



# Plant Archives

Journal homepage: <http://www.plantarchives.org>

DOI Url : <https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.321>

## FROM TRADITIONAL SELECTION TO NEXT-GEN PADDY: A LOOK AT PADDY ADVANCEMENTS

Dibyendu Seth<sup>1\*</sup>, Rahul Dhaniya<sup>1</sup>, Amitava Paul<sup>2</sup> and Pummy Kumari<sup>3\*</sup>

<sup>1</sup>Department of Genetics and Plant Breeding, CCS Haryana Agricultural University (CCSHAU), Hisar - 125 004, Haryana, India.

<sup>2</sup>Department of Genetics and Plant Breeding, Palli Siksha Bhavana, Visva Bharati University, Sriniketan - 731 236, India.

<sup>3</sup>Forage Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University - 125 004, Haryana, India.

\*Corresponding authors E-mails : [deep032002@gmail.com](mailto:deep032002@gmail.com), [pummy.hau@gmail.com](mailto:pummy.hau@gmail.com)

(Date of Receiving-09-03-2024; Date of Acceptance-26-05-2024)

### ABSTRACT

Rice, as a staple food for over half of the world's population, plays a pivotal role in global food security. The challenges of population growth, climate change and diminishing agricultural resources necessitate continuous innovation in rice breeding. This book chapter delves into the evolution of rice breeding methodologies, emphasizing the synergy between traditional practices and modern biotechnological advancements. It explores classical breeding approaches such as phenotypic selection and hybridization, alongside cutting-edge techniques including molecular markers, genomics, and gene editing. The chapter evaluates the impact of these innovations on enhancing rice yield, quality, and resilience to biotic and abiotic stresses. Furthermore, it discusses the socio-economic implications of rice breeding innovations, highlighting the importance of farmer participation, equitable access to technology, and conservation of genetic diversity. Through an interdisciplinary lens, this chapter aims to provide insights into the dynamic landscape of rice breeding, offering a roadmap for sustainable rice production to meet the demands of the future.

**Key words :** Rice breeding, Crop improvement, Food security, Genomic selection, Gene editing techniques, Biotic & Abiotic stress.

### Introduction

*Oryza sativa* L. ( $2n = 2x = 24$ ), commonly known as rice, holds immense significance as the second most cultivated cereal globally. This grass species serves as the primary dietary source for over half of the world's population. In Asia alone, rice provides a staggering two-thirds of the daily caloric intake for more than three billion people. Domesticated rice has played a central role in human nutrition and culture during the past 10,000 years. It contributes significantly to food security in Africa and Latin America, with nearly 1.5 billion people in these regions relying on rice for one-third of their daily caloric needs. Rice played a pivotal role in the Green Revolution, a period of significant agricultural advancements during the 1960s and 1970s. The development of high-yielding rice varieties, coupled with improved agricultural practices, led to substantial increases in global food production.

Nowadays rice is widely planted in more than 120 countries across the world, from 35°S to 53°N, with a concentration in tropical and subtropical areas of Asia. The continued importance of rice is reflected in the extensive research dedicated to its improvement. Strong breeding programs exist across various geographical regions, actively seeking to enhance rice yield, disease resistance, and other vital characteristics. Additionally, three key centres within the Consultative Group on International Agricultural Research (CGIAR) focus specifically on rice research. The International Rice Research Institute (IRRI) boasts a global mandate, while the West Africa Rice Development Association (WARDA) and the International Centre for Tropical Agriculture (CIAT) address rice research needs in West Africa and Latin America, respectively. These dedicated efforts underscore the crucial role rice plays in global food security and highlight the ongoing research

**Table 1** : Genetic Insights into Rice Domestication and Geographic History.

Gene	Trait affected	Impact	Predominant in	Discovery/Notes	Reference
sh4	Seed shattering	Reduces grain shattering	All rice cultivars	Identified in indica and <i>O. nivara</i> ; explains 69% variation in shattering reduction	Li <i>et al.</i> (2006a), Zhang <i>et al.</i> (2009)
qSH1	Seed shattering	Reduces grain shattering	Japonica rice	Linked to hard-to-shatter level in <i>japonica</i> vs. <i>indica</i>	Konishi <i>et al.</i> (2006), Li <i>et al.</i> (2006a)
PROG1	Growth habit	Promotes erect growth	All erect growth rice varieties	Mutation led to erect growth; found in <i>O. rufipogon</i>	Jin <i>et al.</i> (2008), Tan <i>et al.</i> (2008)
Rc	Seed coat colour	Changes from red to white pericarp	Not selected in all cultivated rice	Encodes a basic helix-loop-helix transcription factor	Sweeney <i>et al.</i> (2006)
Bh4, Waxy, TAC1, qSW5/GW5, Sdr4, Sd1, OsLG1, An-1, LABA1, GAD1	Various (plant architecture, seed width, etc.)	Affects multiple domestication traits	Variations in rice	Show wide variation; not fixed like sh4 and prog1	
Photoperiod genes (e.g., Hd1, Ehd1, Hd3a, Ghd7, Ghd8, DTH2)	Flowering time, adaptation	Extends rice cultivation to temperate areas	-	Key for rice becoming a worldwide plant	Takahashi <i>et al.</i> (2009), Wu <i>et al.</i> (2013)

aimed at ensuring its continued importance for future generations.

The domestication centres of rice are geographically distinct, with indica primarily originating in eastern India, Myanmar and Thailand, while japonica traces its roots back to Southern China, particularly the Pearl River Valley region. Molecular clock analysis estimates that the process of rice domestication occurred approximately between 8200 to 13,500 years ago, marking a significant turning point in human agriculture. Furthermore, the divergence between indica and japonica varieties is believed to have taken place around 3900 to 6700 years ago. This long history of human cultivation has fundamentally altered domesticated rice from its wild ancestors. Culturally, japonica rice was initially domesticated in South China before later interbreeding with wild rice species in South Asia, ultimately leading to the development of indica rice varieties. Unlike its wild progenitors, domesticated rice lacks the ability to survive on its own in the wild, a testament to the intense selection pressure exerted by humans for desired traits. While unintentional at first, early human cultivation practices initiated the first rice breeding programs. These early farmers likely began by favouring plants with beneficial traits such as larger seeds, increased yields, or improved resilience. Table 1 encapsulates crucial aspects of rice

domestication and its genetic and geographical history, providing a concise reference for understanding this complex process.

There are alternative theories suggesting a southern Indian origin, with rice dispersing northwards to China before reaching Korea and the Philippines around 2000 BC. Subsequent spread included Japan and Indonesia by 1000 BC. Historical accounts suggest that Alexander the Great may have introduced rice to Greece following his Indian invasion in 327 BC. Arab travellers are credited with bringing rice to North Africa and Europe via Egypt, Morocco, and Spain. European colonization further facilitated rice dispersal. Portugal and the Netherlands introduced rice to their West African colonies, and the Columbian Exchange ultimately brought rice to the Americas. However, rice cultivation in the Americas proved slow to establish, taking nearly two centuries to gain a foothold after Columbus' voyages. The story of rice continues with the Moors introducing it to Spain in 700 ADE, followed by the Spanish bringing it to South America in the early 17th century.

