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ASSESSING SALINITY STRESS TOLERANCE IN RICE: A REVIEW OF SCREENING METHODS AND BREEDING APPROACHES

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Abstract

The salinity stress poses one of the most serious problems in the global rice production, affecting the yield and quality of rice. This review article systematically covers the current status of salinity stress tolerance in rice, highlighting various screening methods and breeding approaches. Traditional screening techniques, such as field-based, hydroponic, and in vitro methods, are all addressed and their advantages and disadvantages are discussed. Furthermore, review goes into the modern screening systems like molecular markers and high-throughput phenotyping, which are powerful tools for the rapid identification of salt-tolerant genotypes. Breeding methods, from the traditional hybridization and selection to the cutting-edge transgenic and genome editing techniques, are discussed in the chapter, showing the successful cases of salt-tolerant rice varieties. Finally, the review is wrapped up when key findings are summarized and future research directions are recommended. Particularly, integration of traditional knowledge with modern technologies is encouraged for the development of sustainable solutions to salinity stress in rice and the assurance of a food secure world.

Keywords: Salinity stress, Rice, Screening methods, Breeding approaches, Salt tolerance.

Introduction

Salinity stress is a serious abiotic stress which is a major concern to rice production throughout the world, as it is estimated to affect about 20% of the world's irrigated lands, and poses a threat to the global food security (Munns & Tester, 2008). This stress that is caused by the accumulation of excessive salts in the soil is in the nature of disrupting the essential physiological and biochemical processes in rice plants, leading to reduced growth, yield and quality of rice grains (Mahajan & Tuteja, 2005). With the increasing global population, the demand for rice is also growing, therefore, it is vital to create salt-tolerant rice varieties that can survive in saline environments and contribute to sustainable agriculture. The conventional breeding techniques that has so far been used to introduce salinity tolerance has largely been phenotypic selection where one had to study how the plants react to salinity. However, the latter is slow, intensive and is highly likely to be affected by several factors in the environment, thus makes the result unpredictable (Gregorio et al., 2002). In the last years, for research in molecular biology and genomics has advanced rapidly to provide new rice breeders with new tools and techniques for the development of salt-tolerant varieties of rice. These current techniques include but are not limited to MAS (marker-assisted selection), transgenic approach and CRISPR-Cas9 which is a genome editing technology (Ismail & Horie, 2017). The effectiveness of these breeding methods in the achievement of the goal depends on the ability to identify and select high performing genotypes that have higher salinity tolerance. Thus, the rapid screening of subs trains for markers of salt tolerance is the way to shortening the time necessary for breeding and ensuring that the tolerant rice varieties reach the farmers on time (Flowers et al., 2010). The current review article will only provide a concise approach on the various screening and breeding techniques and their newer approaches for identification of salinity stress tolerance in rice and their future trend.

Importance of developing salt-tolerant rice varieties

The ability to grow rice on saline land is, therefore, critical for increased production in response to the increasing global demand and food security in view of climate change atmosphere degradation (Ismail & Horie, 2017). Growing salinity stress in the arable land affects several traditional rice varieties much with an incurred several negative impacts resulting from high salt concentrations hence reduced yield rates (Gregorio et al., 2002). The salt-tolerant varieties can grow in saline areas as they relish to saline environments, which is a suitable technique of growing rice in water logged areas (Flowers et al., 2010).

Understanding Salinity Stress in Rice

Physiological and biochemical responses of rice to salinity stress

High salinity stress results in the complex of changes at physiological and biochemical levels in the rice plants, affecting the different processes of their growth and development. These responses are multilateral and involve a number of elaborated processes which allow the plant to survive in the aggressive saline conditions. In the physiological level, salinity stress affects the osmotic concentrations in cells of the plants, thus the water relation and ion homeostasis are distorted (Munns & Tester, 2008).

In response to the biochemical changes caused by salinity stress, rice plants initiate several defense strategies to produce desired changes. One of the most significant responses is the synthesis of compatible solutes that will maintain cell osmotic potential and protect part of the cell from damage: for example, proline, glycine betaine, and sugars (Chen & Murata, 2011). Readily, salinity stress produces ROS that can cause oxidative damage to the plant tissue, but the plant organs develop antioxidant defence mechanisms such as SOD, catalase and peroxidase to neutralise these toxic substances (Gill & Tuteja, 2010).

