Multi-omic Techniques and Nitrogen (N) Induced Drought Tolerance in Rice Crop

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Abstract:

The present review highlights the growth and productivity of a rice crop that is an issue due to global climate change and Nitrogen deficiency that induce different stresses in a crop. Researchers reported a reduction in rice productivity due to drought stress worldwide. A comprehensive bioinformatics analysis, including Transcriptional Factors, was developed to analyze multi-omics data. Multi-omics technique provides a better understanding of cellular dynamics under many different kinds of stresses or either against biotic or abiotic stresses. The most important multi-omic techniques, Genomics, Proteomics, Transcriptomics and Metabolomics, have been recently studied. Breeding drought-tolerant rice varieties are more adaptive due to multigenic control of drought-tolerant traits. Drought significantly decreased agronomic traits, with biomass at 25.2% and yield at 25.4%. Nitrate Transporters play a critical role in nitrogen absorption. Nitrogen induces leaf production and expansion, shoots to root distance, accelerates transpiration and photosynthesis and promotes yield, especially under abiotic stresses in which drought is more effective. Plants use nitrogen as ammonium (NH₄⁺) and Nitrate (NO₃⁻), which can affect various aspects of a plant. This review provides an extensive outline of breeding methods and mineral induction from conventional to the latest innovation in molecular development of drought-tolerant rice varieties. This information could serve as a guideline for researchers and rice breeders.

Keywords: Multi-omics, Nitrate transporter, Agronomic traits, Transcriptional factors, Bioinformatics

Highlights

- Growth and productivity of a rice crop are affected in drought conditions.
- Nitrogen deficiency and efficiency both create a huge problem in rice crop productivity.
- Omic technology solves the problem of rice growth in water deficit conditions.

1. Introduction

Rice (*Oryza sativa* L.) is the monocot most important staple food crop for human nutrition and is widely consumed worldwide, especially in Asia (Asia, 2021; Samal et al., 2018). Rice Knowledge Bank (RKB) helps to talk the chief trial to farming progress by supporting the rapid and active transference of technologies from the research test site to the agricultural field for farmers. Rice (*Oryza sativa* L.) provides minerals, vitamins, and fiber. However, all constituent except carbohydrates is reduced by milling (knowledgebank.irri.org), providing 21% of global human per capita energy and 15% of per capita protein. According to the FAO report (2016-2020), the average production of Rice (*Oryza sativa* L.) is estimated as 1.6×10^7 t. But due to the rising population, the average production of Rice (Oryza sativa L.) will increase upto 2.9×10^9 t by the year 2030 (Panda et al 2021). Rice is a semiaquatic plant that requires constant irrigation all season to grow. The main growing season of Rice is June to November, followed by November to May. Beneficial crop system improves by crop rotation, and mineral also improves crop in which nitrogen plays an important role because it is part of amino acid, protein, nucleic acid, nucleotide, DNA, RNA etc. and involves soil fertility, soil properties and increasing organic matter. Nitrogen supply is influenced by crop type also. Nowadays, drought is a major problem in rice growth due to the problematic condition of the ecosystem. Rice production is severely constrained by drought stress, which causes devastating financial repercussions. The main influence of drought stress appears on flowering and grain filling stages, resulting in less rice yield.

About global climate change, it is turning into a more significant concern. It has become crucial to emphasize increasing agricultural output on drought-prone rainfed lands in light of the projected and current world food demand (Juntawong & Bailey-Serres,2012). There is a need for rice varieties that can withstand droughts in rainfed areas, and genetic improvement for drought-tolerant rice should be a top priority issue of study in the future. Due to its complexity, multigenic control of drought tolerance features would be a significant barrier to the existing research. Breeding for drought-tolerant rice varieties is a challenging responsibility that would significantly delay present research. In the last 20 years, significant strides have been taken in comprehending the mechanisms underlying rice adaptability and tolerance to drought stress (Kadam et al. 2017). Several valuable traditional genotypes are evolved in eastern India, Bangladesh, Lao PDR, and Nepal with an adaptation to the specific environmental challenges. Still, they are not used commercially because of low production and undesirable morphological, physiological and grain characteristics.

