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Enhancing abiotic stress tolerance in rice with the help of molecular markers

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Abstract
Abiotic stresses pose significant challenges to rice production worldwide, impacting yield stability and agricultural sustainability. In recent years, molecular markers have emerged as powerful tools for understanding the genetic basis of abiotic stress tolerance in rice and facilitating targeted breeding efforts. This review paper aims to consolidate and analyze the findings from various studies that have utilized molecular markers to enhance abiotic stress tolerance in rice. This review serves as a valuable resource for researchers, breeders, and policymakers involved in enhancing abiotic stress tolerance in rice. By elucidating the role of molecular markers in rice breeding, it contributes to the development of sustainable and stress-tolerant rice varieties, which are essential for ensuring global food security in the face of changing climatic conditions.

Keywords: Rice, abiotic stress, molecular markers

Introduction
Rice is an incredibly important crop that holds significant value for global food security and human nutrition. It serves as a staple food for more than half of the world's population, particularly in Asia, providing a primary source of energy and nourishment (Khush, 2013) [48]. Rice plays a crucial role in ensuring food security and sustenance for billions of people. Economically, rice cultivation is vital as it supports the economies of numerous countries, serving as a source of income for farmers and a major export commodity. The economic importance of rice cultivation helps alleviate poverty and fosters overall economic growth (Timmer, 2010) [43].

Abiotic stress in rice refers to the adverse environmental conditions that can negatively affect rice growth and development. These stresses include temperature extremes, drought, salinity, and nutrient deficiencies (Nakashima *et al*., 2014) [29]. Rice is particularly susceptible to these abiotic stresses, which can lead to reduced yields and compromised crop productivity.

Temperature stress, both heat and cold, can have detrimental effects on rice plants. High temperatures can disrupt various physiological processes, impairing photosynthesis and reproductive development (Wahid *et al*., 2007) [46]. On the other hand, exposure to low temperatures can lead to chilling injury and affect rice growth and yield (Jagadish *et al*., 2010) [49].

Drought stress is a major challenge for rice cultivation, especially in regions with limited water availability. Water deficit negatively impacts plant water relations, photosynthesis, and nutrient uptake, leading to reduced biomass and grain yield (Kumar *et al*., 2008) [50].

Salinity stress, caused by excessive salt in the soil, poses a significant threat to rice production, particularly in coastal and irrigated areas. High salt concentrations can hinder water uptake and disrupt ion balance in rice plants, impairing growth and development (Munns *et al*., 2006) [28].

Furthermore, nutrient deficiencies, such as nitrogen, phosphorus, and potassium, can limit rice growth and yield. Insufficient nutrient availability affects various metabolic processes and leads to stunted growth and nutrient-related disorders. To overcome the challenges posed by abiotic stresses in rice, extensive research is being conducted to develop stress- tolerant varieties through breeding programs. These efforts aim to enhance rice resilience to abiotic stresses and ensure sustainable crop production under adverse environmental conditions.

Molecular markers have emerged as valuable tools in enhancing abiotic stress tolerance in rice through breeding programs (Collard *et al*. 2005) [10]. Various types of molecular markers, including Single Nucleotide Polymorphisms (SNPs), Simple Sequence Repeats (SSRs), and Insertion/Deletion (In Del) markers, have been widely utilized in rice research. These markers aid in pinpointing specific genes or Quantitative Trait Loci (QTLs) linked to abiotic stress

tolerance. By analyzing the genetic variation in different rice accessions or breeding lines, molecular markers assist in unraveling the genetic basis of stress tolerance and identifying potential candidate genes for further investigation (Collard *et al*., 2005) [10].

The use of molecular markers has facilitated marker-assisted selection (MAS) in rice breeding programs. MAS involves selecting plants based on specific markers associated with desired traits, enabling more precise and efficient selection of stress-tolerant genotypes (Thomson, 2014) [42]. This approach has been successfully applied to develop rice varieties with enhanced tolerance to diverse abiotic stresses, including drought, salinity, and submergence etc. In conclusion, the utilization of molecular markers has revolutionized rice breeding for abiotic stress tolerance by enabling the identification of stress-related genes and QTLs (Collard *et al*., 2005) [10]. This advancement has significantly contributed to the development of stress-tolerant rice varieties, enhancing crop resilience and ensuring sustainable production under challenging environmental conditions.

Enhancing rice tolerance to drought stress

Molecular tools offer a promising approach to identify genes responsible for drought resistance and to develop new drought resistant plant varieties (Quarrie, S.A., 1996) [33]. Studies by Champoux *et al*., 1995 [6] discovered a genetic connection between root traits and the ability of rice plants to withstand drought. Verma and Sharma R.N., 2021 [21] found specific regions of the plant's chromosomes that contain genetic markers associated with both root characteristics and drought tolerance. By selectively breeding rice plants with desired root traits, such as thicker roots or higher root-to-shoot ratio, drought resistance in rice can be enhanced. Their research suggests that manipulating the genetic markers can improve root characteristics and contribute to developing rice varieties with stronger, drought-resistant roots. 114 rice genotypes were studied and significant variations were found in root and shoot traits under water stress conditions. Correlations were observed between shoot and root traits and 11 marker-trait associations were detected for root, shoot and drought tolerance traits. Notably, markers RM252 and RM212 showed potential for improving the root system. These findings offer insights for enhancing root and drought tolerance traits through marker- assisted selection (MAS) in rice breeding programs.

A study was conducted by Jalil *et al*., 2018 [18], to introgress root trait gene from aerobic rice variety, AERON1, to a high yielding rice variety of Malaysia, MRQ74, through MABB. The use of foreground markers RM242 and RM263 and background markers was done to track the introgression of desired root traits for drought tolerance. Development of drought tolerant rice lines through marker-assisted backcross breeding helped in understanding the importance of molecular markers in enhancing the ability of rice crops to tolerate abiotic constraints and accumulate development of new varieties.

Ghazy *et al*., 2021 [13], studied a diverse group of 22 rice genotypes which revealed a wide range of genetic variation. Certain traits such as grain yield and sterility percentage were strongly correlated under normal conditions. To understand the genetic factors behind these traits, the researchers conducted a genetic analysis using SSR markers. Specific regions called QTL were found in rice genomes to be associated with different yield traits under normal and drought conditions. Based on phenotypic traits, number of QTL and genetic diversity, five rice genotypes (Giza178, IET1444, GZ1368-S-5-4, Nahda, and Giza 14) were identified as most promising drought tolerant genotypes. Each genotype had specific QTL associated with drought tolerance. This information is valuable for future breeding programs as these genotypes can be used for crossing with other varieties to develop improved drought- tolerant rice varieties.

