) on the development and yield of various plant species. However, scientific data on the interactive effects of exogenous SA and drought on plant-water relationships, external and internal morphological changes, and alteration in endogenous SA content, particularly rice, is rare. The key objective of the present investigation was to evaluate the ameliorating effect of drought stress by exogenous application of SA on two rice cultivars. The experiment was performed as a laboratory test using the seeds of two genotypes, i.e., Sahbhagi dhan (drought tolerant) and Jaya (drought sensitive). It was performed to observe the effect of exogenously applied SA on both cultivar's morphological, physiological, biochemical and molecular attributes under PEG_{6000}-induced moderate and severe water deficit conditions at the vegetative stage. In light of the available literature, research findings of the parameters analysed are discussed in this chapter.

4.1.Interactive effect of drought and salicylic acid on plant growth and biomass

Water deficit, a major environmental constraint, decreases plant growth and development more than any other abiotic factor. Plant responses to water stress significantly depend on plant species, stress duration, intensity, and growth stage (Ghafar et al., 2021; Nawaz et al., 2021). Our results exhibited a significant effect of moderate and severe drought stress on the growth of both DT and DS rice cultivars due to their water-loving nature. The outcomes of the present study indicate the harmful effects of water deficit on the overall growth of rice plants subjected to drought stress. A significant decline in the shoot length and prolific increase in root growth in both rice cultivars was observed due to drought conditions. This indicates that the overall growth of rice plants is hindered by high osmotic

stress, leading to an oxidative burst. Due to reduced turgor pressure, cell growth is considered one of the most drought-sensitive processes. The well-watered control, i.e. optimal hydration necessary for the development and metabolic processes, maintains greater RWC and, thus, accounted for the highest biomass and increased growth in both DT and DS rice cultivars. These results are consistent with the earlier study by Audebert et al. (2000). According to Bañon et al. (2006), the reduction in shoot and root length occurring as a consequence of drought stress may be caused by decreased cell elongation, cell turgor, cell volume, and ultimately cell growth, along with blocked xylem and phloem channels that prevent any translocation (Lavisolo and Schuber, 1998).

However, SA is vital in regulating plant growth and productivity and also affects the physiological and biochemical activities in plants (Hayat et al., 2010; Naz et al., 2021). Upon foliar application of SA, the plant height has recovered in both genotypes exposed to moderate and severe drought stress conditions compared to untreated control. However, among the SA concentrations used, i.e., 0.1, 0.25 and 0.5 mM, 0.25 mM concentration significantly enhanced the plant height compared to the untreated controls. These findings validate those of Hayat et al. (2007), who reported that SA treatment improved shoot length, shoot diameter, and leaf number in ornamental plants like gloxinia and violet under drought stress. Similar results were seen in soybean plants with respect to shoot growth and an increase in the number of leaves per plant in wheat cultivars (Gutiérrez-Coronado et al., 1998; Hayat et al., 2005; Hussein et al., 2007; Aldesuquy et al., 2012). Ahmad et al. (2021) concluded that applying an adequate amount of SA increased the plant growth attributes of wheat cultivars under drought stress.

Further, Ghazi (2017) reported that drought caused a remarkable reduction in growth and yield parameters in *Zea mays* genotypes. It was observed that the exogenous application of SA enhanced yield parameters such as plant height, plant biomass, number, and grain weight under drought treatment compared to the control. Aldesuquy et al. (2012) observed that under drought stress, the wheat cultivars recorded a reduction in shoot length. Similarly, in *Abelmoschus esculentus* (Sankar et al., 2007 and 2008), *Vigna unguiculata* (Manivannan et al., 2007), soybean (Zhang et al., 2010), and *Petroselinum crispum* (Petropoulos et al., 2008) showed similar outcomes when subjected to drought stress. Shao et al. (2008) suggested that cell expansion and proliferation are significantly suppressed due to the low turgor pressure and water stress. Reduced plant height under water stress is linked to diminished cell expansion and increased leaf senescence (Bhatt and Srinivasa Rao, 2005). Applying SA is known to elicit the meristematic activity of cells, initiating cell elongation and enlargement, consequently enhancing plant vigour under stress (Singh et al., 2021).

Crop plants show reduced fresh and dry biomass under water stress, directly resulting from reduced photosynthesis. In the present study, drought resulted in a substantial reduction in biomass production. Such drought-induced reduction in growth biomass in rice is reported in earlier studies in wheat (Kingsbury et al., 1984), maise (Cramer et al., 1994), and fenugreek (Babar et al., 2014). Drought has been shown to reduce the imbibition of water by roots because of a decrease in solute potentials of the substrate and create changes in the metabolism, which is responsible for decreasing plant growth and development. Reductions in these parameters are linked to less water availability and stomatal factors,

such as low stomatal density and g_s, interfering with CO_2 uptake and reductions in cell division, expansion and differentiation due to water limitations in plant tissues (Idrees et al., 2010). Overall, drought-induced decrease in growth biomass may be due to adverse effects on physiological processes such as photosynthesis, ion homeostasis, and accumulation of osmoprotectants.

Exogenous application of SA efficiently increased the root and shoot dry mass in DT and DS cultivars under water deficit conditions. Foliar-applied SA enhanced vegetative growth by increasing fresh and dry biomass. These findings correlate with earlier observations of El-Tayeb (2005) and Gautam and Singh (2009), who reported that foliar-applied SA enhanced biomass production in barley and corn. It is also observed that the increase in growth biomass in response to SA under drought stress may be due to the protective role of SA on membranes that might be responsible for increasing plant drought tolerance. All these events culminate in less accumulation of dry matter. Our results also revealed that SA positively affected water status, stomatal characteristics, gas exchange, and antioxidant metabolism, contributing to higher root and shoot. Similar observations have been made in earlier studies (Farooq et al., 2010; Gautam and Singh, 2009). Under drought stress, a high dry mass is considered a supportive characteristic for the plant's survivability (Vardharajula et al., 2011). A foliar spray of SA significantly increased the dry weight mass (shoot and root). According to Fariduddin et al. (2003), there was a significant increase in dry matter production in *Brassica juncea* when sprayed with lower concentrations of SA. The process of dry matter and temporal biomass distribution is indirectly related to plant production under water stress (Ahmad et al., 2018; Ijaz et al.,

2021).

Kazemi et al. (2010) evaluated the biomass and antioxidant system of *Brassica napus* plants exposed to nickel toxicity. They described the beneficial effects of SA on shoot and root dry mass, explaining that these results were caused by the reduction of oxidative stress, thus confirming the data obtained in the present study. Similarly, Latif et al. (2016) reported increased shoot and root biomass in *Zea mays* grown under water deficit and treated with SA, confirming the effectiveness of this substance on biomass accumulation.

4.2.Interactive effect of drought and salicylic acid on external and anatomical changes

Under water stress, it was observed that there was an increase in stomatal density along with cuticular papillae (silica bodies) and wax deposition. In contrast, the stomatal length decreased in both DT and DS cultivars. It was observed that the stomatal characteristics were negatively affected by water deficit on the adaxial and abaxial leaf surfaces in both cultivars. According to the function of stomata, the microscopic pore, which directly governs the physiological mechanisms in plants, is known to play a vital role in gas exchange (Chatterjee et al., 2020; Giuliani et al., 2013). Franks and Beerling (2009) suggested that smaller stomatal sizes and higher stomatal densities are associated with maximum stomatal conductance to water.

In the present study, the alterations in physiological mechanisms and stomatal morphology affected growth in DT and DS cultivars. Stomatal closure in drought-stress environments caused transpiration and photosynthetic rate downturns, thereby declining CO_2 fixation (Taiz and Zeiger, 2002). In comparison, foliar application of 0.25 mM concentration of SA

alleviated these effects, leading to increased stomatal density and reduced stomatal length. These external morphological deviations caused by SA possibly have an ecophysiological advantage for these plants during drought, favouring stomatal regulation, leading to decreased water losses and, consequently, increased hydration of the leaf structures (Brito et al., 2019). These results correspond with the RWC values on exogenous SA treatment of DT and DS plants under water deficit in our study.

Additionally, external morphological modulation, such as variations in the number and size of stomata, due to SA treatment ameliorated the impacts provoked by drought stress, resulting in decreased interference of gas exchange, contributive to higher C fixation and increments related to WUE (Bertolino et al., 2019; Dow et al., 2014). Drought stress also adversely affected the stomatal traits of *Leymus chinensis* plants, leading to decline in stomatal density and size (Xu and Zhou, 2008). Ma et al. (2017) verified that varying levels of salt stress led to reductions in stomatal density in *Dianthus superbus* plants, however, SA treatment increased stomatal density, attenuating the adverse effects of salinity. According to Graça et al. (2010), the response of stomata to drought conditions varied in some plant species or cultivars. The photosynthetic process was promoted by increasing stomatal density and decreasing stomatal size (Nawazish et al., 2006), which suggested that Sahabhagi Dhan (DT) had higher drought tolerance than Jaya (DS). Drought stress-tolerant plants also exhibited higher photosynthetic rates. Because of protein protection, PS II functioning remains constant for as long as experimental periods (Lu and Zhang, 1999).

Furthermore, as roots are the frontline organs to sense the decrease in soil water availability, a comparative analysis of differences in root anatomy among the two rice cultivars and the foliar application of SA will possibly help identify the mechanism required for tolerance to drought stress. In this study, drought stress adversely affected the root anatomy by reducing stele diameter, aerenchyma thickness and the CSA in DT and DS cultivars. However, the application of SA was adequate to ameliorate the adverse effects of drought stress, promoting increased division and expansion of cells in root tissues (Shakirova et al., 2003). These structural variations, such as the changes related to stele diameter, are vital in improving the absorption and conduction of water and nutrients and indicate adaptation strategies to drought stress (Hasan et al., 2018; Makbul et al., 2011). In addition, an increase in epidermal thickness is fundamental to avoid water loss through the root surface when the soil water potential becomes more negative. According to Ribeiro et al. (2019), *Glycine max* seedlings exposed to water deficit revealed reductions in the anatomical characteristics of roots, such as epidermal thickness, endodermis thickness, cortex diameter, stele diameter, and metaxylem diameter. Our observations are on par with Agami (2013), who found improvements in the anatomical structures of *Lactuca sativa* roots treated with SA under water deficit.

Plant adaptation concerning water retention was revealed by leaf area reduction. Bosabalidis and Kofdis (2002) reported that the epidermal and mesophyll cells decreased in size, whereas cell density increased due to transpiration and respiration rate reduction. Moreover, reducing water content in plant cells also decreases turgor pressure and cell volume, thus weakening the cell walls. Under drought stress, cell expansion disruption

resulted in deceleration of leaf extension, causing interruption of H^+-ion movement across cell membranes. Cell and leaf expansion postponement also affected the transpiration rate and helped preserve water content within the cells during drought stress (Udomprasert, 2015). Studies have revealed that leaf thickness was associated with photosynthetic rate and plant growth under drought-stress conditions, resulting in increased mesophyll density (Kulya et al., 2018). In the present study, almost all anatomical characteristics of major midrib and lamina vascular bundles were significantly decreased under drought conditions, which may result in reduced water transportation due to leaf area reduction. The transport of water and food via tracheal elements is possibly related to the photosynthetic rate. Taiz and Zeiger (2002) reported that the lack of water during plant growth inhibited the transportation of water and food by reducing turgor pressure.

Under severe drought stress conditions, cells are known to be compact, and higher solute concentrations cause drought stress sensitivity of plant growth (Udomprasert, 2015). Bundle-sheath cells and vascular bundle sizes are known to be associated with respiration and photosynthetic percentage (Wu et al., 2011). Bulliform cell thickness in both DT and DS cultivars significantly decreased on exposure to drought. However, an increase in bulliform length, i.e. the horizontal size, was observed in both the cultivars. This finding concurred with Nawazish et al. (2006), who reported that bulliform plant cells expand as an anatomical adaptation to drought stress in *Cenchrus ciliaris* L. leaf. Nawazish et al. (2006) and Taratima et al. (2019) reported that an increase in bulliform cells and lamina thickness is related to anatomical adaptation under drought. The results of the present study are in agreement with Bosabalidis and Kofdis (2002), Nawazish et al. (2006), and Kulya

et al. (2018), who reported plant adaptation based on anatomical features.

In contrast, exogenous SA application alleviated the damage caused by drought stress to the leaf anatomy. These changes proved the role of SA on leaf structures, contributing to improvements in physiological processes and enhancing P_N and $g_{s.}$ These observations conform with an earlier study by Abd El-Mageed et al. (2016). Higher epidermal thickness (adaxial and abaxial) is adequate in drought situations, as it avoids water loss connected to the leaf surface and maintains a higher Ψw in leaves (Nawazish et al., 2006). In addition, an increase in intercellular spaces provides more space for CO_2 buildup between the substomatal cavity and the surface of mesophyll cells (Ennajeh et al., 2010). Thus, these structural changes, related to increases in stomatal density, favour the photosynthetic capacity of plants subjected to SA and water deficit, as observed in the present study (P_N, Ci, and MC). Cárcamo et al. (2012) assessed anatomical responses in *Zea mays* plants treated with SA exposed to salt stress. They also found that increased epidermal (adaxial, abaxial), leaf thickness, and vascular bundle size enlargement improved water and food transportation efficiency; further, they suggested that it may be caused by stimulation of the defence pathway by SA, inducing substantial defensive effects on cellular structures.

4.3.Physiological responses of plants to the interactive effect of drought and salicylic acid

Drought stress reduces plant growth by altering photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism, and growth promoters (Haider et al., 2022; Kosar et al., 2021; Waseem et al., 2006; Zafar et al., 2021). Water potential indicates plant

water status and is essential in water transport in the soil-plant-atmosphere continuum (Kumar et al., 2019). RWC, an indicator of plant water status and early response to drought stress affects plant water status by building osmotic solutes in the roots and further transportation to shoot, thus causing physiological drought (Barrs and Weatherly, 1962). The RWC decreased in plants facing drought stress and was found to have a high reduction under high-stress intensity. The present study resulted in higher leaf RWC in the DT cultivar than in the DS cultivar, suggesting better water availability to maintain cell turgidity and metabolic process under drought stress. The morphological changes observed in our study may also play a role in maintaining better plant water relations by preventing water loss in DT than in the DS cultivar. Drought stress reduces water content, leaf osmotic potential, turgor pressure, stomatal activity, cell enlargement and plant growth.

Further, higher drought results in a downregulation in photosynthesis, interrupted metabolism, and, finally, causing plant death (Farooq et al., 2020; Nawaz et al., 2021; Shemi et al., 2021; Hussain et al., 2008; Kosar et al., 2021). Mohd Razi et al. (1992) observed a significant reduction in photosynthesis and transpiration due to a drop in leaf water potential below 2.0 MPa in bananas. Similarly, Natarajan and Kumaravelu (1993) reported that drought-resistance rice varieties showed consistently higher leaf water potential in their tissues than susceptible types under soil moisture deficit, supporting our results.

Photosynthesis is particularly sensitive to the effects of water deficiency. Plants' resistance to water deficiency yields metabolic changes and functional and structural rearrangements

of photosynthetic apparatus. Photosynthesis of higher plants decreases with the reduction in the relative water content (RWC) and leaf water potential. Lower photosynthesis rate is a usual effect of water stress in plants and has been attributed primarily to stomatal limitation. In the present study, the exogenous application of SA alleviated the adverse effect of drought stress and enhanced growth and photosynthesis under normal and stressed conditions. It has been suggested that SA-induced enhancement in plant growth under drought stress might be due to changes in biochemical or physiological processes. Due to the water deficit, reductions in Fv/Fm were observed in both DT and DS cultivars. Our results revealed that the application of SA effectively mitigated the negative impact of water deficit on the Fv/Fm ratio.

Additionally, an increase in Fv/Fm indicates a reduction in the excessive photon flow caused by water deficit, consequently lowering photoinhibition (Khoshbakht and Asgharei, 2015). An increase in Fv/Fm was observed in *Phaseolus vulgaris* that received SA treatment and was exposed to cadmium toxicity (Wael et al., 2015), supporting our results. Semida et al. (2017) evaluated the combined effects of SA treatment under water deficit in *Allium cepa* plants and recorded an increase in Fv/Fm, revealing the positive effects of SA during stress. Furthermore, SA treatment efficiently increased ΦPSII and qp in both cultivars under water deficit conditions. These outcomes reinforce the defensive action of SA on PSII, which induces higher values of ΦPSII, representing thermostability and restoration of the number of open reaction centres in PSII, contributing to improvements in the light capture processes (Wang et al., 2010). This result can be confirmed by the increase in qp, representing the fraction of open reaction centres in PSII. The reduced

photoinhibitory damages promoted by SA can also be attributed to reduced loss of excitation energy by thermal dissipation (NPQ), which could compete with the transfer of electrons to the reaction centres of PSII (Brito et al., 2019; Poór et al., 2019). Tang et al. (2017) assessed the photosynthetic performance of antioxidant enzymes in *Glycine max* under water deficit. They found an increase in ΦPSII and qp after exogenous application of SA, confirming the outcomes obtained in the present study. Yotsova et al. (2018) examined responses related to the photosynthetic apparatus. They found an increase in ΦPSII in *Oryza sativa* plants treated with SA subjected to cadmium stress. This may have led to the alleviation of chloroplast membrane damage and the increase of the electron transport rate caused by SA.

Furthermore, stomata play an essential role in gas exchange, directly affecting photosynthesis, stomatal conductance, and transpiration (Chatterjee et al., 2020). Less water availability leads to lower leaf water content and reduced stomatal size by losing turgor pressure (Ψt) in guard cells, resulting in stomatal closure (Karimpour, 2019). At the beginning of drought stress, gs reduced photosynthesis (Nikolaeva et al., 2010), but prolonged drought stress may cause tissue dehydration, leading to metabolic impairment (Mafakheri et al., 2010). In this study, the foliar application of SA restored the g_s in both genotypes under drought stress. Stomatal closure, which is known to reduce CO_2 influx into the mesophyll cells (Farooq et al., 2013), reduces the activity and content of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), as well as ribulose bisphosphate (RuBP) regeneration (Mohamed et al., 2020). Under drought stress, rice attempted to conserve water by lowering g_s, as found in the study of Caine et al. (2019), with the

potential trade-off of reducing carbon assimilation.

Further, the drought stress also showed a decline in P_N, E, g_s, WUE, and MC, with increments in Ci in both DT and DS cultivars. However, treatment with 0.25 mM SA, compared to 0.1 and 0.5 mM SA, could ameliorate the harmful effects of drought stress. These outcomes revealed that SA protects the photosynthetic apparatus, including stomatal and non-stomatal factors, as reported in an earlier study by Stevens et al. (2006). The improvements linked to stomatal characteristics are associated with an increase in g_s after SA treatment, contributing to a higher influx of CO_2 and higher values of P_N compared to plants exposed to water deficit without SA (Habibi, 2012). In parallel, the increase of g_s is related to the positive effects of SA, which may inhibit the ABA concentration in guard cells and minimise stomatal closure (Khan et al., 2013).