1.1 Drought stress promotes Different Mechanisms in Rice.



Drought influences early germination through reduced water uptake and low seedling strength (Xiong et al. 2014). Drought stress causes damage in metabolisms and at the cellular level, resulting in reduced photosynthetic efficiencies, energy currency (ATP) production, and activating different mechanisms in crops. Functional gene products (SS), late embryogenesis abundant (LEA) proteins, and aquaporin (AQP) can be involved in plant metabolism and, thus, affect plant state (Jang et al., 2004; Yang et al., 2021). Drought stress act on the cell membrane because it is sensitive and hydrophobic. ROS generation under the biotic and abiotic influence on the cell wall. All living organisms produce lipids, and their role is significant in forming the cell wall, while the generation of ROS affects lipids, a part of the cell wall; therefore, cell wall damage is observed. They start leakages, increasing MDA content in crop plants that trigger the production of Osmolytes (Proline, Glycine-betaine, Sugar, Alcohols etc.) and disturb plant life (Farooq et al. 2012)

Under drought stress, roots of rice(*Oryza sativa* L.) crops have been shown to respond in three different ways: (i) osmotic adjustment in roots, (ii) improved root penetration into the soil, (iii) root strength and saturation and root to shoot ratio with a large soil water content (Panda et al. 2021; Lian et al. 2006; Bhattacharjee & Dey 2018). Long root penetration is much stronger in water deficit conditions and maintains prolificacy (Venuprasad et al. 2009). The negative impact of drought stress on rice crops' morphological, physiological, biochemical and molecular processes reported by Panda. et al. (2021) **1.2 POS** *C* an analysis of the stress o

1.2 ROS Generation in Rice under Drought Stress

ROS (Reactive oxygen species) generation under biotic and abiotic stress acts as a signal that initiates protective responses by precise indicator transduction pathways in crops. ROS increases oxidative damage leading to retarded growth and ultimate cell death (Hou et al., 2009). ROS include singlet oxygen, superoxides, Hydrogen peroxide, free radicals etc. Low to moderate concentrations of ROS may act as secondary messengers in stress signalling pathways, triggering stressdefensive/adaptive responses (Gould et al. 2018). Plants are shielded against oxidative stress damage by the antioxidant defence system. Plants have very effective enzymatic and nonenzymatic antioxidant defence systems to prevent cascades of uncontrolled oxidation and shield plant cells from oxidative damage. Such systems work together to scavenge ROS and regulate oxidative damage cascades. Moreover, ROS affects several genes' expression, which regulates various activities, including pathogen defence, abiotic stress responses, PCD (Programmed cell death), growth, cell cycle, and development (Harrell et al. 2011).

1.3 Osmolyte Production in Rice under Drought Stress

Osmolyte adjustment occurs under stress that helps to maintain water balance by developing different osmolytes that protect function and cellular structures and maintain physiological mechanisms followed by cell turgor under water scarcity environments (Vibhuti et al. 2015). The accumulation of several osmolytes like proline, polyphenol, proteins, carbohydrates, and soluble sugars under drought conditions was reported to be higher compared to water conditions. Osmolytes such as proline, glycine-betaine, sugar, polyamines, and alcohol all are involved in osmoregulation through maintaining cell turgor. Proline is also involved in free radical quenching and can maintain buffering cellular redox potential and subcellular structures (Ashraf and Foolad, 2007)

1.4 Activation of Antioxidant System under Drought Stress in Rice

Plants have defensive antioxidant systems that play a crucial role under stress and protect against oxidative damage (Gill & Tuteja,2010). Oxidative stress tolerance depends on antioxidant activity; this system is categorized into two classes (Gould et al., 2018). The equilibrium between the reclamation and generation of ROS is sustained by both enzymatic and nonenzymatic antioxidant defence systems under harsh environmental stresses. Generation of reactive oxygen species and its conversions in plants reported by (Hasanuzzaman et al. 2020):