Degenkolbe *et al*., 2013 [51] studies aimed to identify drought tolerance in a diverse population of rice cultivars by analyzing gene expression and metabolite profiling which revealed potential markers of drought tolerance in rice. These markers included specific genes that were upregulated or down regulated in drought-tolerant cultivars as well as metabolites that exhibited significant changes in their levels under drought stress. These findings gave valuable insights and biochemical pathways involved in drought-tolerance in rice. By identifying these markers, breeders and researchers can better understand the genetic basis of drought tolerance and develop strategies to improve drought resistance in rice crops.

Drought resistance in 154 double haploid rice lines were studied by Zhnag *et al*., 2001 [52] to map the components of drought resistance, such as osmotic adjustment and root traits, a genetic linkage map was constructed using 315 DNA markers. 41 QTL associated with osmotic adjustment and root traits were identified. Each QTL explained a phenotypic variance, ranging from 8 to 38 percent. One particular region on chromosome 4 was found to contain major QTLs for multiple root traits. These QTLs were consistent across different genetic backgrounds, making them valuable for marker-assisted selection in breeding programs. By incorporating these QTLs into elite rice lines through breeding, breeders can enhance drought resistance.

Bernier *et al*., 2009 [4], focused on the study of a drought resistance gene in rice, known as qtl12.1. They conducted extensive evaluations to assess the impact of qtl12.1 on various traits and yield in different rice varieties and environments, specifically in the Philippines and India. The findings of the study revealed that the presence of qtl12.1 resulted in an average increase in yield of 30 percent under drought stress conditions. Additionally, qtl12.1 exhibited a positive influence on plant height, biomass production, harvest index, and panicle number. These results emphasize the potential of qtl12.1 as a valuable gene for enhancing drought resistance in rice breeding programs. To accomplish their objectives, the researchers employed molecular markers, specifically simple sequence repeat (SSR) markers. These markers served multiple purposes in the experiment. Firstly, they were used to confirm the presence or absence of qtl12.1 in the rice lines utilized for the field trials. By genotyping the rice lines with SSR markers, the researchers were able to determine whether qtl12.1 was present in each line. Secondly, SSR markers played a crucial role in identifying the flanking markers for qtl12.1 and estimating its physical size. This allowed the researchers to precisely locate qtl12.1 within the rice genome and determine its boundaries. Furthermore, the use of SSR markers facilitated the assessment of the genetic diversity and population structure of the rice lines. By analyzing the SSR marker profiles, they gained insights into the genetic variation present among the different rice varieties and populations under study. Moreover, they performed a linkage disequilibrium analysis utilizing SSR markers. This analysis helped to detect associations between qtl12.1 and yield traits. By examining the patterns of linkage

disequilibrium between qtl12.1 and other genomic regions, the researchers were able to identify potential genetic interactions and relationships that contribute to the observed traits. Overall, the utilization of SSR markers in this study was instrumental in confirming the presence of qtl12.1, identifying its genomic location, assessing genetic diversity, and investigating associations with yield traits. The integration of molecular markers, specifically SSR markers, enabled the researchers to comprehensively evaluate the effects of qtl12.1 on rice plants' performance under drought stress conditions and explore potential interactions with other genes and environmental factors.

Janaki *et al*., 2021 [19], aimed to enhance drought tolerance in rice by introgressing three major quantitative trait loci (QTLs) responsible for increased yield under drought stress (qDTY1.1, qDTY2.2, and qDTY4.1) from two donor parents (IR 96321-1447-561-B- 1 and IR 87707-445-B-B) into the recipient variety Naveen. The pyramided lines were then screened for reproductive stage drought tolerance in two test fields under severe drought stress, and their performance was compared with Naveen, QTL donors, two susceptible checks, and one tolerant check. The findings revealed that certain pyramided lines exhibited higher grain yield than Naveen, donors, and checks under both control and drought stress conditions. Notably, significant differences were observed in agronomic traits and grain quality characteristics among the lines under stress and non-stress conditions. To further evaluate drought tolerance, eight lines developed through forward breeding (MAFB) and twelve lines developed through backcross breeding (MABC) were assessed alongside Naveen, the QTL donors, and the checks Measurements were taken for various traits including days to 50 percent flowering (DTF), plant height (PH), productive tiller number (PT), panicle length (PL), grain yield (GY), and 1000 grain weight (TGW) under control and drought stress conditions. The results indicated that the mean DTF of MAFB and MABC lines increased under drought stress compared to the control, while the other four traits (PH, PT, PL, and TGW) displayed a significant decrease in mean values under stress. Furthermore, specific lines stood out in terms of grain yield under drought stress. Three MAFB lines (MAFB 3, MAFB 4, and MAFB 7) and five MABC lines (MABC 4, MABC 6, MABC 7, MABC 11, and MABC 12) demonstrated higher grain yield than Naveen, donors, and checks under drought stress conditions. Notably, two MABC lines (MABC 6 and MABC 7) exhibited higher grain yield than Naveen, donors, and checks under both control and drought stress conditions. Grain quality characteristics, such as kernel length (KL), kernel breadth (KB), L/B ratio, gel consistency (GC), and amylose content (AC), were also analyzed under control and stress conditions. Significant differences were observed among these traits between the control and drought stress conditions, indicating that drought stress had an impact on grain quality characteristics. Overall, the study successfully introgressed three major QTLs for drought tolerance into the rice variety Naveen, resulting in improved grain yield under drought stress conditions. The evaluation of different breeding lines, both through forward and backcross breeding, provided valuable insights into their performance and highlighted lines with superior grain yield. Additionally, the analysis of grain quality traits demonstrated the influence of drought stress on these characteristics. These findings contribute to the ongoing efforts in developing drought-tolerant rice varieties with enhanced agronomic performance and grain quality.

Enhancing rice tolerance to salinity stress

Salinity stress is a major constraint in rice production, leading to reduced yields and compromised quality. Overcoming this challenge requires the identification and utilization of genetic resources associated with salt tolerance in rice. Several scientific studies have made significant contributions to unravel the genetic mechanisms underlying salt tolerance and develop strategies for marker-assisted selection (MAS) in rice breeding programs.

Mardani *et al*., 2014 [25], conducted a study to identify molecular markers linked to salt-tolerant genes in rice at the germination stage. They evaluated 150 rice genotypes for salt tolerance using a germination test and identified 11 markers significantly associated with salt tolerance. These markers hold potential for MAS in rice breeding programs, facilitating the selection of salt-tolerant genotypes. In a project by Linh *et al*., 2012 [23], marker-assisted backcrossing (MABC) was employed to transfer a salt tolerant gene, Saltol QTL, from the donor parent FL478 to the recipient parent BT7. The project successfully developed a new salt-tolerant rice cultivar, BTR478, which exhibited higher yield and improved quality compared to BT7 under saline conditions. The agronomic and molecular characteristics of BTR478 were evaluated, confirming the presence and stability of the Saltol QTL in the new cultivar.

