



Bridging the gap between quality and quantity of Rice through Marker-Assisted Breeding: A review

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Abstract

An increase in quantity as well as quality are the primary objectives of rice breeding program. Conventional breeding is based of phenotypic selection, time consuming, laborious and has a significant risk of linkage drag. DNA molecular marker methods are faster, easier, and less costly than traditional breeding operations. Marker-assisted selection (MAS) is becoming popular because it improves plant breeding efficiency by precisely transferring gene of interest and speeding up the recurrent parent genome's recovery. MAS is used to target yield and agronomic features, cooking and nutritional quality, and tolerance to biotic- abiotic stress. MAS is frequently used in gene pyramiding for disease and pest resistance. Quality and productivity of rice depends upon various genes that are directly or indirectly affect total outcomes. Selection of genes associated with different traits, MAS contributed to enhance food security and human health. As a model crop with a fully sequenced genome, rice offers excellent chances to investigate the molecular mechanisms of yield, biotic-abiotic stress improvement and to engineer the creation of rice varieties with higher potential that are effective against different types of stresses. In this review, we discussed about different QTLs that helps in MAS for improving quality traits, agronomic traits, and biotic/abiotic stresses of rice.

Keywords: Rice, MAS, stress, quality, molecular marker

Introduction

Rice (*Oryza sativa*) a monocot plant that belongs to the Poaceae family, provides the necessities and livelihoods for people living in Asia's monsoon regions. Nearly 137.8 lakh metric tons of rice is produced in India, and the cultivation area is estimated to be 47.6 million hectares (FAOSTAT 2023). Exponential growth of the human population increases the food demand. In addition, climate change, and urbanization increase the pest and pathogen pressure and decrease crop production due to stress conditions. A large portion of world population primarily depends upon rice as a staple crop. Increasing its production in addition to other cereal crop is necessary to meet the daily requirement for food (Wang *et al.*, 2015). Due to its inadequate eating and cooking quality some rice hybrid creates difficulties in satisfying consumer demands. In order to fulfil the growing market demand and consumption requirement in recent years, quality enhancement is currently promoted as one of the primary objectives in hybrid rice breeding. Conventional breeding methods have historically played a significant part in quality improvement and yield enhancement. However, these methods are time consuming for development of any specific traits. Modern molecular and genomic techniques like genomic selection (GS), marker-assisted selection (MAS), genome editing (CRISPR-Cas), have been applied to conventional breeding methods to enhances the accuracy and efficiency of developing biotic and abiotic stress tolerant varieties. The application of marker-assisted selection (MAS), breeders may now more quickly and accurately identifying the decided character (Haque *et al.*, 2021) [18]. In rice different molecular marker that are closely linked with economically significance trait has been identified by using Marker-Assisted Breeding (MAB) such as resistance to brown plant hopper, gall midges, green leaf

hopper, blast, and tolerances to salinity, submergences, drought, grain quality, and many other factors. The potential implementation of Marker assisted breeding gives effective information for rice breeder to enhance rice quality and create a climate-resilient rice variety that can resist with different types of stress (Hasan *et al.*, 2015) [19]. Marker-assisted breeding in rice bridges the gap between quality and quantity by improving both yield potential and grain quality traits simultaneously. This approach aims to develop superior varieties that combine high yield potential, stress tolerances, disease resistance with excellent grain quality. It provides more precise and effective method as compared to traditional breeding method which allows breeder to full fill the consumer demands and overcome the challenges of food security.

QTL mapping

The Green Revolution gives a great impression on rice and wheat crop production in 1960s. In the recent time plant breeder try to decrease the biotic-abiotic stress and increase the yield by adopting new breeding methods. To enhance the selection intensity based on phenotypes along with genes, marker-assisted selection is the best way where DNA markers are polymorphic, specialised and reliable. There are different types of markers based on polymorphisms that are amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), single nucleotide polymorphisms (SNPs), cleavable amplified polymorphic sequences (CAPS) *etc.* Quantitative trait loci (QTL) region is where one or several linked genes present and influence a particular trait. QTL mapping helps to identify region of chromosome that affect the variation. Molecular markers consist of nucleotide sequences that may be investigated by examining the

variations among different individuals' nucleotide sequences. Characteristics of a DNA marker are co-dominant, genome-wide, evenly distributed, and capable of identifying a higher degree of polymorphism (Hasan *et al.*, 2021) [20]. Rice is a monocot plant which has the potential for molecular studies for its best developed system. The rice genome is composed of 12 gametic chromosomes and has the smallest genome of any monocot known (430Mb). Rice is diversified due to its large collection of wild species and cultivated varieties. Molecular markers are used to trace the linkage of targeted genes/QTLs of rice, as well as to help in their introgression or even pyramiding into previously proven elite cultivars. Markers can increase selection for target genes that are hard to evaluate in individual plants, decrease linkage drag near the gene of interest, and shorten the cycle of generations needed to regain a significant amount of the recurrent parent genetic background.