Effects of salinity on growth, development, and yield of rice

The effects of salinity stress become observed in many activities within the growth and development of the rice plant leading to yield reduction. Some of the ways salinity negatively affects plants include; reduced germination rates during germination and in early seedling stages, less vigor and delayed emergence (Lutts et al., 2016). Most of these effects are osmotic stress because it limits water availability and compromises other metabolic processes critical for initial growth (Munns & Tester, 2008). These stress factors reduce shoot growth during the vegetative stage by minimising plant height, tillers, leaf size and biomass production in the plant (Negrão et al., 2017). This is partly due to osmotic stress, ion toxicity especially sodium and chloride ions, and nutrient derangement due to the ability to absorb nutrients (Roy et al., 2021). Photosynthesis an important process in plant growth and development is also influenced badly in high salinity because stomata closure, low chlorophyll content and damage to the photosynthetic apparatus (Liang et al., 2018).

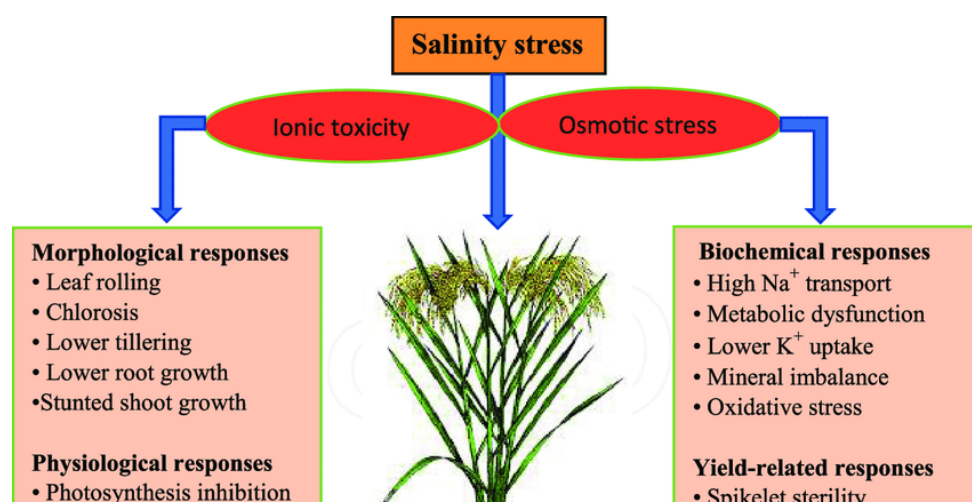


Figure 1: Schematic representation of physiological and biochemical responses of rice to salinity stress (Hasanuzzaman, 2022).

It elucidates all the aspects influenced by salinity stress in rice plant comprising morphological, physiological, biochemical and yield aspects. Salinity stress is divided into two main components: Ions toxicity and osmotic imbalance.

High level causes ionic toxicity especially increases concentration of Na^+ and Cl^- global biochemical effects comprises of increase in Na^+ transport, metabolic derangement, low K^+ uptake, alteration in mineral homeostasis, and oxidative stress. These biochemical changes are reflected morphologically in form and function through rolling of the leaves, chlorosis, reduced tillering, and Short shoots and roots.

Osmotically induced stress refers to the effects of high solute concentration in the environment outside parenchyma cells such as inhibition of photosynthesis, stomatal closure, low osmotic potential and low water potential. In order to oppose this, plants synthesise higher osmolytes. All these physiological changes in conjunction with morphological and biochemical changes resulting in yield-related responses such as reduced spikelet sterility, fewer florets per panicle, reduced grain weight, reduced harvest index and reduced biological yield.

Mechanisms of salinity tolerance in rice

Rice plants have evolved various mechanisms to cope with salinity stress, enabling them to survive and reproduce in saline environments. These mechanisms can be broadly categorized into three main types: tolerance, avoidance, and escape (Munns & Tester, 2008).

1. Tolerance mechanisms: These involve the plant's ability to withstand high salt concentrations within its tissues. This can be achieved through several strategies:

Osmotic adjustment: Accumulation of compatible solutes (e.g., proline, glycine betaine, sugars) in the cytoplasm to maintain cell turgor and protect enzymes and cellular structures from damage (Chen & Murata, 2011).

Ion exclusion: Selective uptake and transport of ions, particularly sodium (Na^+) and chloride (Cl^-), to minimize their accumulation in the cytoplasm and prevent toxic effects (Roy et al., 2021).

Tissue tolerance: Compartmentalization of toxic ions into vacuoles, older leaves, or sheaths, thus protecting actively growing tissues (Munns & Tester, 2008).

