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Rice crop improvement through conventional breeding and modern molecular techniques

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Abstract
Production of rice (*Oryza sativa* L.) and the application of conventional and modern breeding technologies for rice improvement. It emphasizes that increasing productivity and resilience to biotic and abiotic stresses requires moving beyond conventional breeding toward molecular and genomic approaches. The paper discusses conventional breeding, in which hybridization, selection, recombination, and progeny evaluation are used to develop locally adapted, high-yielding rice varieties. It also describes marker-assisted selection (MAS), in which DNA markers linked to desirable genes or QTLs allow breeders to identify and select useful genotypes at an early stage. Important examples include markers associated with bacterial blight resistance, drought tolerance, fragrance, submergence tolerance, salinity tolerance, grain size, and grain number. A major focus is marker-assisted backcrossing (MABC), which enables the transfer of specific target genes or QTLs into an elite recurrent parent while retaining most of its desirable genetic background. The process involves foreground selection, recombinant selection, and background selection, with recombinant selection helping to minimize linkage drag. The review also explains gene pyramiding, where multiple resistance genes are combined into a single cultivar to provide broader and more durable disease resistance. Finally, it discusses CRISPR/Cas9-mediated genome editing as a precise and rapid technology for modifying specific rice genes. CRISPR/Cas9 uses guide RNA to direct Cas9 to a target DNA sequence, producing a double-strand break that can be repaired through NHEJ or HDR, thereby enabling gene knockout or precise genetic modification.

Keywords: Rice breeding, QTL analysis, marker-assisted backcrossing (MABC)

Introduction

Rice (*Oryza sativa* L.), a member of the Poaceae family, is one of the most important and widely eaten staple food crops in the world. It originated in Southeast Asia and is mainly grown in the piedmont of the far eastern Himalayas and various climate zones. In India, rice is grown extensively during the kharif season and is one of the most important cereal crops and a major component of food security, particularly in Asia (Gowda *et al*., 2025) [17]. Rice is the second most-produced cereal in the world after wheat (Luz *et al*., 2016) [27]. Among the six continents worldwide, Asia alone accounted for approximately 454 million tons of rice (90% of total rice production) from approximately 141 million hectares (86% of total production area) (Abdullah *et al*., 2025) [1]. West Bengal, known as the "rice bowl" of India, is a leader in rice production and is home to a remarkably diverse range of cultivated folk rice genotypes (Debnath *et al*., 2020) [9]. Among these, the distinctly scented landrace Kanakchur is locally known as aromatic popped rice or 'khoi' (Deb *et al*., 2021) [8]. This article aims to synthesize information on recent practical utility successes of gene editing technology in developing multiple high-yielding rice varieties in India and to discuss the possibility of using gene editing technology to improve various components of rice. New Rice is one of the target commodities that has received adequate attention in Supporting agricultural growth (Moncada *et al*., 2001) [33]. Programs for crop breeding gather, induce and combine desirable phenotypes across crop species to meet human needs (Fu *et al*., 2015). Because of this, breeders focus on developing crop varieties that protect against yield loss while pushing overall harvest potential higher. Today, several modern breeding techniques make it possible to significantly boost these production limits. Such as Conventional breeding, CRISPR, Marker-Assisted Backcrossing (MABC), QTL, and Conventional breeding have made major contributions to rice improvement, but the requirement for higher productivity and greater resilience has increased the importance of molecular and genomic approaches. (Singh and Solanki 2026) [43]. Conventional breeding depends mainly on the exploitation of existing variation through selection, hybridization, recombination and evaluation of progenies. Although these approaches remain fundamental to crop improvement, they are relatively slow and strongly influenced by environmental conditions (Chouksey *et al*., 2025) [5]. With the advent of genetic engineering, transgenic technologies, and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) genome editing, these tools are becoming the preferred options for rice improvement (Westra *et al*., 2016) [48]. Marker-assisted backcrossing (MABC) is a smart DNA-based tool for breeding, mainly used in a large number of research. Its aim is to identify genomic regions of interest. Molecular markers that are strongly associated with economically important traits have been identified or used for MABC help breeders quickly breed varieties that can withstand major diseases in rice, including resistance of BB, blast, brown plant hopper, green leaf hopper, gall midge, virus infections, submergence, drought, cold, semi-dwarf, grain quality, etc. (Neeraja *et al*, 2007) [34]. Many important traits in rice, including yield, drought tolerance, root architecture, and other stress Responses, have quantitative