The genus *Oryza*, as documented by Vaughan in 1994, encompasses 22 species. However, only two species, *Oryza sativa* and *Oryza glaberrima* have been domesticated for cultivation. The chromosome number within *Oryza* varies from 24 to 48, with a basic

chromosome number (n) of 12. Morishima’s 1984 work, based on meiotic chromosome pairing, identified several genome types within *Oryza*: AA, BB, CC, EE and FF for diploid species and BBCC and CCDD for tetraploid species. Intriguingly, the two cultivated rice species, both diploid with  $2n = 24$  chromosomes, underwent domestication under distinct environmental pressures. *Oryza sativa*, originating from South and Southeast Asia, traces its ancestry back to *Oryza rufipogon* and *Oryza nivara*. Conversely, *Oryza glaberrima*, native to tropical West Africa was domesticated from *Oryza barthii*. *Oryza sativa* enjoys widespread cultivation across global rice-growing regions, while *Oryza glaberrima*’s cultivation remains largely confined to its centre of origin in West Africa.

The rice-wheat cropping system, consisting of rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.), dominates agricultural practices in South Asia (Nawaz *et al.*, 2019). This system covers a vast area, with approximately 85% concentrated in the fertile Indo-Gangetic Plains (IGP) (Saharawat *et al.* 2012). Notably a significant portion (76%) of the IGP’s rice-wheat are aisencom passed by states including Punjab, Haryana, Uttar Pradesh, Bihar and West Bengal. Rice and wheat, serve as the foundation of South Asian diets, and their

cultivation has been instrumental in bridging the gap between food grain demand and production in the region. In recent years, India has achieved a surplus in food grain production through a comprehensive approach. This approach incorporates the use of high-yielding crop varieties, effective disease and pest management strategies, optimized nutrient management practices, improved irrigation water management techniques and the adoption of advanced mechanization technologies. The productivity of rice in various countries as per FAO (2023) have been show in Fig. 1. India and China are the biggest rice production countries, which produced approximately 50% of the world’s rice. However, the average productivity of China (7.07 t/ha) is much higher than that of India (4.3 t/ha). In India rice provides 43% of calorie requirement for more than 70% of population. The trend in area under paddy cultivation along with the productivity is shown in Fig. 2. This chapter delves into the exciting world of rice breeding, exploring the significant advancements that have shaped this crucial field. We’ll not only look back at the traditional methods that have ensured food security for centuries, but also peer into the future, examining the cutting-edge tools and techniques poised to revolutionize rice production.

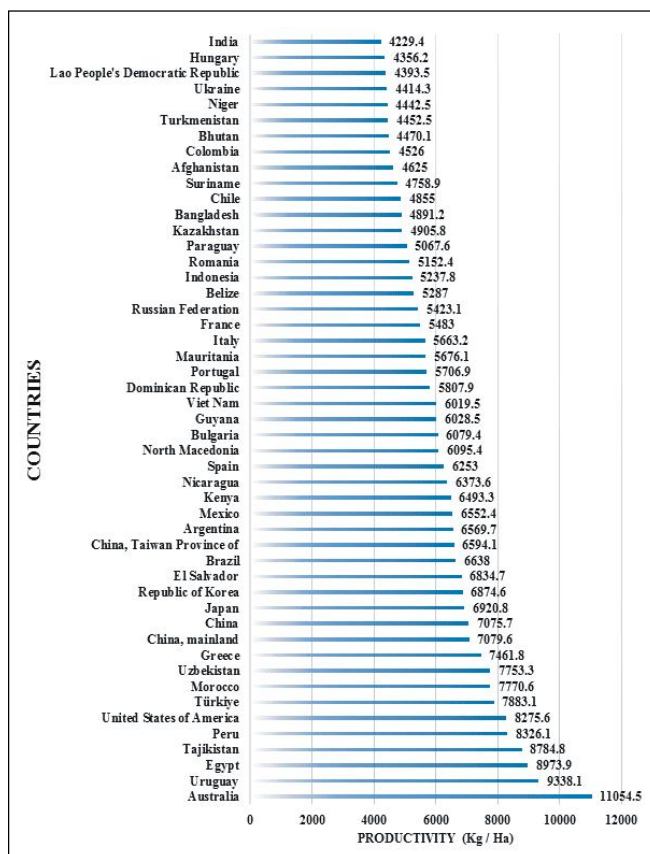


Fig. 1 : Productivity of rice in various countries of the world (FAO, 2023).

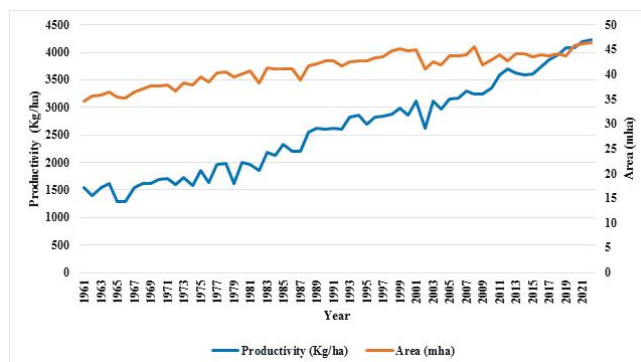


Fig. 2 : Trends in area and productivity of paddy in India.

### Current Cultivation Practices and Challenges

**Current cultivation practices :** A wide variety of soils, including loam, sandy loam, and clay loam, can be used for cultivating paddy. However, clay loam soils with good water retention capacity are considered best suited for optimal paddy growth. Following summer ploughing, the field undergoes additional ploughing 2-3 times to ensure proper preparation. Bunding is constructed around the field to conserve and retain irrigation water. In cases where the previous crop was green manure (daincha), it is incorporated with phosphorus before ploughing. To facilitate weed removal, the field is irrigated at least one week prior to transplanting. Finally, puddling (wet ploughing) is performed just before transplanting to create a semi-aquatic environment suitable for paddy.

The selection of paddy varieties is critical. Recently developed varieties that have been demonstrated to perform well in the specific region are recommended for cultivation. Consultation with local agricultural authorities is highly recommended to determine the most suitable options. The appropriate seed quantity (kg/hectare) is determined by grain size (fine, medium, coarse) and the area being planted (nursery and final field). Proper nursery management is essential for healthy seedlings. This involves preparing raised nursery beds with specific dimensions and applying recommended amounts of nitrogen and phosphorus fertilizers. Seed treatment with fungicides and biocontrol agents is carried out to control diseases. It is often recommended to adopt preventative measures to address specific deficiencies (zinc, iron) and strategies for pest control within the nursery. Before seeds are sown in the nursery, seed treatment with antibiotics or fungicides is conducted to control diseases. A differentiated NPK fertilization schedule is recommended based on paddy variety and maturity time (early, medium and scented). The appropriate transplanting window depends on variety maturity (early/medium vs. long duration/scented). Seedlings aged 20-22 days are considered ideal for transplanting. Specific spacing (plant-to-plant distance) and depth of sowing are followed to optimize plant population. Strategic irrigation is implemented during critical growth stages (tillering, ear emergence, flowering, grain filling) in the absence of sufficient rainfall. It is also recommended to use safe and effective insecticides, pesticides, fungicides and bactericides for control of various biotic stresses upon their occurrence.