Moreover, the increments of P_N also reveal the action of SA on photochemical and biochemical processes involved in the photosynthetic machinery (Khoshbakht and Asgharei, 2015), such as an upsurge in ΦPSII and qp as well as reductions in Ci and an increase in MC due to the probable increases in RuBisCO activity (Khan et al., 2003). Faried et al. (2017) assessed the effects of SA in two cultivars of *Solanum tuberosum* under salt stress and found increases in P_N and g_s compared to SA untreated plants. Similar to our results, Shao et al. (2018) described that the adverse effects of water deficit on P_N, g_s, Ci, and WUE were mitigated in *Zea mays* plants treated with SA, revealing the efficiency of exogenous SA against the harmful interference caused by drought stress.

In the DT cultivar, the Chl a, Chl b, Total Chl, and Car levels were reduced due to water deficit. In comparison, the Chl a/Chl b and Total Chl/Car ratios recorded an increase in the DS cultivar with the increase in the drought levels. However, these parameters improved after SA application compared to plants subjected to water deficit without SA treatment. The enhancement of chlorophyll degradation in stressed plant leaves can probably be due to the disturbance in hormonal balance. Such disturbance may be manifested by diminished kinetin biosynthesis and increased abscisic acid, with the former inhibiting chlorophyllase activity while the latter accelerating it (Drazkiewicz, 1994).

The adverse effects of water deficit on photosynthetic pigments may be due to the increase in the activity of the chlorophyllase enzyme, which aggravates the degradation of chlorophyll in addition to the destruction of chloroplasts and instability of the protein complex due to an upsurge in ROS levels (Askari and Ehsanzadeh, 2015; Mibei et al., 2017). Foliar application of SA possibly decreased the activity of chlorophyllase combined with reduced ROS concentrations and increased Car contents. Carotenoids protect chlorophylls under water deficit conditions (Razmi et al., 2017).

In the present study, the chlorophyll levels increased in the DS cultivar only under moderate stress. Increasing drought stress induces oxidative stress in plant cells, followed by a photoprotection mechanism known as osmotic adjustment (Blum, 2017) and activation of the antioxidant system (Khaleghi et al., 2019). Osmotic adjustment is an essential element in the drought response strategy of plants (Weithmann et al., 2022).

Photosynthetic capacity is remarkably altered by abiotic stress like drought and their respective intensities and durations (Elferjani and Soolanayakanahally, 2018). Photosynthetic pigments are involved in photoprotective processes and the activities of antioxidants, which contribute to oxygen and biomass production (Kuczynska et al., 2015). However, reducing chlorophyll content is a typical response to drought stress. Similar to our findings, Rolando et al. (2015) illustrated increased Chlorophyll a and b content following water restriction. Chlorophyll and carotenoid contents are controlled by genotypes (Rustioni and Bianchi, 2021). It has been suggested that water-stressed plants that maintain high chlorophyll content use light more efficiently than other plants and have enhanced resistance to drought stress (Fang and Xiong, 2015). However, our results contradict the earlier finding of Khan et al. (2017), who indicated that chlorophyll content reduction was more pronounced in drought-sensitive varieties.

Enhancing chlorophyll content followed by increasing drought results from slowing cellular growth relative to chloroplast development or water-deficit shock (Garcı´a-Valenzuela et al., 2005). The defensive mechanism may act primarily under conditions of biotic and abiotic stress, such as drought, helping to reduce their toxicity. This phenomenon is a type of conditioning mode of defence where adaptive responses (higher chlorophyll concentrations) are activated by moderate stress (Calabrese et al., 2007; Agathokleous et al., 2019). This may reduce chlorophyll degradation or the inhibition of chlorophyll biosynthesis (Alscher and Castelfranco, 1972). Higher concentrations of chlorophylls may also represent a strategy for increasing CO_2 assimilation (Sanchez-Zabala et al., 2015), thus mitigating accumulated stress.

Additionally, the reduction in the Chl a/Chl b ratio suggests a decrease in the conversion rate of Chl b to Chl a, indicating a reduction of the chlorophyll degradation process (Azizpour et al., 2010), supporting our observation that SA mitigated the effect of water deficit. Similarly, Vaisnad and Talebi (2015) studied four *Cicer arietinum* genotypes under water deficit that were treated with SA and reported an increase in chloroplast pigments as observed in the present study (Chl a, Chl b, and Car). Loutfy et al. (2012) also reported that the application of SA was able to promote an increase in Chl a, Chl b, Total Chl, and Car levels in four cultivars of *Triticum aestivum* under water deficit conditions, as observed in the present study.

The results of our study are consistent with a study by Song et al. (2020), who showed that the SA treatment caused an increase in chlorophyll content in *P. chinensis*. SA either induces the chlorophyll and carotenoid biosynthesis pathway or reduces their degradation, consequently increasing photosynthetic pigments. These results indicated that the increased chlorophyll and carotenoid contents in the plants treated with SA may play an essential role in alleviating drought stress damage in DT and DS cultivars.

4.4.Biochemical response of plants to the interactive effect of drought and salicylic acid

Water deficit induced increases in OH• and H_2O_2, with consequent upsurge in the MDA and EL concentrations in both DT and DS cultivars. In general, stress environments, such as drought, trigger the extreme formation of ROS because of the intersection of O_2 molecules with electrons that escape the regular route in the respiratory pathways and the electron transport chain of photosynthesis (Ali, 2017). One of the primary cellular targets

under various stress circumstances is the cell membrane (Levitt, 1980). The degree of membrane damage represents the plant's resistance to stressors like drought (Aldesuquy et al., 2013). In this context, electrolyte leakage (EL) and lipid peroxidation may be the two common plant membrane stress indicators. Stress-related situations in the current study significantly increased lipid peroxidation and EL in DT and DS cultivars. These findings concur with those of Fazeli et al. (2007), who showed that dryness possibly raises malondialdehyde (MDA) levels in the leaves of two different cultivars of sesame, leading to a considerable increase in lipid peroxidation. It is well known that abiotic stress increases the formation of free radicals, which causes biomembrane lipid peroxidation and reflects stress-induced tissue damage (Harinasut et al., 2003). ROS can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in plasma lemma or intracellular organelles, resulting in cellular content leakage, rapid desiccation, and cell death (Sridharan et al., 2009). Evidence suggests that membranes are the primary sites of injury to cells and organelles (Candan and Tarhan, 2003).

Malondialdehyde (MDA) is one of the breakdown products of polyunsaturated fatty acids (PUFA), the primary membrane lipid components susceptible to peroxidation and degradation, used in the current investigation to quantify the amount of lipid peroxidation. A drop in leaf CO_2 concentration resulting from stomatal closure may cause a rise in MDA content. As a result, there may be less $NADP^+$ available to absorb electrons from PSI and PSII, which initiates the O_2 reduction process and produces ROS (Hernandez et al., 2000). Cell membrane damage and increased permeability caused by water stress may lead to electrolyte leakage (Almeselmani et al., 2012). Valentovic et al. (2006) reported that

drought treatment in two maize cultivars increased the electrolyte leakage under water shortage conditions. Stress can denature bio-membrane proteins and decrease the activity of plasma membrane H^+-ATPase, resulting in lower membrane stability, as shown by increased leakage under water deficiency situations. Similarly, Lin and Wu (1996) reported that the plasma membrane H^+-ATPase activity and total plasma membrane protein content in buffalograss were significantly reduced due to stress.

The production of reactive oxygen species (ROS) should be prohibited, and ROS-induced oxidative damage should be mitigated through lipid peroxidation (MDA), protein and DNA oxidation (Akram et al., 2022). However, our results demonstrated that SA had a stabilizer effect on the enzymatic antioxidant system (SOD, APX and GR), declining the levels of OH• and H_2O_2 in plants under drought stress. These variations mitigated the impairment of structures and functions of cell membranes in plants exposed to stressed conditions, as observed by the lower values of MDA, EL and PC in plants treated with SA (Sayyari et al., 2013). Our results are in par with La et al. (2019), who observed reductions in the O_2^- and H_2O_2 levels in *Brassica rapa* plants exposed to water deficit and treated with SA. Fayez and Bazaid (2014) also found a beneficial effect of SA on *Hordeum vulgare* under water deficit and salinity conditions, which promoted a reduction of the MDA level, confirming the results obtained in this study. SA applied to drought-stressed rice cultivars significantly reduced lipid peroxidation, protein oxidation and EL compared to SA-untreated water-stressed plants. Thus, SA treatment considerably reduced MDA formation, protein carbonyl content and electrolyte leakage, as observed in an earlier study by Sayyari et al. (2013). Additionally, under salt stress, SA treatment dramatically reduced lipid

peroxidation in sweet basil (Delavari et al., 2010).

In the present study, the content of protein carbonyl in the leaves of two rice cultivars was significantly elevated due to drought stress, and it was concurrent with the increased accumulation of H_2O_2 and OH•. At the same time, exogenous application of 0.25 mM SA markedly declined contents of protein carbonyls, H_2O_2 and OH•. However, the DT cultivar appeared to have a more significant buildup of protein carbonyl content than the DS cultivar. Protein carbonylation is a post-translational modification brought about by ROS, which can cause protein degradation. Unlike other protein breakdown processes like ubiquitination, protein carbonylation does not require ATP or enzymes. An energy-efficient strategy to rapidly recycle amino acids during nutritional deprivation or stress is the breakdown of carbonylated proteins by the 20S proteasome system. Protein carbonylation may be seen as a process that supports protein turnover and aids in the tolerance to oxidative stress, helping plants deal with short-term stress (Tola et al., 2021). The present study confirms that both rice cultivars could develop tolerance to the damaging effects of oxidative stress due to the exogenous application of SA.

In the present study, it was observed that there was a significant increase in proline content in drought-treated DT and DS cultivar plants, and SA application enhanced the proline levels in these plants. This may be attributed to strategies adopted by plants to cope with drought stress conditions. Our results are in conformity with Mafakheri et al. (2010) and Pospisilova et al. (2011), who reported that exogenous application of SA increased the proline content under drought stress conditions. In the present study, the application of SA

results in the accumulation of proline, which protects the photosynthetic machinery from drought stress by stabilising the structure of Rubisco, increasing water and osmotic potential and reducing oxidative stress. Thus, proline accumulation is a potential indicator of stress tolerance (Ashraf and Foolad, 2007), protecting the enzymes by stabilising the structure of proteins such as Rubisco (Zhu, 2001) and protecting membrane structures (Maggio et al., 2002). It has been reported that proline accumulation increases under drought stress due to the increased production of P5C reductase (P5CR), which is then converted to proline by the average or enhanced level of P5CR activity (Ábrahám et al., 2003). Studies on the green gram (Misra and Gupta, 2005) and *B. juncea* (Madan et al., 1995) have shown a correlation between increased P5CR activity and proline accumulation. Also, H_2O_2-mediated oxidative stress was alleviated by proline in grape wine leaves and almonds (Sorkheh et al., 2012), supporting our finding. Our results also suggest that the reduction of oxidative stress under drought conditions is attributed to the increased proline content by SA application, which protects photosynthesis. Proline accumulation under stress is correlated with osmotic adjustment and improves plant drought tolerance (Vinocur and Altman, 2005). Hussain et al. (2009) and Saruhan et al. (2012) have shown that plants overcome drought stress by improvement in leaf water potential via the accumulation of compatible solutes, which then enhance the osmoregulation ability of crops that are influenced by SA. Proline regulates the accumulation of useable nitrogen (N), and is osmotically active and contributes to membrane stability (Bandurska, 2000; Bandurska et al., 2008; DaCosta and Huang, 2006). It may also act as a signalling regulatory molecule able to activate multiple responses that are components of the adaptation process (Maggio et al., 2002).

In addition to the enzymatic antioxidant machinery, antioxidant metabolites are also strongly involved in alleviating drought stress by regulating the osmotic stress. In the present study, it was observed that the total Glutathione (TGSH) concentration upsurged upon exogenous SA treatment due to elevation in GR activity, leading to alleviated oxidative stress and enhanced plant growth. These observations are consistent with Dat et al. (1998), who reported the application of SA-enhanced levels of antioxidant substances in plants. The enhanced GR and GSH activity is known to play a decisive role in preserving the antioxidant pool in its redox state and reducing the levels of carbonylated proteins by inducing an antioxidant defence system under various environmental stresses (Ignatenko et al., 2021; Song et al., 2011).

Glutathione is an excellent antioxidant molecule having protective roles in different cells, e.g. as redox and also functions as a redox signalling molecule for biotic and abiotic stress and is also linked to C, N and sulphur metabolism (Noctor et al., 2012; Hernández et al., 2015; Salbitani et al., 2015). In plant cells, the ratio of reduced and oxidised forms of glutathione (GSH/GSSG) plays a vital role in the signalling and activation of defence mechanisms (Foyer and Noctor, 2012; Salbitani et al., 2015). The Glutathione (GSH) to Glutathione disulfide (GSSG) ratio predominates in controlled or normal conditions. In contrast, a brief shift in the oxidised value, i.e., increased GSSG content, occurs during stress conditions (Tausz et al., 2004).

4.5. Interactive effect of drought and salicylic acid on molecular aspects of plants

Homeostasis of ROS is known to be a convergence point to assess plant stress status. Fortunately, plants have evolved several defence mechanisms, including enzymatic antioxidant systems that may effectively decrease or remove ROS at varying levels of stress-induced degradation. A coordinated chain of antioxidant enzyme synthesis, such as SOD, CAT, and APX, is triggered to neutralise the damaging effects of ROS on plant growth. In the present study, the tolerant cultivar outperformed the sensitive cultivar in enzyme activity under water deficit conditions. Our results suggest that the antioxidative response is well associated with growth sensitivity and tolerance of the two cultivars to water stress. It may thus be concluded that DT induces the antioxidative system more efficiently than DS, resulting in slow growth suppression and lipid peroxidation under drought stress.

On the other hand, exogenous application of SA increased the expression of antioxidant enzyme gene expression (Cu/Zn-SOD, APX-1) than plants subjected to drought stress alone and improved their enzymatic activity. This suggests that exogenous SA can enhance the antioxidant enzyme activity of both rice cultivars under drought stress, hence reducing ROS accumulation and preventing oxidative damage to the plants. However, there was a decrease in SOD activity in both cultivars due to drought. This observation is in agreement with the previous findings, which also recorded reduced SOD activity in plants exposed to drought and cold stress (Zhang and Kirkham, 1994; Ignatenko et al., 2021). The dismutation of superoxide anion radical ($O_2^{\bullet -}$) is effectively facilitated by SOD, leading to the production of H_2O_2 and O_2 (Biczak, 2016; Razmi et al., 2017). SOD also provides

tolerance to stress conditions by binding with singlet oxygen (Kliebenstein et al., 1999). In different plant species, including *Arabidopsis*, rice, and tomato, several SOD genes have been analysed during stress tolerance which showed enhanced resistance to stress conditions (Kliebenstein et al., 1999). On exposure to drought stress, the activity of the antioxidative enzyme (SOD) decreased and continued to decrease with more prolonged exposure. A decline in SOD function could hinder the cells' ability to remove $O_2^{\bullet -}$ and result in its buildup. The decline in net SOD activity during drought stress could be attributed to reduced synthesis or enhanced enzyme degradation. Moreover, increased H_2O_2 levels during drought stress may also decrease SOD action. The decline in SOD activity during drought stress could significantly affect the cellular antioxidant defence system (Zhang and Kirkham, 1994).

In the present study, drought stress significantly increased GR activity. The increased activity of the GR enzyme was inadequate to quench the excessively generated ROS caused by drought stress. This antioxidant machinery (SOD and GR) was enhanced in both rice cultivars upon foliar treatment of SA under drought-stressed conditions. These results are in agreement with Zafar et al. (2021), who demonstrated the foliar application of SA on *Conocarpus erectus* L. and *Populus deltoides* L. saplings. They observed that the foliar application of SA boosted the antioxidant enzyme activity of SOD, POD, APX and CAT on exposure to drought compared to the control, which curtailed the oxidative damage under stress and correlated it with the maintenance of cellular integrity by reducing the activities of ROS.

Applying SA increases the enzymatic and non-enzymatic components (Kadioglu et al., 2011). SA increased abscisic acid (ABA) content under stress, reduced the harmful effects of stress on plants (Ianovici, 2011), and caused plants to re-grow. According to Flexas et al. (2006), plants with high photosynthesis and antioxidant capacity show tolerance and rapid recovery. The increase in ABA may be connected to the activity of antioxidant enzymes (SOD, APX, GR) in DT and DS cultivars treated with SA and exposed to water deficit, indicating a defensive role of this phenolic compound (SA) against oxidative damage. Higher enzymatic activities, such as SOD, APX, and GR, are intrinsically related to the antioxidant system. They are vital to alleviate the damage produced by water deficit at the cellular level because they act as a primary pathway in converting H_2O_2 into H_2O and O_2.

In addition, these outcomes confirm the role of SA as a signalling molecule in the defence system of plants under stress, contributing to the balance between the production of ROS and cell detoxification via enzymatic processes, consequently avoiding photo-oxidative damage to proteins, lipids, DNA and cells (Chandrakar et al., 2016; Demidchik, 2015). The application of SA also caused an increase in the activities of SOD, CAT and POX in *Zoysia japonica* plants under water deficit, which was elucidated by the fact that the antioxidant responses induced by SA are crucial to shield the plant from oxidative stress (Chen et al., 2014). El-Esawi et al. (2017) also observed an increase in SOD, CAT, and APX activity in *Rosmarinus officinallis* plants treated with SA and under salt stress, confirming that SA may stimulate the antioxidant enzymatic mechanism.

Furthermore, potassium (K) nutrition is closely associated with plant water homeostasis and water use efficiency (Ahmad et al., 2016a). Improved K uptake is a crucial response of plants exposed to water deficit (Wang et al., 2004; Ahmad et al., 2016b) as limiting K deficiency improves water retention, confirms appropriate stomatal regulation, and aids preserve photosynthetic activity via photoassimilate translocation (Römheld and Kirkby, 2010; Zörb et al., 2014). Improved K retention lowers cellular water potential and prevents added water loss, for example, from roots to soil. OsTPKb alters K concentration in small vacuoles, determining the overall cellular K homeostasis and influencing stress tolerance (Ahmad et al., 2016a). It has been reported that overexpression of OsAKT1 in rice improves osmotic and drought stress tolerance by boosting tissue K levels, particularly in the roots, which can stimulate drought tolerance in rice (Ahmad et al., 2016b). In the present study, it was observed that overexpression of OsHAK1 on foliar application of SA is driven by its native promoter, significantly increasing the growth of roots and aerial parts. K is known to maintain both root and shoot growth in plants, including cell cycle regulation (Sano et al., 2007) and the completion of cell death programs (Peters and Chin, 2007).

