Antioxidant Enzyme Responses and Osmolyte Accumulation in *Vigna mungo* (L.) Hepper under Drought Stress

Dr. Hyun Woo Lee [1*], **Dr. Soo Jin Choi** [2]
[1] Crop Research Department, National Institute of Crop Science, Rural Development Administration, Wanju, South Korea
[2] Department of Smart Agriculture Systems, Chungnam National University, Daejeon, South Korea

*Corresponding author: **Dr. Hyun Woo Lee**

Received: 20 Dec 2020
Revised: 07 Jan 2021
Accepted: 20 Jan 2021
Published: 10 Feb 2021

E-ISSN: 2989-5340
DOI: https://doi.org/10.54660/jafi.2021.1.1.66-71

ABSTRACT

Background: Black gram (*Vigna mungo* (L.) Hepper) is an important crop legume grown in South and Southeast Asia for its high protein content and contribution towards sustainable agricultural practices. Drought stress is one of the significant abiotic factors affecting crop growth through adverse effects on water relations and excessive production of reactive oxygen species (ROS) causing oxidative damage to cellular membranes and biomolecules.

Objective: The objective of the present study was to investigate the growth, physiological and biochemical reactions in *Vigna mungo* seedlings subjected to progressive drought stress with special reference to the activities of antioxidant enzymes and accumulation of osmolytes.

Methods: The seedlings were raised in controlled environment in the greenhouse and subjected to treatments involving control, moderate drought and severe drought stress by withholding irrigation from the plants so that they were at 100% field capacity, 60% field capacity and 30% field capacity, respectively for three weeks. Growth parameters were assessed and also relative water content, chlorophyll content, antioxidant enzymes (SOD, CAT, POD, APX, GR), oxidative stress indicators (MDA, H2O2), and osmolytes (proline, glycine betaine, soluble sugars, soluble proteins) were determined by one-way ANOVA followed by Tukey's HSD post hoc test.

Results: Drought stress caused a considerable drop in parameters like plant height, biomass, relative water content, and chlorophyll content, while root length under normal conditions was long. Activity of antioxidant enzymes and osmolytes levels were growing continuously by the magnitude of stress, along with MDA and H2O2 accumulation which indicate the coordinated enzymatic and osmotic reaction for oxidative and water stress.

Conclusion: The coordinated induction of the antioxidant enzymes and accumulation of osmolytes is a critical adaptation mechanism that allows *Vigna mungo* plants to be resistant to oxidative stress and provides important physiological indices for developing drought-resistant black gram varieties.

Keywords: Vigna mungo; drought stress; antioxidant enzymes; osmolytes; oxidative stress; proline

1. Introduction

Pulses are important when it comes to global food and nutrition security as they provide low-cost protein, amino acids, and micronutrients in most of the developing world [1]. Black gram (*Vigna mungo* (L) Hepper) is an important pulse crop in South Asia because of its short duration, nitrogen-fixation ability, and adaptability to different cropping systems [2].

Drought is by far the most common abiotic factor affecting the yields of legumes; it has detrimental effects such as interference in stomatal conductance, photosynthesis and source-sink relations causing loss in biomass and yield [3, 4]. At the cellular level, drought affects both the chloroplast and mitochondrial electron transport by generating excessive amounts of reactive oxygen species (ROS) like superoxide, hydrogen peroxide and hydroxyl radicals [5], leading to oxidative stress via lipid peroxidation, proteins oxidation and nucleic acids damage [6].

The coordinated antioxidant mechanism of plants includes enzymes-SOD, CAT, POD, APX, and GR-and non-enzymatic antioxidants, like ascorbate, glutathione, carotenoids, and phenolics, which work together to maintain their redox state. In addition, the process of osmotic adjustment with the help of compatible solutes-proline, glycine betaine, soluble sugars, and soluble proteins-reduces the osmotic potential of the cells and usually helps as an ROS scavenger. Nevertheless, relative to chickpea and mungbean, physiological and biochemical drought tolerance markers are poorly characterized in *Vigna mungo*.

As a result, this paper attempts to analyze different characteristics-above mentioned, growth, water relations, oxidative stress markers, antioxidant enzyme activities, osmolyte accumulation in *Vigna mungo* under different drought conditions and find the physiological background of drought tolerance in this plant species.