**Vertical Farming for Rice :** Rice (*Oryza sativa* L.) is the world's most important staple food, feeding over half of the global population. With a growing population and looming threats from climate change, ensuring sustainable rice production is of paramount importance. Traditional rice cultivation faces challenges like land scarcity, water depletion, and pesticide pollution. Vertical farming (VF) has emerged as a potential solution, promising to revolutionize food production by utilizing vertical space in controlled environments. VF presents a promising avenue for addressing the challenges of traditional rice production. However, the feasibility of cultivating rice in vertical farms remains an ongoing discussion. Advantages of VF for Rice Production includes

- ❖ **Increased Land-Use Efficiency:** VF utilizes vertical space, significantly reducing the land footprint required for rice production. This is particularly beneficial in urban areas with limited

horizontal space.

- ❖ **Reduced Water Consumption:** VF employs hydroponic or aeroponic systems, minimizing water usage compared to traditional flooded paddy fields. This is crucial in regions facing water scarcity.
- ❖ **Controlled Environment:** VF allows for year-round production with precise control over temperature, light, humidity, and nutrient delivery. This minimizes crop losses due to weather fluctuations and pests/diseases, potentially increasing yields.
- ❖ **Reduced Environmental Impact:** VF significantly reduces greenhouse gas emissions associated with traditional rice cultivation (e.g., methane from flooded fields). Additionally, it eliminates the need for pesticides and herbicides in controlled environments.
- ❖ **Potential for Automation:** VF systems are highly amenable to automation, minimizing human labour requirements and potentially reducing overall production costs.

#### **Challenges of vertical farming for Rice**

- ❖ **High Energy Consumption:** VF systems rely heavily on artificial lighting and climate control, leading to high energy demands. This raises concerns about sustainability and cost-effectiveness, particularly with current reliance on non-renewable energy sources.
- ❖ **Technical Challenges:** Rice, typically grown in flooded fields, requires significant water compared to other crops being successfully grown vertically. Adapting existing hydroponic or aeroponic systems for efficient rice cultivation is an ongoing research area.
- ❖ **Economic Feasibility:** The initial capital investment for establishing a vertical farm is currently high. Until energy costs are reduced and production efficiencies optimized, VF may not be commercially viable for rice cultivation compared to traditional methods.
- ❖ **Limited Research:** While research on VF for various crops is ongoing, studies specifically focused on rice are scarce. More research is needed to determine optimal growth parameters, identify suitable rice varieties for VF, and develop cost-effective systems.
- ❖ **Consumer Acceptance:** Consumers may have

concerns regarding the taste and quality of rice grown in controlled environments compared to traditionally cultivated rice.

Addressing these concerns through research and consumer education is crucial for successful market adoption. Despite the challenges, several research initiatives are exploring the potential of VF for rice production. Studies in China, Japan and South Korea have shown promising results in cultivating rice varieties under controlled environments. Researchers are investigating modifications to existing VF systems to accommodate the water requirements of rice. Additionally, research is focusing on developing energy-efficient LED lighting and utilizing renewable energy sources to power VF operations. The future of VF for rice cultivation likely lies in a hybrid approach, integrating it with traditional methods. VF could be used for high-value seed production or producing rice in urban areas with limited land resources. As technology advances and production costs decrease, VF may become a more viable option for large-scale rice production, particularly in regions facing water scarcity or limited land availability.

**Digital farming :** Digital farming and artificial intelligence (AI) technologies are emerging as powerful tools to transform traditional rice farming practices into a more precise and data-driven approach known as “smart rice farming.” Digital farming and AI represent a paradigm shift in rice production, offering significant potential for sustainable intensification and increased food security. Overcoming the existing challenges and focusing on farmer-centric solutions will be crucial for widespread adoption of these technologies. By embracing these advancements, rice farmers can transform their practices, enhance resource efficiency and contribute to a more resilient and sustainable global food system.

Digital farming utilizes a suite of technologies like sensors, robotics, and communication networks. These tools collect and analyse real-time data on various aspects of the rice field, including soil moisture, nutrient levels, plant health, weather conditions, and crop growth stages. AI algorithms then process this data to generate insights and recommendations for optimizing rice production practices. AI algorithms analyse field data to create customized maps. These maps show soil fertility, nutrient requirements and irrigation needs. This allows for targeted application of fertilizers, pesticides, and water resources, minimizing waste and maximizing efficiency. AI models can predict rice yield based on historical data, weather forecasts and real-time field conditions. This information helps farmers make informed decisions about resource

allocation and harvest planning. Another advantage is AI-powered image recognition tools can identify pests and diseases in rice crops. These tools use drone-captured images or field sensors. Early detection allows for targeted interventions, minimizing yield losses. AI can also optimize irrigation schedules by analysing soil moisture data and weather forecasts. This reduces water waste, a crucial consideration in water-scarce regions. Autonomous robots equipped with AI can automate tasks like weed removal, fertilizer application, and crop monitoring. This reduces reliance on manual labour and improves farm efficiency.

Digital farming and AI offer significant benefits including increased productivity and yield, enhanced resource efficiency (water, fertilizer, pesticides), improved crop health and reduced disease/pest damage, reduced reliance on manual labour, improved decision making and farm management and enhanced traceability and food safety. However, several challenges also exist. High initial investment costs for infrastructure and technology along with limited access to technology and internet connectivity in rural areas are the major drawbacks. Also, adoption of this technology needs for training and capacity building for farmers and also raises concerns for data privacy and security apart from ethical considerations surrounding automation and potential job displacement

The integration of digital farming and AI with other advancements like big data analytics, Internet of Things (IoT) and blockchain technology holds immense potential for the future of rice production. Further research is needed in areas like developing affordable and user-friendly AI tools for smallholder farmers, enhancing the explainability and transparency of AI models to increase farmer trust, improving data security and privacy protocols for agricultural data collection and analysis, developing AI algorithms that can adapt to diverse farming conditions and rice varieties and research on the socio-economic implications of AI adoption in rice farming communities

**Current agricultural challenges :** Rice demonstrates remarkable adaptability, thriving in diverse ecological settings. However, its growth and yield are significantly impacted by various abiotic stresses. These stresses, including heat and cold extremes, salinity, flooding, and drought, are demonstrably intensifying due to climate change (Biswal *et al.*, 2019). Predictive models paint a concerning picture, with substantial rice yield losses anticipated under extreme warming scenarios (Zhao *et al.*, 2016). Temperature stands as one of the most critical abiotic factors influencing rice production. It directly affects growth, overall productivity and final grain quality