Antioxidant defense: The up regulation of enzyme and metabolites that help in the detoxification of the super oxide radicals that are synthesized under stress (Gill & Tuteja, 2010).

2. Avoidance mechanisms: These strategies are intended to avoid or at least reduce the absorption and depositing of salt within the vegetal cell. This can be achieved through:

Root architecture modification: Inversion of root distribution patterns to plants and expansion of root system in the upper, less saline layers of the soil (Negrão et al., 2017).

Salt secretion: Efflux of osmotic excess through salt glands or bladders (Flowers et al., 2010).

Reduced transpiration: Reducing transpiration and uptake of toxic concentration of salt by rolling of the leaves or closing of the stomata.

3. Escape mechanisms: These include either the completion of the life cycle before the development of a severe salinity stress or the production of seeds able to germinate at high salt concentrations (Flowers et al., 2010).

First, salinity tolerance in rice has been reported as a multi-gene and a multi-phenotype trait which may or may not be differentially effective depending on the type of genotype of the rice plant, the specific environment of either growth chamber or field, and developmental stage of the plant (Roy et al., 2021). Such mechanisms come in handy when it comes to

breeding salt tolerant rice varieties that would stand the occasion due to the on- Going salinization of fertile agricultural soils.

Conventional Screening Methods for Salinity Tolerance

Field-based screening

Field based screening is an efficient strategy for selection of resistance to salinity stress in rice germplasm. It entails using naturally or preferentially saline field or plots to grow selected rice genotypes in order to allow near nature interactions (Ahmadi et al., 2022). The performance of the various genotypes is thus a product of agronomic factors such as; fate, growth characteristics; plant height, tiller numbers and leaf area, yield component; panicle plus grain weight; grain yield under saline regime (Roy et al., 2021).

Hydroponic screening

Hydroponic screening is a controlled environment method widely used for assessing salinity tolerance in rice. It involves growing rice seedlings in nutrient solutions with varying levels of salinity, typically achieved by adding NaCl to the solution (Negrao et al., 2017). The seedlings' growth and physiological responses are then monitored and compared to identify tolerant genotypes.