Ming-zhe 2005 [26], employed a mixed genetic model to investigate the inheritance of three salt tolerance- related traits in rice: salt tolerance rating (STR), Na+/K+ ratio in roots, and dry matter weight of shoots (DWS). The study revealed that these traits were controlled by two major genes with modification by polygenes. Additionally, they constructed a linkage map using 62 SSR molecular markers and detected three QTLs for STR, two QTLs for DWS, and two QTLs for Na+/K+ ratio, each explaining a significant proportion of the phenotypic variation. They emphasized the potential of molecular markers for MAS in the improvement of salt tolerance in rice.

Bizimana *et al*., 2017 [5], conducted a study to identify QTLs for salinity tolerance in rice using a mapping population derived from IR29 and Hasawi varieties. The mapping population displayed significant variation for salinity tolerance and related traits. They identified 18 QTLs across nine chromosomes, explaining a notable proportion of the phenotypic variation for each trait. Among these QTLs, six were specifically associated with salinity tolerance index (STI), which serves as a comprehensive measure of salinity tolerance. The study highlighted QTLs with substantial effects and consistent expression across environments as potential candidates for marker-assisted selection.

Thi-Lang 2008 [40], investigated the use of microsatellite markers to identify and tag genes associated with salt tolerance in rice. Two different crosses of rice varieties were screened and evaluated for salt tolerance under greenhouse conditions. The study identified a significant association between salt tolerance and a microsatellite marker (RM223) on chromosome 8 in both crosses. Moreover, this marker demonstrated high accuracy (over 95 percent) in predicting the phenotype of salt tolerance in 93 improved rice varieties. They suggested that this marker could be utilized for selecting salt tolerance genes in rice breeding programs, thereby enhancing salt tolerance by incorporating QTLs from the two crosses.

Adak *et al*., 2020 [1] investigated the genetic diversity and salinity tolerance of rice landraces. They found that the rice

landraces exhibited high genetic diversity and population structure, reflecting their adaptation to diverse agro-climatic conditions. The study also revealed varying levels of salinity tolerance among the landraces, with four landraces (Kalabhat, Kalaikata, Kalo Nunia, and Dudheswar) identified as highly tolerant. These landraces were linked to four molecular markers (RM336, RM510, RM3412, and RM253), which can serve as valuable tools for marker-assisted selection.

Chen *et al*., 2020 [8] published a scientific paper in the Journal of Agronomy and Crop Science, reporting the identification of new QTLs for salt tolerance in rice using the Pokkali variety. They employed a recombinant inbred line population derived from Pokkali and IR29 to map QTLs for salt tolerance traits. They successfully identified 12 QTLs, including four novel QTLs on chromosomes 1, 2, 6, and 10. These QTLs hold potential for improving salt tolerance in rice breeding programs.

Jahan *et al*. 2020 [17] conducted a QTL analysis for rice salinity tolerance using a recombinant inbred line (RIL) population derived from the super hybrid rice LYP9. The study identified 38 QTLs associated with six salinity tolerance-related traits under different salt concentrations. One major QTL, qSL7, located on chromosome 7, was fine mapped and found to be associated with increased shoot length, higher potassium concentration, and lower sodium concentration under salt stress. This QTL harbored 40 annotated genes, including a potential candidate gene, providing valuable genetic resources and insights for improving salinity tolerance in rice breeding.

Valarmathi *et al*., 2019 [44] developed a salinity-tolerant version of the popular rice variety called Improved White Ponni (IWP) using marker-assisted backcross breeding (MABC). They utilized the salt-tolerant donor parent FL478 and the recurrent parent White Ponni to produce three backcross generations and two selfed generations. They selected two promising lines, IWP-Sal-1 and IWP-Sal-2, which exhibited higher yield and improved quality under salinity stress compared to the recurrent parent and the check variety Pokkali. The study highlighted the effectiveness of MABC in improving salinity tolerance in rice and the potential application of the developed lines in salt-affected regions.

Rana *et al*. 2019 [35] developed a new salt-tolerant rice germplasm through speed-breeding and SNP marker-assisted selection. They transferred the hst1 gene, conferring salinity tolerance, from Kaijin to Yukinko-mai, a high-yielding rice cultivar. They employed biotron speed-breeding techniques to rapidly produce six generations in 17 months and selected the best-performing line, YNU31- 2-4, based on whole-genome sequencing and phenotypic evaluation. YNU31-2-4 exhibited similar agronomic traits to Yukinko-mai under normal conditions and demonstrated higher survival rate, biomass, photosynthesis, and yield under salt stress. The study provided insights into the regulation of ion transport and uptake, indicating the potential of YNU31-2-4 in improving salinity tolerance in rice.

Ahmed *et al*., 2019 [19], focused on screening rice genotypes for salinity tolerance using both morphological and molecular markers. They conducted an evaluation of 30 rice genotypes of diverse origins to assess their salt tolerance at the seedling stage, employing a hydroponic system and visual scoring methods. In addition to the morphological evaluation, the researchers utilized 16 SSR markers, which are highly polymorphic molecular markers known as simple sequence repeats or microsatellites. These markers enable the assessment of genetic diversity and relatedness among the rice genotypes. They amplified the DNA samples of the genotypes using PCR and separated the fragments on polyacrylamide gels. By scoring the presence or absence of bands, they calculated various parameters such as the number of alleles, gene diversity, polymorphism information content (PIC), and genetic distance for each marker and genotype. The results of the study revealed distinct differences in salt tolerance among the rice genotypes. Several genotypes, including Balam, THDB, Q-31, Ab.Hai, BR-5, and FR13A, exhibited salt tolerance, while Moulota, Super hybrid, Y-1281, and Binadhan-16 demonstrated moderate salt tolerance. On the other hand, some genotypes were found to be susceptible or highly susceptible to salinity stress. Furthermore, based on the genetic distance calculated using the SSR markers, they constructed a UPGMA dendrogram, which indicated the relatedness and clustering patterns among the genotypes. Four major clusters were identified, and it was suggested that the salt-tolerant genotypes could serve as valuable donors for developing salt-tolerant rice varieties through molecular breeding approaches. The use of molecular markers provided additional insights into the genetic diversity of the rice genotypes under study. The analysis of the 16 SSR markers revealed a total of 65 alleles, with an average of 4.06 alleles per locus, indicating considerable genetic variation. The PIC values, ranging from 0.24 to 0.86 with an average of 0.51, further supported the high level of polymorphism observed. Additionally, the genetic distance analysis displayed a wide range of diversity, with values spanning from 0.36 to 0.88. In conclusion, the research successfully screened rice genotypes for salinity tolerance, employing both morphological and molecular markers. The findings highlighted specific genotypes with varying levels of salt tolerance, while also elucidating the genetic diversity and relatedness among the studied genotypes using SSR markers. These results have important implications for the development of salt-tolerant rice varieties through molecular breeding strategies, ultimately contributing to enhanced productivity and quality in salinity-affected environments.