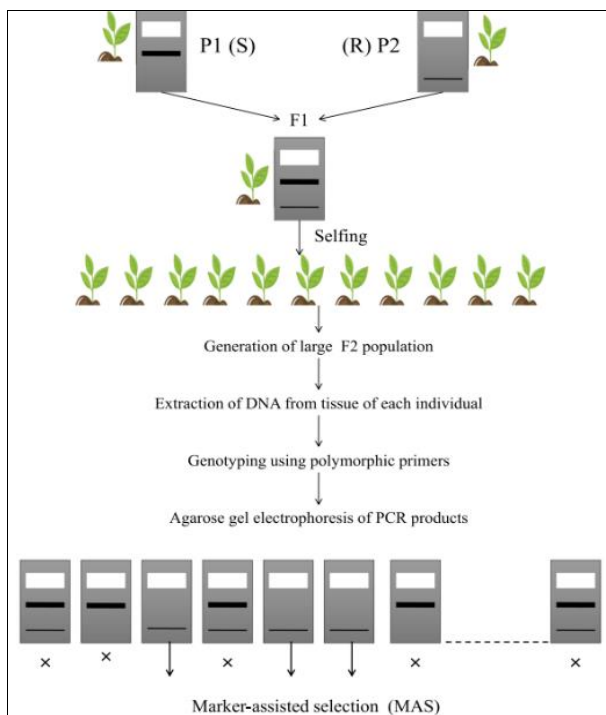


Fig 1: Steps of selection by using polymorphic primers (Hasan *et al.*, 2021) [20]

MAS for agronomic traits

Marker assisted selection is a useful plant breeding strategy that can use molecular marker to identify a specific trait that linked with the marker such as various agronomic trait, yield contributing trait, quality improvement trait and different breeding related traits, due to the genetic complexity of various traits like yield, marker assisted selection is useful method for improving the quantitative traits.

Yield improvement

Yield is a convoluted character that is directly or indirectly depended upon several traits, so it is hard to identify the potential marker for MAS. Primary hybrid rice considered as a more acceptable approach to increasing the yield potential in rice instead of existing rice cultivar. In the case of *Japonica* Nipon bare a QTL for yield potential on chromosome 5, rg5 indicated an allele from the indica cultivar Kasalath (Abdeta *et al.*, 2022) [1]. Various

agronomic traits, like the number of grains, plant height, panicle length, culm length, no of tillers, days to 50% flowering, number of panicles, days to heading (DTH), plant biomass, *etc.*, affect the total production of rice. Among these, grain yield has a greater effect on the total production of rice.

Grain Number

A large no of mapping population derived from inter specific Indica-Indica, Indica-Japonica, Japonica-Japonica crosses used to identify several quantitative trait loci (QTL) responsible for increasing rice yield like Gn1a, DEP1, Ghd8, OSPL14, OsMADS17 and APO1 (Li *et al.*, 2023). Gn1a is the first mapping-based grain number QTL identified by utilizing a near isogenic line (NIL); it is cloned from Habatkai, which produced cytokinin oxidase/dehydrogenase /dehydrogenase (CKX2), responsible for increasing the grain numbers and branches, which leads to enhanced grain yield (Gouda *et al.*, 2019) [15].

Tiller number

Panicle number is the important component in rice grain it is determine by number of tillers. Tiller development is affected by several environmental factors like climate conditions, planting density, daylight, temperature, and availability of water. A number of genes, particularly MONOCLUM1 (moc1), LAX1, LAX2, and OSH1 were identified for tiller number (Yan *et al.*, 2023) [46]. Genes that are controlling branches of panicle have been recently identified; among these, DEP1 and IPA1 are the most important dominant, semidormant regulators associated with panicles and branches and therefore affect the grain yield.

Days to heading

Days to heading (DTH) is an essential component in rice breeding programs for increasing the grain yield. QTLs for Days to heading, like qDTH-5, have been identified by crossing between Milyang 23 and *O. rufipogon* in the BC₂F₂ population (Gouda *et al.*, 2019) [15]. Heading date 1(Hd1) and early heading date 1(Ed1) are the two regulatory pathway that can regulated two flowering networks of homologous florigen genes HEADING DATE3a and FLOWERING LOCUS 1(RFT1). Under short-day and long-day conditions, Hd1 controls the expression of Hd3a/RFT1 and the interaction with Ghd7 and DTH8; it controls the expression of Ehd1 and Hd3a/RFT1 (Zhang *et al.*, 2019).

Plant height

Plant height is an agronomic trait that has a direct effect on crop yield. MYB-like transcription factor, OSMPH1 regulates the plant height in rice, and overexpression of OSMPH1 increases plant height and grain yield (Zhang *et al.*, 2017) [47]. Semi-dwarf 1 (SD1) plant height regulating gene, which encodes an enzyme GA20ox-2 important for gibberellin biosynthesis.

Spikelet fertility

To increase rice yield, breeder is emphasized on panicle morphology of rice and the floret numbers of each spikelet to increase the grain yield, typically rice spikelet consist single fertile floret which produced only one grain. In rice, interspecific and inter-subspecific crosses are important methods for the genetic enhancement of rice using near-isogenic line spikelet fertility QTLs (qss12, qss8, qss11) that have been identified (Lee *et al.*, 2021) [22].