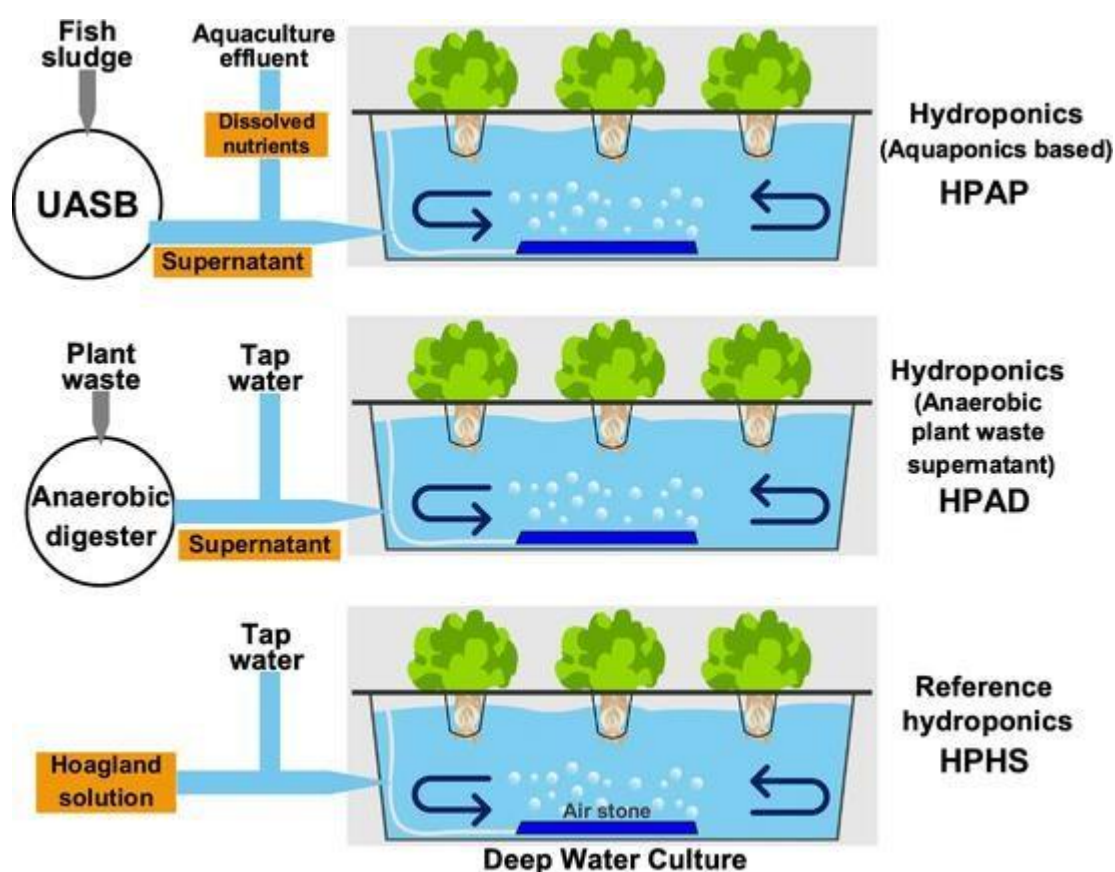


Figure 2: Schematic representation of three hydroponics treatment systems and the reference hydroponics setup (Zhu et al, 2023).

The figure shows three types of hydroponic systems intended for treating and recycling waste products produced in fish farming (aquaculture) and plant growing. Both systems use the nutrient rich effluent or supernatant, which results from the waste treatment processes for fertilizing hydroponically grown plants.

The first system, HPAP (Hydroponics (Aquaponics based)), employs nutrient solution derived from the supernatant of UASB (Upflow Anaerobic Sludge Blanket) reactor treating fish sludge. The second system, HPAD (Hydroponics (Anaerobic plant waste supernatant)), applies the supernatant from an anaerobic digester treating plant waste regarding the same objective. The third system is the standard one, the Reference hydroponics or HPHS where nutrient source is the Hoagland solution. All three systems utilize the DWC system in which root of plant is immersed in nutrient solution and air stone for aeration. Accompanying this figure is a new method in practicing efficient agricultural system with emphasis on waste recycling and lowered need for external fertilizers.

Invitro Screening

The application of in vitro screening technique has therefore proved more relevant in the screening for salinity tolerance in rice than field and hydroponic methods. It encompasses culturing rice tissues including callus, embryos or even seedlings on basal media containing different concentration of salts (Lutts et al., 2016). This makes it easy to manipulate the salinity and analyze all physiological and biochemical changes at the cellular and molecular level (Negrão et al., 2017).

Table 1: Comparison of Conventional Screening Methods for Salinity Tolerance in Rice.

Screening Method	Advantages	Disadvantages	Applications
Field-based	Realistic assessment, Holistic evaluation, Direct selection	Time and resource intensive, Environmental variability, Limited control	Validation of promising genotypes, Final evaluation of performance under field conditions

Hydroponic	Precise control of salinity, Reduced space and time, Ease of observation and measurement	Artificial environment, Resource intensive, Limited root development	Early-stage screening, Physiological studies, Mechanistic studies
In vitro	Rapid and high-throughput, Controlled environment, Mechanistic studies, Genetic transformation	Artificial conditions, Genotype dependence, Limited traits assessed	Early-stage screening, Biochemical studies, Molecular studies, Genetic studies

Table 1 presents a comparative analysis of three conventional screening methods for salinity tolerance in rice: It can be field-based, hydroponic and In-vitro. Both methods are defined by the specific strengths and weaknesses of the applied techniques and their application in research and breeding work. Field screening is effective at providing details into the performance of plants under natural conditions, but is time consuming and depends on the conditions under which the plants are grown. Screening for wheat under hydroponic system is an efficient way of maintaining and controlling the salinity of the growing solution conducive for physiological investigations but the conditions may not be closer to field conditions. A rapid and high throughout technique it is suitable for mechanism and genetics but data obtained may not reveal the whole plant performance in the field. The table works as a manual where depending on the targets and available resources, the proper method for screening is chosen by the researchers and breeders.

Tissue Culture Techniques:

Various tissue culture techniques can be employed for in vitro screening of salinity tolerance in rice:

1. **Callus Culture:** Rice callus is induced from the explants such as mature embryo or young leaf and maintained on basal media containing appropriate concentration of salt. Tolerance is based on the measurement of relative growth rate and callus morphology at various concentrations of salinity (Lutts et al., 2016).
2. **Embryo Culture:** Rice embryos are removed and incubated on basal media with different salt concentrations. In the present study, germination percentage, seed vigor, and seedling physiological attributes were measured to assess the salinity stress tolerance.
3. **Suspension Culture:** Sterile water with salt is added to the rice cells and their growth, viability, and biochemical analysis in media are studied (Roy et al., 2021).
4. **Anther Culture:** Its mature anthers with pollen grains are cultured leading to haploid plants that can be used in screening for salt tolerance (Negrão et al., 2017).