inheritance. QTL mapping allows researchers to identify genomic regions associated with phenotypic variation Chouksey *et al*. (2025) [5]. The following review provides a detailed description of rice production and modern breeding techniques.

Conventional Breeding

The main objective of breeding is particular agro-ecological areas to develop new rice varieties suited for local environmental conditions. The locally adaptive varieties mainly show improvement of yield (Sabar *et al*., 2024) [39]. Conventional rice breeding originated from the age old practice of artificial selection from local landraces. Farmers choose plants with favorable traits such as high yield, good grain quality, greater adaptability, salinity tolerance, and drought tolerance (Xie *et al*., 2021) [50]. These hybridization techniques require many years to develop a plant with desired traits, and they can sometimes create new diseases, while climate shifts can also hinder rapid responses (Peng *et al*., 2008) [36].

First step involves crossing of different parents to get genetic variation, followed by identify and selection of superior individuals based on the field performance (Altaf *et al*., 2021) [2]. Prevalent rice breeding method is pedigree method. Pedigree or mass selection is adopted when the selection is unable to effectively differ superior genotypes from inferior one. This method combines five segregating generations from the F2 generation and is bulked before initiating. Using conventional breeding, IRRI successfully developed agronomically superior rice varieties with high tolerance to flooding (Khan *et al*., 2015) [23].

Marker Assisted Breeding

Marker-assisted molecular breeding uses mainly DNA markers linked with specific genes

(Nadeem *et al*., 2017) or sometimes QTLs controlling traits. At first, identifying and validating the markers linked to genes to target traits. Then, we need to breed populations by genotypes, followed by the selection of individual alleles that carry favorable markers for posterior crossing (Chouksey *et al*., 2025) [5]. Marker-assisted selection is mainly useful for selecting desirable traits that are expressed only in the later stages of reproduction. But through DNA markers those desirable traits showed their characters in genotype at early stage (Madhusudhana 2019). Some of the most used marker-assisted breeding are RFLP, AFLP, RAPD, SSR, and SNP (Jiang *et al*. 2013) [21].

Table 1: List of some agronomically important major genes and QTLs governing important traits in rice and their chromosomal locations.