Iqbal *et al*., 2015 [15], performed DNA fingerprinting of rice lines for salinity tolerance at the reproductive stage using microsatellite markers (SSR). The study involved 22 rice lines sourced from different origins. The methods employed included salinity screening, DNA extraction, PCR amplification, electrophoresis, and data analysis. The results of the SSR analysis provided valuable insights into the genetic characteristics of the rice lines. The analysis revealed information such as the number of alleles, gene diversity, polymorphism information content (PIC), major allele frequency, and genetic similarity among the rice lines. These findings shed light on the genetic diversity and relationships among the tested rice lines. The research emphasized the usefulness of SSR markers for assessing genetic diversity and identifying potential parents for developing salt-tolerant rice varieties through molecular breeding. The SSR markers enabled the detection of genetic variations and polymorphisms associated with salt tolerance, including the presence of the Saltol gene, which serves as a valuable marker for selection. Furthermore, the SSR markers facilitated the measurement of genetic similarity and distance between rice lines, aiding in the determination of their potential for hybridization and gene transfer. The grouping of rice lines into clusters based on their genetic relationships provided

insights into the choice of suitable parents for crossbreeding and breeding programs. Overall, the application of molecular markers, specifically SSR markers, played a crucial role in the research. They helped in identifying salt-tolerant rice lines by detecting genetic variations, identifying markers associated with salt tolerance, assessing genetic relationships, and providing a means for fingerprinting and verification of salt-tolerant lines.

Rasel *et al*., 2020 [37], aimed to evaluate the salt tolerance of various rice genotypes through the assessment of their growth, morphology, and molecular responses under salt stress. A total of 28 rice genotypes, including landraces and high-yielding cultivars, were subjected to a salinity level of 12 ds per meter in hydroponic media for 18 days. The experiment measured morpho-physiological traits such as live leaves, survival rate, and root number, as well as biochemical responses including Na+/K+ ratio, proline accumulation, and antioxidant enzymes' activity. Additionally, three microsatellite markers were employed to classify the genotypes into salt-tolerant, moderately tolerant, and susceptible groups. The results revealed that Nonabokra, Hogla, Ghunsi, Holdegotal, and Kanchon were identified as salt-tolerant genotypes. These genotypes exhibited less reduction in growth, higher survival rates, lower proline accumulation, higher antioxidant enzymes' activity, and lower Na+/K+ ratios compared to the susceptible genotypes. The molecular screening using three microsatellite markers closely linked to the saltol QTL (RM493, RM3412b, and RM140) further supported the grouping of genotypes based on their genetic similarity. This molecular grouping aligned with the morpho-physiological clustering and biochemical responses observed under salinity stress. In conclusion, this experiment identified five salt-tolerant rice landraces (Nonabokra, Hogla, Ghunsi, Holdegotal, and Kanchon) that hold potential for enhancing breeding programs aimed at developing high-yielding, salt-tolerant rice cultivars. The integration of morpho-physiological, biochemical, and molecular analyses provided a comprehensive understanding of salt tolerance in rice genotypes and paved the way for future advancements in rice breeding for improved salinity tolerance.

Enhancing rice tolerance to submergence stress

Mohapatra *et al*., 2023 [27] developed a new version of Ranidhan, a popular rice variety in Odisha state, India, with enhanced tolerance to submergence and resistance to bacterial blight disease. They employed marker-assisted backcross breeding to transfer four target genes (Sub1A, xa5, xa13, and Xa21) from two donor parents (Swarna-Sub1 and CR Dhan 800) into the Ranidhan variety. Molecular markers facilitated the precise identification of the progenies carrying all four target genes through foreground selection in each backcross generation. Through this approach, they identified four elite pyramided lines that exhibited not only high yield, submergence tolerance, and broad-spectrum resistance to bacterial blight pathogens but also retained the desirable agro-morphologic and grain quality traits of Ranidhan. This newly developed version of Ranidhan holds great promise for farmers in eastern India, where flash floods and bacterial blight disease outbreaks are prevalent. Additionally, the introduction of this variety is expected to contribute to reduced chemical usage and environmental pollution. By utilizing molecular markers, this study accelerated the breeding process, reducing both time and cost compared to conventional breeding methods. The successful development of these elite pyramided lines demonstrates the effectiveness of marker-assisted backcross breeding in enhancing important traits in rice varieties, offering a valuable approach for sustainable agriculture and addressing the challenges faced by farmers in flood-prone regions.

The research of Luu *et al*., 2021 [24] presents the successful development of a submergence-tolerant rice variety in Vietnam using marker-assisted backcrossing (MABC) method. The study focuses on the transfer of a submergence tolerance gene (SUB1) from the donor variety (IR64 SUB1) to the popular recipient variety (AS996), while maintaining the essential characteristics of the recipient variety. The research describes the process of MABC and highlights the utilization of allele- specific molecular markers for SUB1A and SUB1C during the backcrossing. These molecular markers played a crucial role in identifying and selecting plants that possessed the SUB1 gene from IR64 SUB1 and had a high percentage of recipient alleles from AS996. This enabled the researchers to minimize the introgression size and minimize the risk of introducing undesirable genes that could alter the essential traits of AS996. The molecular markers also facilitated the confirmation of the presence and expression of SUB1 in the selected plants and allowed for the evaluation of their submergence tolerance under greenhouse and field conditions. Notably, after three generations of backcrossing, the best plant (P422-14-177) exhibited 100 percent recipient alleles from AS996 and only a small introgression size of SUB1. The selected plants demonstrated submergence tolerance comparable to IR64 SUB1 and superior to AS996 after prolonged submergence and recovery periods. As a result, the new variety (AS996-SUB1) was successfully developed and is now ready for field testing and validation in areas prone to submergence in Vietnam. Overall, this study highlights the effectiveness of the marker-assisted backcrossing approach in developing submergence-tolerant rice varieties. It offers promising prospects for enhancing rice productivity in flood-prone regions and addressing the challenges posed by climate change. The development of AS996-SUB1 provides a valuable resource for Vietnamese farmers and contributes to sustainable agricultural practices in submergence-affected areas.