5. Protoplast Culture: Protoplasts (plant cells without cell walls) are isolated and cultured in the presence of salt to study their responses and identify tolerant lines (Roy et al., 2021).

In vitro screening, when combined with other methods, can provide valuable insights into the genetic and physiological basis of salt tolerance in rice. This knowledge can then be used to develop improved breeding strategies and accelerate the development of salt-tolerant rice varieties.

Modern Screening Methods for Salinity Tolerance

Molecular markers

Molecular typing has been influential in plant breeding in particular due to the important role it plays in the selection of useful characteristics, such as tolerance to salt accumulation in rice. These markers are DNA sequences containing variation between individuals or populations so that they are known to be polymorphic (Collard & Mackill, 2008). Since different markers point towards specific segments of a gene, breeders are able to get an estimate of the genetics of the same individual and the likelihood it will express certain characteristics (Jena & Mackill, 2008).

There are various types of molecular markers used in rice breeding for salinity tolerance, including:

- Simple Sequence Repeats (SSRs): SSRs – short for simple sequence repeat – are 1 to 6 base pair DNA sequences that are repeated multiple times. They are present in large numbers and are dispersed literally over the entire genome, which makes them useful for genetic relationships, mapping, and MAS (McCouch et al., 2016).
- Single Nucleotide Polymorphisms (SNPs): SNPs are one nucleotide changes in the DNA sequence. They are by far the most numerous type in the genome and thanks to their density and easy genotyping have recently gained a lot of attention in genetic researches and MAS (Thomson et al., 2012).
- Insertion/Deletion (InDel) Markers: These are small-scale resulting from the insertions or deletions in the DNA sequences between two specified genes. They are moderately frequent and highly polymorphic owing to which they are often employed for genetic mapping and for assessing the diversity (Gupta et al., 2002).

Molecular markers offer several advantages in the screening and breeding of salt-tolerant rice:

- **Early Selection:** Molecular markers linked to salinity tolerance can be employed to identify seedlings or the young plants to enhance the choice of tolerant genotypes and time as well as capital intensive salinity field tests (Jena & Mackill, 2008).
- **Precise Selection:** Molecular markers are more accurate and efficient as bio-metric DNA tags to select for a gene governing salinity tolerance or QTLs than the phenotypic selection method.
- **Increased Efficiency:** The use of molecular markers can significantly increase the efficiency of breeding programs by accelerating the development of salt-tolerant varieties (Thomson et al., 2012).

However, the development of high-throughput sequencing and genotyping by sequencing (GBS) technologies in the past few years had provided additional opportunities of effectively using molecular marker in rice breeding for salinity tolerance.

Marker-assisted selection for salinity tolerance

MAS is a worldwide breeding method that employed molecular markers to choose individuals with important traits such as salinity tolerance in rice (Collard & Mackill, 2008). This approach involves selection of DNA markers that are genetically in linkages with genes or QTLs controlling salt tolerance and use these markers to select plants with the preferential alleles (Jena & Mackill, 2008).

The process of MAS for salinity tolerance typically involves the following steps:

- **Identification of markers:** The identification of DNA markers that are closely linked to genes or QTLs already known to provide salt tolerance in rice. This can be done by use of different strategies including the linkage mapping, candidate gene association and Genome-wide association studies (GWAS) (Ismail & Horie, 2017).
- **Development of marker assays:** Afterwards, it is possible to design the assays for indicating the presence of specific alleles at these markers. This can be done with one or techniques including the polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and single nucleotide polymorphism (SNP) genotyping (Collard & Mackill, 2008).

- Genotyping of breeding populations: The developed marker assays are applied to score a large number of individuals in a breeding population, most probably seedlings or young plants (Jena & Mackill, 2008).
- Selection of superior genotypes: Those possessing the desired alleles in the identified markers are elite, to be used for further breeding depending on their perceived salinity tolerance (Collard & Mackill, 2008).