Furthermore, overexpression of OsHAK1 improved root surface and total root length, leading to an enhanced absorption surface for K when grown under stress conditions. Another sign of oxidative stress is H_2O_2 buildup. The damage due to the buildup of H_2O_2 because of lack of moisture has been documented in rice (Cai et al., 2015; Jiang et al., 2016). Proline accumulation was also measured, an osmoprotection response associated with membrane and protein stability (Xiang et al., 2007; Zhao et al., 2014). Although still

debatable, several studies on rice support the idea that proline functions in the osmotic adjustment under drought stress (Cai et al., 2015; Jiang et al., 2016). In the present study, OsHAK1-Ox plants accumulated significantly more Pro under drought than control.

MIPs (Membrane Intrinsic Proteins) such as AQP are thought to play a part in controlling cell turgor as they act as regulators of intracellular water flow by forming channels in the tonoplast. TIPs can accurately control the flow of a few small neutral molecules, including glycerol, urea, ammonia, H_2O_2, formamide, and water. Since aquaporins are in charge of precisely controlling water flow, they may be essential for drought-stress response and tolerance (Golldack et al., 2014). Significant variations in the AQP gene expression in response to drought stress and exogenous SA application were studied by conducting a transcriptome study on the leaf tissues of the two cultivars. Downregulating the AQP gene can reduce the tonoplast's water permeability under drought stress. This may help minimise water loss and restrict water flow through the cell membranes, stopping the further loss of leaf turgor. This expression pattern has been demonstrated in several investigations. The TIP homologue genes NgMIP2 and NgMIP3 were down-regulated in *Nicotiana glauca* leaves during drought stress (Smart et al., 2001) and after 12 days of drought, the levels of AtTIP1;1, AtTIP1;2, AtTIP2;1, and AtTIP2;2 in *Arabidopsis* were more than four times down-regulated (Alexandersson et al., 2005). By evaluating the TIP gene expression in drought-stressed leaf and root tissues, researchers have investigated the potential functional significance of TIPs in controlling the water balance in *Coffea arabica* L. (Miniussi et al., 2015).

Interestingly, PvTIP4;1 gene expression during drought differed depending on the cultivar, with more downregulation of these genes in the drought-tolerant cultivar (Zupin et al., 2017). The tolerant cultivar appears to limit water loss during droughts to a higher extent based on variations in RWC and the water potential of leaves between these cultivars. This may be related to the prompt and sufficient down-regulation of the AQPs (Zupin et al., 2017). It has been suggested that the most significant and well-acknowledged function of all AQPs, including TIPs during drought stress, is the control of water transport. Their participation in the transport of H_2O_2, one of the ROS, which also includes the unstable, highly reactive molecules formed by abiotic stress, such as drought, might serve a second crucial role (Fahad et al., 2017; Choudhury et al., 2017). Due to this action, the AQPs become significant participants in the redox signalling network and H_2O_2 detoxification (Bienert and Chaumont, 2014). TIPs' *in vitro* expression in yeast or oocytes has previously demonstrated that they can make it easier for H_2O_2 to pass membranes (Kurowska et al., 2019). Based on antioxidant enzyme activity and gene expression, the study concludes that SA has strong scavenging activity and helps remove the toxic effect of drought stress.

4.6.Interactive effect of drought and salicylic acid on endogenous SA

SA is a hormone and signalling molecule in plants, regulating plant responses against different environmental stresses. Its biosynthetic pathway is induced in response to different stresses. It has been reported that the ICS gene plays an essential role in SA biosynthesis in some plant species (Boatwright and Pajerowska-Mukhtar, 2013). With reference to our finding, it appears that the AtICS1 gene may play an essential role in the stress-activated production of SA in *Arabidopsis thaliana* (Wildermuth et al. 2001; Ogawa

et al. 2007). Initially, the SA treatment under drought conditions may act as a positive regulator of the ICS gene, leading to increased SA content under drought stress.

We observed that the endogenous SA levels in the shoot, induced under drought conditions, were alleviated by exogenously supplied SA. This suggests that the endogenous SA concentration in the treated plants was not critical because the exogenously applied SA mitigated the drought stress, as evident by improved plant growth and lowered MDA levels under these conditions. Besides, the exogenous SA regulates the ROS generation and activates antioxidant defence mechanism to combat stress conditions. However, lower levels of endogenous SA during severe drought also reveal that SA signalling has often been involved with antagonistic and synergistic responses with other endogenous phytohormones, which-are yet to be identified.

CONCLUSION

The present investigation aimed to evaluate the ameliorative effects of SA on the growth, physiological, biochemical, and molecular characteristics of DT and DS cultivars under drought stress. Drought stress alone significantly affected root and shoot length, whole plant fresh and dry weight of DT and DS cultivar at seedling stage compared to control. However, the foliar application of SA in drought stress enhanced these growth and yield parameters but did not exceed the control. RWC in the treated plants significantly increased as compared to drought-stress plants. Drought stress decreased the photosynthetic pigments and carotenoid contents in the leaves of the DT cultivar, while the pigment content increased in the DS cultivar. However, exogenous foliar application of SA enhanced the photosynthetic pigment contents in the leaves, along with morphological changes such as increased number and size of stomata and epidermal thickness. Besides, it prevented water loss to increase the RWC and plant water status. Upregulation of plasma membrane Intrinsic Proteins (PIPs) such as AQPs may also account for better RWC in DT cultivar under drought stress. DT cultivar also showed better chlorophyll a/b and carotenoids to chlorophyll ratio and better RWC, resulting in higher CO_2 assimilation and productivity. Our results on chlorophyll fluorescence also suggest the role of drought in pigment content composition, thereby influencing photosynthesis.

Our results also showed that a water-deficit environment induced severe toxicity in rice genotypes by increasing ROS generation through oxidative stress. This leads to oxidative damage to membrane lipids and protein, more so in DS than in DS cultivar. This observation suggested better membrane integrity, which is seen as a low lipid (MDA)

oxidation level and electrolyte leakage in DT. This was further confirmed by a fatty acid composition that showed biosynthesis of unsaturated fatty acid, contributing to the better maintenance of membrane integrity in the DT cultivar. Our findings show that the effects of drought exposure were apparent in membrane properties, enzymatic antioxidant activity, and non-enzymatic antioxidant activity. The adverse effects of stress on membrane properties on the investigated rice plants are also seen in the increased lipid peroxidation and higher EL compared to the unstressed rice cultivars.

In comparison, the exogenous application of salicylic acid (SA) appeared to mitigate the toxic effects of drought with varying magnitude through stimulation of the enzymatic and non-antioxidant systems. These results were further supported by higher gene expression of SOD and APX in the DT cultivar, resulting in decreased oxidative damage induced by MDA and ROS. Compared to the control, the compatible solutes like proline increased in the DT and DS cultivars under drought stress. The foliar application of SA in drought stress further enhanced the accumulation of these compatible solutes compared to untreated drought-stressed and control plants **(Fig. 4.1**).

From the above findings, it may be concluded that the 'Sahbhagi Dhan' (drought tolerant) showed higher tolerance to drought stress than 'Jaya' (drought sensitive) owing to better plant water status, morphological changes such as an increased number and size of stomata and increasing the epidermal thickness and prevented water loss to increase the RWC and plant water status. Upregulation of Plasma membrane Intrinsic Proteins (PIPs) such as AQPs may also account for better RWC in the DT cultivar to tolerate water deficit than in

the DS cultivar. Drought stress affected the growth and yield of *Oryza sativa* L. cv. 'Jaya' (drought sensitive) by affecting morphological and physiological processes. However, exogenously applied SA through foliar spray ameliorated the adverse effects of drought stress in the selected rice cultivars by inducing morphological growth such as root and shoot length, total biomass, improved RWC, photosynthetic pigments, biochemical components, compatible solutes and antioxidant system and inhibited lipid peroxidation and ROS production. Further, the discussion of drought-resistance features and drought recovery caused by exogenously applied SA will help understand how plants can withstand, survive, and restore productivity in a constantly changing, unpredictable environment. Enhancing survival during drought stress through drought-resistance mechanisms is essential for maintaining efficient metabolic processes and ensuring the plant can recover following SA treatments. The effects of drought on different plant species and their coping mechanisms have been the subject of numerous studies. However, it is essential to note that additional research is still required to fully comprehend the mechanisms underlying plants' recovery from drought stress following foliar applications of SA. Moreover, proteomic and genomic techniques must be used to identify the essential players in SA-induced drought tolerance. Still requiring much investigation are its functions in plant design, development, and metabolism, along with the cross-talk between different endogenous phytohormones due to exogenous SA application under various stressors. Thus, using an optimum dose of SA specific to plant species would help increase crop yield and photosynthetic efficiency in a drought-stressed environment may be recommended.

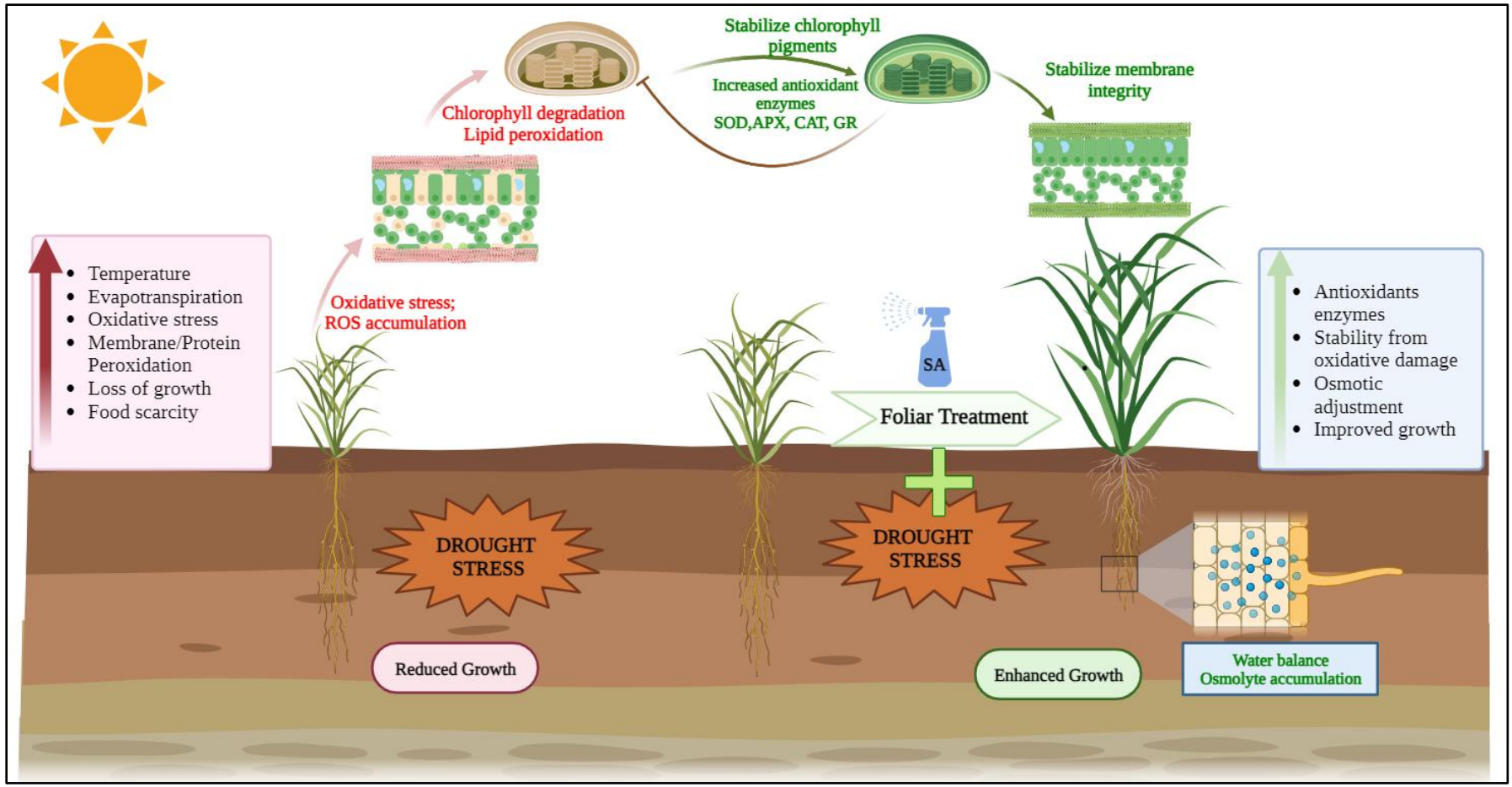


Fig. 4.1: Diagram illustrating the possible mechanism of foliar applied salicylic acid alleviating drought effects in rice (*Oryza sativa* L.).

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Presentation at conferences

1. Attended National Conference On Vistas In Biodiversity, Biology, Biotechnology And Nanotechnology Of Algae, held from 20th- 22nd September 2018, Organized by the Department of Botany, Madras Christian College (Autonomous), Chennai, Tamil Nadu.
2. National Virtual Conference on "Current Trends and Challenges in Plant Biochemistry and Biotechnology", held from 20th - 21st November 2020, organized by SPBB, New Delhi and BITS. Birla Goa Campus. (Oral Presentation)
3. International Conference on "Environmental Sustainability & Biotechnology: Opportunities & Challenges" held from 16th -19th November 2022, at Goa, India. (Poster Presentation).
4. National Conference on Recent Trends in Plant Sciences & Biotechnology, held on 3rd & 4th November 2022, organized by Botany discipline, School of Biological Sciences and Biotechnology, Goa University, Goa. (Poster Presentation).
5. International Conference on Natural Science and Green Technologies For Sustainable Development (NTSD- 2022), held from 30th November- 2nd December 2022, organized by National Environmental Science Academy, New Delhi & Goa University, Goa. (Oral Presentation).
6. National Conference on Recent Adavances in Plant Sciences & Biotechnology, held during 26th & 27th October, 2023 organised by the School of Biological Sciences and Biotechnology (Botany), Goa University, Goa. (Best Poster Presentation).

Workshops attended:

1. E-Workshop on MS Office Tools and Resources for Thesis Writing held on, December 24th 2020, organized by Swayam.
2. One week online training program on Statistical Data Analysis using "R" Software during December 21st-27th , 2021, Organized by Science Tech Institute , Lucknow, India.
3. Two days Online skill development program "Data interpretation of GCMS & LCMS" on 12th and 13th of March, 2022, organized by CytoGene Research & Development, Lucknow.
4. One day workshop on "Writing Manuscripts & Publishing in Quality Research Journals", held on 27th August 2022.
5. International workshop on "Basic Statistical Analysis an its Interpretation using SPSS" from September 24th to 26th 2022, Organized by Global Institute of Statistical Solutions (GISS).
6. "Application of Real Time PCR in Disease Diagnosis and Research" held on 18th October 2022.organized by School of Biological Sciences and Biotechnology, Goa University in association with Molbio Diagnostics Private Limited
7. One day workshop on "LaTeX Scientific Editing with hands-on practice", held on 15th September 2023, organised by Goa University.
8. International workshop and hands-on training on 'Chloroplast Bioenergetics' held on January 4th to 10th, 2024, Organized by the Department of Plant Sciences School of Life Science, Universitty of Hyderabad.

Research Publications:

1. **Korgaonker, S**., & Bhandari, R. (2021). Response of *Oryza sativa* L. To the interactive effect of drought and salicylic acid. Journal of Stress Physiology & Biochemistry, 17(3), 95-104.
2. **Korgaonker, S.**, & Bhandari, R. (2023). Alleviation of Drought Stress Effects in Two Rice (*Oryza sativa* L.) Cultivars by Foliar Application of Salicylic Acid. Russian Journal of Plant Physiology, 70(6), 131. (IF 1.6)
3. **Korgaonkar, S.,** & Bhandari, R. (2023). Drought Stress in Plants: Effects and Tolerance. Journal of Stress Physiology & Biochemistry, 19(1), 5-17 (Review).

Communicated:

1. Anatomical And Physiological Responses Of Rice Cultivars To Salicylic Acid Under Drought Stress (Rice Science)

National Biodiversity Authority & Department of Science and Technology (DST-SERB) sponsored

NATIONAL CONFERENCE ON VISTAS IN BIODIVERSITY, BIOLOGY, BIOTECHNOLOGY AND NANOTECHNOLOGY OF ALGAE (VBBBNA2018)

(20 - 22 SEPTEMBER 2018)

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for short oral talk on the topic, "Salicylic acid enhances the growth of rice (Oryza sativa L.) plants under drought stress conditions" during the Young Scientist Session of the Conference

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National Conference on Recent Trends in Plant Sciences & Biotechnology

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This is to certify that Dr./Mr/Ms ✓ Shravani Kargaonker has attended/ ~~Delivered Lead Lecture/~~ Presented a paper (~~Oral~~/Poster ✓) entitled "The mitigation effect of salicylic acid on physiological and biochemical characteristics of rice (Oryza sativa L.) under drought stress" in the National Conference on Recent Trends in Plant Sciences & Biotechnology, held during 3rd & 4th November 2022, at the School of Biological Sciences and Biotechnology (Botany), Goa University, Goa

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has participated in the International Conference on

"Environmental Sustainability & Biotechnology: Opportunities & Challenges"

held at Goa, India from 16-19 November 2022 & delivered Oral ☐ /Poster ☑ presentation on

SALICYLIC ACID: A PHENOLIC ANTIOXIDANT TO ALLEVIATE DROUGHT STRESS IN RICE PLANTS

Prof. Purnakala V. Samant
Principal,G.C.A.S.C.
Khandola, Goa

Prof. Vikas J. Pissurlekar
Principal, P.E.S's R.S.N. College
Farmagudi, Goa

Prof. Naveen Kumar Arora
Dean, S.E.E.S., B.B.A.U.,Lucknow
General Secretary, S.E.S.

International Conference on

NATURAL SCIENCE AND GREEN TECHNOLOGIES FOR SUSTAINABLE DEVELOPMENT (NTSD-2022)

30th November - 2nd December, 2022

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Goa University, Goa

Certificate of Participation

This is to certify that

SHRAVANI NARAYAN KORGAONKAR

actively participated as delegate and presented a paper (**Oral**) entitled Mitigating effects of drought by foliar application of salicylic acid on rice cultivars: a comparative study

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This is to certify that Dr./Mr/Ms. Shravani Korgaonkar

has participated in an ~~oral~~/**poster** entitled (1st PLACE)

"Response of rice (Oryza sativa L.) to salicylic acid and water deficit"

at the **National Conference on Recent Advances in Plant Sciences & Biotechnology,** held during **26th & 27th October 2023,**

at the School of Biological Sciences & Biotechnology (Botany),

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Organising Secretary

Prof. S. Krishnan
Convenor

Certificate

OF PARTICIPATION

awarded to

SHRAVANI NARAYAN KORGAONKAR

GOA UNIVERSITY

for participating in an

E workshop on MS Office Tools and Resources for Thesis Writing

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December 24th, 2020 at 11:00 AM to 02:00 PM IST

Ajay Semalty

Dr. Ajay Semalty

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PRESENTED TO PROF./DR./MRS./MISS./MR.

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Research Scholar, Department of Botany, Goa University, Taleigão (Goa)

has actively participated in the two days
Online Skill Development Program
"DATA INTERPRETATION OF GCMS & LCMS"
on 12 - 13 of March, 2022
organized by CytoGene Research & Development, Lucknow.