$$\begin{array}{c} O_{2} + e^{-} \rightarrow O_{2}^{+-} & (1) \\ O_{2}^{-} + H^{+} \rightleftharpoons HO_{2}^{+-} & (2) \\ H_{2}O_{2} + HO^{\bullet} \rightleftharpoons HO_{2}^{-} + H_{2}O & (3) \\ OH + OH \rightleftharpoons O^{\bullet} \Rightarrow HO_{2}^{-} + H_{2}O & (4) \\ O_{2}^{-} + 2H^{+} + e^{-} \rightarrow H_{2}O_{2} & (5) \\ O_{2}^{-} + HO_{2}^{--} + H_{2}O \rightarrow H_{2}O_{2} + O_{2} + OH & (6) \\ HOOH \rightarrow HO^{\bullet} + OH & (7) \\ ROOH \rightarrow RH^{\bullet} + OH & (8) \\ Fe^{3+}/Cu^{2+}/Mn^{3+} + H_{2}O_{2} \rightarrow Fe^{2+}/Cu^{+}/Mn^{2+} OH + OH & (9) \\ Fe^{2+}/Cu^{+}/Mn^{2+} + H_{2}O_{2} \rightarrow Fe^{3+}/Cu^{2+}/Mn^{3+} + HO_{2}^{--} + H^{+} (10) \end{array}$$

1.4.1 Enzymatic Antioxidant System

Primarily, maximum reactive oxygen species (ROS) have short half-lives, are disposed to chemical reactions, and are converted into water or secondary ROS. The enzymatic antioxidant system consists of various enzymes having strong

activities. It includes catalase (CAT) that, catalyzes the dismutation of H_2O_2 into H_2O and O_2 and is a heme-containing enzyme (Pandhair & Sekhon, 2006).

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Superoxide dismutase (SOD) acts as a first-line defence against oxidative stress and involves the disintegration of superoxide anions (O_2^{-}) .

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

Ascorbate peroxidase (APOX) normalizes ROS levels and main cellular or sub-cellular structures under stress. It catalyzes the reduction of H_2O_2 to convert it into H_2O and O_2

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Glutathione reductase (GR) maintains redox potential. It provides antioxidant defence systems to plants and participates in the cell's enzymatic and nonenzymatic oxidation-reduction processes. It is also H_2O_2 scavenger. Guaiacol peroxidase (GPX) reduces H_2O_2 into H_2O and O_2 , increasing GPX activity in rice under drought stress. The activity is universally studied and established as a good screening tool for tolerance characters (Bai et al. 2017; Luo et al. 2022). Dehydroascorbate reductase (DHAR) regenerates a pool of reduced ascorbate and detoxifies reactive oxygen species (ROS) (Gill & Tuteja,2010; Do et al 2016). Monodehydroascorbate reductase (MDHAR) protects against the damage produced by reactive oxygen species (ROS) (Das & Roychoudhury,2014; Sharma & Dubey, 2005).

1.4.2 Nonenzymatic Antioxidant System

The strong antioxidant system is significant in enduring severe dehydration stages faced by plants and encountered in the revival of plants. Both osmolytes and non-protein antioxidants with higher levels under drought stress reprogrammed their metabolism and augmented their antioxidant capacity (Jan et al. 2022). Furthermore, the antioxidant activity is important during acute drought stress and interferes with recovery from water limitation and reappearance from dehydration (Laxa et al. 2019). Nonenzymatic antioxidants include Ascorbate (AsA) and Glutathione (GSH) (Ahmad et al. 2009). Also include Melatonin, Polyamines that involve in the degradation of chlorophyll and maintain physiological activities (Tiwari et al. 2021), and induced oxidative damage in rice under drought stress (Fig.1)

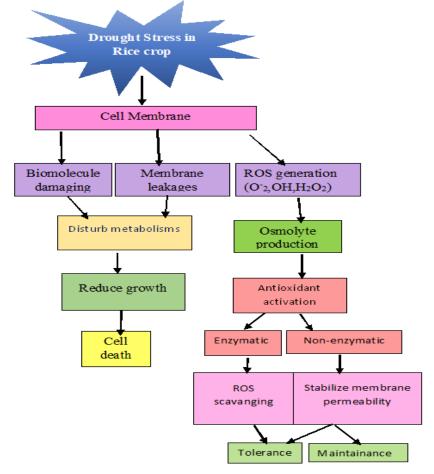


Figure 1: The different mechanisms by cell wall adopted by rice crops under drought stress