- **High-throughput phenotyping**

High-throughput phenotyping (HTP) has recently been recognized as a breakthrough technology for phenotyping in regard to salinity stress tolerance in rice because it allows for quick and accurate assessment of phenotypic parameters under saline environmental conditions (Campbell et al., 2023). This integrated strategy applies point technologies to acquire phenotypic data for multiple genotypes at once, enhancing tenfold the speed at which salt tolerance traits and genes are being discovered (Roy et al., 2021).

Cultivar phenotypes are measured using different non-destructive sensors and imaging techniques at HTP platforms, these include growth rate, biomass accumulation, chlorophyll content, stomatal conductance, and photosynthesis rates etc. These platforms can be used for experiments carried out in growth chambers or greenhouses or for experiments under field conditions that yield a comprehensive appraisal of salinity tolerance under both environments (Campbell et al., 2023).

Table 2: Examples of Salt-Tolerant Rice Varieties Developed Through Different Breeding Approaches

Variety	Breeding Approach	Origin	Salient Features
FL478	Marker-assisted backcrossing	India	High yield potential under saline conditions, derived from Pokkali landrace
CSR 10, CSR 13, CSR 27, CSR 30	Conventional breeding, marker-assisted selection, mutation breeding	India	Good adaptability and yield potential in different salt-affected regions of India
BRRI Dhan 47, BRRI Dhan 50, BRRI Dhan 51	Conventional breeding	Bangladesh	Improved yield potential and grain quality in saline coastal areas of Bangladesh
IR 64-Sub1	Submergence tolerance breeding with moderate salt tolerance	IRRI (Philippines)	Widely adopted in flood-prone areas, shows promise in saline conditions
CSR36	Conventional breeding and marker-assisted selection	IRRI (Philippines)	High-yielding variety developed for saline-prone areas in Kenya

Table 2 displays achieved breeding strategies and salt-tolerant rice varieties released in various parts of the world. It is also the method under which each of the varieties was developed and a brief description based on data as to the tolerance to salinity and yield of each of the varieties. Some released varieties are from India (FL478, CSR series), from Bangladesh (BRRI Dhan series), and the International Rice Research Institute, the Philippines (IR 64-Sub1, CSR36). This table shows the various mitigation measures that can be used to cope with the salinity stress in growing rice and the success achieved when those approaches were used to develop rice varieties that are suitable for different particulars of various states.

Chlorophyll Fluorescence:

Fluorescence of chlorophyll is a non-invasive technique to measure the efficiency of photosynthesis and stress in plants (Baker, 2008). It measures the amount of light returns to the chlorophyll molecules as it looks for a light energy organism. During stress situations such as salinity, the photosynthetic system is impacted resulting to changes in chlorophyll fluorescence patterns (Baker, 2008). Such changes may be expressed in terms of some performance and stress-related descriptors like maximum quantum yield of photosystem II (Fv/Fm), which presents a given plant's integrated efficiency of photosynthetic machinery and its condition (Baker, 2008). Measuring of chlorophyll fluorescence is used to assess the intensity of stress response in large plants and hence has practical aspects of high throughput screening of salinity tolerance in rice (Roy et al., 2021).

Hyperspectral Imaging:

Other non-destructive techniques are hyperspectral imaging which is the imaging of plants across a range of wavelengths in the visible and near invisible near infrared regions (Humplík et al., 2015). From the spectra reflectance by plants, biochemical composition, physiologic state and stress levels can be described by Humplík et al., (2015). For example, alteration in pigment concentration, water state and cell organization affected by high salinity level using hyperspectral imaging (Roy et al., 2021).

Advantages of Non-Destructive Methods:

- **Rapid and High-Throughput:** Both chlorophyll fluorescence and hyperspectral imaging allow for rapid assessment of large numbers of plants, enabling high-throughput screening of salinity tolerance.
- **Non-Invasive:** These methods do not harm the plants, allowing for repeated measurements over time to monitor the dynamic responses to salinity stress.
- **Early Detection:** Non-destructive methods can detect early signs of stress before visible symptoms appear, enabling timely interventions and selection of tolerant genotypes.

Molecular technologies collectively known as omics have become instrumental in elucidating the intricate processes governing rice salt tolerance. These approaches occupy the large scale analysis of genes (genomics), transcripts (transcript omics), proteins (proteomics) and metabolites (metabolomics) for the systematic understanding of molecular level responses of the rice plants under salt stress (Roy et al., 2021).