Dr. Umashankar
Director R&D
CytoGene Research & Development
Lucknow

Er. Sujeet Kr. Singh
Director
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Azadi Ka Amrit Mahotsav

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ON
WRITING MANUSCRIPTS & PUBLISHING IN QUALITY RESEARCH JOURNALS

This is to certify that Ms. *Shravani N Korgaonker,* from *Goa University* has participated in the One Day Workshop on "Writing Manuscripts & Publishing in Quality Research Journals" *(Offline)* held on 27.08.2022.

Prof. Sunder N. Dhuri
Director, DIQA & Convener

Date: 27.08.2022

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Conference Chair
University of Lucknow
Lucknow, UP, India

Dr. Susheel Kumar Singh
Convenor
Science Tech Institute
Lucknow, UP, India

Dr. Dilshad Ahmad Ansari
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AIDC, Lucknow UP, India

Mrs. Shweta Singh
Chairman
MKSES, Lucknow
UP, India

GLOBAL INSTITUTE OF STATISTICAL SOLUTIONS (GISS)

CERTIFICATE OF PARTICIPATION

This is to Certify that SHRAVANI N KORGAONKER , Research scholar Botany , Goa university

has participated in the International online workshop on **"General Paper on Teaching and Research Aptitude (UGC NET: Paper 1)** from November 10th to 14th 2022.

Dr.K.NARAYANAN
Resource person

Dr.E.KUMAR
Organizing Secretary

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This is to certify that

~~Dr./Mr/Mrs.~~Ms. Shravani Kougoankar ....from

School of Biological Sciences and Biotechnology, Goa university

has attended one day workshop on "Application of Real Time PCR in Disease Diagnosis and Research" organized by School of Biological Sciences and Biotechnology (SBSB), Goa University in association with Molbio Diagnostics Private Limited on 18th October 2022. The workshop included lectures on Real Time PCR & hands on training.

Dr. Milind Naik	**Prof. Sandeep Garg**	**Prof. Savita Kerkar**	**Dr. Shivraj Bhoite**
Organizing Secretary SBSB	Convenor SBSB	Dean SBSB	Manager Application Services
Goa University	Goa University	Goa University	Molbio Diagnostics Private Limited

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DIRECTORATE OF INTERNAL QUALITY ASSURANCE (DIQA)

CERTIFICATE OF PARTICIPATION

This is to certify that

Ms. SHRAVANI N. KORGAONKER

participated in a one-day workshop titled "LaTeX Scientific Editing with hands-on practice" on 15th September 2023 at Goa University.

DR. NARAYAN T. VETREKAR
Assistant Professor
School of Physical and Applied Sciences

PROF. SUNDER N. DHURI
Director
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DR. APARNA LOLAYEKAR
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PROF. RAJENDRA S. GAD
Dean
Manohar Parrikar School of Law, Governance and Public Policy

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ORIGINAL ARTICLE

OPEN ACCESS

Response of *Oryza sativa* L. to the Interactive Effect of Drought and Salicylic Acid

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Plant growth and rice productivity are negatively affected by the alarming rise of abiotic and biotic stress factors. Drought stress is a significant factor that directly affects numerous physiological, biochemical and molecular responses in plants. The exogenous application of plant growth regulators such as salicylic acid is a crucial route to alleviate the detrimental effects of water scarcity and plant efficacy. The research was conducted to evaluate the impact of foliar-applied salicylic acid of 0.25 mM concentration on morphological, physiological and biochemical alterations in rice plants under two levels of polyethylene glycol 6000 induced drought stress (8%, 16%). Drought stress increased lipid peroxidation, ion leakage, proline accumulation but decreased the leaf relative water content, root and shoot biomass. In contrast, foliar application of 0.25 mM SA mitigated PEG-induced drought stress by enhancing the LRWC, proline accumulation, decreasing the lipid peroxidation and electrolyte leakage. It was observed that SA treatment led to substantial improvement in plant biomass at both the drought stress levels, thereby increasing the plant acclimation under water deficit conditions.

Key words: Drought stress, Exogenous salicylic acid, lipid peroxidation, Proline, Rice

Crop plants are continuously exposed to different abiotic stress conditions both in natural and agricultural conditions, affecting their growth and development, thus enabling the plant to develop unique and sophisticated tolerance mechanisms to cope with the environmental constraints. The short-term physiological response or long-term structural, morphological, and physiological modifications help the plant minimize stress. Drought stress is one of the most common and damaging environmental stress factors, decreasing crop productivity compared to other abiotic stress (Lambers and Oliveira, 2019). According to OECD (Organization for Economic Cooperation and Development) estimates, the water demand will be more than double by 2050 and water systems have to provide water for around 9.6 billion people in 2050 (Anon, 2012). The rising population, drastic climatic change, and farming development, where water demand has amplified several folds, will make water scarce in several parts of the world (Mishra and Singh, 2010). The decrease in soil water availability and changing climatic conditions leads to continuous water loss by transpiration or evaporation, causing drought stress (Jaleel *et al*., 2008).

Plants on exposure to water stress cause reduced yield and shift themselves in survival mode, ultimately reduce its yield. Therefore, it is essential to control the stress-induced damages to the plant's average growth and development. Many techniques have been used to induce drought mitigation, such as gene transformation and the cultivation of drought-tolerant varieties. However, such methods need specific environmental and edaphic conditions according to individual plants, high financing, technology as it is time-consuming. Thus, exogenous treatment with plant growth regulator (PGR) can be an economical, manageable and uncomplicated substitute as by using the defined application of PGR, the plant will upturn the water use efficiency and acclimatize to drought stress.

Salicylic acid (SA) is a common phenolic compound synthesized by the plant and has diverse physiological roles in photosynthesis, ion uptake and transport, stomatal movement and membrane permeability (Hayat *et al*., 2008). An individual plant's response to the exogenous application of SA depends entirely on the plant's developmental stage, mode of application, SA concentration, and its endogenous SA level in the plant (Horvath *et al*., 2007). Singh and Usha (2003) reported that SA's application on drought-stressed wheat seedlings showed higher moisture content, dry matter accumulation, carboxylase activity of RuBisCo, SOD, chlorophyll content compared to control.

Rice (*Oryza sativa* L.) is one of the important cereal crops and the staple food for approximately half the world's population (Carriger and Vallee, 2007) and requires water throughout its life cycle compared to other crops. Hence, water stress is a vital threat to the loss of plant productivity in the agricultural system worldwide. The drought stress affects rice at morphological, physiological, and biochemical levels, thereby affecting its yield. The improvement in drought stress tolerance of major food crops is of utmost importance, and hence detailed understanding of various processes that regulate the yield of rice grown under drought stress induced by exogenous application of salicylic acid is a prerequisite. Thus, this experiment was carried out to understand the interactive effect of exogenously applied salicylic acid and drought stress on morphological, physiological and biochemical responses in rice plants from the application point of view.

MATERIALS AND METHODS

Plant material and growth conditions

Rice seeds (*Oryza sativa* L.cv. Jaya) were obtained from ICAR - Central Coastal Agricultural Research Institute, Goa. Seeds were surface sterilized and allowed to germinate in plastic pots, 10 cm in diameter containing vermiculite at 25℃±1 at 16/8 h light/dark periodicity with the light intensity of 200 µmol $m^{-2}s^{-1}$ and the relative humidity was maintained at 60% -70%.

Induction of drought stress

Rice seeds were subjected to 0% (control), 8% and 16% of water potential using PEG 6000 dissolved in Hoagland's solution (pH 6.4) to fulfil nutrient requirements.

Treatment of plants with salicylic acid

Plants were subjected to salicylic acid with 0.25 mM

concentration (pH 7), foliar sprayed on ten-day-old plants for three consecutive days. The concentrations of SA were selected based on earlier findings.

Relative Water Content (RWC) measurement

The RWC is an important index to evaluate plants' physiological water content, was carried out by the standard method (Barrs and Weatherley, 1962). Fresh weight (FW) of randomly chosen leaves was measured and allowed to immerse in distilled water at room temperature in closed petri plates for 24 h to obtain the turgid weight (TW). Tissues were then placed in a pre-heated oven at 80℃ for 48 h to measure the dry weight (DW). RWC was further calculated using the formula:

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

Biomass analysis

The fresh and dry weight of shoots and roots were recorded by randomly selecting ten plantlets from respective concentrations. For obtaining the dry weight, the samples were oven-dried at 70℃ for 72 h (Chen *et al*., 2014).

Estimation of electrolyte leakage

Total ion leakage from fully expanded leaves was determined using Shi *et al*., (2006). Twenty leaf discs were placed in 50 ml glass vials, rinsed with distilled water to eliminate the electrolytes released during leaf disc excision. Vials then filled with 20 ml of distilled water were allowed to stand in the dark for 24 h at room temperature. The electrical conductivity (EC1) of the solution was determined at the end of the incubation period. Vials were then heated in a temperature-controlled water bath at 95°C for 30 min, cooled at room temperature, and measured electrical conductivity (EC2) using Waterproof PCTestr 35 pH/Conductivity. Electrolyte leakage was calculated as the percentage of (EC1/EC2) x 100.

Determination of lipid peroxidation

The extent of lipid peroxidation was assessed by quantifying the malondialdehyde (MDA) content, the end product of lipid peroxidation, according to Sharma, (2002). Leaf samples (0.5g) was homogenized with 5 ml of 1% trichloroacetic acid (TCA) in an ice bath. The homogenate was centrifuged at 12000 g for 10 min, and the supernatant was used for lipid peroxidation analysis. To the 1 ml aliquot of supernatant, 2.5 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA and 2.5 ml of incubation buffer was added. The mixture was incubated for 30 min at 95℃. MDA content was detected spectrophotometrically at 532 nm and altered for nonspecific turbidity at 600 nm. The extinction coefficient of 155 mM^{-1} cm^{-1} was used for MDA quantification.

Determination of Proline content

The free proline content in leaf tissue was determined by Bates *et al*., (1973). 0.5 g of leaf tissue was homogenized in 5 ml of 3% (v/v) aqueous sulfosalicylic acid followed by centrifugation at 5000 rpm for 5 min. 1 ml of supernatant was mixed with 1 ml of freshly prepared acid ninhydrin and glacial acetic acid. The mixture was heated at 90°C for an hour; the developed colour was extracted in 5 ml of toluene with vigorous shaking. The tubes were set aside to stand for 10 min to allow separation of toluene and aqueous phase. The absorbance of the toluene phase was measured spectrophotometrically at 520 nm. A standard curve prepared with analytical grade proline was used for the final calculations.

Data analysis

Each experiment was repeated thrice and the data presented are with a mean of n=3. The data analysis was carried out using standard error ± in MS Excel.

RESULTS

Effect of drought and SA on plant biomass:

Our results in Fig. 1 and Table 1 indicate that increasing drought stress levels from 8% to 16% progressively decreased plant biomass [above (57% and 74%) and below (4% and 17%) -ground] compared with the control plants. Exogenous application of SA increased the drought-affected growth in 8% drought-stressed rice by 14% and 23% in above and below-ground biomass as compared to SA untreated 8% rice plants, whereas as 16% drought-stressed rice plants showed an increase in above-ground biomass by 135% as compared to SA untreated 16% stressed plants.

Effect of drought and SA on Relative Water Content in rice:

We observed that drought stress reduced the leaf RWC as the PEG level increased (8% by 89%; 16% by

79%) (Fig. 2 and Table 1). The well-watered or drought-stressed seedlings showed higher RWC with SA treatment than without SA. The application of 0.25 mM SA caused an increase in RWC (14%) in stressed seedlings, which seems to be more evident at the highest drought stress level of 16% compared to 16% untreated plants.

Effect of drought and SA on membrane integrity:

As drought stress boosts free radicals levels in plants, the damage caused to the membrane was examined by monitoring total ion leakage (Fig. 3 and Table 1). Our findings show that electrolyte leakage increased from 25% to 46% as the stress level elevated from 8% to 16 %. The exogenous SA treatment of 0.25 mM led to electrolyte leakage from 26% to 39%, respectively from 8% to 16% in drought-stressed rice seedlings compared to well-watered plants.

Effect of drought and SA on lipid peroxidation:

Our study indicates the level of membrane damage as the product of lipid peroxidation caused by the increased level of free radicals due to induced drought stress (Fig. 4 and Table 1). Lipid peroxidation increased as the stress level elevated, i.e. in 8% by 56% and in 16% by 350%. The exogenous SA treatment of 0.25 mM led to downregulation of MDA content by 25% and 15% in 8% and 16% drought-stressed rice seedlings compared to well-watered rice seedlings.

Effect of drought and SA on proline content:

Our findings indicate that increased proline content is associated with increasing drought stress levels (Fig. 5 and Table 1). The highest level of proline content in plants is directly related to the severity of drought stress to which plants are exposed, i.e. 16% of PEG-induced drought stress in combination with 0.25 mM SA (1.2 folds increase) and the minimum amount of proline obtained in plants exposed to well-watered conditions without SA. The application of SA improved the resistance of plants to water stress. This increased defence was predominant under 0.25 mM SA in 8% (1.6 folds increase).

Table 1: The effect of exogenous salicylic acid on rice (*Oryza sativa* L.cv. Jaya) under PEG 6000 induced drought stress

TREATMENT	Biomass		RWC	EL	MDA	Proline
	AG	BG				
CONTROL	0.519±0.06	0.434±0.03	94±0.04	13.8±0.06	0.0021±0.0002	0.552±0.002
CONTROL + 0.25 SA	0.551±0.03	0.449±0.04	97±0.08	12.8±0.07	0.0018±0.0001	0.460±0.006
8% PEG	0.225±0.03	0.418±0.06	89±0.20	14.3±0.04	0.0032±0.0002	2.335±0.010
8% + 0.25 SA	0.256±0.02	0.516±0.03	93±0.25	13.2±0.20	0.0025±0.0001	3.811±0.111
16% PEG	0.134±0.01	0.360±0.03	79±0.36	17.8±0.06	0.0093±0.0006	12.160±0.081
16% + 0.25 SA	0.152±0.01	0.324±0.05	90±0.01	16.6±0.03	0.0080±0.0006	14.573±0.088

Data are given as mean, n=3. Error bars ± indicate standard error (SE). ***AG****(Above ground);* ***BG*** *(Below ground);* ***RWC*** *(Relative Water Content);* ***EL*** *(Electrolyte Leakage);* ***MDA****(Malondialdehyde);*

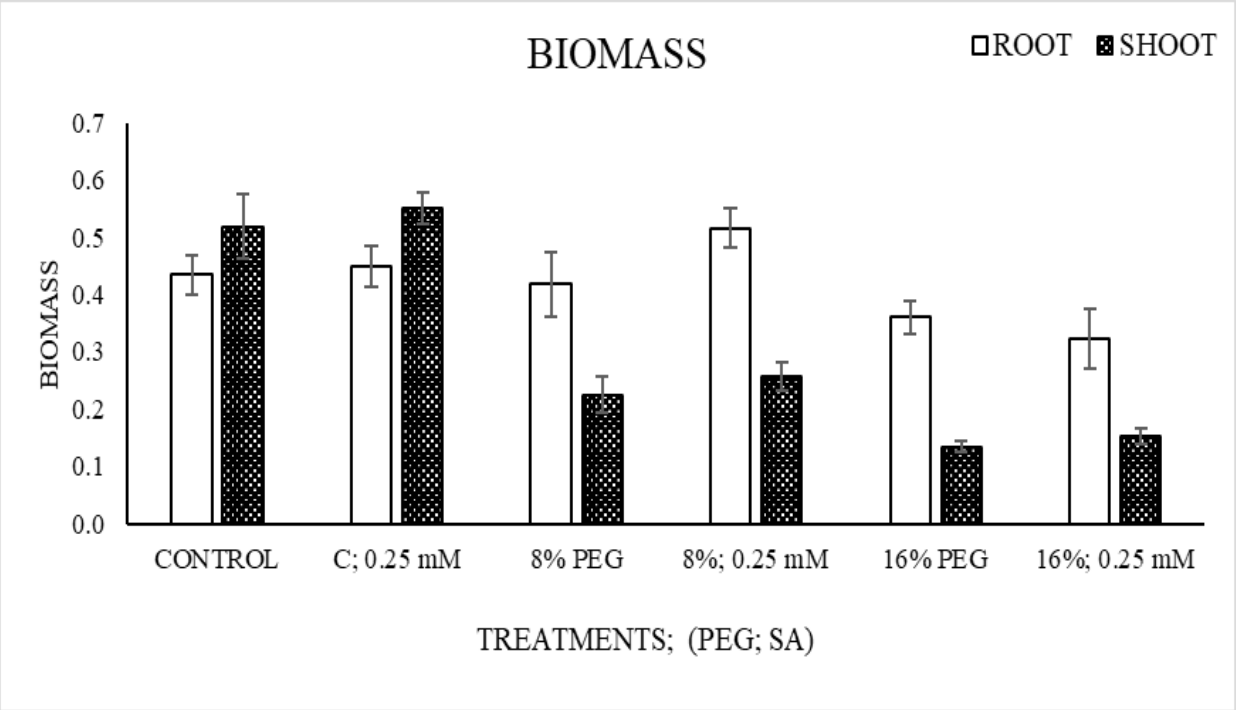

Figure1. Effect of PEG induced drought and SA on plant biomass in rice (*Oryza sativa* L.). Data are given as mean, n=3. Error bars ± indicate standard error (SE).

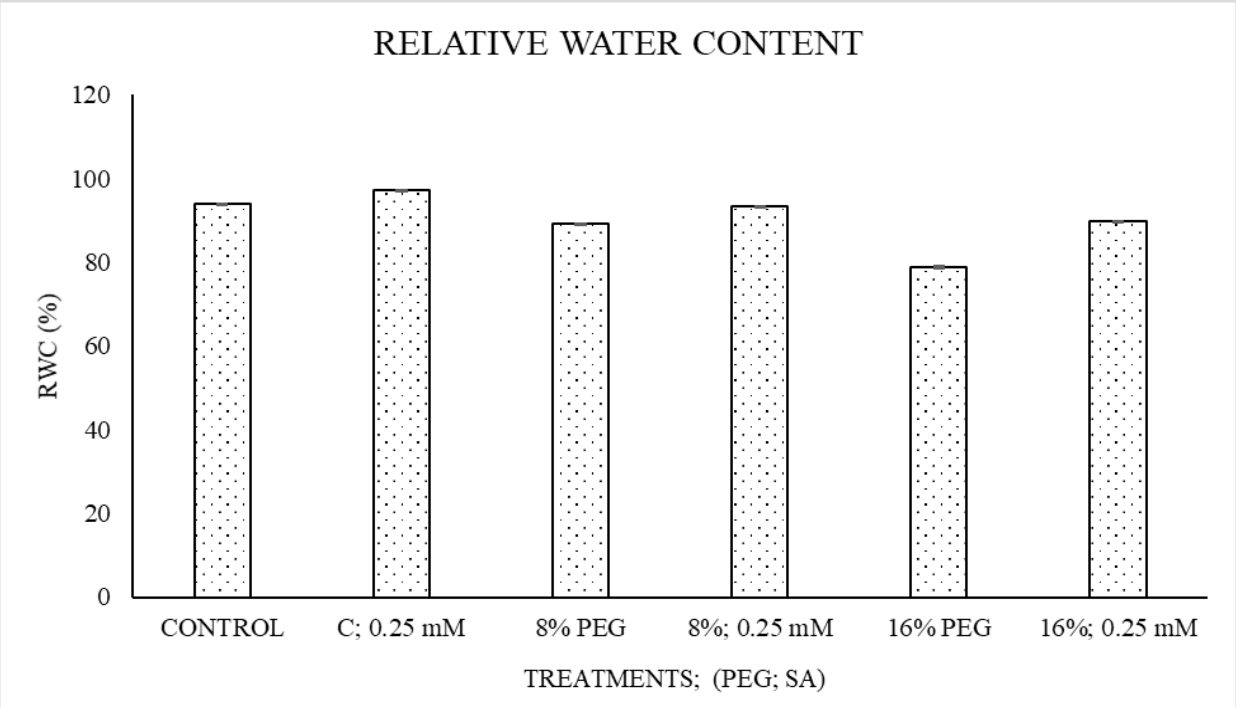

Figure 2. Effect of PEG induced drought and SA on Relative water content (RWC) in rice (*Oryza sativa* L.). Data are given as mean, n=3. Error bars ± indicate standard error (SE).