1.4.3 Drought Stress Develop Adaptations in Rice through Multi-omic Techniques.

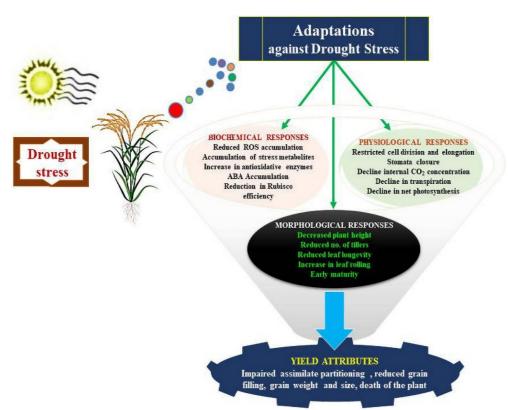


Figure 2 Adaptive features induced by Drought Stress in Rice [Zargar et al. 2022].

Drought stress response and adaptation in plants involves an array of pathways for signal perception, transduction, gene expression and synthesis of protein and other stress metabolites (Lata et al. 2015). Recent advancements in drought stress-related data support the role of omics technologies in understanding and influencing the mechanical development of plants in several crop varieties that are resilient to drought stress. Most of these studies have demonstrated the critical role of post-transcriptional and post-translational modifications of proteins in defensive mechanisms that allow the plant to adapt to a diverse range of abiotic stressors (Miller et al 2010;, Bernier et al 2004). Zargar et al. (2022) reported self-defence induced under drought Stress in Rice (Fig.2).

1.4.4 Genomic Responses under Drought Stress of Rice

Chromosome and heme proteins are involved in genomics studies. Chromosomes have specific positions for genes on which genes are located, called locus (plural loci) (Swamy & Kumar, 2013). Different genes are responsible for drought tolerance against stress in Rice (*Oryza sativa* L.). Drought-responsive gene scans are classified into two groups, as shown in Fig.(3). Some genes provide tolerance through osmotolerance, and some through signal transduction mechanisms. But some genes provide tolerance by ABA signalling.

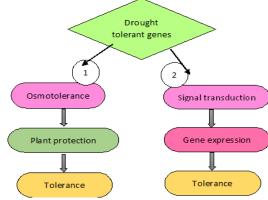


Figure 3 Group of Drought tolerant genes shows tolerance by two different mechanisms.

1.4.5 Abscisic acid (ABA) activates Drought Responsive Genes through Signal perception.

These are the OsSYT-5 gene that produces more yield during water deficit conditions by ABA signalling (Shanmugam et al. 2021). Zhang et al. 2006). Plants manifest various biochemical, molecular and physiological changes in response to drought stress. Level of ABA increase under drought, leading to the activation of several stress-responsive genes

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(Schraut et al., 2005; Bhargava & Sawant, 2013). ABA reduces transpiration rate by reducing stomatal conductance and gradually upsurges hydraulic conductivity, and inspires root cell elongation, permitting plants retrieval under drought conditions. The OsSYT-5 gene inhibits the growth promoters that encode Ca^{2+} in Rice. It promotes photosynthesis but reduces stomatal conductance and transpiration under drought stress. ABA regulates drought-tolerant gene expression (Shanmugam et al., 2021; Teng et al., 2014; Panda et al., 2021; Mahdieh & Mostajeran, (2009); Lian et al. (2006); Qureshi et al., 2018)

1.4.6 Nitrogen Transporters induce Drought Tolerance by ABA signalling, Promote Nitrogen uptake in Rice

OsNAR2.1 Transporter involves in Nitrogen uptake in the form of $NH^{15}_{4}NO_3$ and OsNAR2.1 overexpress under drought stress. OsNAR2.1 also enhance photosynthesis under water deficit condition. Ammonium uptake increased by OsNAR2.1 but decreased with the treatment of OsNAR2.1RNAi. Furthermore, OsNAR2.1 only involves nitrogen uptake but doesn't maintain a ratio of $^{15}NH_4^+$ to $^{15}NO_3^-$ (Chen et al., 2019; Parent et al., 2009.). Plants consume nitrogen for their grain formation under water deficit conditions. Therefore, it provides a tolerance strategy against abiotic stress like drought stress in rice (Fig.4) (Ding et al. 2016; Feng et al. 2011).

