

Antioxidant Defence System of Rice (*Oryza sativa* L.) Induced by Waterlogging Stress

Dr. Vibha Tiwari¹

¹Assistant Professor, Botany Department, Dr. Ram Manohar Lohia Awadh University, Ayodhya

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Abstract: Waterlogging is an important abiotic stress that adversely affects plant growth, metabolism and productivity by restricting oxygen diffusion into the root zone. Rice (*Oryza sativa* L.) is comparatively tolerant to flooding; however, prolonged waterlogging can cause hypoxia or anoxia, impaired respiration, reduced photosynthesis, nutrient imbalance and excessive generation of reactive oxygen species (ROS). Enhanced ROS production results in oxidative stress and can damage lipids, proteins, nucleic acids and cellular membranes. Rice plants counteract this oxidative damage through a complex antioxidant defence system consisting of enzymatic and non-enzymatic components. Major antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POD), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR). Non-enzymatic antioxidants include ascorbate, glutathione, tocopherols, carotenoids, phenolic compounds and flavonoids. The present paper reviews the physiological and biochemical responses of rice to waterlogging, with particular emphasis on ROS generation and antioxidant defence mechanisms. Understanding these mechanisms is important for developing waterlogging-tolerant rice cultivars through conventional breeding, molecular approaches and biotechnological interventions.

Keywords: Rice, Waterlogging, Hypoxia, Reactive Oxygen Species, Antioxidant Defence, SOD, CAT, APX, Glutathione, Oxidative Stress.

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I. INTRODUCTION

Waterlogging occurs when soil becomes saturated with water for a prolonged period, resulting in a substantial reduction in gaseous diffusion and oxygen availability around plant roots. Oxygen diffuses approximately 10,000 times more slowly through water than through air; consequently, oxygen consumption by roots and soil microorganisms rapidly creates hypoxic or anoxic conditions.

Rice is one of the major cereal crops cultivated under flooded conditions and possesses several structural and physiological adaptations that enable it to tolerate low-oxygen environments. These adaptations include aerenchyma formation, enhanced internal oxygen transport, regulation of carbohydrate metabolism and activation of anaerobic fermentation pathways. Nevertheless, prolonged or severe waterlogging can adversely affect even tolerant rice genotypes.

A major biochemical consequence of waterlogging is disturbance of cellular redox homeostasis. Oxygen deficiency restricts mitochondrial oxidative phosphorylation and modifies electron transport processes.

When oxygen availability changes during flooding and subsequent reoxygenation, uncontrolled electron transfer may result in enhanced production of reactive oxygen species (ROS). These include superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($\bullet OH$) and singlet oxygen (1O_2).

At moderate concentrations, ROS function as signalling molecules and participate in stress acclimation.

However, excessive ROS accumulation causes oxidative damage. Plants therefore maintain a sophisticated antioxidant defence network that controls ROS concentration and protects cellular components.

The antioxidant system is particularly important during waterlogging followed by reoxygenation, when the sudden restoration of oxygen can produce a rapid oxidative burst. The efficiency of this antioxidant machinery may therefore contribute significantly to differences in waterlogging tolerance among rice genotypes.

II. OBJECTIVES

The major objectives of this study/review are:

- To understand the physiological effects of waterlogging stress in rice.
- To examine ROS generation under waterlogging and reoxygenation.
- To evaluate the role of antioxidant enzymes in ROS detoxification.
- To understand the contribution of non-enzymatic antioxidants.
- To assess the relationship between antioxidant defence and waterlogging tolerance.
- To identify potential antioxidant traits useful for developing stress-tolerant rice cultivars.

III. MATERIALS AND METHODS

➤ *Experimental Material*

For an experimental investigation, healthy seeds of selected rice (*Oryza sativa* L.) genotypes representing waterlogging-tolerant and waterlogging-sensitive varieties may be used. Seeds should be surface sterilized and germinated under controlled conditions. Seedlings may be grown in pots containing a suitable mixture of soil and organic substrate under controlled environmental conditions.

➤ *Application of Waterlogging Stress*

Waterlogging treatment may be imposed at the desired growth stage by maintaining the water level above the soil surface for a predetermined period. Control plants should be maintained under normal soil-water conditions.

Plant samples may be collected at different time intervals, for example: Control, Early waterlogging, Prolonged waterlogging, and Recovery after removal of waterlogging. Root and leaf tissues should be collected separately because roots experience oxygen deficiency directly, whereas leaves respond indirectly through changes in photosynthesis and systemic signalling.

➤ *Biochemical Analysis*

The following biochemical parameters may be determined:

Antioxidant enzymes: Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX), Peroxidase (POD), Glutathione reductase (GR), Dehydroascorbate reductase (DHAR), and Monodehydroascorbate reductase (MDHAR). Enzyme activities may be expressed on a fresh-weight or protein basis.

Non-enzymatic antioxidants: The concentrations of ascorbate, reduced glutathione (GSH), oxidized glutathione (GSSG), carotenoids, tocopherols, and total phenolics may be estimated using established biochemical protocols.

➤ *Determination of Oxidative Stress*

Oxidative damage may be assessed by estimating hydrogen peroxide (H_2O_2), malondialdehyde (MDA),

electrolyte leakage, and membrane stability. MDA is commonly used as an indicator of lipid peroxidation and membrane damage.

➤ *Statistical Analysis*

Experiments should preferably be conducted using an appropriate number of biological replicates. Data may be expressed as a mean $\pm$ standard error. Statistical significance can be evaluated using analysis of variance (ANOVA), followed by an appropriate post-hoc test. Correlation analysis may be used to determine relationships between antioxidant activity and stress tolerance.