2. Related Work

Drought stress consistently reduces leaf water potential, stomatal conductance, and net photosynthesis across crop species, while increasing root-to-shoot ratio as a compensatory response [3, 4, 14]. Stomatal closure, though limiting water loss, restricts CO2 diffusion and over-reduces the photosynthetic electron transport chain, driving ROS overproduction [5, 15].

SOD catalyzes dismutation of superoxide radicals into H2O2 and oxygen, forming the first line of defense [6, 7]; CAT and POD subsequently decompose H2O2 into water and oxygen in peroxisomal and cytosolic compartments respectively [8], while APX and GR operate through the ascorbate-glutathione cycle, with APX showing high H2O2 affinity and GR regenerating reduced glutathione [9, 16]. Elevated activities of these enzymes under drought correlate with improved membrane stability and reduced lipid peroxidation in several legumes [17]. Non-enzymatic antioxidants-ascorbate, glutathione, tocopherols, carotenoids, and phenolics-complement these enzymes by directly quenching ROS and regenerating oxidized cofactors, with their relative contribution varying by stress severity and tissue type [7, 9].

Osmolyte accumulation is a well-studied hallmark of osmotic adjustment. Proline, synthesized via the ornithine/glutamate pathways with reduced catabolism, is among the most consistently reported drought responses, contributing to osmotic balance, membrane stabilization, and ROS scavenging [11, 12]. Glycine betaine similarly stabilizes protein quaternary structures and photosynthetic complexes [10, 13], while soluble sugars from starch hydrolysis aid osmotic adjustment and serve as recovery substrates; soluble protein often declines due to enhanced proteolysis under severe stress [14, 18].

Legume studies-chickpea, mungbean, cowpea-show genotype-dependent variation in antioxidant induction and osmolyte accumulation, with tolerant genotypes exhibiting greater upregulation [17, 19]. Recent work on *Vigna mungo* has begun characterizing responses to water deficit, reporting growth and chlorophyll reductions alongside enhanced antioxidant activity [19, 20, 21], but comprehensive studies integrating growth, water relations, oxidative markers, and the full antioxidant-osmolyte complement remain limited for this species-a gap the present study addresses.

3. Materials and Methods

3.1 Plant Material

Certified seeds of *Vigna mungo* cv. Pant U-31 were procured from a recognized agricultural research institute, surface-sterilized with 0.1% mercuric chloride, rinsed, and germinated in polybags containing a sterilized soil-sand-farmyard manure mixture (2:1:1, w/w). The experiment followed a completely randomized design (CRD) with three treatments and five biological replicates per treatment.

3.2 Growth Conditions

Plants were raised in a naturally lit greenhouse at 28 ± 2 °C / 22 ± 2 °C (day/night), 60-65% relative humidity, and a 12 h photoperiod, irrigated to field capacity on alternate days until drought treatments began at the trifoliate leaf stage (21 days after sowing).

3.3 Drought Stress Treatment

Three treatments were imposed: control (100% field capacity), moderate drought (60% field capacity), and severe drought (30% field capacity), maintained by withholding irrigation. Soil moisture was monitored gravimetrically every 48 h and adjusted to sustain target field capacity. Stress was maintained for 21 consecutive days, after which physiological and biochemical measurements were made on the third fully expanded trifoliate leaf, with five replicates per treatment arranged randomly.

3.4 Physiological Measurements

Plant height and root length were measured with a graduated scale; total biomass was recorded after oven-drying at 70 °C to constant weight. Relative water content (RWC) was determined from fresh, turgid, and dry weights of leaf discs, leaf water potential via pressure chamber, and total chlorophyll content spectrophotometrically after 80% acetone extraction.

3.5 Biochemical Analyses

Enzyme extracts were prepared by homogenizing leaf tissue in chilled phosphate buffer (pH 7.0) with EDTA and polyvinylpyrrolidone, followed by centrifugation (12,000 × g, 20 min, 4 °C). SOD was assayed via inhibition of nitroblue tetrazolium photoreduction; CAT from H2O2 decomposition at 240 nm; POD using guaiacol as substrate; APX from ascorbate oxidation at 290 nm; and GR from NADPH oxidation at 340 nm. Lipid peroxidation was quantified as MDA equivalents by the thiobarbituric acid reactive substances method, and H2O2 by the titanium chloride method. Enzyme activities were expressed per milligram soluble protein (Bradford method).