(Sailaja *et al.*, 2015; Bhogireddy *et al.*, 2021). Heat stress disrupts rice growth and metabolic processes, negatively impacting all growth stages, particularly the seedling and reproductive phases (Sailaja *et al.*, 2015). A recent study compiled extensive research and alarmingly estimated a global yield loss of 3.2% for every 1°C increase in global mean temperature. Interestingly, some studies suggest potential benefits. Gerardeaux *et al.* (2012) predicted that temperature increases and elevated CO<sub>2</sub> levels could positively influence rice growth in Madagascar, hinting at a potentially improved cultivation scenario in specific regions. While abiotic stresses pose a significant challenge, rice also faces significant biotic threats. Five major diseases (blast, bacterial blight, sheath blight, tungro, and grassy stunt) and four prevalent insect pests (brown planthopper, green leafhopper, stem borers and gall midge) commonly plague rice crops in tropical and subtropical Asia. Fortunately, breeding programs are actively developing germplasm with robust multiple resistance to these major diseases and insects, aiming to safeguard future rice production.

**Breeding objectives :** Rice breeding programs embark on a complex mission: to create a delicate equilibrium between three crucial objectives. The first priority is maximizing yield. With a rapidly growing global population, ensuring sufficient rice production is paramount to achieving food security. This is achieved by selecting for varieties with higher yield potential. However, simply maximizing yield isn't enough. To ensure long-term sustainability and food security, rice varieties must also exhibit resistance to a multitude of threats. This multifaceted approach is crucial in the face of a changing climate and emerging challenges. Breeders must also prioritize developing varieties with robust resistance to various threats. These threats encompass a multitude of factors, including abiotic stresses like heat, drought, and salinity, as well as biotic stresses like diseases and insect pests. A variety highly susceptible to such stresses will ultimately result in significant yield losses, defeating the purpose of high yield potential.

A significant portion of breeding efforts focuses on resistance to biotic stresses, specifically major fungal diseases and insect pests. Breeding programs target these diseases, including viral disease by incorporating genes that provide resistance, often derived from wild rice relatives. By focusing on both high yield potential and resistance to biotic stresses, rice breeders aim to create varieties that are not only productive but also resilient in the face of diverse challenges. This multi-pronged approach is critical for ensuring global food security in a changing environment.

Beyond biotic threats, rice breeding programs prioritize tolerance to abiotic stresses, which are environmental factors that limit growth and yield. These include drought, salinity, iron toxicity and low temperatures. Each stressor can have a devastating impact on rice production in specific regions. By developing varieties with tolerance to these abiotic stresses, breeders aim to ensure stable yields even under harsh environmental conditions. A growing area of concern is heat stress tolerance. With rising global temperatures, heat stress poses a significant threat to rice production. While breeding programs have addressed other abiotic stresses, research on mapping quantitative trait loci (QTLs) associated with heat stress tolerance remains limited (Kilasi *et al.*, 2018). Increased focus on this area is essential to develop future rice varieties resilient to a warming climate.

Finally, rice breeding programs must consider the diverse quality demands of farmers and consumers. Grain size, shape, aroma, cooking characteristics and nutritional content are all important quality traits that influence marketability and consumer preference. A prime example is the upland rice program, which initially focused on varieties with grain types not preferred by farmers. Only after releasing upland varieties with long and slender grains, favoured by the market, did upland rice regain popularity. This highlights the importance of incorporating consumer preferences and market demands into breeding objectives. Specialty grain quality rice types, catering to specific culinary uses or nutritional needs are also gaining traction in many breeding programs around the world. Balancing these sometimes-conflicting demands necessitates a multifaceted approach. By meticulously addressing these interconnected objectives, rice breeding programs aim to develop superior rice varieties. These varieties are not only high-yielding but also possess robust resistance to a multitude of threats. Additionally, they cater to the diverse quality demands of the market, ensuring not only food security but also consumer satisfaction in a continuously changing global environment.

### **Germplasm Biodiversity and Conservation**

Rice, as a crop, boasts remarkable adaptability owing to its extensive genetic diversity and the presence of wild relatives (Singh *et al.*, 2018). Within the genus *Oryza*, there exist 22 wild species alongside two cultivated species, namely *Oryza sativa* and *Oryza glaberrima* (Ge *et al.*, 1999). *Oryza sativa*, encompassing the majority of rice cultivation areas, has been categorized into five principal groups: indica, aromatic japonica, tropical japonica, temperate japonica, and aus (Garris *et*



al., 2005). The efficacy of breeding strategies crucially depends on leveraging the genetic diversity inherent in the crop. Public germplasm repositories have amassed a vast collection of rice accessions, exceeding 400,000, sourced from farmers' cultivars, landraces and the diverse genetic composition of the 22 *Oryza* species. Specifically, the International Rice Genebank Collection Information System (IRGCIS) of the International Rice Research Institute (IRRI) conserves approximately 132,000 accessions of rice, as of December 2019 (Neeraj *et al.*, 2021). Moreover, other CGIAR centres, including WARDA, CIAT, and the International Institute for Tropical Agriculture (IITA), harbour numerous rice accessions in trust. Furthermore, the conservation efforts extend to national institutions, such as the National Crop Genebank of China (housing >80,000 accessions) and the National Plant Germplasm System of the United States Department of Agriculture (USDA) (>18,000 accessions). The National Gene Bank of India, operated by the National Bureau of Plant Genetic Resources (NBPGR) in New Delhi, preserves an extensive assortment of rice germplasm resources, including indigenous, exotic, and wild accessions, amounting to over 60,000 specimens. These repositories play a pivotal role in safeguarding the genetic diversity of rice, facilitating research, breeding programs, and ensuring the long-term sustainability of rice cultivation globally.

Based on geographical distribution, Morinaga (1954) delineated three morphological groups termed japonica, javanica, and indica. Subsequently, Oka (1958) asserted that the japonica and javanica groups could be regarded as tropical and temperate japonicas, respectively. Notably, the tropical japonica varieties exhibit taller stature with heavier panicles, as highlighted by Glaszmann and Arraudeau (1986). Moving beyond morphological categorizations, advancements in karyotype and molecular analyses have provided deeper insights into the taxonomic structure of the *Oryza* genus. According to these studies, *Oryza* is classified into four complexes, comprising *O. sativa*, *O. officinalis*, *O. ridleyi*, and *O. meyeriana*. Within these complexes, ten genome types (A, B, BC, C, CD, E, F, G, HJ and HK) have been identified, each containing a varying number of species. Specifically, the cultivated species and their wild relatives are characterized as AA genome diploid species, encompassing two cultivated species and six wild relatives. Additionally, 17 wild species are distributed across nine other genome types. This taxonomic framework, elucidated by Morinaga, Oka and subsequent molecular studies, provides essential insights into the classification, distribution and genetic relationships within

the *Oryza* genus. It serves as the foundation for understanding the evolutionary dynamics and potential applications in rice breeding and conservation programs. Table 2 encapsulates the salient features of *Oryza* genus species, including their classification, geographical distribution, life cycle characteristics, and genetic relationships. Such comprehensive information is invaluable for researchers and breeders alike, facilitating informed decision-making in efforts aimed at conserving genetic diversity and harnessing the genetic potential of rice varieties for agricultural advancement.