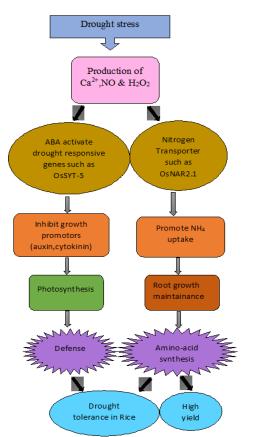


Figure 4 shows Nitrogen uptake & ABA activates drought genes to provide tolerance in Rice Crops.

1.4.7 Breeding Techniques Understand Molecular Mechanisms

Molecular breeding techniques include Marker Assisted Selection (MAS), Genome-wide assisted selection (GWAS), SNP marker application, Genome editing Technologies like CRISPR Cas, Quantitative trait loci (QTL_s)etc. (Zargar et al, 2022). All these are used to understand molecular mechanisms (Fig.4). Quantitative trait loci (QTL_s) such as AQHP069, CQAI49, AQAN001, AQHP079, AQHP082 were identified to resolve drought stress, responsible for high grain yield (Fleury et al. 2010, Bernier et al. 2004, Venuprasad, et al 2009, Zargar et al 2022). Quantitative trait loci (QTL_s) are selected based on Genomic background, Climatic condition etc. (Zargar et al. 2022). These techniques will be further modified and used in the upcoming year to understand molecular mechanisms better.

1.4.8 Transcriptomic Techniques Induce Tolerance under Water Deficit Condition

Transcriptomics involves the study of total transcripts found in an individual's cell. Under drought stress, Rice varieties' situation was identified through RNA-sequencing (Xiong et al. (2014). Transcriptomic investigation of rice under water deficit conditions could help to elucidate the possible molecular switches adapted by the Rice crop (Gould et al. 2018; Zargar 2022)._Transcriptomic techniques include differential display reverse transcription polymerase chain reaction (DDRT-PCR). Numerous methods, including Serial analysis of gene expression(SAGE), Quantitative real-time PCR (QRT-PCR), RNA sequencing (RNA-seq), Microarrays, and whole Transcriptome analysis, are used to identify

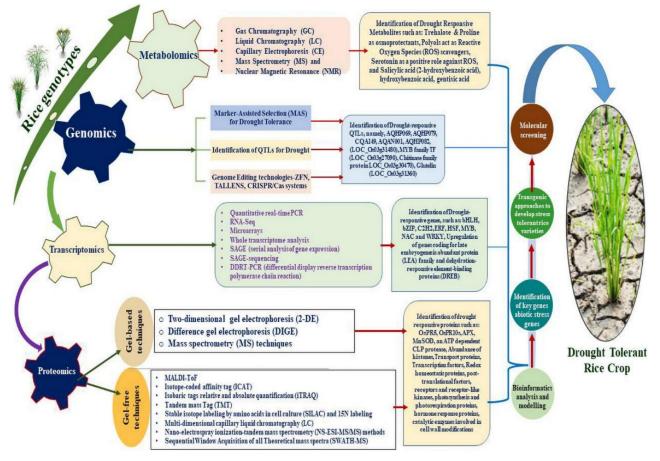
drought-responsive genes and proteins. These Transgenic approaches develop stress-tolerance varieties of rice. Nowadays, researchers use these techniques strongly to avoid abiotic stresses.