Genes/ QTLs	Chromosome	Locus	Trait/Function	Citation
Xa21, Xa5, Xa13	11 5 8	Os11g0569733 Os05g0108600 OsSWEET11	Bacterial blight resistance	Fiyaz *et al*., 2022
qDTY1.1	1	RM431–RM12091	Drought tolerance	Ghimire *et al*., 2012
Badh2	8	Os08g0424500	Fragrance	Shi *et al*., 2008
Sub1A	9	SUB1	Submergence tolerance	
SUB 1A-1	9	SUB1		Fukao *et al*., 2009
SUB 1B	9	SUB1		
SUB1C-1	9	SUB1		
OsSAPK2	7	LOC_Os07g42940	Drought stress tolerance	Lou *et al*., 2017
Pup1	12	Pup1	Tolerance to phosphorus (P) deficiency	Chin et al., 2011
Bph14	3	Bph14	Brown planthopper resistance	Zhou et al., 2013
Awn3-1	3	Os03g0418600	Awn	Li *et al*., 2016
OsCIPK03	7	Os07g0687000	cold	Xiang et al., 2007
qLTG3-1	3	Os03g0103300		Fujino *et al*., 2008
MYBS3	3	Os03g0407400		Su *et al*., 2010
GIF1	4	Os04g0413500	Grain filling	Wang et al., 2008
NOG1	1	Os01g0752200	Grain number	Huo *et al*., 2017
WFP	8	Os08g0509600		Miura et al., 2010
GS3	3	Os12G0528400	Grain size	Fan *et al*., 2006
GW2	2	Os02g0244100		Song et al., 2007
GS2	2	Os02g0701300		Zhang et al., 2013
BBH/Lsi 1	2	Os02g0745100	Hull colour	Yang et al., 2018
Lsi6	6	Os06g0228200		
LP1	9	Os09g228300	Panicle length	Li *et al*., 2016
SP1	11	Os11g0235200		Li *et al*., 2009
OsKATI	1	Os01g0756700	Salt tolerance	Obata et al., 2007
OsNOH1	2	Os02g0168200	Plant length	Zhang et al., 2017
MOC1	6	Os06g0610300	tillering	Li *et al*., 2003
qSH1	1	Os01g0848400	Seed shattering	Konishi *et al*.,2006
Hd6	3	Os03g0762000	Heading date	Takahashi *et al*.,2001

MAS can be applied to both biotic and abiotic traits. The Sub1 and Saltol regions have been particularly important in breeding for submergence and salinity tolerance, respectively. Similarly, bacterial blight resistance genes, including Xa21, Xa5, and Xa13, have been transferred into elite rice backgrounds through MAS (Sabar *et al*., 2024) [39].