Indica and japonica rice varieties exhibit distinct characteristics that extend beyond seed shape and texture. Indica rice, characterized by long grains and relatively lower stickiness, contains less amylopectin compared to japonica varieties, which are known for shorter grains and stickier texture. Additionally, substantial differences between indica and japonica are observed in various agronomic traits such as plant height, leaf shape, leaf color, plant type, awn length, density of glume pubescence, germinating rate, cold tolerance, lodging resistance, disease resistance, seed shattering and tiller number. For instance, japonica varieties typically feature shorter plant height, sharper leaf shape, lighter leaf color, stronger cold tolerance, robust lodging resistance and non-shattering seeds. Conversely, indica varieties are characterized by taller plant height, broader leaf shape, darker leaf color, and susceptibility to lodging, but exhibit higher tiller numbers and faster germinating rates. Moreover, japonica rice is primarily cultivated in regions with less water availability, such as northern latitudes of East Asia, upland areas of Southeast Asia and high elevations in South Asia, encompassing approximately 13 million hectares, constituting less than 10% of the total planting area. Notably, the majority of japonica cultivation occurs in China, predominantly in Northeast China, East China, and the Yunnan-Guizhou Plateau, accounting for about 60% of the total japonica cultivation area.

The concept of an ideal plant type, as proposed by Khush (1994), aims to harness the genetic variability within the indica group to enhance rice yields by up to 30%. Notably, rice stands as the first crop to have its entire genome sequenced, a milestone that significantly contributed to the development of genetic resources for gene discovery, molecular markers and crop improvement. Recent initiatives, such as the sequencing of 3,000 rice accessions from 89 countries, have facilitated the identification of superior alleles and haplotypes crucial for rice breeding programs. The genomic information obtained from 3,010 diverse Asian cultivated rice accessions, including those from the 3K rice genome

**Table 2 :** *Oryza* Genus species Overview.

Species	Genome	Type	Geographical Distribution	Annual/Perennial	Notable Phylogenetic Insights
<i>O. sativa</i> (Asian)	AA	Cultivated	Global, concentrated in Asia	Annual	Progenitor species: <i>O. rufipogon</i> , <i>O. nivara</i> . Supported by molecular analyses (Chloroplast DNA, RFLP, Microsatellites).
<i>O. glaberrima</i> (African)	AA	Cultivated	West Africa	Annual	Ancestor: <i>O. barthii</i> . Supported by molecular and crossing analyses.
<i>O. rufipogon</i>	AA	Wild	Asia	Perennial	Progenitor of <i>O. sativa</i> . Phylogenetic relations analysed by multiple molecular approaches.
<i>O. nivara</i>	AA	Wild	Asia	Annual	Progenitor of <i>O. sativa</i> . Supported by phylogenetic analyses.
<i>O. barthii</i>	AA	Wild	Tropical Africa	Annual	Ancestor of <i>O. glaberrima</i> . Supported by molecular analyses.
<i>O. longistaminata</i>	AA	Wild	Tropical Africa	Perennial	Phylogenetic relations analysed.
<i>O. meridionalis</i>	AA	Wild	Australia	Perennial (Recent annual form found)	Molecular phylogenetic analyses conducted.
<i>O. glumaepatula</i>	AA	Wild	South America	Perennial	Part of <i>O. sativa</i> complex. Phylogenetic analyses support distinct classification.

project, has enabled the identification of millions of single nucleotide polymorphisms (SNPs), small insertions and deletions (indels), novel protein-coding genes, and structural variations, serving as invaluable genetic resources for breeding and biotechnology research. (Wang *et al.*, 2018).

The importance of having genetic diversity available is the possibility of making it useful to develop products that will have an impact at farmers' field level. Rice breeders have been exploiting this potential in many different and creative ways. The choice of parents for crossing, when having such wide genetic diversity available, requires careful characterization and evaluation of the germplasm as well as good knowledge and breeding skills to make the right decisions. Systematic screening of rice germplasm has unveiled the presence of exceptional flood-tolerant rice varieties, such as "FR13A" and "FR43B" from India, "Kurkaruppan" from Sri Lanka, and "Goda Heenati" from Indonesia. Extensive studies on these flood-tolerant varieties have revealed that flooding tolerance is primarily governed by one or a few genes with major effects, supplemented by additional genes with minor modifying effects. Such insights hold significant implications for the development of flood-tolerant rice varieties, thereby contributing to global food security efforts. Rice breeders have achieved remarkable success in enhancing the crop through several significant

milestones. These milestones include the pivotal contribution to the Green Revolution with the development of semi-dwarf varieties, the introduction of the new rice plant type, the innovation of hybrid rice, and the creation of NERICA rice.

Rice stands as a reservoir of genetic diversity, offering breeders a vast array of parental materials to select from. However, despite its genetic richness, literature reports highlight a concerning trend where varieties released by breeding programs across the globe exhibit a narrow genetic diversity base. This shift has been particularly pronounced in the post-IR8 era, characterized by significant changes in varietal composition and cultural practices. Following this era, a relatively limited number of improved varieties have supplanted thousands of traditional cultivars, resulting in a substantial reduction in the genetic variability of the crop. Studies by Cuevas-Pérez *et al.* (1992) and Montalban *et al.* (1998) in Latin America, focusing on irrigated and upland rice, respectively, have underscored this trend, revealing that commercial varieties released for both systems often originate from a narrow genetic base. The dwindling genetic variability in rice has been exacerbated by factors such as intensified agricultural practices and the prevalence of continuous cropping with rice. These factors collectively contribute to the heightened genetic vulnerability of the crop. Nevertheless, it is important to



Table 3 : Phylogenetic Analysis and Genetic differentiation in Asian Cultivated rice.