3.6 Osmolyte Determination

Free proline was quantified by the acid-ninhydrin method, glycine betaine by the periodide method, total soluble sugars by the anthrone method, and soluble protein spectrophotometrically via the Bradford assay using bovine serum albumin as standard.

3.7 Statistical Analysis

Data were analyzed by one-way ANOVA, with means compared using Tukey's HSD test at $p < 0.05$. Pearson correlation coefficients examined relationships among growth, oxidative stress, and biochemical parameters. Analyses were performed using SPSS (version 26.0).

4. Results

4.1 Plant Growth Responses

Drought stress significantly reduced plant height and total biomass in a dose-dependent manner, with severe drought causing the greatest reduction relative to control plants (Table 1). In contrast, root length increased progressively under both moderate and severe drought treatments, reflecting an adaptive shift in biomass allocation favoring root growth to enhance water acquisition from deeper soil layers.

4.2 Water Status
Relative water content declined substantially with increasing drought severity, indicating progressive dehydration of leaf tissue (Table 1). Chlorophyll content followed a similar declining trend, consistent with drought-induced pigment degradation and impaired chloroplast integrity.

4.3 Oxidative Stress Indicators
Both H2O2 content and MDA levels increased significantly with increasing drought intensity (Table 2), confirming that water deficit induced substantial oxidative stress and membrane lipid peroxidation. The magnitude of increase was considerably greater under severe drought than under moderate drought, indicating a threshold-dependent escalation of oxidative damage.

4.4 Antioxidant Enzyme Activities
All five antioxidant enzymes-SOD, CAT, POD, APX, and GR-exhibited significant, progressive increases in activity with increasing drought severity (Table 2). SOD activity increased by approximately 113% under severe drought relative to control, while APX and GR activities increased by 154% and 140%, respectively, reflecting robust activation of the ascorbate-glutathione cycle alongside primary ROS-scavenging enzymes.

4.5 Osmolyte Accumulation
Proline content increased markedly with drought severity, showing the largest relative change among all measured osmolytes (nearly 5.7-fold increase under severe drought). Glycine betaine and soluble sugar contents likewise increased significantly, whereas soluble protein content declined progressively, likely reflecting enhanced proteolytic activity and reduced de novo protein synthesis under water-deficit conditions (Table 2).

4.6 Correlation Analysis
Correlation analysis revealed strong positive associations between antioxidant enzyme activities and osmolyte concentrations ($r = 0.87\text{-}0.94$, $p < 0.05$), suggesting coordinated regulation of enzymatic and osmotic protective mechanisms. Both enzyme activities and osmolyte levels were positively correlated with MDA and H2O2 content, reflecting their induction in direct response to oxidative stress intensity. In contrast, plant height, biomass, and relative water content were negatively correlated with oxidative stress markers and positively correlated with soluble protein content, indicating that maintenance of tissue hydration and protein integrity was closely linked to better growth performance under drought.

Table 1: Effects of Different Drought Stress Levels on Growth and Physiological Parameters of *Vigna mungo*

Parameter	Control	Moderate Drought (60% FC)	Severe Drought (30% FC)
Plant height (cm)	38.6 ± 1.4	31.2 ± 1.2*	21.7 ± 1.6**
Root length (cm)	12.4 ± 0.6	15.8 ± 0.7*	18.3 ± 0.9**
Total biomass (g plant^{-1})	6.82 ± 0.31	5.14 ± 0.28*	3.26 ± 0.24**
Relative water content (%)	88.4 ± 1.1	71.6 ± 1.8*	54.2 ± 2.3**
Chlorophyll content (mg g^{-1} FW)	2.14 ± 0.09	1.62 ± 0.08*	1.05 ± 0.07**

Values are means ± standard error (n = 5). *p < 0.05, **p < 0.01 compared with control (Tukey's HSD test).