Panicle length

Panicle length is an essential trait for increasing rice grain yield. A recombinant inbred line derived through a cross between Xiushui79 (short panicle) and C-bao (long panicle) a major locus LONG PANICLE 1 (LP1) present on chromosome no 9 identified using linkage and association mapping (Liu *et al.*, 2016). Heading date and panicle length QTL are commonly co-identified, and some genes are cloned, such as EHD4, Hd6, DTH7, Hd1, and Ghd7 (Sun *et al.*, 2022).

Grain Quality

Rice is a staple food for the world and improving rice grain quality is key for improving its nutritional value and consumer acceptance. Conventional breeding approaches for improving rice grain quality are time-consuming and less effective. Marker assisted selection is very much effective as well as important tool for direct selection of desirable alleles linked with grain quality traits. In the late 1900s, the eating quality of rice significantly improved in the rice breeding program, and several quality traits, such as amylose content, aroma, whiteness (white/dark with blight), cooking quality, grain size, and hardness (soft, hard), in rice had a significant effect on both the quality of eating and cooking.

Amylose Content

In the case of rice starch comprise of amylose and amylopectin. Granule -bond starch synthetase I (GBSSI) synthesis amylose that is responsible for the biosynthesis of extra-long branches of amylopectin which is synthesized by starch-branching enzymes (SBEs) and starch debranching enzyme (DBE). Addressing the genetics and genomics of starch biosynthesis and its variation such as f-starch branching enzyme, WX (waxy) gene mutation, and soluble starch synthase III genes, has become primary focus of recent studies on quality of eating and cooking of rice. The *osRING315* gene considered as a potential gene for both *qGC9-2* and *qAC9-2* which expressing E3 ubiquitin ligase increased the expression of *osRING315* related to the lower amylose content (AC) and higher gel consistency (GC) (Nie *et al.*, 2024) [24].

Aroma

Developing aromatic rice by using marker assisted selection (MAS) is a cost effective and useful strategy to enhance the elite genetic background with delight aroma. Crossing among (MR84) a non-aromatic variety and a low-yielding but high aromatic variety (MRQ74) helps to identify the most effective marker for aroma biofortification. The important chemical compound 2-acetyl-1-pyrroline (2AP) is associated with aroma in rice and is the most important QTL for aroma, *Qaro8.1*, which is located on chromosome no. 8 (Hajiaqatabar *et al.*, 2019) [17].

Cooking quality

While characterizing rice germplasm, the quality of eating and cooking is essential. The two primary factors, temperature of gelatinization (GT) and amylose content (AC), determine the cooked rice quality. High amylose content (AC) rice is good for diabetics and metabolic condition due to its slower to digest and low glycaemic index (Ishfaq *et al.*, 2023). Using transcriptome analysis, two candidate genes, *LOC_Os08g30550* and *LOC_Os08g29370*, are identified (Zhu *et al.*, 2024) [50].

Grain whiteness

The grain whiteness of rice is an important indicator of visual quality that has a direct impact on its commercial value, japonica rice. The recipient parent, Koshi Hikari and Nona Bokra as the donor parent identified major QTLs, *qWRG9* for grain whiteness of rice by chromosome substitution line (CSSL) (Chen *et al.*, 2024) [8].

Grain Size

Grain size is an important rice breeding trait that can affect grain yield and quality and controlled by multiple QTLs. A semi dominant QTL *GS2* which encode a transcription factor *OsGRF4* and regulate many traits of rice like grain number, grain size and grain quality. Various number of QTL identified for grain size like *GS3*, *GS5* (Zhang *et al.*, 2021) [48].

MAS for biotic stresses

Global rice production is severely hampered by severe yield reduction caused by various biotic stresses including Blast, Bacterial Blight, Brown Plant Hopper (BPH) etc. The primary objectives of breeding are to improve quality, increase yield and increase tolerance to stresses. Precision and efficiency in breeding could be improved by employing MAS. "Biotic Stress" is a condition that is caused by living things mostly bacteria, fungus, viruses, insect, nematode that affects plant's regular metabolism. There are 2 types of Biotic stress that includes stress due to disease and stress due to pest.

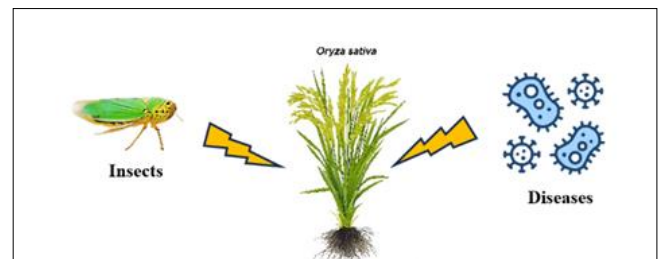


Fig 2: Different biotic stresses on Rice

Disease stress

The productivity, physiological function and growth of plants are impacted by the organisms that cause plant diseases. In plant breeding, resistant plants are found and crossed with susceptible ones to create disease-resistant cultivars. However, traditional breeding takes around ten years and might not identify different types of pathogens, which could result in susceptibility. By using marker-assisted selection (MAS), DNA markers can increase plant breeding's accuracy and efficiency while decreasing the need for time-consuming screening processes. These markers are useful for features that need maintenance of recessive alleles during backcrossing, that require particular environmental or host developmental stages, or that are difficult to control through traditional phenotypic selection.