Study	Year	Method	Sample Size	Genetic Markers Used	Identified Groups/Subpopulations	Key Findings
Glazmann	1987	Isozyme loci	1688 traditional	15 isozyme loci rices	Indica, Japonica, Aus, Basmati, Deepwater (Bangladesh and NE India)	Initial classification into six groups, highlighting the distinct genetic backgrounds of indica and japonica.
Nakano <i>et al.</i>	1992	RFLP	Not specified	Not specified	Indica and Japonica differentiation detected	Supported the differentiation between indica and japonica using RFLP.
Garris <i>et al.</i>	2005	SSRs and chloroplast loci	234 accessions	169 nuclear SSRs, 2 chloroplast loci	Indica, Aus, Aromatic, Temperate Japonica, Tropical Japonica	Identified five divergent groups, providing a more detailed phylogenetic analysis.
Agrama <i>et al.</i>	2010	SSR markers	1763 accessions from USDA world rice collections	72 genome-wide SSR markers	35% Indica, 27% Temperate Japonica, 24% Tropical Japonica, 10% Aus, 4% Aromatic	Quantitative distribution of major rice subpopulations in a significant rice collection.
McNally <i>et al.</i>	2009	Resequencing microarrays	20 diverse varieties and landraces	160,000 non-redundant SNPs	Temperate Japonica, Tropical Japonica, Aromatic (closely allied); Aus and Indica	Highlighted a close relationship between temperate japonica, tropical japonica, and aromatic types.
Zhao <i>et al.</i>	2011	Affymetrix SNP array	413 inbred accessions from 82 countries	44,100 SNPs	Indica, Aus, Temperate Japonica, Tropical Japonica, Aromatic	Clustering based on principal components analysis, affirming five main subpopulations.
Huang <i>et al.</i>	2012b	NGS: Whole-genome resequencing	950 worldwide germplasms	High-quality SNPs	Indica, Aus, Temperate Japonica, Tropical Japonica, Intermediate	Introduced an “intermediate” group, refined classification within indica and japonica subspecies.
Xie <i>et al.</i>	2015	NGS: Whole-genome resequencing	1483 accessions (combined)	6,551,358 high-quality SNPs	Indica I, Indica II, Temperate Japonica, Tropical Japonica, Aus, Intermediate	Detailed subdivision of indica into Indica I and Indica II, highlighting diversity within indica and japonica.

note that these observations pertain specifically to cultivated rice varieties and do not necessarily reflect the genetic diversity within the broader rice species. For instance, Sun *et al.* (2001) conducted an analysis on *Oryza rufipogon* (wild rice) and *Oryza sativa* (cultivated rice) using molecular markers. Their findings contradicted the notion of dwindling genetic diversity, revealing that both species still exhibit a broad genetic variability. Their analysis encompassed parameters such as the number of alleles, number of genotypes, heterozygosity, and diversity among genes, demonstrating that wild and cultivated rice varieties continue to maintain significant genetic diversity.

**Phylogenetics of Asian cultivated rice :** Previous phylogenetic and population analyses have delineated five principal rice groups: indica, aus, aromatic, temperate japonica, and tropical japonica, with subsequent studies further dividing indica into indica I and II groups. This classification reflects the geographical distribution and adaptation of these groups to varied climatic and light conditions across Asia. Table 3 outlines significant milestones in the phylogenetic analysis of Asian cultivated rice, focusing on the methodologies used, sample sizes, genetic markers, identified groups and key findings. From isozyme loci analysis in the 1980s to advanced next-generation sequencing (NGS) and whole-genome resequencing in the 2010s, the classification of rice into distinct genetic



**Fig. 3 :** Plant genetic resource map of *Oryza* sp. in India.

subpopulations has become increasingly refined. These studies underline the complexity of rice genetics and the influence of domestication, artificial selection, and natural distribution on the phylogeny of rice.

**IRGSP & OMAP :** The International Rice Genome Sequencing Project (IRGSP), a collaborative effort launched in 1998, marked a significant milestone in plant genomics. This ambitious project brought together sequencing teams from 10 nations with a shared goal: to decipher the complete and precise map of the rice genome. The chosen reference genome for this project was Nipponbare, a widely studied japonica rice cultivar. In 2005, the IRGSP achieved a major breakthrough by releasing a high-quality, finished genome sequence of japonica rice. This accomplishment held immense significance. The rice genome sequence represented the first complete picture of the genetic makeup for a monocotyledonous (monocot) plant species. Monocots, which include important crops like maize and wheat, differ significantly from the more extensively studied dicotyledonous (dicot) plants like *Arabidopsis*. Understanding the rice genome provided valuable insights into the unique genetic architecture of monocots. Following the successful sequencing of the *Arabidopsis thaliana* genome, the rice genome became the second fully sequenced plant genome overall. This accomplishment significantly accelerated plant genomics research, paving the way for comparative studies between plant species and the identification of genes with conserved functions

across the plant kingdom.

The highly accurate and publicly available IRGSP rice genome sequence served as a powerful resource for researchers. It opened doors for functional characterization of the rice genome. This involved linking specific genes to their biological functions and roles in various plant processes. The complete rice genome sequence empowered rice geneticists to identify the genes underlying complex agronomic traits, such as grain yield, disease resistance, and abiotic stress tolerance. This new found knowledge became a critical tool for breeding programs to develop improved rice varieties with enhanced characteristics. The IRGSP's achievement laid the foundation for a new era in rice research. By providing a comprehensive reference genome, the project facilitated a deeper understanding of rice biology and paved the way for targeted approaches to improve this vital food crop. Through comparative analyses, the domestication and evolution research of rice and other cereal crops was also largely promoted (Paterson *et al.*, 2004; Salse *et al.*, 2008).

The *Oryza* Map Alignment Project (OMAP), launched in 2005, represents a significant initiative in understanding the wild relatives of rice (*Oryza sativa* L.). This project emerged from the need for a deeper exploration of the genetic diversity within the *Oryza* genus, particularly focusing on wild rice species. OMAP's initial objectives were multifaceted (Wing *et al.*, 2005):

- **Genome Sequencing:** The project aimed to sequence the complete genomes of African cultivated rice and eleven wild *Oryza* species.
- **BAC Library Construction:** Bacterial artificial chromosome (BAC) libraries were constructed for each targeted species. BAC libraries are essentially collections of DNA fragments cloned within bacterial artificial chromosomes, facilitating large-scale DNA sequencing and analysis.
- **Physical Map Construction:** OMAP focused on creating high-resolution physical maps for each sequenced genome. Physical maps represent the linear organization of chromosomes, depicting the order and relative positions of genes and other DNA features.
- **Genome Alignment:** The physical maps generated for the wild rice species were then aligned to the reference genome sequence of *Oryza sativa* ssp. *japonica*, known as the IRGSP genome sequence. This alignment allows researchers to identify regions of similarity and

difference between the genomes of cultivated rice and its wild relatives.

The overarching goal of OMAP is to establish a comprehensive research platform for understanding various aspects of rice biology and improvement. This platform holds immense potential for advancements in several key areas. By comparing the genomes of wild and cultivated rice, researchers can gain insights into the evolutionary history of rice and identify the genetic changes that occurred during domestication. OMAP can contribute to a better understanding of the impact of polyploidy on rice genomes. The project can shed light on the genetic pathways regulating rice development at different stages, from seed germination to grain formation and hence by leveraging the vast genetic diversity present in wild rice species, OMAP can pave the way for the development of improved rice varieties with enhanced traits like disease resistance, stress tolerance, and yield potential.