IV. RESULTS

Because the present article is a literature-based research/review framework and no original experimental dataset has been supplied, the following results represent the expected and commonly reported response pattern rather than fabricated numerical observations.

➤ *Effect of Waterlogging on Plant Growth*

Waterlogging is expected to reduce root growth, biomass accumulation and, under severe conditions, shoot growth. Prolonged waterlogging may cause chlorosis and reduced leaf expansion. Tolerant genotypes generally maintain better growth than sensitive genotypes.

➤ *Effect on ROS Production*

Waterlogging and subsequent reoxygenation are expected to increase ROS accumulation, particularly H_2O_2 and superoxide radicals. Increased ROS production is associated with disruption of electron transport and cellular redox balance.

➤ *Antioxidant Enzyme Response*

Waterlogging-tolerant plants are generally expected to maintain stronger antioxidant responses. SOD provides an initial line of defence by converting superoxide radicals into H_2O_2 . CAT and APX subsequently detoxify H_2O_2 . APX is particularly important in chloroplasts and other cellular compartments, whereas CAT has a major role in removing relatively high concentrations of H_2O_2 . POD and GR contribute to peroxide detoxification and maintenance of cellular redox status.

➤ *Changes in Non-Enzymatic Antioxidants*

Waterlogging may modify the concentrations and redox status of ascorbate and glutathione. Increased availability of reduced ascorbate and glutathione can improve the capacity of plants to detoxify ROS and maintain cellular redox balance.

➤ *Oxidative Damage*

Sensitive rice genotypes are expected to show greater accumulation of MDA, H_2O_2 and membrane leakage compared with tolerant genotypes. Higher oxidative damage generally indicates insufficient ROS-scavenging capacity.

V. DISCUSSION

Waterlogging creates a complex physiological environment in which oxygen deficiency, altered energy metabolism and oxidative stress interact. The antioxidant defence system is therefore not an isolated mechanism but an important component of the overall flooding-response network.

➤ *ROS as Both Toxic Molecules and Signals*

ROS should not be considered exclusively harmful. At controlled concentrations, H₂O₂ and other ROS can function as signalling molecules that activate stress-responsive genes and promote acclimation. Problems arise when ROS production exceeds the detoxification capacity of the plant. Therefore, waterlogging tolerance depends not simply on eliminating ROS but on maintaining an appropriate ROS homeostasis.

➤ *Role of SOD, CAT, and APX*

SOD is considered one of the first enzymatic barriers against oxidative stress. It converts highly reactive superoxide radicals into H₂O₂. Although H₂O₂ is less reactive than superoxide, excessive accumulation is also toxic. Consequently, SOD activity must be coordinated with CAT, APX and other peroxide-scavenging enzymes. CAT and APX represent two major pathways of H₂O₂ detoxification. CAT can efficiently remove high concentrations of H₂O₂, whereas APX has a high affinity for H₂O₂ and functions effectively at relatively low concentrations. The coordinated operation of these enzymes is particularly important during the transition from hypoxia to reoxygenation.

➤ *Ascorbate–Glutathione Cycle*

The ascorbate–glutathione cycle is a major ROS-scavenging pathway in plants. APX uses ascorbate to reduce H₂O₂ to water. Oxidized ascorbate is subsequently regenerated through reactions involving MDHAR and DHAR. Glutathione participates in the regeneration of ascorbate, while GR maintains the reduced glutathione pool. Thus, efficient operation of this cycle is essential for maintaining cellular redox homeostasis during environmental stress.

➤ *Antioxidants and Membrane Protection*

Excess ROS can initiate lipid peroxidation, leading to the formation of MDA and disruption of membrane integrity. Tocopherols, carotenoids, phenolic compounds and glutathione contribute to protection against lipid and membrane oxidation. Maintenance of membrane stability is especially important for root tissues exposed directly to waterlogging.

➤ *Differences Between Tolerant and Sensitive Genotypes*

Genotypic variation is an important feature of rice waterlogging tolerance. Tolerant genotypes may exhibit better antioxidant capacity, improved ROS regulation, more effective carbohydrate conservation and superior oxygen transport. However, antioxidant activity alone cannot completely explain waterlogging tolerance. It interacts with morphological adaptations, carbohydrate metabolism,

hormonal regulation, transcriptional responses and recovery mechanisms.

➤ *Molecular Regulation*

Waterlogging induces extensive transcriptional changes. Hypoxia-responsive pathways regulate genes associated with anaerobic metabolism, carbohydrate utilization, ROS detoxification and cell survival.

Ethylene accumulation under submerged conditions also participates in morphological and molecular responses. Genes involved in ROS detoxification and redox regulation may therefore serve as potential molecular targets for improving stress tolerance.

VI. CONCLUSION

Waterlogging stress significantly alters oxygen availability, energy metabolism and cellular redox homeostasis in rice.

Excessive ROS production can result in lipid peroxidation, membrane damage, protein oxidation and impaired cellular functions. Rice responds through a coordinated antioxidant defence system involving SOD, CAT, APX, POD, GR, MDHAR and DHAR together with non-enzymatic antioxidants such as ascorbate, glutathione, carotenoids, tocopherols and phenolic compounds.

The balance between ROS production and antioxidant scavenging capacity is an important determinant of waterlogging tolerance. Future research integrating physiological, biochemical, molecular and genomic approaches can help identify reliable antioxidant markers and develop rice cultivars capable of maintaining productivity under increasingly variable water regimes.

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