Ray *et al*., 2018 [38] focused on the development of submergence- tolerant rice lines using marker-assisted selection (MAS). The study employed molecular markers, backcrossing, and submergence screening to introgress the SUB1 QTL into high- yielding rice varieties. They reported the outcomes of the experiment, which evaluated the performance of two sub1 introgression lines (BPR6 and BPR7) in comparison to two check varieties (BINAdhan-11 and BRRI dhan52) under both artificial and natural submergence conditions. The assessment included parameters such as survival rates, shoot length, root length, and biomass. Molecular markers played a crucial role in the experiment by enabling the identification and selection of plants carrying the desired submergence tolerance gene (Sub1) from the donor parent (BRRI dhan52). Additionally, the markers facilitated the elimination of unwanted donor segments from the carrier chromosome. They were instrumental in assessing the genetic diversity and similarity among the submergence-tolerant rice lines and constructing a linkage map near the Sub1 locus. The results demonstrated that the two sub1 introgression lines, BPR6 and BPR7, exhibited significantly higher survival rates, shoot length, root length, and biomass compared to the check varieties under both artificial and natural submergence

conditions. Notably, the BPR6 line emerged as the most promising sub1 line, displaying the ability to produce high yields and withstand submergence for three to four weeks in flash flood-prone areas of Bangladesh. This study highlights the effectiveness of marker-assisted selection (MAS) in developing submergence-tolerant rice lines. The integration of molecular markers, backcrossing, and submergence screening proved successful in introgressing the SUB1 QTL and enhancing the performance of high-yielding rice varieties. The identified BPR6 sub1 line holds significant potential for improving agricultural productivity and resilience in areas prone to flash floods.

Septiningsih *et al*., 2012 [39] presented the findings of a study Aimed at identifying novel QTLs (quantitative trait loci) associated with submergence tolerance in rice cultivars IR72 and Madabaru. The research employed a recombinant inbred line (RIL) population generated from a cross between IR72 (submergence intolerant) and Madabaru (submergence tolerant) to map the QTLs related to submergence tolerance. The study successfully identified four novel QTLs located on chromosomes 1, 4, 7, and 9, which accounted for 7.8 to 15.7 percent of the phenotypic variation observed in submergence tolerance. Furthermore, the presence of a major QTL on chromosome 9, known as Sub1, was confirmed. This Sub1 QTL explained a significant proportion, 66.1 percent, of the phenotypic variation for submergence tolerance. Molecular markers played a crucial role in the experiment, as the researchers utilized 200 SSR (simple sequence repeat) markers to genotype the RIL population and construct a genetic linkage map. Additionally, 10 STS (sequence-tagged site) markers were employed to validate the presence of the Sub1 QTL on chromosome 9. The utilization of molecular markers enabled the precise identification and localization of QTLs associated with submergence tolerance on specific rice chromosomes. Key findings of the study revealed that the RIL population exhibited a wide range of variation in submergence tolerance, with certain lines displaying higher tolerance than Madabaru and others being more intolerant than IR72. The researchers successfully detected five QTLs for submergence tolerance, including the four newly identified QTLs and the previously reported Sub1 QTL. The Sub1 QTL exhibited the most substantial effect on submergence tolerance, explaining 66.1 percent of the observed phenotypic variation. The novel QTLs had comparatively smaller effects, explaining 7.8 to 15.7 percent of the phenotypic variation. The presence of the Sub1 QTL was confirmed using STS markers, demonstrating its presence in all tolerant lines and absence in all intolerant lines. Based on these outcomes, the researchers proposed that the novel QTLs could be incorporated alongside the Sub1 QTL to further enhance submergence tolerance in rice breeding programs. The identification of these novel QTLs provides valuable insights for the development of improved rice varieties with enhanced submergence tolerance, contributing to the advancement of agricultural practices and food security in flood-prone regions.

Prasad *et al*., 2011 [32] presented the results of a study focused on characterizing submergence tolerance in rice genotypes through biochemical and molecular approaches, with the aim of facilitating crop improvement strategies. The study involved screening 10 rice genotypes for submergence tolerance using survival percentage, alcohol dehydrogenase (ADH) activity, isozyme analysis, and random amplified polymorphic DNA (RAPD) markers. Based on the findings, the rice genotypes were categorized into three groups according to their submergence tolerance. FR13A, Jalashree, and Jalkunwari were identified as tolerant genotypes, while Bahadur and Ranjit exhibited moderate tolerance. Luit, Keteki, Lachit, Chilarai, and Mahsuri were classified as susceptible genotypes. Molecular markers, specifically RAPD markers, were employed in the experiment to assess genetic variation among the rice genotypes and construct a dendrogram based on their genetic similarity. They used 15 RAPD primers, which generated 62 polymorphic bands among the rice genotypes. Notably, four specific primers (OPD-06, OPH-07, OPN-04, and OPS-03) effectively discriminated the rice genotypes based on their submergence tolerance. The use of molecular markers played a pivotal role in this study by providing a rapid and reliable method for evaluating genetic diversity and relationships among the rice genotypes. Molecular markers are independent of environmental influences and phenotypic variations, enabling the identification of underlying genetic differences. Furthermore, molecular markers can aid in the identification of specific genes or loci associated with submergence tolerance, such as the Sub1A gene mentioned in the context. The results obtained from the experiment yielded several key findings. The survival percentage of the rice genotypes under submergence displayed a wide range, with FR13A exhibiting the highest survival rate and Mahsuri displaying the lowest. ADH activity, an indicator of submergence tolerance, increased with the duration of submergence and varied among the genotypes. FR13A, Jalashree, and Jalkunwari exhibited the highest ADH activity and the most isozymes, while Mahsuri showed the lowest ADH activity and the least isozymes. The RAPD analysis further delineated two major clusters of rice genotypes based on their submergence tolerance. FR13A, Jalashree, and Jalkunwari formed one cluster, while all other genotypes, excluding Bahadur, constituted the second cluster. Bahadur showed closer proximity to the tolerant cluster. Importantly, the four RAPD primers (OPD-06, OPH-07, OPN-04, and OPS-03) effectively differentiated the rice genotypes based on their submergence tolerance. In conclusion, the study demonstrated the utility of biochemical and molecular markers for characterizing rice genotypes with respect to submergence tolerance. The findings underscore the potential of these markers to aid plant breeders in developing high-yielding rice cultivars suitable for lowland ecosystems. The identification of submergence tolerance indicators, novel QTLs, and genetic markers opens avenues for targeted crop improvement strategies, contributing to enhanced resilience and productivity in flood-prone regions.

Enhancing rice tolerance to heat stress

Heat stress poses a significant challenge to agricultural productivity, particularly in crops like rice that are highly sensitive to elevated temperatures during crucial growth stages. To address this issue, researchers have conducted extensive studies to understand the genetic basis of heat tolerance in rice and identify relevant genetic factors that can aid in the development of heat-tolerant varieties.

Chang-lan *et al*., 200 [7], undertook a comprehensive investigation to map QTLs controlling heat tolerance during the grain filling stage in rice. Their study employed a backcross inbred lines (BILs) population derived from a cross of Nippon bare/Kasalath//Nipponbare and utilized a mixed linear- model approach to detect and assess the effects of

QTLs. The researchers identified three QTLs located on chromosomes 1, 4, and 7 that played crucial roles in heat tolerance. Additionally, they conducted a detailed analysis of the additive, epistatic, and QTL×environment interactions of these loci. Notably, they found that the QTL on chromosome 4 exhibited stability and held promise for improving heat tolerance in rice breeding, while the other two QTLs displayed significant interactions with environmental factors and genetic background.