Blast Disease

Blast disease is caused by the filamentous heterothallic ascomycetous hemi biotrophic fungus *Magnaporthe grisea*. It is the most destructive fungal disease that affects rice, resulting in significant yield losses and endangering the world's food security. More than 350 quantitative trait loci (QTLs) and 100 blast resistance genes have been discovered to date; 21 of these have been cloned and described in

detail. The backcross breeding approach with marker assistance was used to insert blast resistance genes (Pi2 and Pi9) into Vandana and Kalinga III (Kar *et al.*, 2018). Four QTLs: qRbr7 on chromosome 7, qRbr8 on chromosome 8, qRbr9 on chromosome 9, and qRbr12 on chromosome 12. qRbr9 and qRbr12, which accounted for phenotypic variance, showed a favourable correlation with blast resistance (Zhao *et al.*, 2024) ^[49].

Bacterial Leaf Blight (BLB)

BLB is caused by *Xanthomonas oryzae*, a dangerous disease in the rice-growing countries. This disease infects at the maximum tillering stage, resulting in yellowing of leaves, causes yield losses of between 20 and 50% in areas that are badly affected and even 100% under very drastic conditions. The development of varieties that carry major resistance (R) genes has been the most effective strategy to control BLB disease. Resistance genes (Xa4, xa13, xa5, and Xa21) pyramided lines were developed through MAB breeding in the genetic background of IR64 and Swarna under the Asian Rice Biotechnology Network (Singh *et al.*, 2001). Some major QTLs for BLB-resistant are Xa1, Xa2, Xa31, Xa45(t)/CGS-Xo1, Xa13/OsSWEET11/Os8N3, Xa21, Xa23, Xa47(t), and Xa41(t)/OsSWEET14/Os11N3.

Sheath Blight

The most harmful fungal disease affecting rice is called sheath blight (SB), and it is brought on by the soil-borne pathogenic fungus *Rhizoctonia solani*. qShB9-2 and qSBR11-1 are the two main loci where the ShB QTLs have been identified (Channamallikarjuna *et al.*, 2010) ^[7]. The primary genes utilized in resistance breeding are qSB-9TQ, qSB-11LE, and qSB-11HJX. Twelve candidate genes have been found in the qSB-9Tq region, and 26 potential disease-related candidate genes have been identified in the qSBR11-1 region (Molla *et al.*, 2020) ^[27]. One of the most efficient resistance QTLs, qSB-11HJX, is found on chromosome 11 and can lower the disease level of ShB by roughly 1.4%. Next-generation sequencing and GWAS are effective complementary approaches for mapping complicated rice characteristics. By increasing the putative resistance alleles aggregation efficiency, association mapping can shorten the ShB resistance breeding cycle. Additionally, this approach offers a fresh approach to basic resistance studies on ShB and marker-assisted breeding.

Brown Spot

Brown spot (BS) is a fungal disease that affects several areas of rice plants and causes large losses in grain quality and yield. It is caused by the necrotrophic fungus *Cochliobolus miyabeanus*. Some natural genetic resources, including "Tadukan," "CH45," "Dawn," and "Tetep," are resistant to BS, and found some QTLs linked to resistance genes. On chromosome 11, a significant BS resistance QTL, qBSfR11, was identified by using recombinant inbred lines produced from crossings between the susceptible "Hinohikari" and the resistant "Tadukan," revealing location of qBSfR11, on chromosome 11 (Sato *et al.*, 2015) ^[33]. Near qBSfR11, two more QTLs were discovered: BSq11.2v from IR62266 and qBSR11-kc from "CH45" (Matsumoto *et al.*, 2017) ^[25].

Sheath Rot

Sheath rot (ShR) disease caused by *Sarocladium oryzae* is one of the major diseases affecting most rice in the world. Marker-assisted selection (MAS) is a powerful tool used to develop rice varieties that are resistant to sheath rot. Researchers identify genes or quantitative trait loci (QTLs) associated with sheath rot resistance. Some major QTLs for sheath rot resistance are qShR-1, qShR-2, qShR-4, *etc.*

Insect stress

The productivity, physiological function, and growth of plants are hampered by insect and pest attacks. In plant breeding, resistant plants are found and crossed with susceptible ones to create insect-resistant cultivars. MAS allows suitable introduction of resistant genes without introducing unwanted traits.

Leaf folder

Rice leaf folder is *Cnaphalocrocis medinalis* is a moth belonging to the family Crambidae. Leaf folder's larvae feed on the rice leaves, causing them to fold and turn white, also reducing the photosynthetic area of the leaves and increasing yield loss. Scientists identified a gene called Pi-d2 that confers resistance to leaf folder in rice (Biswal *et al.*, 2017) ^[6]. Several quantitative trait loci (QTLs) have been identified in rice that are linked with resistance to leaf folder. Some of these QTLs include: qLF1, located on chromosome 1, qLF3 located on chromosome 3, qLF5 this QTL, found on chromosome 5.