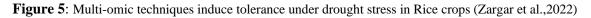
1.4.9 Proteomic Approaches Produce Tolerance Against Drought Stress

Proteomics involves the study of total protein found in an individual's cell. Proteins are found in biological systems influenced by abiotic factors such as Temperature, Light, Drought, salinity, etc. This review focused on drought stress; therefore, under water deficit conditions in rice (*Oryza sativa*), a wide range of protein i-e genomes is produced responsive to drought. Almost 31 drought-responsive proteins have been identified in crop plants (Raorane et al., 2015; Muthurajan et al., 2011; Zargar et al., 2022). A tandem mass tag (TMT)-based proteomic approach was applied to compare the roots and its near-isogenic lines(NIL) harbouring QTLqDTY12.1, a large effect QTL for rice yield under drought (Rahayu et al., 2005; Zargar et al., 2022). Comparative Proteomic analysis is used to understand the metabolic networks that root proteomes regulate in wild-type and transgenic rice plants (Zargar et al., 2022). Proteomic studies based on Gel based techniques and Gel free techniques involve identifying drought-responsive proteins, and transcriptional factors, maintaining redox homeostasis, and creating a tolerance against drought stress in rice.

1.4.10 Metabolomic Techniques Induce Drought Tolerance in Rice

Metabolomics involves the study of metabolites found in the cell of an individual. These metabolites are found in a biological system collectively known as Metablomes. Metabolomic techniques identify gene function determination and phenotyping of genetically modified plants. Metabolomic techniques include Nuclear Magnetic Resonance images (NMR), Mass Spectrometry (MS), Chromatography, and Electrophoresis used to understand the molecular mechanisms under drought stress. Drought-responsive metabolites and ROS scavengers identified through Metabolomic techniques create a tolerance strategy in Rice crops against abiotic stresses (Lawas et al., 2018; Michaletti et al., 2018).





1.4.11 Nitrogen Improves Rice Yield

Nitrogen deficiency is the most common problem in Asia, where modern rice varieties are grown with insufficient nitrogen minerals (<u>Rice Knowledge Bank,2019</u>). Their excessive amount is also a big problem resulting in reduced stem strength (<u>Rice Knowledge Bank,2019</u>). Rice yield is 150 Kg/ha without nitrogen but with nitrogen treatment. The rate exceeds 150 kg/ha (Harrell et al. 2011). Under the vegetative stage, nitrogen promotes Rice growth. Still, under the reproductive stage, nitrogen involves grain formation because it is the main constituent of amino acids and protein (Fig.

5 & 6). Different concentrations of Nitrogen application in rice diminish economic returns suggesting less influence of the cultivar on optimum fertilizer rates than originally anticipated (Zhao, et al 2022).

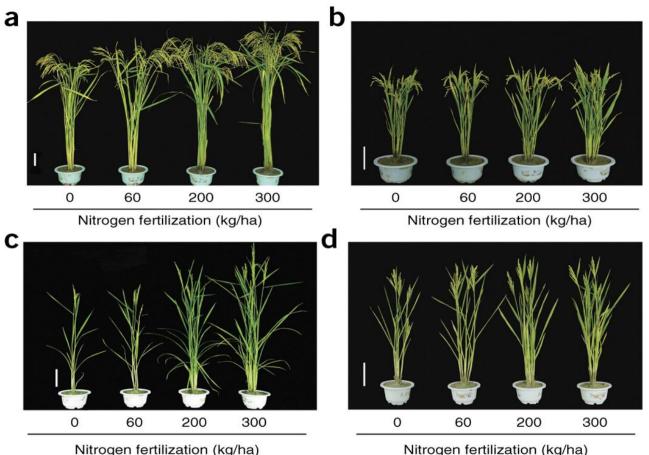


Figure 6: Nitrogen fertilizers application under drought conditions in Rice crops (Sun, et al., 2014)

Conclusion

Multi-omic technologies induce drought tolerance in rice through different techniques, including Quantitative trait loci, Nuclear Magnetic Resonance images, Chromatography, Electrophoresis, etc. These are involved in identifying Metabolites, Drought responsive key genes (found in the rate-limiting step), and determination of QTL sand to provide tolerance in Rice crops by molecular screening.

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Conflict of interest

No conflict of interest among Authors

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