Hu *et al*., 2022 [14], conducted a genome-wide association study (GWAS) to map QTLs associated with heat tolerance during the anthesis stage in rice. Their study focused on the assessment of relative spikelet fertility (RSF) as an indicator of heat tolerance using a diverse set of 173 rice accessions. Through GWAS analysis with a massive dataset of 1.2 million single nucleotide polymorphisms (SNPs), they successfully identified five QTLs associated with RSF, among which they discovered a novel QTL named qRSF9.2. To narrow down the QTL region, they employed various techniques and identified 16 candidate genes, ultimately pinpointing LOC_O *s*09*g*38500 as the most promising candidate based on no synonymous SNPs, expression levels, and haplotypes. Their findings hold significant potential for facilitating the cloning of qRSF9.2 and advancing the breeding of heat-tolerant rice varieties through marker-assisted selection.

Pradhan *et al*., 2016 [53] investigated the population structure, genetic diversity, and molecular marker- trait associations for high temperature stress tolerance in rice. Initially, the researchers screened 240 rice germplasm lines to identify eight highly tolerant genotypes with spikelet fertility exceeding 40 percent under stress conditions. To assess the genetic diversity, population structure, and marker-trait associations, they employed 20 molecular markers associated with high temperature stress tolerance, genotyping a selected panel of 60 genotypes. Through their analysis, they observed a moderate level of genetic diversity within the panel, along with the presence of three distinct sub-populations. These findings suggested a common primary ancestor, with only a few admixed individuals among the landraces. Utilizing the 20 molecular markers, the authors successfully identified seven markers that exhibited a strong association with spikelet fertility and other phenotypic traits related to high temperature stress tolerance. These markers hold potential for marker-assisted breeding programs. The application of the molecular markers served several purposes. They enabled the genotyping of the 60 rice genotypes, using a combination of two INDEL and 18 SSR markers specifically linked to high temperature stress tolerance. Additionally, the markers facilitated the estimation of various genetic parameters, such as the number of alleles, allele frequency, gene diversity, heterozygosity, and polymorphic information content for each marker.

To further analyze the genetic relationships and ancestry among the genotypes, the researchers constructed an unrooted tree based on the dissimilarity index and conducted a population structure analysis using a Bayesian clustering approach. This provided valuable insights into the genetic structure within the panel and the presence of distinct sub-populations. To assess the genetic variation within and between the identified sub-populations, they performed an analysis of molecular variance. This analysis allowed them to quantify the extent of genetic diversity within each sub-population and examine the differentiation between them. Furthermore, they conducted marker-trait association analysis using both general linear models and mixed linear models. Through these analyses, they identified seven markers that demonstrated a significant association with spikelet fertility and other high temperature stress tolerance- related traits. These markers have the potential to be utilized in marker-assisted breeding programs, facilitating the selection of genotypes with enhanced tolerance to high temperature stress. In conclusion, it presents a comprehensive investigation into population structure, genetic diversity, and marker-trait associations for high temperature stress tolerance in rice. By screening a large number of germplasm lines and employing molecular markers, the authors identified tolerant genotypes, assessed genetic diversity, inferred population structure, and identified markers associated with key traits. These findings contribute to the development of rice varieties with improved tolerance to high temperature stress, benefiting rice breeding programs aimed at enhancing crop resilience and productivity in challenging environments.

Enhancing rice tolerance to cold stress

Cold stress poses a significant threat to rice production, particularly during the seedling stage, affecting crop yield and quality. Over the years, researchers have made substantial efforts to understand the genetic mechanisms underlying cold tolerance in rice and identify potential targets for molecular breeding strategies. In this context, several notable studies have contributed valuable insights into cold stress tolerance in rice by investigating various aspects such as genotypic screening, identification of functional genes, and mapping quantitative trait loci (QTLs).

Thippeswamy *et al*., A 2015 [41], conducted a study focusing on the identification of cold-tolerant rice genotypes and exploring the potential of linked markers for marker-assisted pyramiding of resistance genes. Through the evaluation of leaf yellowing scores and SPAD chlorophyll meter readings (SCMR), they identified nine rice genotypes that exhibited tolerance to cold stress. Furthermore, their investigation revealed specific banding patterns of a microsatellite marker (RM85) in cold-susceptible and cold-tolerant genotypes, suggesting the potential use of linked markers to enhance cold stress resilience through the pyramiding of biotic and abiotic resistance genes.

In a separate study by Zhao *et al*., 2017 [47], a novel functional gene associated with cold tolerance at the seedling stage in rice was identified. The researchers successfully cloned the gene Os09g0410300, which underlies the cold-tolerant QTL qCTS-9. They further developed a gene-specific marker and demonstrated its effectiveness in molecular breeding approaches for improving cold tolerance in rice. Molecular markers played a crucial role in this study, serving two main purposes. First, SSR markers were used to genotype the RIL population, enabling the mapping of the QTL for cold tolerance on chromosome 9. These markers aided in identifying the qCTS-9 region and narrowing down the candidate gene region to a 534 kb interval. Second, the authors developed an In Del marker based on insertion-deletion polymorphism in the promoter of Os09g0410300. This marker was utilized to validate the association between the gene and cold tolerance in the RIL population. The findings demonstrated a significant correlation between the In Del marker and cold tolerance, confirming Os09g0410300 as the functional gene underlying qCTS-9. Consequently, the In Del marker holds promise for facilitating marker-assisted

selection in molecular breeding programs targeting cold tolerance at the seedling stage in rice. Overall, this study contributes to the understanding of the genetic mechanisms governing cold tolerance in rice at the seedling stage. The identification and characterization of the novel gene, Os09g0410300, provide valuable insights for the development of improved cold-tolerant rice varieties. Leveraging molecular markers and the potential involvement of the brassinosteroid signaling pathway, researchers and breeders now have a promising target to enhance cold tolerance and enhance the productivity and resilience of rice crops in challenging environments.

Kim *et al*., 2014 [21], delved into a comprehensive investigation of the genetic factors contributing to cold tolerance in rice. Their study involved a cold tolerance test on recombinant inbred lines (RILs) derived from a cross between rice varieties with contrasting cold tolerance levels. By employing a quantitative trait analysis, they identified multiple QTLs associated with cold tolerance on different chromosomes. Furthermore, through the selection of candidate genes within the QTL regions and subsequent marker validation, they confirmed the correlation of several markers with cold tolerance across a diverse panel of rice accessions.