Gall midge

A number of pests, diseases, and other dangerous species, such as the rice gall midge (GM, *Orseolia oryzae*) attacked during production of rice. Using molecular markers, the 11 GM resistance genes are identified in different cultivated rice varieties—Gm11(t), Gm2, Gm6, Gm3, Gm1, Gm4, and Gm8—have been mapped to distinct rice chromosomes. Gm1, which is found on chromosome 9, was discovered in "W1263" rice. Gm2 was discovered to be present in "Phalguna" rice and was connected to a 0.66-Mb segment of chromosome number 4. The resistance gene gm3 is found on chromosome 4 and was discovered in the rice line "RP2068-18-3-5.". One potential gene for gm3 has been identified as Os04g52970 (Sama *et al.*, 2014) ^[32]. Gene pyramiding is made easier by MAS. Within the hybrid rice paternal line RPHR-1005, Gm4, Gm8, and Xa21 are pyramided (Kumar *et al.*, 2017). To create a number of resistance lines, three genes that resist bacterial blight, two that resist blast, and four that resist genetic modification were pyramided together.

Green leafhopper

Nephotettix cincticeps uhler, also known as the green rice leafhopper (GRH), is a significant insect pest of cultivated rice. The GRH extracts sap from the phloem and xylem of rice cultivars that are vulnerable. Marker-assisted selection (MAS) has been used to create near-isogenic lines (NILs) for qGRH4, Grh2, Grh4, Grh1, Grh6, and Grh5. The resistance levels of four pyramided lines with the combinations Grh2 and Grh6, Grh2 and Grh4, Grh4 and Grh6, and, Grh5 and qGRH4 were greater than those of NILs with just one gene. GRH resistance regulated by qGRH9 exhibits a distinct developmental pattern that distinguishes it from previously discovered GRH-resistance

genes. At the seedling stage, the resistant cultivars and the NILs harbouring Grh6, Grh5, and Grh2 demonstrated strong resistance to the GHR. The novel GRH resistance pattern in qGRH9-IL (high during the booting stage, lacking in seedlings) raises the possibility that qGRH9 regulates the host plant's resistance to leafhoppers in rice during the adult stage (Fujita *et al.*, 2010) [13].

Brown planthopper

One of the most damaging insect pests of rice is BPH (*Nilaparvata lugens*), which causes severe yield loss and an estimated 300 million USD in economic losses in Asia each year. Five QTLs linked to BPH resistance in ARC10550 were found: qBphNp1, qBphDw3, qBphDs6, qBphDw8, and qBphNp12. The two main QTLs, qBphDw8 for days to wilt and qBphDs6 for damage score, were crucial for additional research and breeding program application (Deen *et al.*, 2017) [10]. Through marker-assisted breeding, the BPH resistance genes Bph1 and Bph2 have been effectively pyramided. It is currently in progress to molecularly map the resistance genes and QTLs from these two landraces, Dhobanumberi and Salkathi. From the Salkathi landrace, two QTLs were found, qBph4.3 and qBph4.4, of which qBph4.3 is novel (Mohanty *et al.*, 2017) [26]. BPH31 is the only dominant gene found on the long arm of chromosome 3 in CR2711-76, according to recent IRRI research.

Yellow stem borer

Yellow Stem Borer is a major threat during rice production in tropical and subtropical rice-growing countries. Since genes for resistance to YSB have not been found in the primary gene pool of rice, it tries to incorporate alien genes from wild species belonging to the secondary gene pool, which are stores of such traits (Singh *et al.*, 2020) [37]. The cytogenetic analysis of the chromosomal variants helps to develop monosomic alien addition lines (MAALs). Of the 8 MAALs screened, MAAL 11 was found to be moderately resistant to YSB (Narain *et al.*, 2020) [28]. Some key QTLs for YSB resistance include qYSB1 located on chromosome 1, qYSB5 located on chromosome 5, and qYSB11 found on chromosome 11.

MAS for abiotic stresses

Rice is a crucial food crop globally, and its production is significantly threatened by various environmental stresses among these, abiotic stress such as drought, salinity, heat, cold are particularly detrimental, which causes substantial yield losses, making it essential for rice breeders to developed strategies to enhance abiotic stress tolerance in rice varieties.