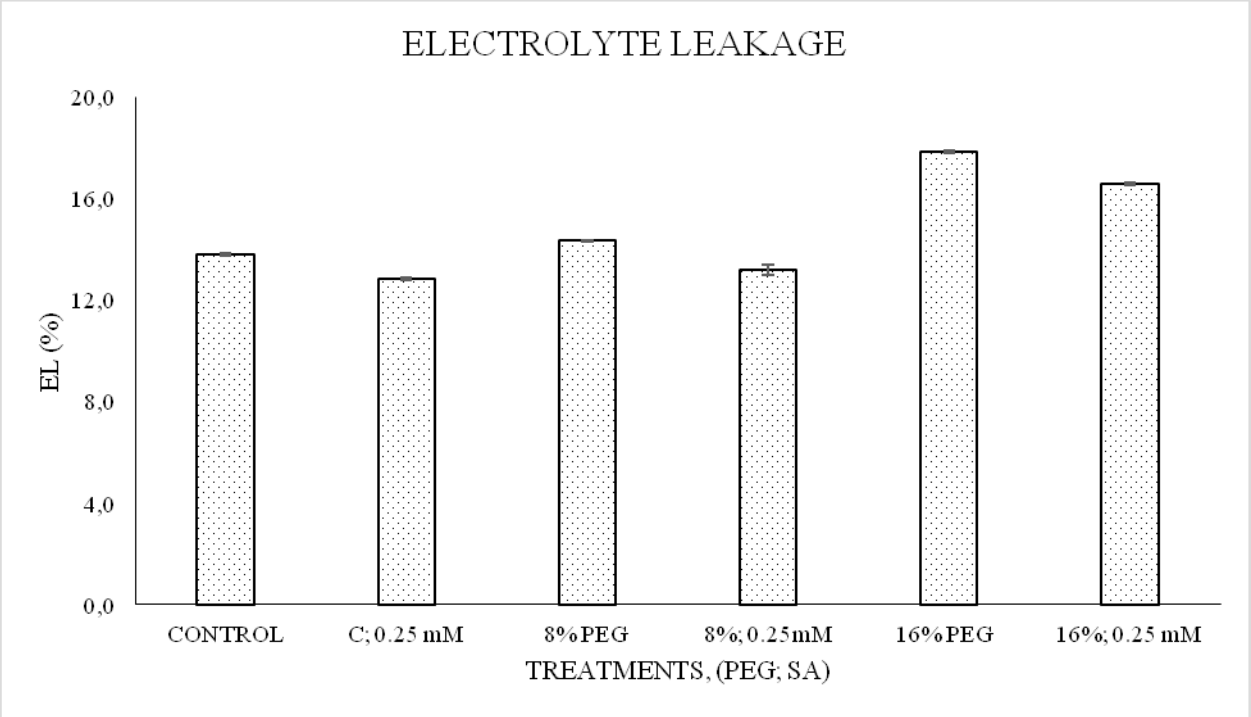

Figure 3. Effect of PEG induced drought and SA on electrolyte leakage in rice (*Oryza sativa* L.). Data are given as mean, n=3. Error bars ± indicate standard error (SE).

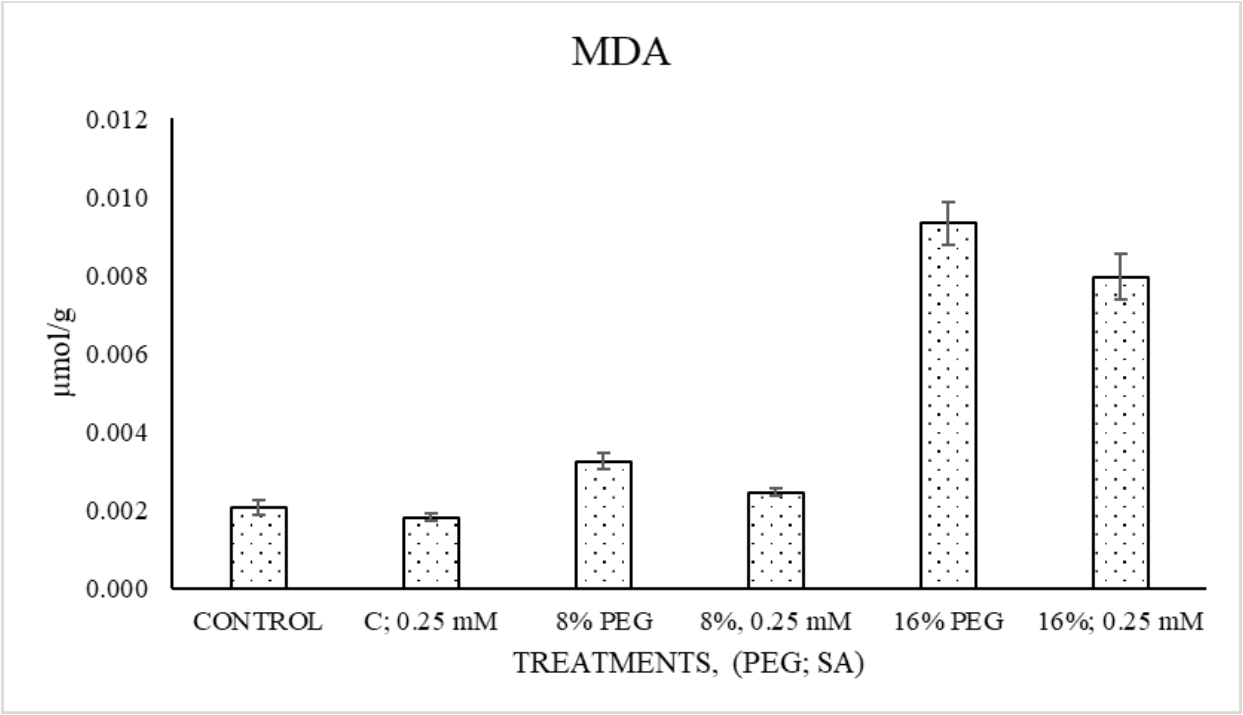

Figure 4. Effect of PEG induced drought and SA on lipid peroxidation in rice (*Oryza sativa* L.). Data are given as mean, n=3. Error bars ± indicate standard error (SE).

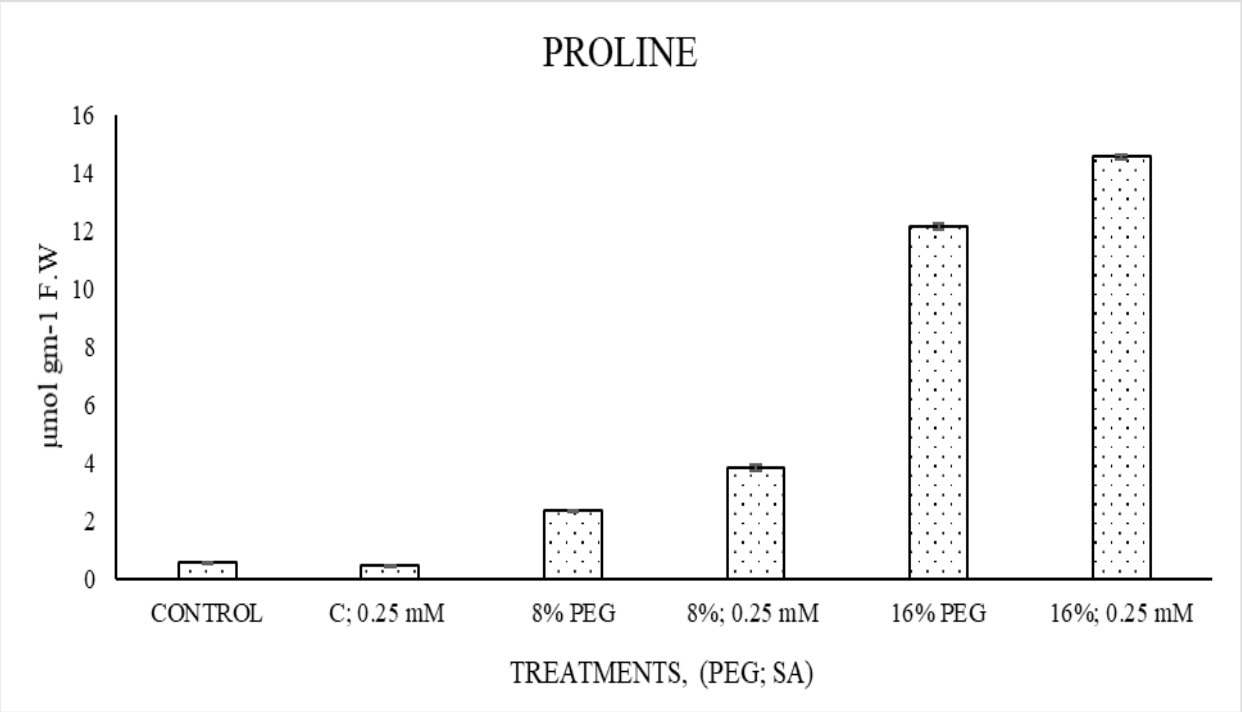

Figure 6. Effect of PEG induced drought and SA on proline content in rice (*Oryza sativa* L.). Data are given as mean, n=3. Error bars ± indicate standard error (SE).

DISCUSSION

Drought is a crucial environmental factor that affects plants' growth and physiological characteristics (Xiangwen *et al.,* 2009). According to Bray (1997), plant response to drought stress has been reported is based on various factors such as period, the severity of water stress, age, individual development stages, species and genotype (Kabiri *et al*., 2014).

Our study on the foliar application of SA on rice (*Oryza sativa* L.) seedlings alleviated the effects of drought stress. PEG induced drought stress caused a drastic reduction in growth and biomass (FW, DW) of root and shoot in rice plants, while the degree of reduction was more in 16% as compared to 8% and well-watered plants, however, recovered with exogenous SA application (Fig.1). This drastic interference in growth and biomass is due to the reduction in cell division and elongation. Similar findings were documented by Hu and Schmidhalter (2005). According to Loutfy *et al*., (2012), plant treatment with SA may confer resistance to drought since SA is attributed to stimulating growth. Boyer (1970) reported that the growth and expansion of young leaves' are sensitive to water deficit rather than photosynthesis. Therefore we can deduce that water-sensitive activities determine the growth rate of the plant.

LRWC is a vital indicator to verify the plant's water status, reflecting on the absolute amount of water, which the plant requires to carry out its metabolic activity influencing growth (Sinclair and Ludlow, 1986). In the present study, we observed that drought stress down-regulated LRWC in rice plants and exogenous SA treatment improved the drought stress effect on LRWC (Fig. 2). Here we could analyze that rice leaves' water status was subjected to 8% and 16% stress and SA, which depends on the respective shoot and root biomasses. This can also suggest that rice plants with the greater biomass of root or shoot can maintain the higher water content in the leaf and be more acclimatized to drought.

This study observed that as drought stress increased from 8% to16%, it enhanced free radicals levels in plants, causing damaging effects on the membranes, which was investigated by monitoring electrolyte

leakage and MDA content (Fig. 3 and 4). Electrolyte leakage facilitates the assessment of cell membrane injury caused to plants when exposed to drought stress. Sairam *et al*., (2001) have reported that drought-stressed condition is considered an integral part of drought tolerance mechanism, thereby maintaining cell membrane integrity. Our study also reported an increase in electrolyte leakage. However, SA treatments lowered the leakage in 8% and 16% drought-stressed rice plants (Fig. 3). According to Jaleel *et al*., (2007), a reduced electrolyte leakage level indicates the leaf membrane's stability. It was observed by Yusuf *et al*., (2008) that SA application enhanced the antioxidant system necessary in reducing oxidative damage and ion leakage from the membrane. Thus, SA upsurges the accumulated Ca^{+2}, thereby maintaining the membrane integrity (Khan *et al*., 2010). Our findings suggest that exogenously applied SA may positively affect plant growth and development under stress conditions.

The amount of MDA content is used as a marker for lipid peroxidation in oxidative stress studies mainly focused on plant responses to abiotic and biotic stresses (Al-whaibi *et al*., 2012). Our study also showed that the level of MDA content in rice plants increased as drought stress increased (Fig. 4). Our observations were on par with previous studies in barley subjected to salt and water deficit reported by Fayez and Bazaid, (2014). Bor *et al*., (2003) also reported that salt stress increased lipid peroxidation in beet species. The application of 0.25 mM SA remarkably decreased MDA content in both drought stress conditions, i.e., 8% showed a decrease by 25% and 16% by 15%. Similar results were obtained by Chen *et al*., (2014), who showed that SA treatment might decrease the damaging effects of drought stress on the membrane.

Xue *et al*., (2008) observed that accumulation of proline in plants is one of the firm strategies for osmotic adjustment for coping with water shortage. Under unfavourable conditions, proline acts as a compatible solute for osmotic adjustment and protects the macromolecular structures and cell membrane. We have observed an upregulation of proline content in drought-stressed rice plants (8% and 16%), and its value increased by applying SA, which helps ameliorate the detrimental effects of unfavourable conditions (Fig. 5). Similar elevation in proline content was observed by Tasgin *et al*., (2006) in bean, wheat, and tomato under drought stress condition. Our observations were in conformity with studies reported by (Raskin, 1992; Shah, 2003), suggesting that SA application alleviates detrimental effects of drought stress by accumulating proline. Nazar et al., (2015) also reported the amelioration of drought-induced oxidative stress due to the accumulation of proline in mustard.

CONCLUSION

Our study has proven that on the application of SA exogenously, plants can mitigate the damaging effects of drought stress in rice plants. SA of 0.25 mM concentration alleviated the adverse effects of drought stress on rice. According to the results obtained, we can suggest that SA may, in the future, have applications in improving plant growth and yield in drought-prone areas.

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CONFLICTS OF INTEREST

All authors have declared that they do not have any conflict of interest for publishing this research.

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REVIEW

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Drought Stress in Plants: Effects and Tolerance

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Water is the catalyst of life and plays a profound role in plant physiological processes ranging from photosynthesis to intermolecular interactions through a hydrophobic bond. Because of the alterations due to changing environmental conditions, the plants are continuously exposed to a lack of optimum water availability, leading to impaired growth and disturbance in water transport and uptake. Drought is a prominent environmental factor that triggers various plant processes from morphological, physiological, biochemical, and molecular. Plants portray an array of drought tolerance mechanisms; these responses differ based on the type of plant species and may involve the functions of various stress genes. Reduction in plant growth and productivity due to stomatal closure affects photosynthetic efficiency, altering membrane integrity and several enzymes involved in adenosine triphosphate synthesis. Plants exhibit a range of drought tolerance mechanisms and undergo several phenological, morphological, physiological, biochemical, and molecular adaptations at the cellular, subcellular and whole plant levels. Also, drought stress induces the production of reactive oxygen species at the cellular level and is strongly protected by the increase in the enzymatic and non-enzymatic antioxidative system. This chapter/ review provides a glimpse of the effects and tolerance strategies adapted by the plant under drought stress.

Key words: Abiotic stress, drought, phytohormone, reactive oxygen species, antioxidants

By 2050, it is hypothesized that the world's population will overdo 9.7 billion, and over 65% will solely rely on agriculture (Castañeda *et al*., 2016). Consequently, food supply and also nation's financial system will be influenced mainly by agronomy. On the other hand, agronomic practices combat hurdles such as inadequate irrigation systems, scarcity of good trait seeds, the excessive course of chemical pesticides and fertilizers leading to unsuitable soils, and soil erosions (Dev 2012). Besides the above constraints, plants both in the natural and agricultural environment are exposed to a wide array of biotic (insects, bacteria, viruses, fungi) and abiotic (temperature, water, salinity, chilling, freezing) stresses, which will affect their growth and development, thereby hampering crop productivity (Seki *et al*., 2003), in extreme cases, leads to the death of the plant. Due to changing climatic conditions, water scarcity is one of the most severe threats to food security for the rapidly increasing population (Farooq *et al*., 2009a). As a result, a 30% downfall in global crop production is estimated by 2025 compared to current productivity as per World economic forum Q2 (Hasanuzzaman *et al*., 2013).

From an agricultural point of view, alterations in rainfall, light and temperature are the factors to induce water deficit in plants. Also, a shortfall in meteorological drought coupled with an increased evapotranspiration rate leads to a lack of optimum soil moisture required for plant's average growth and development, leading to agricultural drought (Mishra and Cherkauer 2010 Manivannan *et al*., 2008). As a result, plant growth, yield, water relations, membrane integrity, pigment composition, and photosynthetic efficiency is drastically affected by drought (Praba *et al*., 2009). The water content's downturn characterizes the leaf water potential, causing stomatal closure, thereby decreasing cell growth and elongation (Anjum *et al*., 2011a). Lowered water content affects the entire physiological and biochemical mechanisms such as ion uptake and its translocation, photosynthesis, respiration, and nutrient metabolism, thus reducing plant growth (Farooq *et al*., 2009a) and causing the death of the plant (Jaleel *et al*., 2008). Hence, the production of drought-tolerant crop plants may help meet the food demands. On the other hand, several drought-tolerant traits are used to assess the plant tolerance to drought stress, such as root-leaf traits, the ability for osmotic adjustment, water potential, synthesis of abscisic acid and cell membrane stability (Ha *et al*., 2012). In addition, several molecular mechanisms, including signal transduction, also help the plant in response to drought stress (Nishiyama *et al*., 2013; Osakabe *et al*., 2014). It is of utmost significance to comprehend the effects of drought on plant's morphological, anatomical, physiological and biochemical adaptations to cope with changing climatic challenges. This review is an overview of plant responses and tolerance mechanisms to drought stress and suggests some management strategies and possibilities to cope with drought effects, especially regarding field crops.