Raharinivo *et al*., 2016 [34], focused on QTL mapping for cold tolerance in rice at the seedling stage. The experiment involved the use of a BC1F2 population derived from a cross between a cold-tolerant japonica variety and a cold-susceptible indica variety of rice. Molecular markers were employed to identify quantitative trait loci (QTLs) associated with cold tolerance. Specifically, a rice 6K SNP chip containing 4606 SNP markers evenly distributed across the 12 rice chromosomes was utilized to genotype the BC1F2 plants and their parents. The polymorphic markers identified through parental polymorphism survey accounted for 34 percent of the total markers and were employed for QTL mapping for cold tolerance at the seedling stage. QTL analysis using inclusive composite interval mapping led to the detection of four QTLs on chromosomes 2 and 10. These QTLs exhibited phenotypic variances (R2) of 11.11 percent, 7.55 percent, 12.8 percent, and 8.8 percent, respectively, highlighting their contribution to cold tolerance in rice at the seedling stage. The findings from this study have significant implications for marker-assisted breeding programs aiming to enhance cold tolerance in rice varieties. The identified QTLs can be utilized to facilitate the introgression of cold tolerance genes into rice varieties suitable for high-altitude regions of Madagascar, as well as other tropical and subtropical countries. By employing molecular markers and conducting QTL mapping, this research provides valuable insights into the genetic basis of cold tolerance in rice at the seedling stage. The identified QTLs offer potential targets for breeding programs aimed at developing cold-tolerant rice varieties, thereby contributing to improved productivity and resilience in cold-prone environments. These collective efforts contribute to our understanding of the genetic basis of cold tolerance in rice. The identification of cold-tolerant genotypes, the discovery of novel functional genes, and the mapping of QTLs provide crucial insights for developing molecular breeding strategies aimed at enhancing cold stress resilience in rice crops. By harnessing these findings, researchers and breeders can strive towards developing cold-tolerant rice varieties that are better equipped to withstand the challenges posed by cold stress, ultimately ensuring stable and productive rice cultivation in diverse environmental conditions.

Enhancing rice tolerance to nutrient deficiency

Improving nutrient use efficiency in crops is crucial for sustainable agriculture, particularly in the context of nutrient deficiencies such as nitrogen and phosphorus. Rice, as a staple food crop, has been the focus of extensive research aiming to enhance its performance under nutrient-limited conditions. In this regard, two significant studies by Anis *et al*., 2019 [3] and Chin *et al*., 2011 [9] have contributed valuable insights into the genetic mechanisms underlying nutrient response in rice, specifically nitrogen and phosphorus deficiencies, respectively.

Anis *et al*. 2019 [3]. Conducted a study to identify quantitative trait loci (QTLs) associated with root traits under nitrogen deficiency in rice. Their investigation identified a major QTL on chromosome 6, designated as RDWN6XB, which exhibited a significant influence on root dry weight, length, surface area, and volume under nitrogen-deficient conditions. To confirm the effect of RDWN6XB, near-isogenic lines (NILs) were utilized, and it was found that the NIL with RDWN6XB had a remarkable 23.8 percent increase in root dry weight compared to the NIL lacking this QTL under nitrogen deficiency. Further fine-mapping efforts narrowed down RDWN6XB to a 95-kb region containing 11 candidate genes. Gene expression analysis enabled the researchers to identify two genes, LOC_O *s*06*g*11010 and LOC_O *s*06*g*11020, as the most likely candidates within this region. Additionally, a single nucleotide polymorphism (SNP) was detected in the promoter region of LOC_O *s*06*g*11010.

Which exhibited associations with both gene expression levels and root traits under nitrogen deficiency. These findings shed light on the genetic control of root traits in response to nitrogen deficiency and provide potential targets for molecular breeding approaches aimed at improving nitrogen use efficiency in rice.

In a parallel effort, Chin *et al*., 2011 [9], focused on the development of rice varieties with high yield under phosphorus deficiency. The authors highlighted a major QTL called Pup1, which confers tolerance to low phosphorus in soil. The study encompassed the development of gene-based molecular markers for Pup1, the validation of gene models, and an extensive survey of Pup1 alleles in diverse rice accessions. Furthermore, marker-assisted backcrossing techniques were employed to introgress Pup1 into five different rice varieties, and the resulting introgression lines were evaluated in various environments for phenotypic performance. The study revealed the significant potential of Pup1 in enhancing grain yield under phosphorus-deficient conditions across different genetic backgrounds and environments. These findings offer valuable insights into the genetic basis of phosphorus tolerance in rice and pave the way for the development of improved rice varieties with enhanced productivity in phosphorus-limited soils.

The amalgamation of these studies by Anis *et al*., 2019 [3] and Chin *et al*., 2011 [9] contributes to our understanding of the genetic factors governing nutrient response in rice. By identifying key QTLs, elucidating candidate genes, and developing molecular markers, these studies provide valuable resources for molecular breeding efforts aimed at enhancing nutrient use efficiency and crop productivity in rice. By harnessing the potential of these genetic factors, researchers and breeders can work towards developing rice varieties that thrive under nutrient-deficient conditions, ultimately

contributing to sustainable and resilient agricultural practices.

Enhancing rice tolerance to toxicity stress

Rice production is significantly affected by abiotic stresses such as iron toxicity and aluminum toxicity, which lead to reduced yields in many countries. Overcoming these challenges requires a thorough understanding of the genetic factors underlying tolerance to these stresses. Several research studies have made significant contributions to unraveling the genetic mechanisms associated with iron and aluminum toxicity tolerance in rice.

Pawar *et al*., 2021 [31] conducted a comprehensive study to investigate iron toxicity tolerance in rice. They screened 352 rice germplasm lines under field and hydroponic conditions, and subsequently genotyped 119 selected genotypes using 51 molecular markers. The use of molecular markers, including SSR and gene-specific markers, allowed for the estimation of genetic diversity, population structure, linkage disequilibrium, and marker-trait association. The findings of the study revealed a wide genetic variation for iron toxicity tolerance among the panel population, as determined by the leaf bronzing index and other agronomic traits. The genotypes were classified into three genetic structure groups, which aligned well with their respective iron toxicity tolerance levels. Through association mapping, four molecular markers (RM471, RM3, RM590, and RM243) were identified as significantly associated with the leaf bronzing index, accounting for 4.2 to 9.8 percent of the phenotypic variation. Furthermore, the researchers detected three novel QTL (qFeTox4.3, qFeTox6.1, and qFeTox10.1) on chromosomes 4, 6, and 10, respectively, which were linked to iron toxicity tolerance. Additionally, a previously reported QTL on chromosome 1 was validated. Notably, certain QTL for iron toxicity tolerance were found to be co-localized with QTL for grain iron content, suggesting a shared pathway for iron homeostasis in rice. Overall, the identified QTL and molecular markers hold significant potential for marker-assisted breeding programs aimed at enhancing iron toxicity tolerance and grain iron content in rice. These molecular tools enable breeders to select plants with desirable traits without relying solely on time-consuming phenotypic evaluations. The study's findings contribute to advancing rice breeding strategies and addressing the challenge of iron toxicity tolerance in rice cultivation.