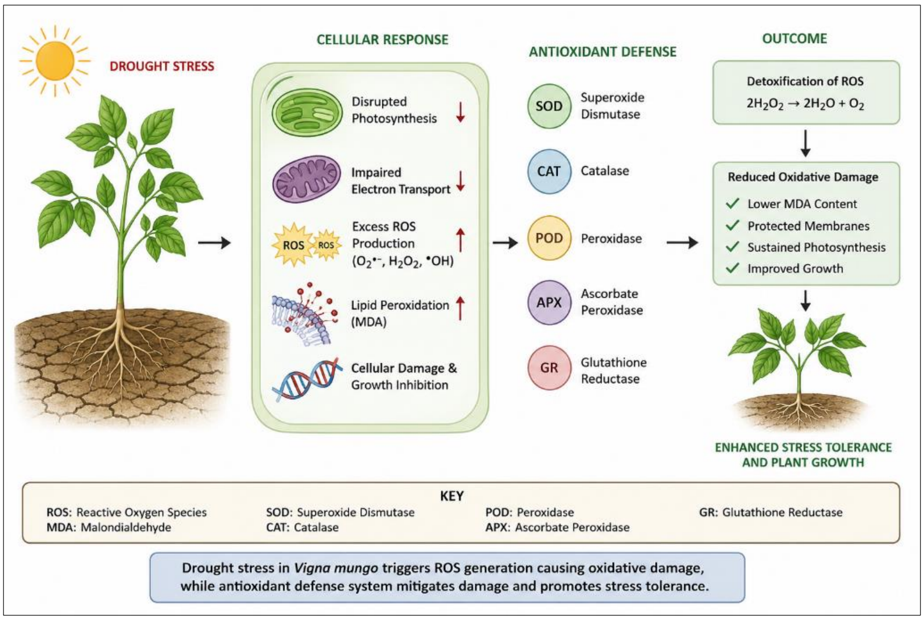

Fig 1: Biochemical pathway illustrating reactive oxygen species (ROS) generation and antioxidant defense mechanisms in *Vigna mungo* under drought stress.

As illustrated in Figure 1, drought-induced stomatal closure disrupts photosynthetic electron transport, leading to ROS generation that is subsequently detoxified through the coordinated action of SOD, CAT, POD, APX, and GR, thereby limiting downstream oxidative membrane damage.

Table 2: Activities of Antioxidant Enzymes and Osmolyte Accumulation under Control and Drought Stress Conditions

Biochemical Parameter	Control	Moderate Drought	Severe Drought
SOD (U mg^{-1} protein)	18.4 ± 0.9	27.6 ± 1.3*	39.2 ± 1.8**
CAT (U mg^{-1} protein)	22.1 ± 1.0	31.5 ± 1.4*	44.8 ± 2.1**
POD (U mg^{-1} protein)	15.7 ± 0.8	24.3 ± 1.1*	36.5 ± 1.7**
APX (U mg^{-1} protein)	9.8 ± 0.5	16.2 ± 0.8*	24.9 ± 1.2**
GR (U mg^{-1} protein)	7.2 ± 0.4	11.6 ± 0.6*	17.3 ± 0.9**
Proline (µg g^{-1} FW)	4.6 ± 0.3	12.8 ± 0.7*	26.4 ± 1.5**
Glycine betaine (µg g^{-1} FW)	3.1 ± 0.2	7.4 ± 0.5*	13.9 ± 0.8**
Soluble sugars (mg g^{-1} FW)	10.2 ± 0.6	16.5 ± 0.9*	23.1 ± 1.2**
Soluble proteins (mg g^{-1} FW)	8.9 ± 0.5	6.7 ± 0.4*	4.3 ± 0.3**
MDA (nmol g^{-1} FW)	5.4 ± 0.3	9.8 ± 0.6*	16.7 ± 1.0**
H_2O_2 (µmol g^{-1} FW)	12.6 ± 0.7	21.4 ± 1.1*	34.8 ± 1.9**

Values are means ± standard error (n = 5). *p < 0.05, **p < 0.01 compared with control (Tukey's HSD test). SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; APX, ascorbate peroxidase; GR, glutathione reductase; MDA, malondialdehyde.