**Genetic resources conservation :** The pursuit of high-yielding rice varieties to meet the demands of growing human populations has resulted in genetic erosion, as landrace varieties are replaced by modern cultivars. This shift, coupled with changes in land use and habitat destruction, poses a threat to wild rice species, pushing them towards extinction. To counteract this trend, international initiatives, spearheaded by organizations like

the International Rice Research Institute (IRRI) have established gene banks across Asia to conserve rice genetic resources. By coordinating efforts among national, regional, and international bodies, these initiatives aim to safeguard the biodiversity of rice. Maintaining germplasm as pure lines facilitates their preservation in gene banks and ensures their availability for global rice research endeavours. The *ex-situ* conservation of seeds is a widely utilized and effective method for preserving plant species, offering both safety and cost-efficiency. Fig 3 shows plant genetic resource map of *Oryza* sp. in India. Rice seeds, in particular, demonstrate orthodox storage behaviour, allowing them to be dried to a low moisture content of approximately 6% and stored at temperatures as low as -20°C. Under these conditions, rice seeds can maintain their viability for decades, if not longer. This preservation method ensures that germplasm stored in gene banks remains readily available for utilization by breeders and researchers. In addition to static conservation in gene banks, there is a global effort to explore on-farm or in situ conservation initiatives for crop varieties, including dedicated projects focused on rice conservation, such as the one conducted at IRRI.

### Conventional Breeding

Conventional hybridization and selection are a time-tested strategy for selecting crop cultivars with higher yield potential. It has two phases. The first phase involves

**Table 4 :** Conventional Rice improvement efforts and Breeding Methods.

Conventional Rice Improvement Work	Breeding Methods	Reference
Development of High-Yielding Varieties (HYVs)	Selection from landraces, mass selection, pedigree breeding	Khush (1995)
Breeding for Biotic Stress Resistance (e.g., Blast, Bacterial Blight)	Marker-assisted backcrossing, recurrent selection	Singh <i>et al.</i> (2012)
Breeding for Abiotic Stress Tolerance (e.g., Drought, Salinity)	QTL mapping, marker-assisted selection, backcross breeding	Bernier <i>et al.</i> (2007)
Improvement of Grain Quality (e.g., Aroma, Cooking, Nutritional Traits)	Germplasm screening, pedigree breeding	Ntanos and Roupakias (2001)
Enhancement of Plant Architecture (e.g., Semi-Dwarf Varieties)	Induced mutations, pedigree breeding	Khush (2001)
Development of Hybrid Varieties	Cytoplasmic male sterility (CMS), three-line breeding system	Rout <i>et al.</i> (2020)
Introduction of Submergence Tolerance	Marker-assisted backcrossing, gene pyramiding	Septiningsih <i>et al.</i> (2009)
Incorporation of Insect Resistance	Genetic engineering, marker-assisted selection	Makkar <i>et al.</i> (2019)
Breeding for Weed Competitiveness	Selection for allelopathic traits, herbicide resistance	Chauhan and Johnson (2009)
Development of Early Maturing Varieties	Early generation selection, line x tester analysis	Singh <i>et al.</i> (2018)

the creation of variability through hybridization between diverse parents. In the second phase desirable individuals are selected on the basis of field observations and yield trials. In general, rice breeding programs have two major different end products. The first and the most common one is a pure line, which will be evaluated and released as a commercial variety. The second one is an inbred line that will be the parent of a commercial hybrid. An intermediate product may be a population with certain desirable characteristics that could be used for further improvement, for cultivars *per se*, or for line extraction. One of the most difficult tasks in carrying out a successful breeding program is the choice of germplasm. To be able to develop a variety with a set of desirable characteristics rice breeders need to be sure that the source germplasm has desirable genetic variability. After the parents are chosen and the crosses are made there are almost no chances of new alleles appearing in the segregating populations. To make the right choice of parental material to be used in a breeding program, breeders must clearly know the type of product to be developed; the characteristics of the species to be bred; the combining ability of the parents in case of hybrid cultivars; the environmental conditions of the target area; the social and economic aspects of the farmers and markets; and the different breeding approaches available to achieve the proposed goals. The choice of parental material depends on the breeders' objectives, the desired type of product, the existing genetic diversity, and the information available, as well as the combining ability of the parents. Table 4 highlights few conventional rice improvement efforts made in late 20<sup>th</sup> and 1<sup>st</sup> decade of 21<sup>st</sup> century. Today, an additional element to be considered is the legal aspect in relation to the materials to be used as parents.

If one makes a global literature review on the breeding methods commonly used to develop rice varieties around the world pedigree selection is always at the top, but development of hybrids and population improvement were added to the breeder's portfolio in recent times. When there are possibilities to carry out more than one generation per year (*e.g.*, winter nurseries) the method is combined with modified bulk or even single-seed descent to speed up the process of having pure lines for agronomic evaluation. Breeders have been taking advantage of biotechnology tools to enhance their breeding capacity; however, many national programs are still struggling on how to integrate them into the breeding programs and how to balance the allocation of resources between conventional and modern tools.

Population improvement through recurrent selection is a well-established breeding method that has been

prominently employed in maize breeding for over half a century (Dudley and Lambert, 2004). Although, historically less common in self-pollinated crops, the potential of recurrent selection in rice breeding was proposed by Fujimaki in 1979, particularly utilizing male sterility mechanisms. This methodology has found widespread application in Latin America but has not gained equivalent popularity elsewhere. Recurrent selection serves as a vital tool to facilitate recombination and enhance the frequencies of favourable genes for quantitatively inherited traits. This cyclic process of plant breeding involves two distinct phases: (a) selecting a cohort of genotypes possessing desirable genes and (b) mating these selected genotypes to foster genetic recombination (Frey, 1982). A central tenet of recurrent selection is its focus on augmenting the frequencies of favourable alleles. The technique's application to self-pollinated species has historically been limited due to challenges associated with intermating. However, the advent of monogenic male sterility, notably in the rice variety IR36 (Singh and Ikehashi, 1981), has overcome this hurdle, enabling the utilization of recurrent selection for the development of rice varieties with heightened yield potential. This breakthrough underscores the significance of advancements in breeding technologies in expanding the repertoire of breeding methodologies, particularly in traditionally challenging crops like rice.

Plant breeders often encounter challenges regarding the low efficiency of visually selecting plants for yield in early segregating generations. Factors such as heterogeneous soil fertility conditions, intergenotypic competition due to population density and micro and macro environmental effects contribute to this inefficiency. Additionally, dominance and epistatic variance within segregating populations further diminish selection efficiency in conventional breeding approaches. Cultural practices, such as employing high fertilizer rates and wider spacing, can enhance selection efficiency by allowing maximum genotype expression for yield while minimizing variability due to environmental factors. Another useful method is the haploid approach using anther culture, which aids in improving selection efficiency for yield and other traits with low heritability. Doubled haploid lines offer advantages over conventional F<sub>2</sub> and F<sub>3</sub> generations, as they exhibit greater additive genetic variance and eliminate dominance variance. In conventional generations like F<sub>3</sub> and F<sub>4</sub>, both additive and dominance effects contribute to phenotypic differences among individuals, whereas doubled haploid progeny variability is primarily due to micro environmental effects. Given these advantages, selection efficiency in doubled

haploid populations tends to be higher when there is significant dominance variation in the cross. While traditional breeding methods, such as pedigree or backcross breeding, have historically improved rice yield potential, further progress necessitates supplementing these approaches with innovative breeding techniques.