DAMAGING EFFECTS OF DROUGHT STRESS

Plants being sessile need to respond and adapt to various unfavourable environmental conditions. Under normal conditions, there is an irreversible increase in size, weight or volume, which comprises cell division, elongation and differentiation, causing plant growth and establishing an optimum crop stand, which is crucial for producing maximum yield (Farooq *et al*., 2012). Global climatic change is the leading cause of the increase in temperatures and atmospheric CO2 levels, increasing the rate of soil water evaporation, thereby altering the rainfall patterns, ultimately triggering drought stress worldwide (Dai 2011; Mishra and Singh 2011), exposing the plants to water stress. The severity of the stress depends entirely on the intensity, duration of stress, onset time, soil physicochemical conditions and the degree of plant susceptibility. The imbalance in water absorption and water loss rate is mainly due to lower soil water potential than plant roots, primarily due to the atmospheric conditions causing continuous water deficit by transpiration or evaporation (Mafakheri *et al*., 2010). Hence, meteorological drought is followed by agricultural drought (Dai 2011). However, in specific conditions, there is ample soil water content, but various edaphic factors, such as low soil temperatures, salinity, and flooding, decrease the water uptake by roots. Subsequently, inducing water stress in plants defined as

pseudo-drought or physiological drought wherein altered atmospheric conditions are not the decisive factors (Arbona *et al*., 2013; Lisar *et al*., 2012).

Drought, a multidimensional stress factor, affects plants from morphological, physiological, and biochemical levels and is apparent at all plant growth phenological phases (Korgaonker and Bhandari 2021; Anjum *et al*., 2011a). Under severe water deficit, plants exhibit a loss in leaf turgor, making it flaccid, leading to chlorosis and premature senescence (Akhtar and Nazir 2013; Sapeta *et al*., 2013). A few uncommon water deficit symptoms include irregular stunted growth, necrosis, cracks in twigs or bark, thinning of shrub and tree canopy, and extreme situations causing plant death (Arbona *et al*., 2013; Sapeta *et al*., 2013). During the early drought, germination and establishment are affected mainly due to reduced water uptake during the imbibition phase, seed germination, eased energy supply, distorted enzymatic activities and downregulated energy supply from photosynthesis (Okcu *et al*., 2005; Taiz and Zeiger 2010; Bhargava and Sawant 2013; Ding *et al*., 2013). Plant growth is reduced when soil water availability is restrained, wherein shoot development is more stunted than root growth (Anithakumari *et al*., 2012). Avramova *et al*. (2015) observed that water deficit reduces leaf expansion and photosynthesis due to impaired cell mitosis, elongation, proliferation, and differentiation (Potopová *et al*., 2016).

The chief response to drought stress is stomatal closure that disrupts leaf gas exchange, phloem loading, assimilate translocation and dry matter partitioning causing a severe deterioration in plant traits (Farooq *et al*., 2009b; Akram 2011). The drought stress also reduced leaf area due to loss of turgor, cut short the number of leaves, and suppressed leaf expansion and tillering (Farooq *et al*., 2010; Kramer and Boyer 1995; Nooden, 1988). All these constituents lowered the accumulation of dry matter and grain yield.

Drought is also associated to alterations in several aspects of morphological and anatomical features, such as the leaf anatomy, crop phenology and its ultrastructure (Hirt and Shinozaki 2003; Rao *et al*., 2006). It is also reported that the early plant development from the vegetative to reproductive phase is suppressed due to limited water supply (Desclaux and Roumet 1996), leading to a substantial decrease in economic yield in the flowering stage (Hussain *et al*., 2008). An elaborative account of various effects of drought stress concerning their responses and adaptational aspects is conferred below.

ADAPTATIONS: PLANT RESPONSES TO DROUGHT STRESS

Plants undergo several phenological, morphological, physiological, biochemical, and molecular adaptations to cope with stress at the cellular, subcellular, and whole plant levels.

MORPHOLOGICAL RESPONSE

Plant resistance to stress conditions is divided into two primary strategies: stress avoidance and stress tolerance (Bhargava and Sawant 2013; Khan *et al*., 2011; Nezhadahmadi *et al*., 2013). These plant responses to withstand drought stress can range from molecular to entire plant level by escape, avoidance and tolerance, which is further explained:

Drought Escape: The drought escape shortens the life cycle or grows seasonally and allows the plants to reproduce in the presence of water (Akhtar and Nazir 2013; Bray 2007). A plant's life cycle mainly depends on the individual's genotype and environmental conditions. The synchrony of the plant phenological growth and development with soil moisture availability helps the plant escape drought conditions. A shortened life cycle can lead to drought escape as the plant matures and flowers early, although the yield is negatively affected (Akhtar and Nazir 2013; Farooq *et al*., 2009b).

Drought Avoidance: The principal objective used by the plant is to maintain higher water potential by reducing loss of water and preserving the water uptake by developing an extensive and prolific rooting system (Dai 2011; Farooq *et al*., 2009b), enabling the plant to absorb water from a considerable soil depth and far away distance from the plant. The xeromorphic trait such as hairy leaves and thick cuticle layers will assist the plant in maintaining high tissue water potential, but producing such xeromorphic structures consumes high energy and leads to decreased yield. Consequently, plants which use this strategy to uphold an optimum

water potential are generally small because of their adaptation to harsh environments (Khan *et al*., 2011; Lisar *et al*., 2012; Farooq *et al*., 2009b).

Drought Tolerance: The plants that adapt tolerance strategy restrict the number of leaves and leaf area in response to drought stress and thus decrease the yield (Akhtar and Nazir 2013; Bray 2007). Also, these plants show some xeromorphic characteristics, such as the presence of trichomes on the adaxial and abaxial surface of leaves, thereby reducing the leaf temperature and minimizing the loss of water by creating a layer of resistance to the movement of water away from the leaf surface (Lisar *et al*., 2012; Farooq *et al*., 2009d, Bray 2007). The root growth rate, size, density and proliferation are the key features of a plant's response to drought stress. However, other mechanisms involved in plant tolerance are the accumulation of compatible solutes, osmotic adjustments, activation of antioxidant systems, modifications in metabolic pathways, increased plant biomass, and stomatal closure (Bray 2007).

PHYSIOLOGICAL RESPONSE

Inadequate water availability alters crop growth and throughput due to declined water status and turgor. The drought-tolerant plants maintain their metabolic activities at low tissue water potential by adapting effective strategies such as osmotic adjustments, antioxidant defence system and changes in concentration of phytohormones (Kiani *et al*., 2007; Hussain *et al*., 2009). The increased accumulation of organic and inorganic solutes under stress aids in lowering water potential without decreasing actual water contents and is defined as osmotic adjustment or osmoregulation (Serraj and Sinclair 2002) and is one of the dynamic routes in plant adaptation to drought stress, minimizing the effects of drought-induced damage (Blum 2005). Osmo-protectants are compatible solutes with low molecular weight, are highly soluble and non-toxic even at higher concentrations, and confer protection to plants under oxidative damage by stabilizing membranes and maintaining primary structures of enzymes and proteins (Farooq *et al*., 2008). Compatible solutes involve soluble sugars such as fructans, sucrose, sugar alcohols, amino acids that include proline, aspartic acid, glutamic acid, glycine betaine (GB), organic acids, trehalose, cyclitols such as mannitol and pinitol (Kiani *et al*., 2007; Kaur and Asthir 2015). Synthesis of osmotic compounds helps maintain leaf turgor, improves stomatal conductance for efficient carbon dioxide intake (Kiani *et al*., 2007), and promotes the root's ability for water uptake (Chimenti *et al*., 2006). As a result, an upsurge in water influx, turgor, and cell wall elasticity is obtained to maintain the physiological activity at an average pace helping the plant to attain growth from the vegetative stage until the reproductive stage during the drought phase (Kramer and Boyer 1995; Ludlow and Muchow 1990; Subbarao *et al*., 2000).

The synthesis of proline, a crucial compatible solute at low water potential in plants, is combined with enhanced biosynthesis and ceasing oxidation in mitochondria (Zhu 2002). Proline is seen to accumulate in bacteria, algae and animals to lower water potential, exposed to dehydration stress having a physiological role in stabilizing macromolecules (proteins), maintaining membrane integrity and quenching reactive oxygen species (Perez-Perez *et al*., 2009; Wahid and Close, 2007; Verbruggen and Hermans 2008; Verslues and Sharma 2010; Kaur and Asthir 2015). During post-drought, proline also serves as energy for carbon and nitrogen source (Szabados and Savouré 2009). Transgenic plants, resistant to osmotic stress, accumulate proline due to overexpression of the pyrroline-5-carboxylase synthase (P5CS) gene (Khan *et al*., 2015). Glycinebetaine (N, N, N-trimethyl glycine), an amphoteric quaternary amine, is yet another widely studied compatible solute in plants, animals and bacteria (Wahid *et al*., 2007). Glycinebetaine is critical in strengthening plant growth under various abiotic stresses by participating in the signal transduction pathway. Further, it safeguards the plant cells either by depositing hydration shells or by directly interacting with the macromolecules, thereby preventing them from unfolding and denaturation (Giri 2011; Quan *et al*., 2004; Subbarao *et al*., 2000). According to findings by Nuccio *et al*. (2015), Trehalose, a non-reducing glucose disaccharide, plays a significant role in plant growth and development by effectively preserving the biological structures and stabilizing enzymes, proteins and lipid

bilayer rather than adjusting the water potential under desiccation stress (Goddijn *et al*., 1997; Wingler, 2002; Lee *et al*., 2003). It is reported that the expression of heterologous genes from some eukaryotic species, such as Escherichia coli and Saccharomyces cerevisiae leads to trehalose synthesis and develops tolerance to drought stress in various species of plants (Iordachescu and Imai 2008). Li *et al*. (2011) observed that the overexpression of multiple isoforms of trehalose- 6-phosphate synthase aids in boosting drought tolerance in rice. Akram *et al*. (2016) emphasized the enhanced expression of SOD and POX in radish due to trehalose synthesis under drought stress. Turner *et al*. (2001) highlighted the maintenance of high tissue water potential by synthesizing abscisic acid and the generation of dehydrins that may confer protection to the plant against drought injuries.

BIOCHEMICAL RESPONSE

The first biochemical response of plants on exposure to any environmental stress that causes a shift from normal ecological conditions such as drought leads to the generation of reactive oxygen species (ROS), also known as oxidative burst, thereby acting as a secondary messenger to induce successive defense responses in plants (Apel and Hirt 2004). ROS radicals such as superoxide radical (O2−), hydroxyl radical (OH.), hydrogen peroxide (H2O2), alkoxy radicals (RO), and singlet oxygen causes oxidative stress in plant cell compartments such as chloroplasts, mitochondria, peroxisomes. On the other hand, under normal metabolism, ROS is formed as a natural byproduct having an essential role in cell signalling. However, ROS being highly reactive, denatures the structure and function of macromolecules such as nucleic acid, oxidation of amino acids, protein, and photosynthetic pigments, and boosts up malondialdehyde (MDA) content, which is a crucial marker for oxidative damage (Labudda and Safiul 2014; Osakabe *et al*., 2014; Farooq *et al*., 2009c; Nezhadahmadi *et al*., 2013, Moller *et al*., 2007). Thus, to deal with continuous oxidative bursts under stress, plants have an internal protective enzymatic and non-enzymatic cleanup system, which is enough to deflect injuries, ensuring normal functioning of the plant cell (Horváth *et al*., 2007). The non-enzymatic antioxidants are low molecular mass, water and lipid-soluble compounds such as glutathione, ascorbic acid, carotenoids, and α-tocopherol. The enzymatic ROS scavenging defense system includes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), and glutathione reductase (GR) (Apel and Hirt 2004; Lisar *et al*., 2012; Hasegawa *et al*., 2000). During the oxidative burst the non-enzymatic defense system upholds the integrity of the photosynthetic membrane, whereas the enzymatic system may scavenge ROS directly, being the most effective mechanism (Farooq *et al*., 2008). According to the preceding literature, an upregulated antioxidant activity was noticed under water deficit conditions (Chugh *et al*., 2011; Chakraborty and Pradhan 2012; Marok *et al*., 2013). Marok *et al*. (2013) observed higher activity of CAT and SOD in drought-tolerant barley genotypes on exposure to drought compared to its drought-sensitive genotype. Carotenoids and other non-enzymatic compounds form vital components of the plant's antioxidant defense system (Havaux, 1998; Wahid 2007), scavenging singlet oxygen and lipid peroxy radicals inhibiting superoxide generation and lipid peroxidation under dehydrative forces (Deltoro *et al*., 1998). Ascorbate peroxidase is a crucial antioxidant enzyme in plants, whereas the fundamental role of preserving the glutathione pool is carried out by glutathione reductase under stress (Orvar and Ellis 1997; Pastori *et al*., 2000). Superoxide radicals with a half-life of less than one second are rapidly dismutased into H2O2 by SOD, a moderate stable product eliminated by catalase and peroxidase (Apel and Hirt 2004; Farooq *et al*., 2009b). SOD, a metalloenzyme, is clubbed up as the main line of defense against generating free superoxide radicals under drought conditions. Thus, increased superoxide dismutase activity confers tolerance against oxidative stress (Pan *et al*., 2006).

MOLECULAR MECHANISMS

Plants exposed to drought undergo several adaptive mechanisms at molecular levels that include signal transduction resulting in the expression of drought-responsive genes and adaptation to drought to curb the water balance (Nishiyama *et al*., 2013;

Osakabe *et al*., 2014; Kaur and Asthir 2017). Sensing stress and activation of defense and acclimation pathways are associated with complex signalling events involving ABA, Ca2+ and calcium-regulated proteins, ROS, phosphoglycerol, diacylglycerols, and signal transduction pathways (Kovtun *et al*., 2000; Kaur and Asthir 2017). These ROS, calcium, and phytohormones are the chemical indicators involved in inducing stress tolerance via transduction cascade and activating genomic reprogramming (Joyce *et al*., 2003). Recent research shows that drought-responsive genes primarily encode proteins involved in transcriptional regulation (mitogen-activated protein kinase cascades, protein phosphatases and cross-talk between diverse transcription factors), signalling cascades and functional proteins that aid the protection to cellular membranes, late embryogenesis abundant (LEA) proteins, antioxidants, proteins related to water uptake such as aquaporins and sugar transporters (Chen *et al*., 2002; Nakashima *et al*., 2014).

Aquaporins are present in the plasma membrane, and vacuoles are a group of crucial intrinsic membrane proteins that assist the passive water exchange across the membranes; their structural analysis reveals the familiar mechanism of protein-mediated water movement (Tyerman *et al*., 2002). These can potentiate a 10 to 20-fold upsurge in water permeability by regulating the hydraulic conductivity of the membranes (Maurel and Chrispeels 2001). Aquaporin activity is significantly controlled by phosphorylation (Johansson *et al*., 1998), calcium and pH (Tournaire- Roux *et al*., 2003). The function of aquaporins is expressed primarily in roots, where they mediate soil water uptake controlled by transcellular water transport (Javot and Maurel 2002).

Production of ABA is triggered in roots under water deficit and transported to shoot, causing restricted growth due to stomatal closure (Mittler and Blumwald 2015). Horváth *et al*. (2014) indicated that ABA compartmentalization and expanse of ABA reaching the stomata are influenced by xylem/apoplastic pH. Underwater deficit condition, xylem/apoplast pH being alkaline results in alkaline ABA trapping; that is, there is a decline in ABA exclusion from xylem and leaf apoplast to symplast, due to which added ABA on reaching guard cells, enables stomatal aperture modulation in response to various stress factors (Shatil- Cohen *et al*., 2011). It was also observed by Le Gall *et al*. (2015) in drought-stressed plants that translocation of sugars through xylem exerts a significant impact on the sensitivity of stomata to ABA.

Many dehydration-responsive element-binding genes are involved in signalling pathways in response to drought and other abiotic stresses (Agarwal *et al*., 2006). The dehydration-responsive element/C-repeat (DRE/CRT) cis-acting element and its DNA-binding protein constitute a significant transcription system modulating ABA-independent gene expression in response to drought and includes dehydration-responsive element-binding proteins (DREB)/C-repeat binding factors (CBF) family of proteins. DREB2 subclass of DREB/CBF family proteins is expressed under drought to articulate genes involved in stress tolerance (Seki *et al*., 2003). Also, an early warning response mechanism exists in plant roots to activate the hydrogen pump ATPase protein (H+-ATPase) on the plasma membrane of root hairs before a substantial decline in plant RWC. The activation further triggers amplified biosynthesis of leaf proline and GB, the key osmolytes that maintain the water budget of plants (Gong *et al*., 2010). Poly Amines (PA) have been associated with the drought response of plants via signalling and are also involved in playing a role in other stresses (Bae *et al*., 2008). In addition, upregulation and downregulation of various gene transcripts and stress protein accumulation are crucial, and stress-driven forces, secondary stress signals, and plant response to injury may trigger gene expression (Kavar *et al*., 2008).

IMPROVING DROUGHT TOLERANCE AND MANAGEMENT STRATEGY

The cultivation of drought-tolerant crops is one of the options to meet the needs of the escalating world population. The production of transgenic plants is one of the well-known approaches for tolerance as a wide range of genes in the plant genome has opened up excellent prospects for crop improvement (Lisar *et al*., 2012; Xoconostle-Cazares *et al*., 2010). On the other hand, the generation of transgenic plants cannot be

entirely operative for producing drought-tolerant crop plants as it requires detailed and expensive protocol, and the success rate is primarily low (Nezhadahmadi *et al*., 2013; Nakashima *et al*., 2014). In the traditional breeding method, two plant individuals with desirable traits are selected and crossed to exchange genetic material; therefore, the offspring result from a new genetic combination (Khan *et al*., 2011; Nezhadahmadi *et al*., 2013). Some crucial traits used in plant breeding include water use efficiency, hydraulic conductance, osmotic and elastic adjustments, and variation in leaf area (Bhargava and Sawant 2013; Farooq *et al*., 2009b; Ding *et al*., 2013). Genetic data can improve plant breeding efficiency by using suitable tags for the target gene, known as polymorphisms, based on the naturally present sequence in DNA (Xoconostle-Cazares *et al*., 2010). The unique approaches are employed to distinguish linked markers, including restriction fragment length polymorphisms (RFLPs), sequence characteristic amplified regions (SCARs), random amplified polymorphic DNA (RAPDs), simple sequence repeats (SSRs), amplified fragment length polymorphism (AFLPs), and others (Khan *et al*., 2013; Xoconostle-Cazares *et al*., 2010).

Plant breeding methods have a massive potential to fasten the production of drought-tolerant plants, which helps in drought management. However, there are various strategies for managing drought in agricultural fields at different levels, such as irrigation at low soil moisture during germination and crop stand establishment, mulch to maintain the soil moisture level, eliminating attacks by insects and herbivores, appropriate planting practices, native plant individual based on the edaphic conditions, and plant inoculation by symbiotic microorganisms such as AM fungi (Nezhadahmadi *et al*., 2013; Khan *et al*., 2011; Farooq *et al*., 2009b).