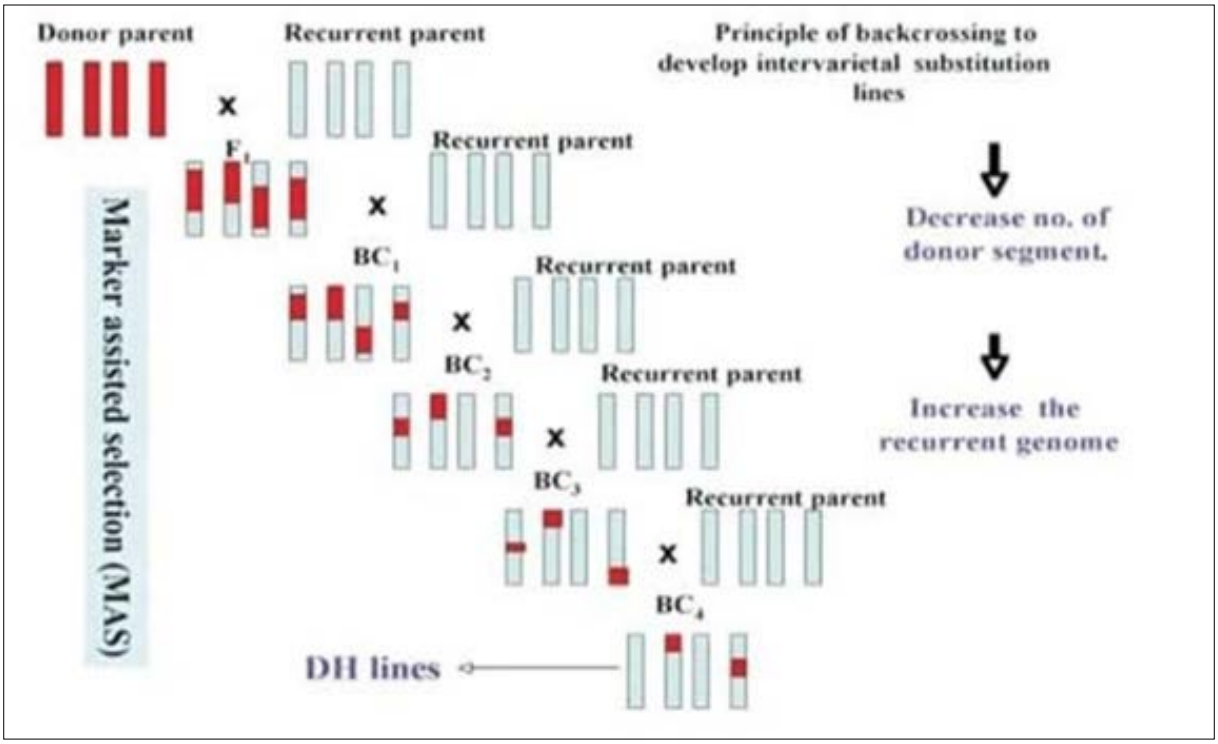


Fig 1: Schematic representation of marker-assisted selection. Source: (Malav *et al*., 2016) [30].

Marker-Assisted Backcrossing (MABC)

MABC is one of the most precise and efficient techniques to introgress a specific trait controlling locus, thereby preserving the essential characteristics (such as genetic background) of the Recurrent Parent (RP) (Collard *et al*., 2008) [6]. It is a process in which a marker based on morphological, biochemical or DNA/RNA polymorphisms is utilized for indirect genetic selection of a desired trait (e.g., disease resistance, abiotic stress tolerance, productivity and quality). This method has been widely adopted to enhance important traits such as disease resistance, tolerance to abiotic stresses, grain yield and quality with other agronomic characteristics (Hasan *et al*., 2015) [18].

MABC is generally carried in three main steps:

Step 1: Foreground selection for the target gene(s)

The main aim is to manage the target locus in a heterozygous state (one donor allele and one RP allele) until the completion of the final backcross. Marker-assisted foreground selection was proposed by Tanksley (1983) and investigated by Melchinger (1990) [31, 46] in the context of the introgression of resistance genes.

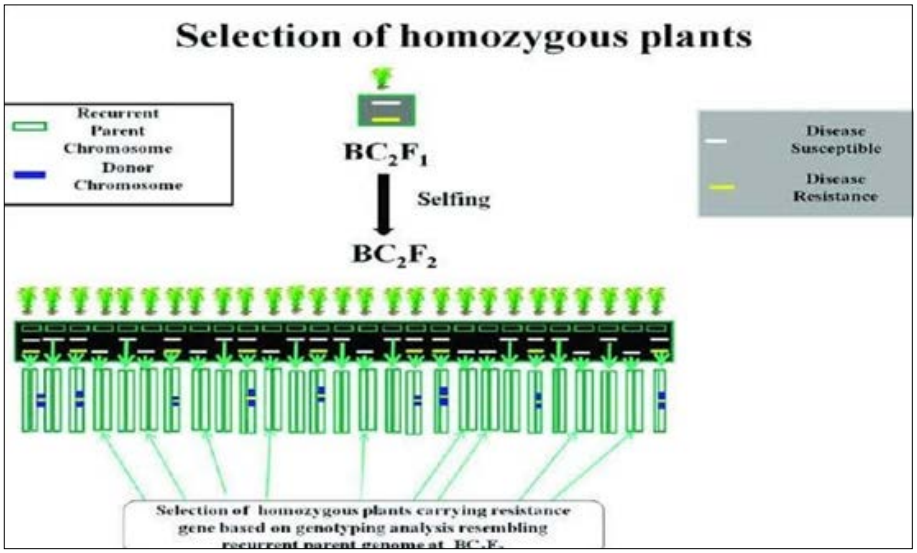
It is the first step in which the marker allele of the parental donor plant at the target locus are selected and is followed by the self-pollination of the selected plant. The resultant progeny plants are homozygous for the donor allele. Molecular markers located near the targeted gene or QTL are used to identify plants carrying the required gene at an early stage of breeding. This method if known as foreground selection (Schulthess & Schwember, 2013) [41].