1. **Genomics:** Genomics is concerned on the discovery and analysis of genes that play role in salinity tolerance in rice. This includes: GWAS for finding the genetic bases for salt tolerant traits-Comparative genomics for finding the differentially expressed genes between salt and salt sensitive varieties (Ismail &Horie, 2017). Molecular techniques have identified a plethora of candidate genes associated with ion transport and osmotic regulation, and stress-responsive pathways (Roy et al., 2021).

2. **Transcriptomics:** Transcriptomics studied the alterations in the gene expression due to salinity stress. This is done normally employing microarray or RNA sequencing technologies to assess the total amount of mRNA transcripts in different tissues or in different saline concentrations (Negrão et al., 2017). Different salt stress responsive genes have been both suggested and discovered through transcriptomic analysis, with intricate gene regulatory networks tuned into salinity response mechanisms in plants (Roy et al., 2021).

3. **Proteomics:** Proteomics investigates the changes in protein abundance and modifications that occur under salt stress. This involves techniques like two-dimensional gel electrophoresis (2D-GE) and mass spectrometry to identify and quantify proteins (Negrão et al., 2017). Proteomic studies have identified proteins involved in various stress response pathways, including ion transport, antioxidant defense, and osmoprotection (Roy et al., 2021).

4. **Metabolomics:** Metabolomics focuses on the analysis of metabolites, small molecules that are products or intermediates of metabolism. By measuring the abundance of metabolites under salt stress, researchers can identify metabolic pathways and biomarkers associated with salinity tolerance (Negrão et al., 2017). Metabolomic studies have revealed changes in the levels of amino acids, sugars, organic acids, and other metabolites in response to salinity stress, providing insights into the plant's metabolic adjustments to cope with the stress (Roy et al., 2021).

Integrating omics data for a comprehensive understanding of salinity tolerance mechanisms

To define a global system regarding stress tolerance to salinity stress in rice, it is necessary to integrate genomic, transcriptomic, proteomic, and metabolomic datasets. Similarly, synthesis of information originating from these organisational levels helps to define gene–transcript–protein–metabolite correlations involved in plant response to salt stress (Roy et al., 2021).

For instance genes linked to salinity tolerance if identified through genomics may be studied using transcript omics to identify stress responses (Ismail &Horie, 2017). Similarly, Proteomics can determine arrays of proteins coded by these genes and differentially abundance or post-translational changes under saline stress (Supplementary Table S1, Negrão et al., 2017). Metabolomics will then identify the pathways as governed by these genes and proteins and demonstrate that there are changes in the concentrations of the metabolites involved in salt tolerance (Roy et al., 2021).

Protein-protein interactionbased study also help to acquire the knowledge of regulatory networks and central orthogonal modules related to various aspect of salinity tolerance other than ion homeostasis, osmotic stress and oxidation stress (Roy et al., 2021). These can then be used to develop systems biology model of plant reaction to the salt stress and the changes this brings due to genetic modification or other changed condition (Ismail &Horie, 2017).

Several approaches have been used to integrate omics data, including:

Correlation analysis: This involves linking two or more datasets; for example gene expression profile with metabolomics to find out relationship between different layers of molecular regulation (Negrão et al., 2017).

Network analysis: This approach is for determining the specific interactome of gene and gene, protein and protein, and metabolite and metabolite for the identification of potential regulators and pathways of salt tolerance (Roy et al., 2021).

Systems biology modelling: This includes the incorporation of the omics data into mathematical models in which the plant's response can be modeled during salt stress and the consequences of genetic or environmental alterations (Ismail & Horie, 2017).

Combining omics data helps researchers to identify key points in regulating the stress response network responsible for salinity tolerance in rice.

Breeding Approaches for Salinity Tolerance

Traditional breeding

Long-term conventional breeding which serves as the basis of crop improvement has greatly contributed to the release of new improved rice varieties with higher yield, per darker value and resistance to a number of biotic and abiotic threats. In a salinity stress, the conventional breeding relies on the two techniques of hybridization and mutation whereby the plant breeders have to cross genetically distinct parent to develop offspring that have a blend of the desired characters, though the breeding lines are selected based on their performance under saline conditions (Gregorio et al., 2002).

Hybridization and Selection:

Hybridization process includes the crossing of two or more of the rice varieties with varying tolerance limits to salinity. This can however be achieved through artificial pollination of the plant through emasculating the plant by taking pollen from one parent and placing it on the stigma of another parent, or through such techniques as embryo rescue and protoplast fusion. The crossbred progeny are known as hybrids, which have an added mix of genes from the two parent plants thus leading a break through new gene/ traits combos and enhanced species/fractional salinity resistance.