Foliar application of assorted PGRs and osmoprotectants can also enhance the drought tolerance of crop plants. The exogenous application of plant hormones and osmoprotectants such as gibberellins (GA3), cytokinin (Ck), abscisic acid (ABA), proline, glycine betaine (GB), brassinolide, polyamine (PA), and salicylic acid (SA) has been recognized to ameliorate stress effects, with high osmotic adjustment to maintain turgor and antioxidants accumulation to detoxify ROS to preserve the stability of membrane structures, enzymes, and other macromolecules (Manivannan *et al*., 2008; Farooq *et al*., 2009b, c; Yuan *et al*., 2010; Alcázar *et al*., 2010; Anjum *et al*., 2011b). Salicylic acid is a secondary metabolite that promotes plant drought tolerance by regulating several physiological processes through signalling (Senaratna *et al*., 2000; Singh and Usha 2003). The foliar application of methyl salicylic acid in water-stressed plants promotes leaf senescence, which contributes to nutrient remobilization, profiting the rest of the plant from the accumulated nutrients of the leaf from its life span (Abreu and Munne-Bosch 2008). Also, exogenously applied glycine betaine involved in osmoregulation acts as an osmoprotectant to protect membranes and enzymes from oxidative stress under drought stress (Ma *et al*., 2006).

CONCLUSION

Under the impact of ongoing global climate change intensifying greenhouse gas emissions, drought onset, frequency, and severity have been foreseen to increase shortly, affecting plant growth, development, and metabolism. The low rainfall, salinity, high temperature, and high light intensity are some of the main causal factors of the drought. Drought as a multidimensional stress factor negatively affects a plant at the molecular level up to the whole plant level. The plant has adopted strategies by shortening its life cycle and yield penalty to withstand drought. Also, the plants respond to drought by maintaining metabolic activities at low tissue water potential. Plants show physiological adaptation to dehydration tolerance by osmotic adjustments such as increased accumulation of proline, glycine betaine, sugars, inducing antioxidant activities (enzymatic and non-enzymatic systems) by scavenging ROS, maintaining cell membrane stability, expression of aquaporins, and altered growth regulators are vital mechanisms of drought tolerance. To curtail the effects of water stress, plants exhibit various signaling pathways and respond by upregulating antioxidant activity, accumulation of osmolytes and changing the growth pattern by producing chaperones and stress

proteins. The production of drought-tolerant transgenic plants by combining traditional breeding methods and gene manipulation is helpful to curtail the adverse effects of drought on plants. Also, several other strategies in agricultural fields for drought management on multiple levels can be effective.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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RESEARCH PAPERS

Alleviation of Drought Stress Effects in Two Rice (*Oryza sativa* L.) Cultivars by Foliar Application of Salicylic Acid

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Abstract—This study evaluated the alleviating effect of salicylic acid (SA) in two rice (*Oryza sativa* L.) cultivars differing in their tolerance to drought in the presence of PEG 6000-induced osmotic stress of 8 and 16%. The results revealed that foliar application with 0.25 mM SA considerably improved the growth parameters in DT and DS cultivars grown under drought. Concurrent with enhanced drought tolerance, the SA treatment showed a substantial increase in antioxidant enzyme activity and metabolite accumulation. Furthermore, PEG 6000-induced drought significantly upsurged the accumulation of hydrogen peroxide and hydroxyl radicals and enhanced the levels of protein carbonyl content. Interestingly, the SA foliar application also markedly declined ROS and protein carbonyl content under drought-stress conditions. These results indicated that the foliar application of salicylic acid proved to be effective in further boosting drought tolerance in DT and DS rice cultivars by overcoming the oxidative effects of drought stress.

Keywords: *Oryza sativa*, antioxidants, drought tolerance, oxidative damage, protein carbonyl, salicylic acid

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INTRODUCTION

Crops are often threatened by unpredicted weather events associated with climate change and land degradation [1]. Drought stress is presently one of the acute stresses compared to other abiotic stresses, significantly affecting crop yield and quality due to an increase in temperature and rate of soil water evaporation, thereby making the crop plant highly vulnerable. The magnitude of drought stress is expected to increase further with rising climate change and water crises [2]. Rice (*Oryza sativa* L.) is the second most important and rapidly growing cereal crop, consumed by more than half of the world's population [3]. According to Food and Agriculture Organization (FAO) [1], India, China, and Indonesia contribute to 50% of the world's rice production, which is affected due to a shortage of water resources [4]. Therefore, the analysis of rice production under water-saving cultivation is of the utmost importance.

Under drought stress, the imbalance in the rate of water absorption and water loss, a key sign of enhanced oxidative stress due to impaired electron transport mechanism in chloroplast and mitochondria, leads to excessive generation of reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($^1O_2$). ROS generation in plants causes severe oxidative injury to various macromolecules, such as DNA fragmentation, lipids, and protein peroxidation, which can ultimately induce programmed cell death pathways [5–7]. To persist the drought-induced oxidative damage, plant cells induce ROS quenchers. This endogenous defence mechanism includes antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione reductase (GR) and non-enzymatic antioxidant metabolites such as proline, carotenoids, glutathione (GSH) and ascorbate (ASC) [8, 9].

Plants induce metabolic instability and activate various defence mechanisms in response to stress-induced environmental stimuli. However, under severe abiotic/biotic stress conditions, the quenching of ROS by antioxidant enzymes is insufficient to limit the damaging effects of oxidative stress. Consequently, plants synthesize signal transducers, such as salicylic acid, Ca^{2+}, ethylene and jasmonic acid in response to environmental stress to limit stress-related damage [10]. Salicylic acid (SA), a phenolic phytohormone, regulates diverse physiological processes and mitigates the detrimental effects of various biotic and abiotic stresses in plants [11, 12]. Previous studies have shown the role of SA in alleviating the effects of low temperature [13], heavy metals, herbicides, and salinity, in addition to mitigating the effects of biotic stresses [14, 15]. However, the explicit role and basic physiological

Abbreviations: DT—drought tolerant; DS—drought sensitive; PVP—polyvinylpyrrolidone; SA—salicylic acid.

mechanisms of SA in abiotic stress have not been fully elucidated [16, 17]. SA application is reported as a non-threatening approach to enhancing plant tolerance to abiotic stress, especially drought.

The primary objective of the present study was to elucidate the possible mechanism and role of SA treatments in improving drought tolerance in rice based on growth, physiological and biochemical characteristics. It was hypothesized that the application of plant growth regulators could mitigate the damaging effects of abiotic stress [18, 19]. To validate this hypothesis, rice cultivars differing in their sensitivity to drought were selected and sprayed with SA under well-watered and drought-stressed conditions to assess the influence of SA application on alleviating drought stress.

MATERIALS AND METHODS

Plant material and growth conditions. The seeds of tested rice cultivars "Sahbhagi Dhan" (IR74371-70-1-1), a drought tolerant (DT) and "Jaya" (IET-723), a drought sensitive (DS), were raised in vermiculite in a growth chamber with 25 ± 2°C for a 16/8 h light/dark period with a mean photosynthetic photon flux density (PPFD) of 200 μmol/(m^2 s) with the average relative humidity of 65–70%. The experiment was designed with two gradients of drought stress; 8% (–0.047 bars) and 16% (–0.107 bars) of polyethylene glycol 6000 (PEG 6000) [20] in Hoagland's nutrient solution (pH 6.4) [21] and optimum watering with Hoagland's nutrient solution served as control.

Plant growth regulator treatment. After ten days of germination, each pot was foliar sprayed on the 11th, 12th, and 13th day with 0.25 mM SA, pH 7.0 (Merck, tissue culture grade) dissolved in water at 55°C. An atomizer was used to spray plants with a steady amount of salicylic acid spray (65 sprays per pot; 20 mL per pot). The entire experimental framework consisted of (a) control (well-watered, without treatment), (b) control + 0.25 mM SA, (c) 8% PEG 6000, (d) 8% PEG 6000 + 0.25 mM SA, (e) 16% PEG 6000, (f) 16% PEG 6000 + 0.25 mM SA. Replicates of three pots per treatment were maintained.

Further, on the 17th day, the fully expanded leaves (second leaf stage) of respective treatments were harvested, and the following indices were analyzed. The effective concentration of SA used in our study was based on the series of screening experiments using 0.1 to 0.5 mM. The concentration of 0.25 mM showed the maximum alleviation of drought effects compared to the control (data not shown).

Determination of shoot and root length. Ten plants were randomly assessed for the shoot length (SL) and root length (RL) from each treatment set using a meter scale.

Determination of physiological indices. The fully expanded rice leaves on harvesting were immediately frozen in liquid nitrogen and used to determine drought-induced changes in the accumulation of ROS, protein carbonyl content, antioxidant enzyme, superoxide dismutase (SOD), glutathione reductase (GR), and antioxidant metabolite, glutathione content (GSH). All the measurements for the physiochemical parameters were recorded in triplicate.

Quantification of reactive oxygen species accumulation. The total hydrogen peroxide (H_2O_2) content was estimated, according to Sagisaka [22]. Concisely, 0.2 g leaf tissue was suspended in 5% (w/v) trichloroacetic acid (TCA), 2.5 mM potassium thiocyanide, and 10 mM ferrous ammonium sulfate. The optical density of the supernatant was considered at 480 nm using UV/VIS spectrophotometer (UV2450, Shimadzu). A calibration curve using 10 mM of H_2O_2 (Merck) as an external standard was used to determine the H_2O_2 concentration expressed as μmol/g fr wt.

For the estimation of hydroxyl radical ($OH^{\cdot}$), the formation of the thiobarbituric acid reactive substances (TBA-RS) on reduced deoxyribose by $OH^{\cdot}$ was spectrophotometrically detected at 532 nm [23]. An increase in $OH^{\cdot}$ concentration is directly related to an increase in absorbance, expressed as AU (absorbance × 1000).

Estimation of protein carbonyls (RC = O). To quantify protein-bound carbonyls, a biomarker of oxidative stress was measured as described by Levine et al. [24]. Briefly, fresh leaf tissue (0.5 g) was extracted and homogenized with 30% (w/v) TCA, followed by centrifugation at 3000 rpm for 10 min. The supernatant (500 μL) was redissolved with an equal volume of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 M hydrochloric acid (HCl). The reagent mixture was then washed three times with 500 μL of 1 : 1 ethanol/ethyl acetate (v/v) after incubation for 1 h at room temperature. Finally, the pellets were dissolved in 6 M urea in 20 mM potassium phosphate buffer (pH 2.5). The absorbance of the resulting stable yellow-coloured product was measured at 370 nm. Carbonyl content was calculated using a molar absorption coefficient for aliphatic hydrazones of 22000 M^{-1} cm^{-1} and expressed in nmol/mg protein.

Antioxidant enzyme assay. Preparation of enzymatic extract. Fresh leaf tissue (0.5 g) was ground in liquid nitrogen. Enzymes were extracted by placing the ground leaf powder in a 2 mL phosphate buffer (50 mM, pH 7) containing 1 mM EDTA and 1% PVP. The protein content of all enzyme preparations was estimated using bovine serum albumin (Sigma) as a standard according to the Bradford method [25].

Superoxide Dismutase (SOD). Superoxide dismutase (EC 1.15.1.1) activity was estimated by assessing its competence to curtail nitroblue tetrazolium (NBT) photo-reduction by forming purple formazone. The reaction mixture (3 mL) contained 75 μM NBT, 2 μM riboflavin, 13 mM methionine, 50 mM phosphate buffer (pH 7.8) and 100 μL extract. Fur-

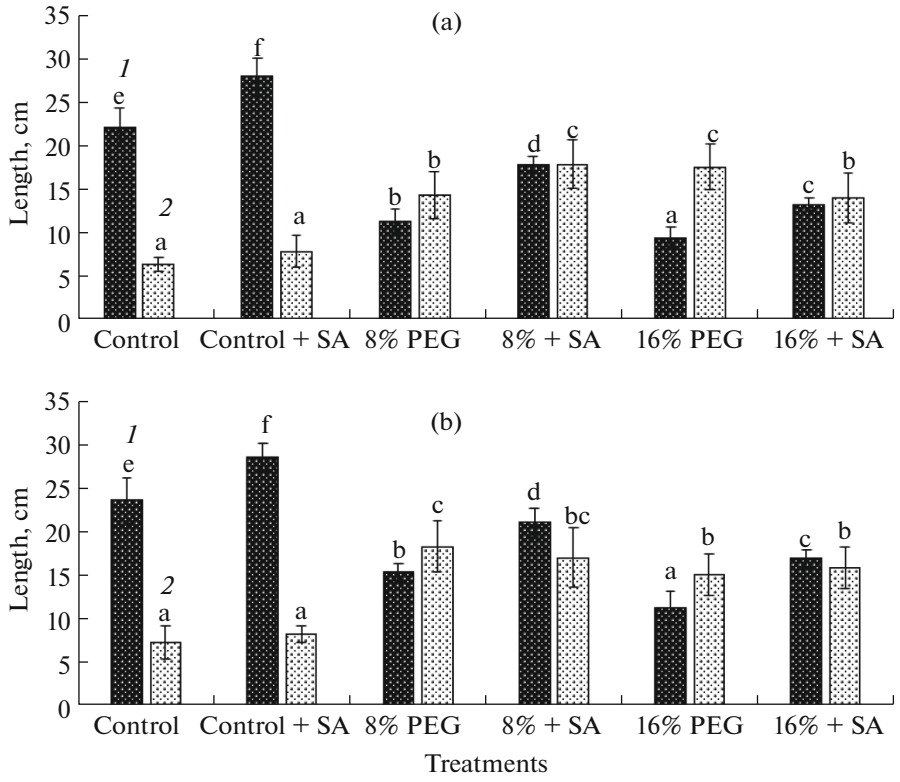

Fig. 1. Variations in (*1*) shoot length and (*2*) root length in leaves of (a) drought-tolerant and (b) drought-sensitive rice (*Oryza sativa* L.) cultivars grown under PEG 6000-induced drought with or without exogenously applied salicylic acid. Bars indicate SE ($n = 9$). Different letters show significant differences between the means at $P \leq 0.05$.

ther, the reaction was illuminated for 30 min under a cool white fluorescent lamp. The absorbance of the reaction mix was read at 560 nm. The reaction mixture without the enzyme extract served as blank. The amount of enzyme essential to cause 50% inhibition of the NBT photoreduction rate is defined as one unit (U) of SOD activity, expressed as U/mg protein [26].

Glutathione reductase (GR). Glutathione reductase (EC1.6.4.2) activity was determined by evaluating the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm at 30°C according to the method of Schaedle and Bassham [27]. The reaction mixture (1 mL) contained 50 mM phosphate buffer with 2 mM EDTA (pH 7.8), 2 mM NADPH, a reducing agent to initiate the reaction, 20 mM oxidized glutathione (GSSG) and 10 µL enzyme extract. Enzyme activity was expressed as U/mg protein.

Determination of antioxidant metabolite: glutathione content (GSH). The content of glutathione, an antioxidant metabolite, was determined by spectrophotometry in accordance with Griffith [28]. The supernatant (1 mL) was treated with 1.5 mL precipitation solution for 10 min and then filtered. Disodium hydrogen phosphate solution (0.3 M Na_2HPO_4) and 250 µL 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) solution were added to 500 µL filtrate. The sample without the filtrate was used as a blank. The absorbance of the resulting yellow color was read at 412 nm within 10 min. The total glutathione content (µg/mL) was obtained by a graphical calculation and was multiplied by the respective dilution factor.

Statistical analysis. To calculate the significant difference between treatments, analysis of variance (ANOVA) was adopted, and data were further subjected to Duncan's Multiple Range Test (DMRT) post hoc in IBM® SPSS® (SPSS Inc., IBM Corporation, USA) Statistics version 26 (2019), with $P < 0.05$ taken as statistically significant. Data are presented as the average value ± standard deviation (SD) based on three independent determinations.

RESULTS

Growth parameters. Drought stress significantly affected the growth of the above-ground parts of both rice cultivars. However, the outcomes showed that the application of SA enhanced the growth parameters under watered and drought-stressed conditions (Figs. 1a, 1b). The results obtained with control, 8 and 16% drought treatment showed a significant reduction in shoot growth of both rice cultivars. Plants treated with 16% drought showed a significant reduction in the shoot length in DT (57%) and DS (52%) cultivars compared to the control. However, compared to con-

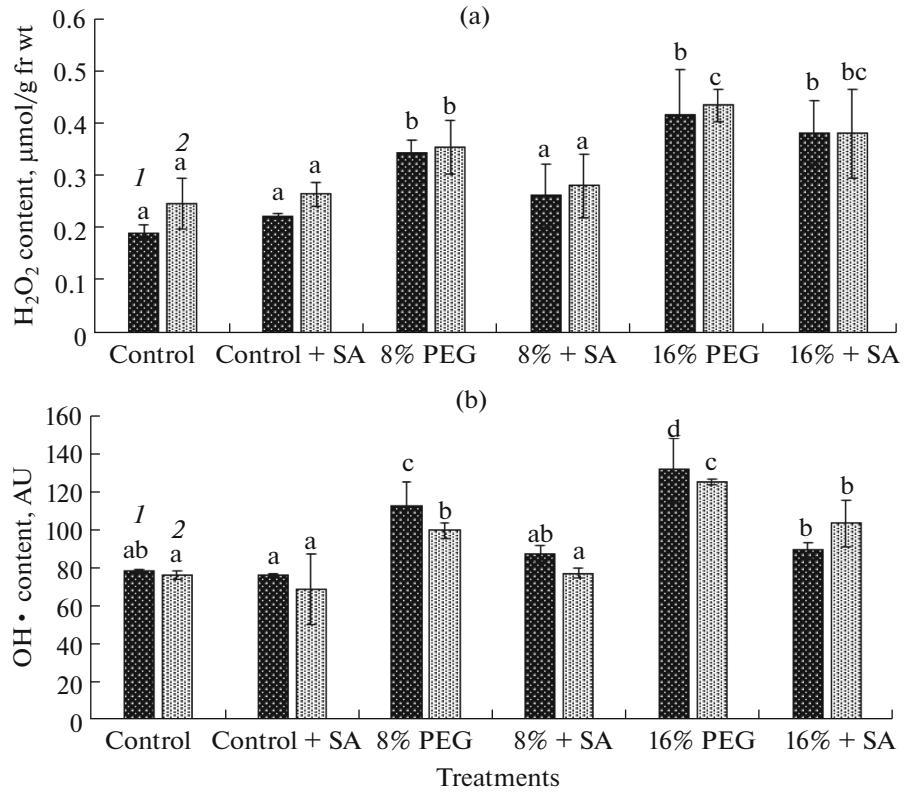

Fig. 2. Measurement of the reactive oxygen species (a) H_2O_2, (b) $OH^{\bullet}$ in leaves of (*1*) drought-tolerant and (*2*) drought-sensitive rice (*Oryza sativa* L.) varieties grown under PEG 6000-induced drought with or without exogenously applied salicylic acid. Bars indicate SE ($n = 9$). Different letters show significant differences between the means at $P \leq 0.05$.

trol plants, increased root lengths were recorded in DT (175%) and DS (109%) cultivars. On the other hand, the spray of SA resulted in inhibiting drought stress effect in both cultivars. The SA treatment on 16% drought stress also increased the shoot length in DT (41%) and DS (49%).