Step 2: Recombinant selection

The second step involves selecting BC progeny lines with the trait of interest. The genetic recombination between the target locus and the adjacent linked markers is referred to as 'recombinant selection' (Collard *et al*., 2008) [6]. By using conventional breeding methods, the donor DNA segment may remain very large even after several backcross generations. However, markers located on both sides of the desired gene can help minimize linkage drag. Recombinant selection is generally carried out after at least two backcross generations, because double recombination at both sides of the target gene is very rare (Frisch *et al*., 1999) [12]. This technique helps to reduce the size of the donor chromosome segment and, therefore, reduce linkage drag (Hospital, 2001) [19].

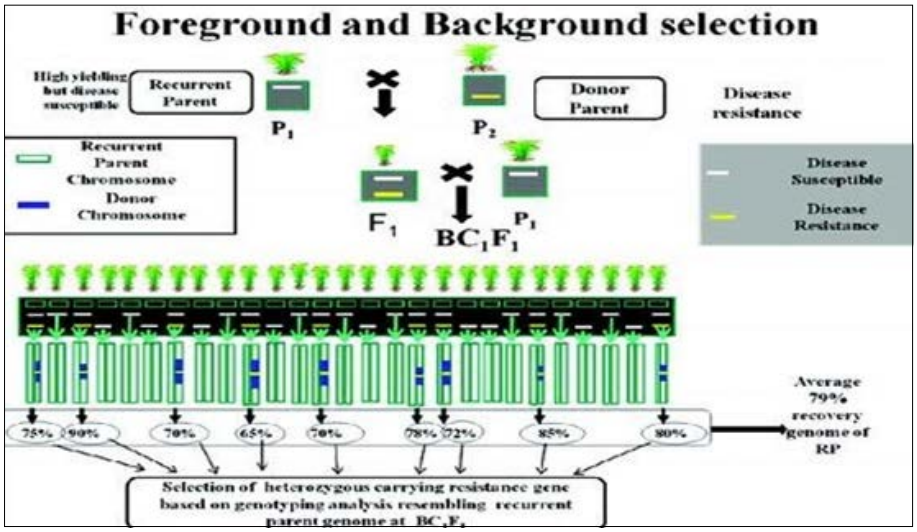
Step 3: Background selection throughout the genome.

In the third step, all genomic regions are selected based on the RP marker alleles, excluding the target locus. This selection is generally based on phenotypic evaluation of the desired locus and is an important step in removing unwanted genes from the donor parent. This technique involves the use of f tightly linked flanking markers for recombinant selection and unlinked markers to select RP. According to Takeuchi *et al*., it is also known as "negative selection." The use of background selection in MABC helps promote the development of a recurrent parent (RP) carrying one or more additional genes. According to Mackill (2006) [28], this has been referred to as "variety development or enhancement" and "complete line conversion" by Ribaut *et al*. (2002) [38].



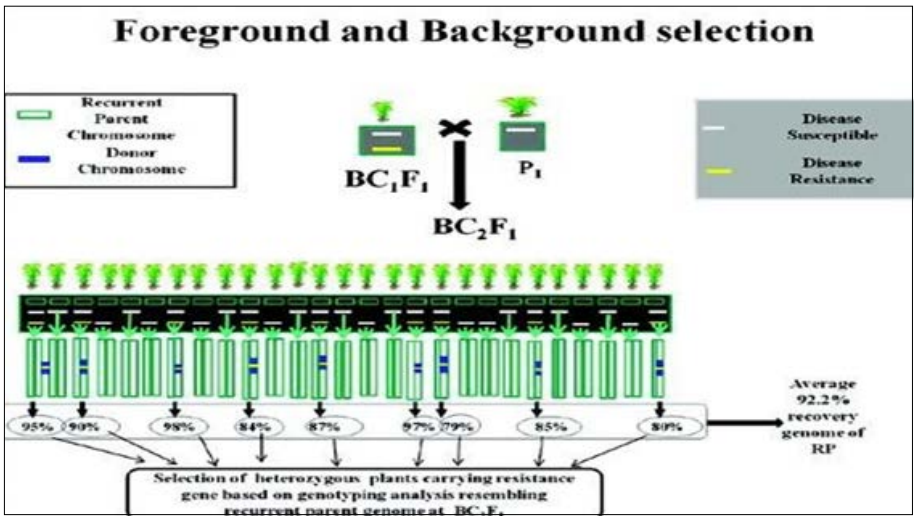
Source: modified from IRRI, (2014) with permission (www.knowledgebank.irri.org).