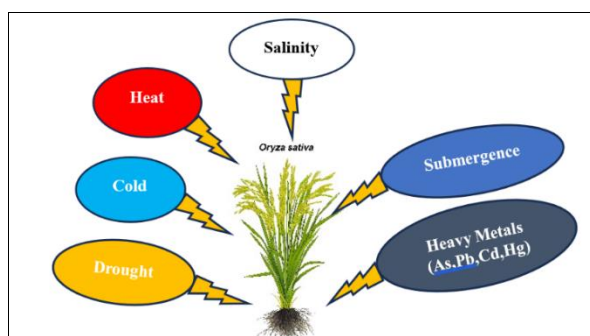


Fig 3: Different abiotic stresses on Rice

Drought stress

Drought is one of the most prevalent stresses that hinder the growth and development of plants under water-limited conditions. Significant improvements have been made through mapping QTLs for drought-resistance traits in rice. Drought resistance is the ability of a plant to produce its maximum yield under water-limited conditions. There is a different mechanism of drought tolerance, the ability of plants to survive under low water content. Under water deficit conditions, the reduction of transpiration is a crucial adaptive mechanism for plants, often achieved through the reduction of leaf area (Fang and Xiong 2015). To adapt to drought stress, plants decrease transpiration by developing a thick, waxy cuticular layer on the leaf surface. Additionally, plants exhibit xeromorphic adaptations, including modifications in leaf structure, such as a reduced number of smaller stomata, increased trichomes density, smaller and more compact leaves and the reinforcement of vascular tissue. Several drought-tolerant QTLs, such as *qDTY9.1*, *qDTY2.2*, *qDTY10.1*, and *qDTY4.1* have been incorporated in high-yielding varieties such as IR64 by marker-assisted backcrossing approaches (Singh *et al.*, 2016). The rice variety TDK1 has been enhanced for more yield under drought conditions by integrating three QTLs-*qDTY3.1*, *qDTY6.1*, and *qDTY6.2* (Dixit *et al.*, 2017) [11]. Developing drought-tolerant rice varieties remains challenging, as most high-yielding cultivars fail to thrive under rainfed conditions. Therefore, greater emphasis should be allocated to breeding climate-resilient and drought-tolerant rice with high yields and the ability to persist in adverse environments.

Salinity stress

Regarding their adaptation to salinity stress, plants can be classified into two groups: halophytes that tolerate relatively high salt concentrations and glycophytes that tolerate low salt concentrations. Among the principal cereal crops, rice exhibits the highest sensitivity to salt stress, whereas rye (*Secale cereale*) exhibits the highest tolerance. Salinity stress affects rice through three main mechanisms: osmotic stress, ionic imbalance, and oxidative stress. Under saline conditions, rice plants undergo morphological, physiological, biochemical, anatomical, and molecular changes. Rice is most sensitive to salinity during the germination to elongation phase and reproductive stages, whereas the germination, tillering, and ripening stages exhibit relatively high tolerance (Singh *et al.*, 2021) [36]. The combination of conventional breeding with molecular and genomic techniques has greatly improve salt tolerant rice variety. Marker-assisted selection, marker-assisted backcrossing (MABC), and gene pyramiding are part of the most effective methods in plant breeding that utilize molecular markers to identify and select genes controlling important traits, including biological or biotic and abiotic or non-biological stress tolerance (Gouda *et al.*, 2021) [14]. Numerous studies have successfully introduced the Saltol quantitative trait locus (QTL) into high-performing rice lines via marker-assisted breeding techniques to improve resistance to salinity. FL478 has been broadly applied as a Saltol QTL donor because of its photoperiod insensitivity, shorter flowering time than Pokkali, and enhanced salinity tolerance during the seedling stage. Several rice varieties, such as AS996, PB112, PB6, BT7, Bacthom 7, BRRI-dhan 49, Q5DB, and Novator, have successfully incorporated the Saltol QTL through SSR markers. (Krishnamurthy *et al.*, 2020) [21].

Heat stress

Climate change and temperature are becoming major issues nowadays, resulting in a shortening of the growing season for rice. The number of hot days and the occurrence of warmer nights are increasing all over major rice-producing countries. Therefore, it is essential to breed rice cultivar that exhibits resistance to temperature stress. Heat stress has multidimensional impacts on every aspect associated with the rice plants, because rising temperatures reduce rice yield during the grain filling period, affect the metabolic process during grain development (Ali *et al.*, 2022) ^[3], poor germination, weak seedlings, leaf yellowing, root deterioration, reduced tillering ability, leaf drooping and rolling, and biomass reduction throughout the vegetative growth period (Pandey *et al.*, 2022) ^[30]. Progress in molecular marker technology has enabled the detection of QTLs associated with heat tolerance, understanding of genetic mechanisms, marker-assisted selection, and QTL cloning in rice. Most heat-tolerant QTLs have been characterized during the flowering stage, particularly for traits such as spikelet sterility, yield, and stay green. These QTLs are mainly located on chromosomes 1, 4, 7, 9, and 10 across all 12 linkage groups. Several heat-tolerant QTLs have been identified at different developmental stages, such as seedling, booting, and flowering stages. Molecular markers associated with QTLs, such as RM11633 and RM11642 for qHTB1-1, enable marker-assisted selection to enhance heat tolerance during the pre-heading or panicle initiation stage (Cao *et al.*, 2020) ^[6]. The goal of molecular breeding is to identify stable QTLs across genetic backgrounds and environments and combine non-allelic QTLs for improved heat resistance.

Submergence stress

After heat and drought, flooding or submergence represents the third significant abiotic stress factor in rice. There are different types of submergence-induced stress in rice: submergence during germination, where soil water logging after heavy rain leads to anaerobic germination (AG), where seeds germinate without oxygen, leading to low germination rate, delayed seedling emergence, and elevated weed pressure in flood-prone areas. The second is flash flooding caused by river overflow, submerging crops for 1-2 weeks, particularly in laying areas where water remains at 25-50 cm for weeks to months, and deep-water flooding, where the water level can reach up to 4 m for months. Rice employs two main strategies to counter flooding: escape, characterized by shoot elongation through the SK1 and SK2 genes in deep-water rice, and quiescence, in which the Sub1A gene limits shoot elongation to conserve energy during flash floods. A breakthrough in submergence tolerance breeding was the identification of Sub1, a QTL responsible for submergence tolerance. Marker-assisted backcrossing (MABC) enables the transfer of stress-tolerant genes such as Sub1, enhancing high-yielding varieties such as Swarna-Sub1, which can withstand 14 days of submergence (Tamang & Fukao, 2015) ^[38]. The identification of the AG1 QTL from Khao Hlan on facilitated the transfer of Tolerance of low-oxygen germination environment into elite varieties such as IR64-Sub1 and PSB Rc18-Sub1 (Toledo *et al.*, 2015) ^[40].