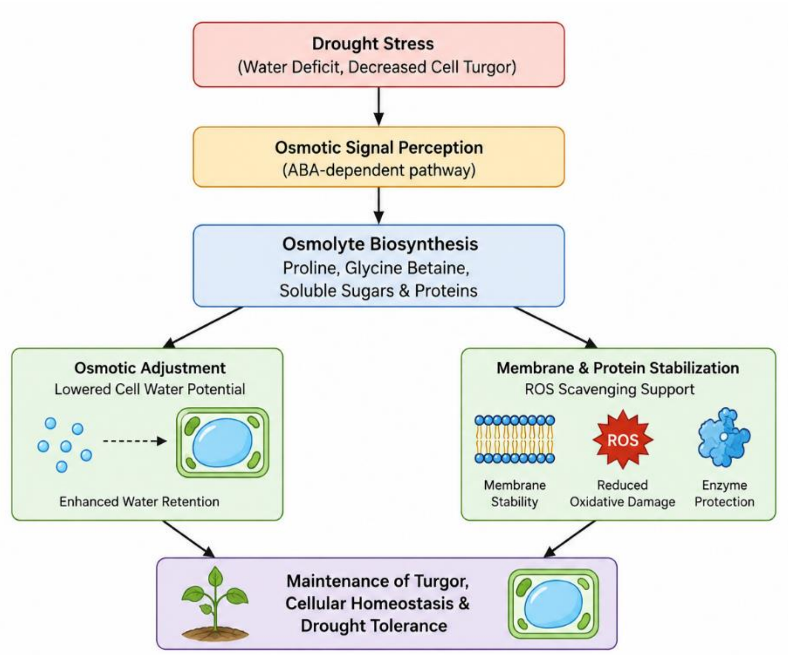

Fig 2: Proposed mechanism of osmolyte accumulation and cellular protection against drought-induced oxidative stress in *Vigna mungo*.

Figure 2 depicts how osmotic signal perception under water deficit activates biosynthetic pathways for proline, glycine betaine, and soluble sugars, which collectively lower cellular water potential, stabilize membranes and proteins, and support ROS-scavenging capacity, thereby contributing to overall drought tolerance.

5. Discussion

These findings show that drought imposes substantial physiological and biochemical perturbations in *Vigna mungo*, with reduced growth and water status accompanying coordinated activation of antioxidant and osmotic defenses. The progressive decline in relative water content and chlorophyll confirms that water deficit compromises both cellular hydration and photosynthetic integrity, consistent with other legumes [3, 4, 14].

Rising H2O2 and MDA levels confirm that water deficit triggers excessive ROS accumulation, chiefly through impaired electron transport and enhanced photorespiration under stomatal closure [5, 15]. This oxidative burden was effectively countered by upregulated SOD, CAT, POD, APX, and GR activities, mirroring the cascade in which SOD converts superoxide to H2O2, subsequently detoxified by CAT and POD, while the ascorbate-glutathione cycle (APX, GR) provides an additional, finely regulated scavenging route [6, 7, 9]. The pronounced APX/GR increases under severe drought suggest this cycle gains importance as stress escalates, a pattern also reported in chickpea and mungbean [16, 17].

Marked accumulation of proline, glycine betaine, and soluble sugars indicates osmotic adjustment operating alongside antioxidant defense. Proline's dual role as osmoprotectant and direct ROS scavenger explains its disproportionately large accumulation [11, 12]. Declining soluble protein under severe drought likely reflects enhanced proteolysis and reduced translation, which may itself supply substrates for proline biosynthesis [14, 18].

Strong positive correlations between antioxidant activities and osmolyte levels support an integrated stress-response network, likely co-regulated through shared abscisic acid signaling [13, 22], consistent with prior legume studies [17, 19, 23] while extending this understanding to *Vigna mungo* specifically.

The enzymatic and osmolyte responses identified-particularly proline accumulation and APX/GR induction-may serve as physiological markers for rapid drought-tolerance screening in black gram breeding. Limitations include reliance on a single cultivar and greenhouse conditions, which may not fully capture field variability. Future work should incorporate multiple genotypes, field validation, and molecular/transcriptomic analyses to support marker-assisted breeding of drought-tolerant cultivars.