Fig 2: Schematic representation of selection of homozygous plants for the donor allele.



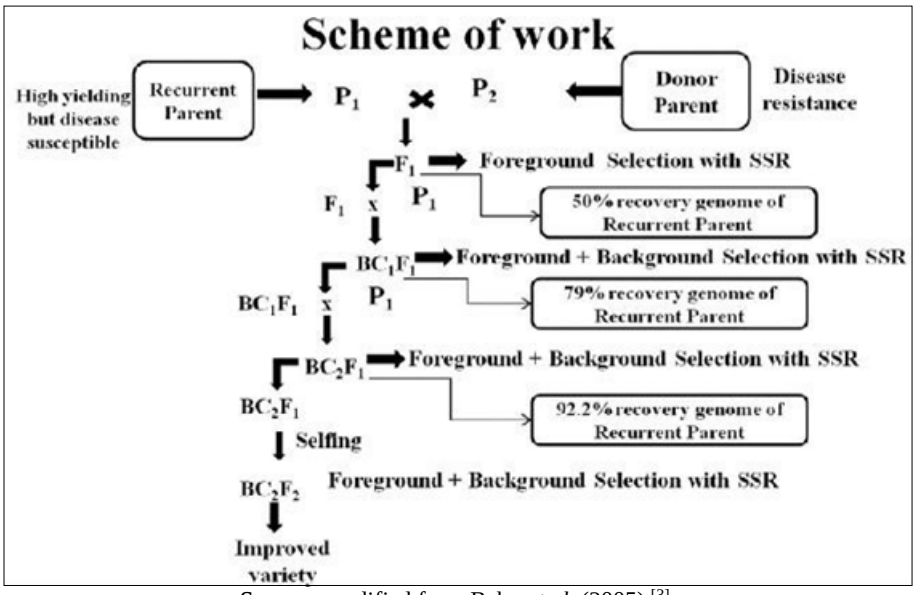
Source: modified from IRRI, (2014) with permission (www.knowledgebank.irri.org).

Fig 3: Schematic representation of selection of heterozygous carrying resistance gene based on genotyping analysis resembling RP genome at BC1F1.



Source: modified from IRRI, (2014) with permission (www.knowledgebank.irri.org).

Fig 4: Schematic representation of selection of heterozygous carrying resistance gene based on genotyping analysis resembling RP genome at BC2F1.



Source: modified from Babu *et al*. (2005) [3].

Fig 5: Schematic representation of the development of a resistant rice variety through marker-assisted backcrossing (MABC).

Gene Pyramiding

Gene pyramiding is a plant breeding method in which breeders combine several resistance genes from various sources into a single plant variety, conferring effective resistance to diseases and pathogens and thereby prolonging the lifespan of resistance genes in cultivars. This technique increases the degree of disease resistance. Its main aims include improving an existing elite cultivar, reducing the need for extensive phenotypic evaluation, minimizing linkage drag, and shortening the overall breeding time (Zhang *et al*., 2006; Malav and Chandrawat, 2016) [28, 30]. The effectiveness of this method depends on several factors, including the number of genes to be transferred, the distance between the target genes and adjacent markers, and the selection of plants at each breeding generation. Marker-assisted selection (MAS) is commonly used to identify and transfer the desired genes from pyramided lines into the target crop variety. This increases the chances of obtaining plants with desired gene of interest (Magar *et al*. 2014; Das and Rao 2015; Pradhan *et al*. 2015; Shamsudin *et al*. 2016) [7, 29, 30].