Cold stress

Predominantly, rice cultivars vary in their tolerance to low-temperature stress (LTS); *indica* subspecies are sensitive to LTS, whereas *japonica* subspecies are known to tolerate cold stress. Rice shows increased vulnerability to temperatures below 15°C, affecting all growth stages. Cold stress reduces seedling vigor, delays germination, causes poor tillering and increases mortality. During the early growth period, it leads to leaf discoloration, slowed growth, and seedling death. The flowering stage is the most vulnerable, leading to failure in anther development and poor pollen shedding (Shakiba *et al.*, 2017) ^[34]. Marker-assisted selection (MAS) plays a crucial role in rice breeding by enabling the accumulation of desirable alleles through inheritance tracking, identification of superior individuals, and integration of key alleles for enhanced cold tolerance. Genes such as COLD1 and CTB4a have been effective in the development of rice cultivars with cold tolerance, whereas genotypic assays targeting markers such as COLD1 and NAC6 have facilitated the identification of cold-resistant accessions (Yongbin *et al.*, 2023) ^[46]. The *japonica* rice cultivar Kongyu 131 was improved by introducing the Gn1a gene for higher yield and transferring an 800 kb chromosomal segment from *indica* rice to enhance adaptability to low-latitude regions (Wang *et al.*, 2019) ^[41, 42].

Light stress

Rice has been seen globally that receive higher solar radiations and longer day lengths during the growing season. Light is an environmental factor that regulates plant development and metabolism and is essential for photosynthesis and photomorphogenesis. However, plants often encounter light stress conditions, such as excessive or insufficient light intensity. This variation in the ideal light condition for plants can have detrimental consequences on photosynthetic activity, ultimately influencing plant growth and yield. Despite this, light stress is considered one of the least characterized and understudied abiotic stresses that plants commonly face throughout their life cycle. However, researchers have recently attempted to understand the significance of light stress and how excess light (EL) and low light (LL) affect plants (Banerjee and Roychoudhury, 2016) ^[4]. Most molecular studies have identified genes controlling traits such as high chlorophyll content, stay-green, erect lamina, and yield under low light, yet natural variation in these genes remains largely unstudied (Rai *et al.*, 2021). Nevertheless, the molecular basis of tolerance to low light (LL) and high light across multiple crop species remains poorly understood, and to date, no gene or marker has been identified that is directly associated with improved performance under low light intensity under field conditions. Consequently, pinpointing genomic loci for high and low light tolerance in key crop plants is crucial. This will facilitate the identification of candidate genes and the development of molecular markers for use in marker-assisted selection breeding programs.

Cadmium stress

Cd is a silvery-white transition metal (atomic number: 48; relative atomic mass: 112.41; density 8.64 g cm⁻³) discovered by German chemist Friedrich Stromeyer in 1817. When Cd accumulates in various organs, it affects human health by first targeting the kidneys and liver, damaging

multiple tissues, and inhibiting DNA synthesis and repair (Liu *et al.*, 2009) ^[24]. “*Itai Itai*” disease, which was a mass Cd poisoning due to ingesting Cd-contaminated rice and drinking water, was reported in Japan in the 1950s. Rice can easily uptake Cd from the field through its roots, and crop nodes play a significant function in Cd distribution in the shoot. During reproductive development, Cd within the grain is transported via the phloem from the leaf blades or xylem-to-phloem transport in the nodes. Researchers have mapped many cadmium (Cd) related quantitative trait loci distributed across all 12 chromosomes (Yan *et al.*, 2019) ^[44]. These QTLs influence traits, such as Cd accumulation, translocation, and tolerance. For example, the QTL qCdT7/qGCd7, located near the SSR marker RM8006 on the p-arm of chromosome 7, has been repeatedly linked to reduced Cd in shoots and grains (Tezuka *et al.*, 2010) ^[39]. Some key genes control Cd uptake in rice, including OsHMA3, OsNRAMP5, OsNRAMP1, OsNRAMP2, and LCD (Wang *et al.*, 2019) ^[41, 42].

Arsenic stress

Arsenic is a common metalloid that occurs in various forms, including inorganic species such as trivalent As (III) and pentavalent As (V) and organic species such as mono- and di-methyl arsenic compounds. Its oxidation state ranges from -III to +V, highlighting its complex chemistry. These diverse forms have harmful effects on human health. However, toxicity depends on the form (inorganic or organic) and oxidation state of As compounds (EFSA2009; WHO 2011). There are at least three complementary options for mitigating the harmful effects of high As concentrations: cropping practices, rice cooking methods, and the adoption of As-tolerant rice varieties. The molecular basis of As tolerance in rice indicates that it represents a complex quantitative trait, as identified through QTL mapping and transcriptome analyses. GWAS experiments have validated several QTLs originally identified in biparental crosses and have refined their positions, making them attractive targets for conventional marker-assisted selection (MAS). The success of MAS for As tolerance relies on the careful selection of donor parents from diverse *Oryza sativa* groups and on targeting QTLs that are robust and have significant effects. Integrating these favorable alleles through marker-assisted recurrent selection (MARS) could further enhance their performance in elite backgrounds. Overall, MAS represents a promising strategy for breeding rice with reduced grain As content (Hassen *et al.*, 2017).