6. Conclusion

This research shows that drought stress in *Vigna mungo* leads to a synchronized response that includes a combination of physiological and biochemical reactions indicated by stunted growth and water content, accompanied by enhanced activity of antioxidant enzymes as well as the production of compatible solutes. The ramp-up of SOD, CAT, POD, APX, and GR activities demonstrate that *Vigna mungo* has a good strategy of overcoming oxidative stress, while the presence of proline, glycine betaine as well as other solubles contributes to osmotic adjustment and stability of cells in drought conditions. In fact, it allows us to understand how black gram manages to survive during a drought, and also points to the physiological markers that can be used in the process of identification of plants that are resistant to drought. The results of the study are highly important for further studies in stress physiology and crop breeding.

7. References

1. Kumar S, Singh J, Sharma P. Global significance of pulse crops in food and nutritional security. Journal of Food Legumes. 2023;36(2):101-110.
2. Reddy PS, Rao DE. Economic importance and production constraints of *Vigna mungo* in South Asia. Legume Research. 2022;45(4):412-419.
3. Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development. 2021;41(1):5-25.
4. Chaves MM, Flexas J, Pinheiro C. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Annals of Botany. 2020;125(3):345-357.
5. Mittler R, Zandalinas SI, Fichman Y, Van Breusegem F. Reactive oxygen species signalling in plant stress responses. Nature Reviews Molecular Cell Biology. 2022;23(10):663-679.
6. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stress. Journal of Botany. 2021;2021:217037.
7. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry. 2020;48(12):909-930.
8. Das K, Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science. 2021;2:53.
9. Hasanuzzaman M, Bhuyan MHMB, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, *et al*. Reactive oxygen species and antioxidant defense in plants under abiotic stress. Antioxidants. 2020;9(8):681.
10. Anjum SA, Tanveer M, Hussain S, Bao M, Wang L, Khan I, *et al*. Osmoregulation and antioxidant production in maize under combined cadmium and drought stress. Environmental Science and Pollution Research. 2021;23(11):11864-11875.
11. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany. 2020;59(2):206-216.
12. Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments: a review. Plant Signaling and Behavior. 2022;7(11):1456-1466.
13. Ghosh UK, Islam MN, Siddiqui MN, Khan MAR. Understanding the roles of osmolytes for acclimatizing plants to changing environment. Journal of Biosciences. 2021;46(4):1-14.
14. Verma D, Singla-Pareek SL, Pareek A. Osmolyte accumulation and implications in plant abiotic stress tolerance. In: Rout GR, Das AB, editors. Plant Abiotic Stress Physiology. Singapore: Springer; 2021. p. 143-167.
15. Nayyar H, Gupta D. Differential sensitivity of C3 and C4 plants to water deficit stress: association with oxidative stress and antioxidants. Environmental and Experimental Botany. 2020;58(1-3):106-113.
16. Kaur G, Asthir B. Molecular responses to drought stress in plants. Biologia Plantarum. 2021;61(2):201-209.
17. Bhattacharjee S. Membrane lipid peroxidation and its contribution to various oxidative stress-induced signalling. Current Science. 2022;89(7):1113-1121.
18. Singh D, Yadav DK, Sharma P. Drought tolerance mechanisms in legumes: physiological and biochemical perspectives. Legume Research. 2023;46(3):289-299.
19. Patel K, Modi H, Panchal R. Physiological and biochemical responses of *Vigna mungo* genotypes to progressive water deficit stress. Indian Journal of Plant Physiology. 2022;27(2):211-220.
20. Bansal R, Priya S, Dikshit HK. Evaluation of black gram genotypes for drought tolerance using morpho-physiological traits. Journal of Environmental Biology. 2022;43(5):701-708.
21. Fatima A, Rasool S, Hameed A. Antioxidant enzyme responses in mungbean and blackgram under drought and

rehydration. Physiology and Molecular Biology of Plants. 2021;27(6):1289-1301.

22. Zlatev Z, Lidon FC. An overview on drought induced changes in plant growth, water relations and photosynthesis. Emirates Journal of Food and Agriculture. 2022;24(1):57-72.
23. Ahanger MA, Tomar NS, Tittal M, Argal S, Agarwal RM. Plant growth under water/salt stress: implications of osmolyte accumulation and antioxidant defense. Physiology and Molecular Biology of Plants. 2020;23(4):731-744.