Rasheed *et al*., 2021 [36], focused on QTL mapping for iron toxicity tolerance in rice using a backcross recombinant inbred lines (BRILs) population, they presented their findings on the identification of 23 QTL associated with various seedling traits under both control and stress conditions. These QTL were mapped using a high-density bin map constructed with the aid of SNP markers, which offer higher resolution and stability compared to other markers. The study highlights the presence of stable and novel QTL that could be utilized for marker-assisted selection and QTL pyramiding to develop iron- resistant rice varieties. By leveraging molecular markers, they successfully linked genotypic variations with phenotypic traits and precisely located the QTL on the relevant chromosomes associated with iron toxicity tolerance in rice. The use of SNP markers also facilitated the estimation of genetic distance, LOD value, phenotypic variance, and additive effect of the QTL, providing valuable insights into the genetic basis of iron toxicity tolerance. Additionally, they observed phenotypic variation, correlation, and heritability of the seedling traits, shedding light on the complex interplay between genetic and environmental factors affecting iron toxicity tolerance in rice. The findings emphasize the need for further research to develop effective breeding strategies for producing iron- resistant rice varieties. Overall, the study underscores the significance of molecular markers, particularly SNP markers, in unraveling the genetic underpinnings of iron toxicity tolerance in rice. The identified QTL and their associated markers offer promising opportunities for marker-assisted selection and QTL pyramiding, enabling the development of rice varieties resilient to iron toxicity. The comprehensive understanding gained from this research contributes to advancing breeding efforts aimed at addressing the challenge of iron toxicity tolerance in rice cultivation.

The genetic basis of aluminum tolerance in rice, an important trait for cultivation in acidic soils, was investigated through the identification of quantitative trait loci (QTLs) and epistatic interactions using a recombinant inbred line (RIL) population derived from two contrasting aluminum-tolerant rice cultivars by Wu *et al*. 2000 [54]. This study employed molecular markers and statistical methods to detect QTLs and epistasis for aluminum tolerance at different seedling stages, including root length, root growth rate, and relative root growth rate. The researchers constructed a genetic linkage map of the rice genome using 164 restriction fragment length polymorphism (RFLP) markers, providing a comprehensive representation of the relative positions and distances of genes or markers on the chromosomes. By utilizing these molecular markers, they successfully identified 21 QTLs associated with aluminum tolerance through interval mapping (IM) or composite interval mapping (CIM) approaches. Additionally, the study revealed the presence of 12 pairs of epistatic QTLs for aluminum tolerance through two-way analysis of variance (ANOVA). The mapping of QTLs and epistasis to specific regions of the rice genome shed light on the genetic basis and variation of aluminum tolerance in rice. Moreover, the effects of QTLs and epistasis on various seedling stages were analyzed, focusing on root length, root growth rate, and relative root growth rate. This comprehensive understanding of the genetic factors influencing aluminum tolerance in rice has important implications for breeding and genetic improvement programs aimed at enhancing the adaptability of rice plants to acidic soils. In conclusion, this research made significant strides in elucidating the genetic mechanisms underlying aluminum tolerance in rice. By employing molecular markers and statistical methods, the study identified QTLs and epistasis associated with aluminum tolerance and mapped them to specific genomic regions. These findings provide valuable insights for rice breeding programs seeking to improve aluminum tolerance and enhance the productivity of rice crops in acidic soils.

Nguyen *et al*., (2001) [30] conducted a study focusing on the mapping of two genes, Alt1 and Alt2, associated with aluminum tolerance in rice. These genes, located on chromosomes 1 and 3, respectively, played a significant role in determining aluminum tolerance in rice plants. Alt1 and Alt2 exhibited independent inheritance and additive effects, explaining a substantial portion of the phenotypic variation for aluminum tolerance. The researchers proposed that these genes may have originated from different germplasm sources, highlighting their importance as valuable genetic resources for rice improvement. Molecular markers played a crucial role in this experiment by enabling the identification of specific DNA sequences linked to aluminum tolerance in rice. These

markers provided a means to detect genetic variation and map the genes responsible for the desired traits. In this study, molecular markers were utilized to precisely locate two major genes, Alt1 and Alt2 that confer aluminum tolerance in rice. Additionally, these markers facilitated an analysis of the genetic diversity and relationships among 21 rice cultivars using 50 molecular markers. This analysis revealed that the cultivars could be grouped into two clusters based on their geographic origins. The results obtained from this study demonstrated the successful identification and mapping of two major genes, Alt1 and Alt2, associated with aluminum tolerance in rice using molecular markers. These genes were found to be located on chromosomes 1 and 3, respectively, and accounted for a substantial proportion of the observed phenotypic variation for aluminum tolerance. Furthermore, the analysis of genetic diversity using molecular markers provided insights into the relationships between different rice cultivars, leading to the identification of distinct clusters based on geographic origin. Altogether, these findings contribute to our understanding of the genetic basis of aluminum tolerance in rice and highlight the potential for utilizing Alt1 and Alt2 as valuable genetic resources in rice breeding and improvement programs.

Conclusion

The future of plant breeding lies in attaining a comprehensive understanding of the genetic control underlying physiological traits and establishing the linkages between these traits and molecular markers on chromosomes, ultimately identifying the genes responsible for these traits. Molecular markers have gained rapid acceptance among researchers worldwide as a valuable and suitable tool for investigating physiological traits in both fundamental and practical studies. By leveraging molecular breeding techniques, researchers can effectively develop improved rice varieties with desirable characteristics such as multiple stress resistance and enhanced nutritional quality.

To achieve these goals, it is essential to unravel the genetic and molecular basis of physiological traits associated with stress resistance and quality in rice. This knowledge provides crucial insights into the underlying mechanisms that govern these traits, enabling breeders to make informed decisions and strategies for targeted breeding efforts. Molecular markers play a pivotal role in this process by facilitating the identification and transfer of desirable genes or quantitative trait loci (QTLs) into rice cultivars. These markers serve as signposts or indicators of the presence of specific genes or genomic regions associated with the desired traits, allowing breeders to select and incorporate these genetic factors into breeding programs more efficiently and accurately.

By harnessing the potential of molecular breeding, it becomes possible to develop rice varieties that possess multiple stress resistances, enabling them to thrive under challenging environmental conditions. Additionally, molecular breeding techniques can contribute to enhancing the nutritional quality of rice, ensuring that the resulting varieties meet the dietary requirements of a growing population. This can be accomplished through the identification and incorporation of genes responsible for improved nutrient content, as well as the manipulation of gene expression and metabolic pathways related to nutritional traits.

Overall, the future prospects of molecular breeding in rice hold great promise. By deepening our understanding of the genetic and molecular basis of physiological traits, exploiting the power of molecular markers, and employing advanced breeding techniques, breeders can develop improved rice varieties that exhibit multiple stress resistance and enhanced nutritional quality. These advancements not only contribute to the sustainable and resilient cultivation of rice but also address the pressing need to provide nutritious food for a growing global population. (Das *et al*., 2017) [11].

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