Phosphorus stress

Phosphorus (P) is a key nutrient required for plant growth and physiological development. In rice, symptoms of phosphorus deficiency appear as early as 30 days after transplanting, resulting in restricted growth, decreased tiller formation, dark green erect leaves, thin stems, and reduced panicle numbers and grain count. The rice genome encodes 26 Pi transporters classified into four subgroups: PHT1 (plasma membrane-localized, involved in uptake), PHT2/4 (plastid transport), PHT3 (mitochondrial transport), and PHT4 (Golgi transport) (Liu *et al.*, 2011) ^[23]. The PHT1 family, particularly OsPT genes, are crucial for Phosphorus acquisition and redistribution, with many members exhibiting phosphate starvation-induced expression (Gu *et al.*, 2016). Additionally, OsSPX-MFS1 and OsSPX-MFS2 regulate Pi transport across the vacuoles. PHO1 and PHO2 control Pi homeostasis by regulating Pi loading in roots and

leaves, respectively. PUP1-based markers are widely utilized in marker-assisted selection (MAS) and marker-assisted backcrossing (MABC) (Chithrameenal *et al.*, 2018) ^[9].

Achievements, Limitations and Future prospects of MAS

Marker assisted selection (MAS) uses DNA markers linked to targeted genes for select plants carrying favourable alleles, which accelerate the breeding process. This process has led to significant achievements in various areas in crop production improvement. MAS has facilitated the development of disease resistance varieties by identifying and selecting for genes resistance to many pathogens. This process decreases yield losses and minimizes the use of chemicals in farming. MAS has also accelerated the breeding process for several complex traits like quality, yield which are controlled by multiple genes. By selecting for favourable alleles at multiple locus Marker Assisted Selection has given access to breeders to make faster progress in improving those traits. Also, MAS helps in the introgression of desirable traits from wild cultivars to cultivated varieties by enhancing their adaptability to diverse environments. MAS also used to develop various varieties with improved nutritional quality, like higher levels of vitamins, minerals and proteins. By selecting for genes associated with these traits, MAS contributed to enhancing food security and human health. Although MAS has emerged as a powerful tool in Plant breeding, given access to breeders to develop improved varieties with also improved productivity and nutritional value. Although MAS has so many limitations, those are also very important for crop improvement. Marker Assisted Selection methods are very much costly and this needs a very well-developed laboratory with modern equipment and expensive chemicals. The different link markers detection (RFLP, RAPD, SNP, AFLP, *etc.*) takes more time, laborious and difficult. Marker assisted selection requires trained labour to handle the advanced equipment, DNA isolation and the study of DNA markers. In MAS uses radioisotopes for DNA labelling, which very much serious to health hazards. From many studies it is reported that MAS may be less effective than phenotype selection in the long term. As a model crop with a fully sequenced genome, rice offers excellent chances to investigate the molecular mechanisms underlying disease resistance and to engineer the creation of rice varieties with a variety of resistance resources that are effective against different diseases. The copy of (R) genes to combat blast, bacterial blight and other diseases has been the significant advancement in our understanding of the molecular processes of disease resistance. In order to find genes or QTLs that will function across populations, GWAS for resistance genes should be added to the effective QTLs or genes that are found using biparental mapping methodologies. Multi-parent populations are thought to be an improvement over association mapping and bi-parental populations because the former only looks at differences between two individuals' genomic regions, while the latter must use very large samples to find gene of interest, despite capturing far more diversity. From a theoretical and practical perspective, multi-parent advanced generation inter-Cross (MAGIC) is a compelling substitute. It's potential to use both pest and host genome sequences to comprehend resistance mechanisms and resistance breakdown has expanded with advances in genome sequencing. It is crucial to identify improved active

haplotypes of resistance genes from both cultivated and wild species to identify functional markers. It would be possible to directly alter effector targets and produce resistant mutants by inducing certain mutations using site-specific nucleases (gene editing). Vertical growth of disease-resistant cultivars in known epidemic regions should receive more attention than horizontal growth into less afflicted areas.

Conclusion

The exponential growth of populations and extreme changes in the environment are becoming a matter of concern for crop improvement. Genetic diversity plays a crucial role in crop improvement programs. Through conventional breeding process it's hard to find genetic involvement but marker assisted selection precisely locate and transfer gene of interest to the targeted location by the help of DNA markers. MAS is effective for identification of alleles, QTLs, and genes responsible for yield enhancement, quality improvement, and stress tolerance of crops. There are many biotic and abiotic stresses that hinder the productivity of rice throughout the world. Quantitative trait loci (QTL) region is where one or several linked genes present and influence a particular trait. Polymorphic DNA marker easily find out the QTLs and helps to select the desired traits present on a particular genotype. Conventional breeding methods along with MAS speed up selection, generation improvement and new variety development. Through background and foreground selection by the help of markers, it's easy to develop disease resistance and stress tolerance rice varieties respectively. Though MAS needs a very well-developed laboratory with modern equipment and expensive chemicals, it's becoming more popular tool in modern plant breeding era.

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