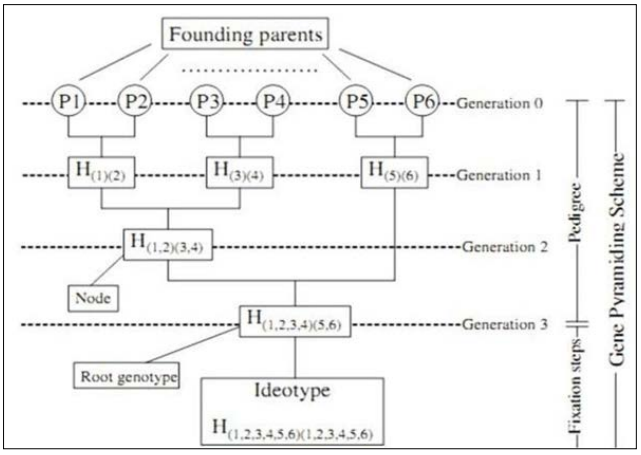


Fig 6: Schematic representation of Gene pyramiding. A distinctive gene pyramiding approach with six target genes (Hospital *et al*., 2004).

CRISPR/Cas9 Mediated Gene Transfer

The introduction of CRISPR/Cas9 genome-editing technology has transformed functional genomics and crop improvement by offering plant breeders a precise, efficient, and flexible method for making targeted genetic changes. The CRISPR/Cas9 system evolved from a natural bacterial defense mechanism. The Cas9 system particularly the one derived from *Streptococcus pyogenes* has become one of the most widely used tools for modifying plant genomes, including rice (Sadhu *et al*., 2025) [40]. The advancing CRISPR/Cas9 base genome editing technology, encompassing knockout, based editing and allele exchange, provides an alternative approach for accelerating crop breeding (Li *et al*., 2022; Rathore *et al*., 2025) [5]. CRISPR/Cas9 introduces a guide RNA that recognizes and directs the Cas9 nucleases to a target DNA sequence, where it generates a double-strand break. The resulting DNA breaks are repaired through either non- homologous end joining (NHEJ) or homology -directed repair (HDR) which can result in gene knockout or precise modification. In rice, CRISPR/Cas9 can target structural or regulatory genes to improve abiotic stress tolerance. It offers precision, rapid and the ability to generate transgene - free rice lines (Rathore *et al*., 2025) [5].

Conclusion

Rice improvement depends mainly on integrating conventional breeding with modern molecular techniques. Conventional breeding is the foundation of rice variety development because it activates naturally available genetic variation through many methods such as selection, hybridization, and recombination. However, it makes rapid crop improvement difficult. Marker-assisted breeding primarily connects conventional and molecular breeding. Using DNA markers linked to genes or QTLs, breeders can identify desirable alleles at early stages. The approach has been applied to important traits such as salt tolerance, salinity tolerance, fragrance, bacterial blight resistance, and many agronomic characters. MABC is particularly valuable for specific genes or QTLs transferring into a prime variety while the recurrent parent retains a desirable genetic background. This allows farmers or breeders to target the

gene properly to reduce linkage drag. Thus, the improvement of rice varieties can be increased by MABC. Efficiency of identifying plants that carry the desired combination of genes while reducing the dependency on extensive phenotypic screening in gene pyramiding. More recently, CRISPR/Cas9 genome editing has extended the possibilities of rice improvement. It enables the modification of specific regions of the genome. This technology uses a guide RNA sequence to generate double-strand breaks, followed by repair through mechanisms such as non-homologous end joining. These approaches are mainly important for rice production and strengthen food security. Combining conventional breeding with modern technologies to achieve faster, more precise rice improvement.

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