Limitations and Challenges:

While traditional breeding has been successful in developing some salt-tolerant rice varieties, it faces several limitations and challenges as Time and Resource Intensive, Limited Genetic Diversity, Complex Inheritance, Environmental Influence and Linkage Drag.

Modern breeding

There is evidence that through modern breeding techniques there are great prospects of developing salt tolerant rice for increased food production due to salinization of arable land due to irrigation and other human induced factors. These techniques use information and knowledge from molecular biology, genomics and genetic engineering to improve the traditional breeding apart from improving the efficiency.

Marker-Assisted Breeding (MAB):

MAB is a very effective device that involves the use of genetic links with various characteristics that enables the up scaling of the selection process and at the same time increases the efficiency of choosing the right genotype (Collard & Mackill, 2008). Regarding the issue of salinity tolerance, MAB aims at establishing the DNA markers associated with genes or QTLs responsible for salt tolerance in rice. These markers can be applied on a very large number of seedlings or young plant populations in which the salt tolerant ones can then be selected without going through the rigours of field trials (Jena & Mackill, 2008). This in essence; cuts the time, effort and capital needed to produce salt-tolerant varieties; thus; making it a better model than phenotypic selection.

Transgenic Approaches:

Transgenic techniques are used in this context to incorporate genes that have traits that allow expression of salt tolerance in the rice plants. This technique makes it possible to introduce other desirable characteristics from other species such as the halophytes into rice without being constrained by the usual breeding techniques. Some of the ion transport related, osmoprotective, and antioxidant genes have been successfully introduced into the rice which impart salt tolerance under both controlled and field environment (Roy et al., 2021).

Genome Editing (CRISPR-Cas9):

In recent years, more advanced techniques to make alterations in the rice genome specifically have been developed with the help of CRISPR-Cas9 method (Zhang et al., 2014). This

Technology	Potential Applications
CRISPR-Cas9	Targeted genome editing for enhancing salt tolerance traits, Creating novel genetic variations for salt tolerance
High-throughput phenotyping	Rapid and accurate assessment of salinity tolerance in large populations, Identification of novel salt tolerance traits and genes
Omics technologies (genomics, transcriptomics, proteomics, metabolomics)	Comprehensive understanding of molecular mechanisms of salt tolerance, Identification of key genes, pathways, and metabolites for targeted breeding
Machine learning	Predictive modeling of salinity tolerance based on phenotypic and genotypic data, Accelerated identification of superior genotypes
Synthetic biology	Engineering novel genetic circuits for enhanced salt tolerance, Developing stress-responsive synthetic promoters and genetic elements

technique enables the generation of desired single nucleotide changes either in addition or removal, thereby providing the user high levels of gene targeting. Even in the frame of the tolerance to salinity a lot has been done using CRISPR-Cas9 for editing the genes of ion transporters and osmotic adjusters and stress signaling in rice in order to enhance the salt tolerance in the plant (Li et al., 2018).

Table 3: Emerging Technologies and Their Potential Applications in Salinity Tolerance Research in Rice.

Table 3 presents a list of emerging technologies and their potential applications in enhancing salinity tolerance research in rice. CRISPR-Cas9 enables precise genome editing to directly modify genes for improved salt tolerance. High-throughput phenotyping allows for the rapid and accurate assessment of numerous rice varieties for salt tolerance, thereby identifying desirable traits and genes. Omics technologies offer a deeper understanding of the molecular mechanisms involved in salt tolerance, aiding in targeted breeding strategies. Machine learning enables predictive modeling, accelerating the selection of superior genotypes. Lastly, synthetic biology holds the promise of engineering novel genetic mechanisms and components for enhanced salt tolerance in rice.

Conclusion

Therefore, this review article systematically assessed salinity stress tolerance in rice including traditional and advanced screening techniques and strategies. Each of the field to molecular and high through-put techniques has its own merits and demerits how this justified the perception of a complex approach towards evaluating salinity tolerance. Although the emphasis on conventional selection strategies has been significant, present day procedures like MAS, TI, and GE are more accurate and effective. Combining of omics technologies has contributed to yield profound understanding of the molecular basis and genetic regulation underpinning salinity tolerance for genetic enhancement. This paper explored the wealth of new technologies including CRISPR-Cas9 for editing the gene and high-throughput phenotyping which could be of significance in the pursuit for salt-tolerant rice in the face of the escalating salinization as well as the enhancement of rice demand around the world.

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