Accumulation of ROS and protein carbonyls. The study revealed that drought stress significantly upsurged the accumulation of ROS and protein carbonyl content (Fig. 2a). However, exogenous SA treatment reduced the H_2O_2, $OH^{\bullet}$, and RC=O content in both treatments. Exogenous SA treatment on plants exposed to 8 and 16% drought stress resulted in a decline in H_2O_2 content by 24 and 8% in the DT cultivar, and a decline by 20 and 12% in the DS cultivar was recorded compared to the control, respectively.

On the other hand, in plants treated with 8% and 16% drought-stressed conditions, SA application decreased the $OH^{\bullet}$ content by 22 and 32% in the DT cultivar, and 23 and 17% in the DS cultivar, respectively, in relation to the control plants (Fig. 2b).

Similarly, an exogenous spray of SA on the drought-stressed plants of 8 and 16% declined the protein carbonyls by 39 and 19% in the DT cultivar, and 12 and 16% DS cultivar, respectively, compared to the control. Therefore, the results of the present study reveal that spraying with SA provides considerable protection to DT and DS rice cultivars under drought-stress conditions (Fig. 3).

Antioxidant enzyme activity. On exposure to drought stress, both the cultivars showed a decline in SOD activities (Fig. 4a). However, spraying with SA increased SOD under both control and drought-stressed conditions. In 8 and 16% drought-stressed conditions, the SA spraying improved the SOD activities by 49 and 26% in the DT variety and 42 and 37% in the DS variety, respectively, compared to the control.

On the other hand, PEG 6000-induced drought stress significantly triggered GR activity. The activity of GR was also enhanced by SA spraying under 8 and 16% drought-stressed conditions, by 33 and 28% in DT, and 61 and 18% in DS variety, respectively, as compared with control (Fig. 4b).

Accumulation of antioxidant metabolite. Drought stress significantly influenced the accumulation of glutathione (TGSH), an antioxidant metabolite. The results suggest that SA treatment significantly increased the accumulation of TGSH under control and drought-stressed conditions in the selected rice cultivars (Fig. 5). Compared to the control, under 8 and 16% drought-stressed conditions, the SA treatment enhanced the accumulation of TGSH by 24 and 3% in the DT variety, and 13 and 32% in the DS cultivars, respectively.

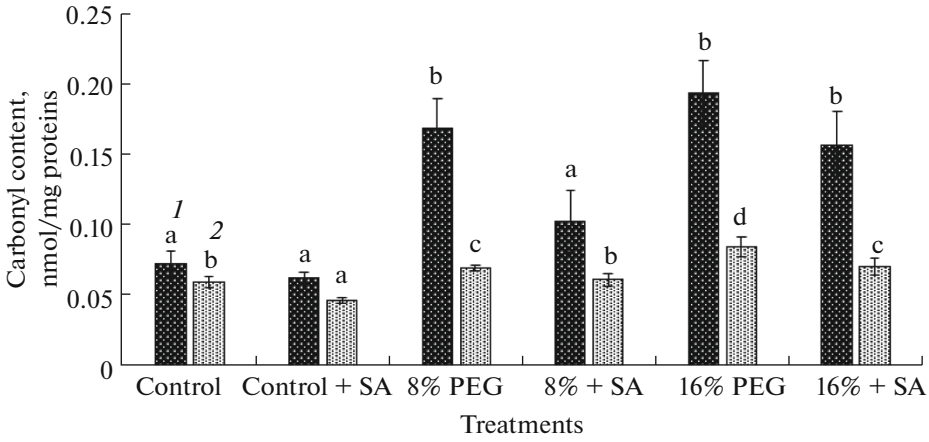

Fig. 3. Variations in protein carbonyl content in leaves of (*1*) drought-tolerant and (*2*) drought-sensitive rice (*Oryza sativa* L.) varieties grown under PEG 600-induced drought with or without exogenously applied salicylic acid. Bars indicate SE ($n = 9$). Different letters show significant differences between the means at $P \le 0.05$.

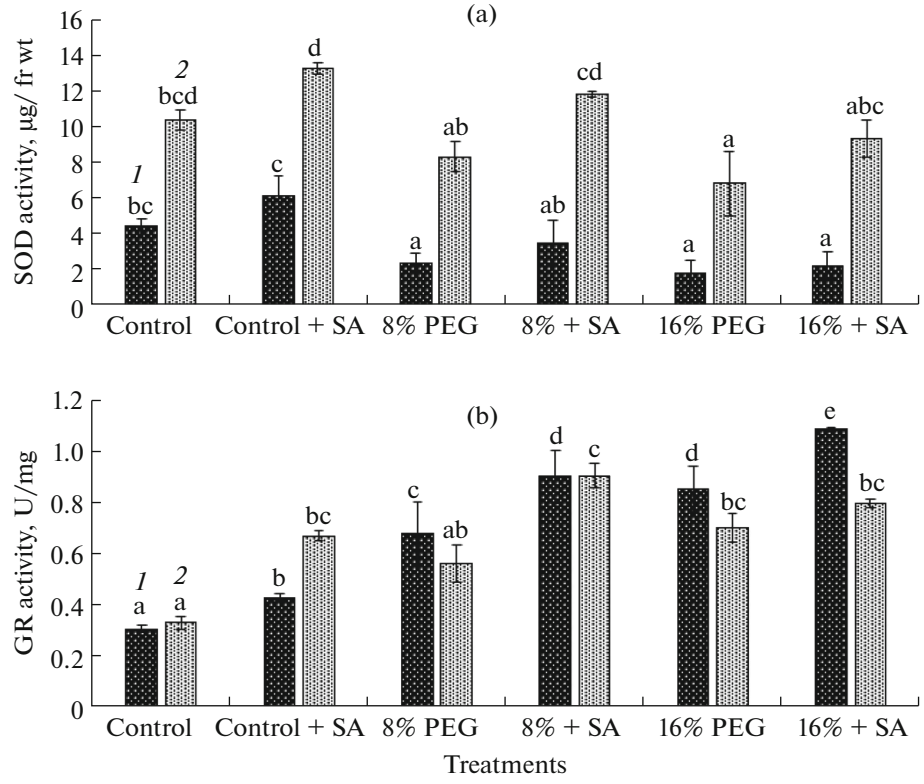

Fig. 4. Measurement of the antioxidant enzymes (a) SOD, (b) GR in leaves of (*1*) drought-tolerant and (*2*) drought-sensitive rice (*Oryza sativa* L.) varieties grown under PEG 6000-induced drought with or without exogenously applied salicylic acid. Bars indicate SE ($n = 9$). Different letters show significant differences between the means at $P \le 0.05$.

DISCUSSION

Results of the present study indicate the adverse effects on the overall growth of rice plants subjected to PEG 6000-induced drought stress (Fig. 1). A significant decline in the shoot length and prolific increase in root growth of both the rice cultivars due to PEG 6000-induced drought stress conditions were observed. The findings of the present study indicate that the overall growth of rice plants is hindered by high osmotic stress, which leads to an oxidative burst. However, SA treatments significantly ameliorated the growth parameters. Ahmad et al. [29] concluded that applying adequate SA treatment increased the plant

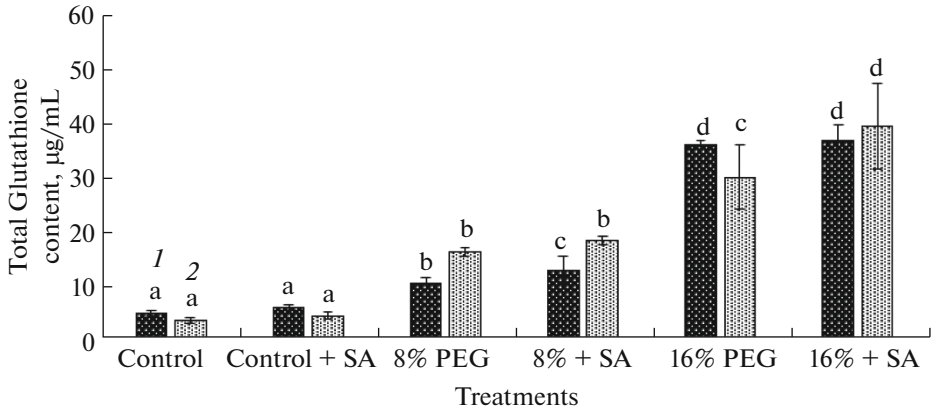

Fig. 5. Measurement of the total glutathione content in leaves of (*1*) drought-tolerant and (*2*) drought-sensitive rice (*Oryza sativa* L.) varieties grown under PEG 6000-induced drought with or without exogenously applied salicylic acid. Bars indicate SE ($n = 9$). Different letters show significant differences between the means at $P \leq 0.05$.

growth attributes of wheat cultivars under drought stress. Furthermore, Ghazi [30] reported that drought caused a remarkable reduction in the growth and yield parameters of maize genotypes. He observed that the exogenous application of SA enhanced yield parameters such as plant height, plant biomass, number, and grain weight under drought treatment compared to the control. The application of SA may elicit the meristematic activity of cells initiating cell elongation and enlargement, consequently enhancing plant vigour under stress [31].

In the present study, the decline in growth of both cultivars under drought stress might be due to the excessive generation of ROS. Hydrogen peroxide and hydroxyl radicals account for oxidative damage and elevated protein carbonyl content, a well-studied and reliable biomarker for oxidative damage. Our previous report documented that drought stress significantly decreased RWC, biomass, membrane integrity, and lipid peroxidation while also causing an increase in proline content [32]. However, supplementation with SA effectively alleviated the adverse effects of drought-induced damages and upheld membrane structural integrity. Previous studies [33] have consistently reported that drought-induced accumulation of reactive oxygen species (ROS) disrupts cell membrane integrity, damaging membrane lipids, proteins, and chlorophylls, ultimately impacting plant biomass. However, the present study uncovered that the application of exogenous SA improved drought tolerance in the chosen rice cultivars. This enhancement seems to be linked to the increased activity of antioxidant enzymes and the accumulation of antioxidant metabolites, which resulted in reduced levels of hydrogen peroxide, hydroxyl radicals, and protein carbonyls. The application of SA may be accounting for the modifications in plant cell physiological and biochemical metabolism that enhances growth parameters under drought stress. Previous reports suggest the damaging effects of drought stress on various crop plants, where the degree of damage varied with the growth phase of the individual and the severity of drought stress [34]. Iqbal et al. [35] reported that SA spraying applications might enhance plant growth characteristics and their attributes under various stresses.

Homeostasis of ROS is known to be a convergence point to assess plant stress status. A coordinated chain of antioxidant enzyme syntheses such as SOD, CAT, and APX is triggered to neutralize the damaging effects of ROS on plant growth. However, the activity of SOD was decreased in both cultivars due to drought (Fig. 4a); these results are in agreement with the previous findings, which also noted reduced SOD activity in plants exposed to drought and cold stress [36, 37]. The dismutation of superoxide anion radical ($O_2^{\bullet-}$) is effectively facilitated by SOD, leading to the production of H_2O_2 and O_2. A decline in SOD function could hinder the cells' ability to remove $O_2^{\bullet-}$ and result in its buildup. The decline in net SOD activity during drought stress could be attributed to reduced synthesis or enhanced enzyme degradation. Moreover, increased H_2O_2 levels during drought stress may also decrease SOD action. The decline in SOD activity during drought stress could significantly affect the cellular antioxidant defence system [36].

Nevertheless, in the present study, PEG 6000-induced drought stress significantly increased the activity of GR. The increased activity of antioxidant enzymes was inadequate to quench the excessively generated ROS caused due to drought stress. This antioxidant machinery was enhanced in both rice cultivars upon foliar treatment of SA under drought-stressed conditions. Our results are in agreement with Zafar et al. [38], who demonstrated that foliar application of SA on *Conocarpus erectus* L. and *Populus deltoides* L. saplings boosted the antioxidant enzyme activity of SOD, POD, APX and CAT on exposure to

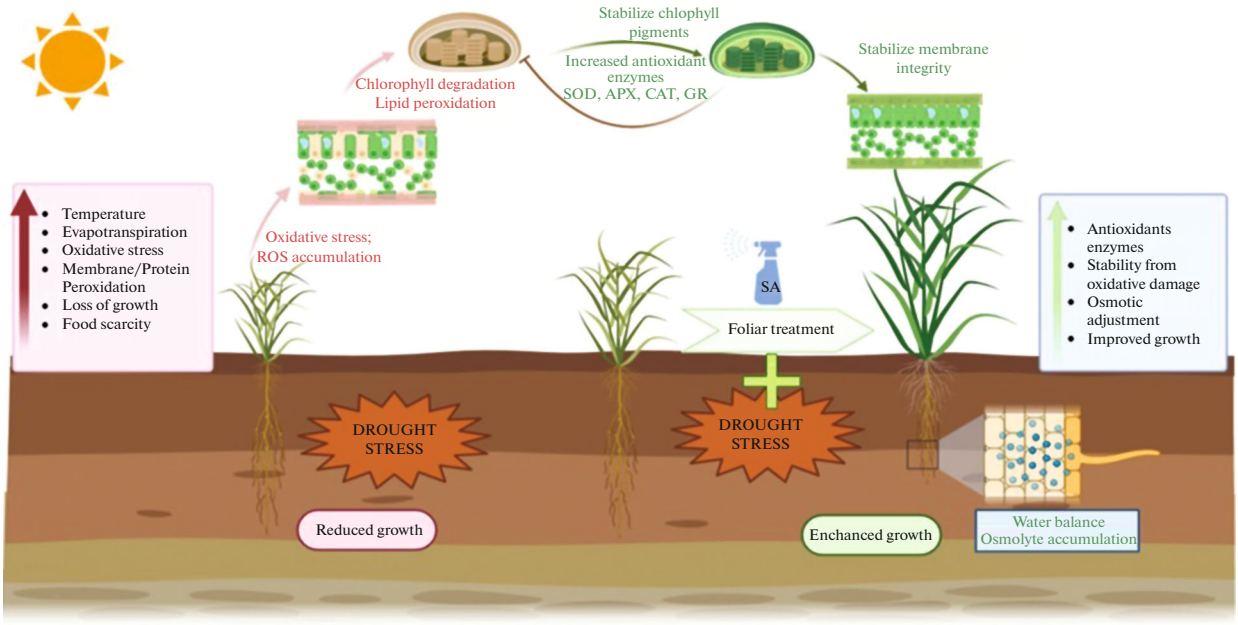

Fig. 6. Diagram illustrating the hypothesis of how exogenous application of salicylic acid mediates drought tolerance in rice (*Oryza sativa* L.). Drought stress hampers plant growth by enhancing the accumulation of ROS (Reactive oxygen species) and peroxidation of lipids and proteins. Exogenously applied SA alleviates the drought stress effects by modulating the antioxidant enzymes; SOD (superoxide dismutase), APX (ascorbate peroxidase), CAT (catalase), GR (glutathione reductase), providing stability from the oxidative damage, helping the rice plant survive the drought stress.

drought compared to the control, which curtailed the oxidative damage under stress and correlated it with the maintenance of cellular integrity by reducing the activities of ROS.

Applying phytohormone could amend the enzymatic antioxidants system in rice cultivars under 8 and 16% drought stress conditions by efficiently maintaining the imbalance of excessive ROS, which was noticeable due to a marked decline in protein carbonyls, H_2O_2, and $OH^{\cdot}$ contents in these cultivars. Previous studies also reported minimizing the oxidative damage on subcellular components and are postulated to be a stress tolerance mechanism by enhancing the antioxidant machinery [36, 37]. An upregulation in the antioxidant enzyme activity in plants supplemented with SA under drought suggested ROS scavenging was more significant. In this study, the content of protein carbonyl in leaves of two rice cultivars was significantly elevated due to drought stress, and it was concurrent with the increased accumulation of H_2O_2 and $OH^{\cdot}$, while exogenous application of 0.25 mM SA markedly declined contents of protein carbonyls, H_2O_2 and $OH^{\cdot}$. However, it appeared that the DT cultivar had a greater buildup of protein carbonyl content than the DS cultivar. Protein carbonylation is a post-translational modification brought on by ROS, which can cause proteins to degrade. Unlike other protein breakdown processes like ubiquitination, protein carbonylation does not require ATP or enzymes. An energy-efficient strategy to rapidly recycle amino acids during nutritional deprivation or stress is the breakdown of carbonylated proteins by the 20S proteasome system. Protein carbonylation may therefore be viewed as a process that supports protein turnover and aids in the tolerance to oxidative stress, helping plants deal with short-term stress [39]. The present study signifies that both rice cultivars could develop tolerance to the damaging effects of oxidative stress due to the exogenous application of SA.

In addition to the enzymatic antioxidant machinery, antioxidant metabolites are also strongly involved in ameliorating the drought stress effects by regulating the osmotic stress. The findings concerning the drought effect on GSH, an antioxidant metabolite in plants, depicted that total Glutathione (TGSH) concentration upsurged upon exogenous SA treatment due to elevation in GR activity, responsible for alleviating the oxidative stress and enhancing plant growth. Our observations are consistent with Dat et al. [18], who reported the application of SA-enhanced levels of antioxidant substances in plants. The enhanced GR and GSH activity plays a decisive role in preserving the antioxidant pool in its redox state and reducing levels of carbonylated proteins by inducing an antioxidant defence system under various environmental stresses [37, 40].

Based on the outcome of the present study, SA, an endogenous signal molecule, is known to play a significant role in the signal transduction network. This suggests that mitigating effects of SA treatment might be

attributed to the downturn in the accretion of hydrogen peroxide, hydroxyl radicals, and protein carbonyl contents. This may be due to an upsurge in the activity of antioxidant enzymes and an elevation in the accumulation of antioxidant metabolites (Fig. 6). These mechanisms are essential to sustain the production of rice plants in water-deficit conditions.

In conclusion, the investigation revealed that PEG 6000-induced drought stress negatively affects rice plants, hindering growth (DT and DS) due to increased levels of ROS and protein oxidation. Our study with conclusive evidence proved that plants on exogenous treatment with SA can mitigate the damaging effects of drought by playing a significant role in scavenging ROS and reducing protein carbonyl accumulation, upsurging the enzymatic activities of SOD and GR and higher accumulation of TGSH, enhancing plant tolerance to drought stress and restoring growth. The impact of SA was more distinct in drought-tolerant varieties under stress conditions, which signifies an improved scavenging system that consecutively helps protect the vital machinery involved in plant growth. Hence, it is a well-thought-out strategy to enhance the plant growth parameters, yield and mechanisms and ensure a comparatively stable rice yield under drought-stressed conditions. Therefore the application of SA in DT and DS cultivars will help to mitigate drought conditions prevalent in tropical countries, and this helps in food security challenges.

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COMPLIANCE WITH ETHICAL STANDARDS

The study does not involve human or animal participants by both authors.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest in publishing this research work.

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