In recent years, the role of biotechnology in rice breeding has become increasingly prominent. Techniques such as marker-assisted selection (MAS) and genetic engineering allow breeders to identify and manipulate genes associated with desirable traits, including yield, disease resistance and stress tolerance. MAS enable precise selection of individuals carrying target genes, thereby accelerating the breeding process and enhancing selection efficiency. Genetic engineering techniques, such as CRISPR/Cas9, offer unprecedented precision in gene editing, allowing for the introduction of beneficial traits or the modification of existing ones. These biotechnological approaches complement traditional breeding methods and offer new avenues for enhancing rice yield potential and addressing emerging challenges in agriculture.

### Hybridization

**Wide hybridization :** The widening of crop gene pools occurs through hybridization, involving crop cultivars with wild species, weedy races, and intra-subspecific crosses, thereby enabling the exploitation of diverse genetic resources to improve various traits, including yield. Lawrence and Frey (1976) observed that a significant proportion of lines derived from BC2-BC4 segregants resulting from crosses between *Avena sativa* and *Avena sterilis* exhibited substantially higher grain yield compared to the cultivated recurrent parent. Additionally, Xiao *et al.* (1996) noted significant yield enhancements in some backcross derivatives originating from a cross between an *Oryza rufipogon* accession from Malaysia and cultivated rice, with yield increases of up to 18%. They also identified two quantitative trait loci (QTLs) from wild species contributing significantly to yield improvement.

Wild rice species serve as valuable reservoirs of genes for resistance breeding. Brar and Khush (1977) utilized *O. nivara* as a source of resistance against grass stunt virus, successfully introducing it into IR28, IR29 and IR30 cultivars. Similarly, resistance to the viral disease “tungro” was acquired from *O. rufipogon*. Resistance to rice bacterial blight was obtained from *O. longistaminata* and subsequently introduced into the commercial variety IR24 (Khush, 1994). Brar and Khush (1997) transferred genes conferring resistance to three biotypes of brown plant hopper from *O. officinalis* to an

elite breeding line. Multani *et al.* (1994) achieved successful transfer of resistance genes against brown plant hopper from *O. australiensis* to cultivated rice. Additionally, genes for resistance to blast and bacterial blight have been transferred from *O. minuta* to improved rice germplasm (Brar and Khush, 1997). Khush (1994) amalgamated different genetic groups, including tropical japonicas, temperate *indicas* and japonicas, with the aim of creating a novel rice type anticipated to augment the grain yield of commercial varieties by approximately 30%.

**Hybrid Rice :** To meet the escalating demand for rice production, it is imperative to rapidly increase yield per unit area, particularly in major rice-producing nations. In 1974, rice hybrids were introduced in China, offering a yield advantage of approximately 10% to 15% over the best inbred varieties. Presently, these hybrids occupy about 45% of rice cultivation areas in the country. The augmented yield advantage observed in tropical rice hybrids stems from heightened biomass, increased spikelet number, and, to some extent, enhanced grain weight. Initially, the breeding strategy for hybrid production relied on three distinct breeding lines: the A line (male sterile), the B line (responsible for maintaining genetic male sterility in the A line) and the R line (used to restore fertility in the A line and produce hybrid seeds). This approach evolved into a two-line process utilizing environmental genetic male sterility (EGMS), which incorporates photoperiod (PGMS) and temperature (TGMS) sensitivity to induce sterility. The development of the first hybrid rice was predicated on a genetic cytoplasmic male sterility system identified in *Oryza sativa* L. f. *spontanea* in China (Shih-Cheng and Loung Ping, 1980). The intricate process of hybrid production involves several key steps: identification and evaluation of male-sterile lines and their restorers, the testcross phase to select heterotic combinations and initiate the conversion of maintainer lines into male-sterile lines, backcrossing to transfer cytoplasmic male sterility to elite maintainer lines, trials to assess the combining ability (both general and specific) of parental lines, and the production of foundation seed for all three lines. However, outside of China, the production of breeding lines for the three- or two-line methods remains a challenging task for most breeding programs.

In hybrid rice breeding, several challenges hinder the development of superior hybrids. These challenges include a limited pool of parental lines possessing specific desirable traits, a scarcity of maintainers and restorers among elite breeding lines, a narrow genetic base, susceptibility to biotic stresses and suboptimal grain quality in some parental lines (Yuan, 2003). Furthermore, the

success of a hybrid is contingent not only on the seed production capacity of the female parent but also on its ability to exhibit heterosis after crossing with its parental varieties. While the initial combination of different varieties sets the foundation for heterosis, its manifestation is heightened when varieties from distinct groups, such as indica and japonica, are interbred. Therefore, enhancing parental lines assumes paramount importance in hybrid rice breeding endeavours aimed at fostering heterotic hybrids and enhancing breeding efficiency.

Efforts to address these challenges have led to the development of diverse parental lines with improved grain quality and resistance to multiple stresses in China and at the International Rice Research Institute (IRRI). The broadening of the genetic base of parental lines has been facilitated by the random mating of composite populations comprising maintainers and restorers developed at IRRI. Introducing restorer genes into tropical japonica lines and integrating wide compatibility genes into promising elite lines are deemed crucial steps toward producing indica/japonica hybrids. An alternative strategy to enhance hybrid potential involves leveraging yield-enhancing genes from other species (Yuan, 2003). Molecular marker technology is being employed to identify restorer genes in japonica backgrounds (Tan *et al.*, 1998) and to isolate thermo-sensitive genetic male sterility genes (Latha *et al.*, 2004). Thermosensitive genic male sterile lines are under development in indica, tropical japonica, and basmati genetic backgrounds. Additionally, marker-assisted selection (MAS) has shown promise in aiding the development of hybrids with enhanced disease and insect resistance in China (He *et al.*, 2004).

### **Ideotype Breeding**

Ideotype breeding endeavours to modify plant architecture to enhance yield potential (Khush, 1995). Selection for shorter statured cereals like wheat, rice, and sorghum has led to a doubling of yield potential. In the case of tropical rice, increasing yield potential necessitated improvements in harvest index and nitrogen responsiveness by enhancing lodging resistance. This was achieved by reducing plant height through the incorporation of the recessive gene *sd1* for short stature. The pioneering short-statured variety, IR8, developed at the International Rice Research Institute (IRRI), possessed a combination of desirable traits, including profuse tillering, dark green and erect leaves for optimal canopy architecture and sturdy stems. It exhibited enhanced responsiveness to nitrogenous fertilizer, higher biomass (approximately 18 tons), and a harvest index of 0.45, resulting in a yield potential of 8 - 9 tons per hectare.

IRRI scientists introduced the ideotype concept termed “New Plant Type” (NPT) as outlined in IRRI’s strategic plan. The NPT architecture featured characteristics such as low tillering (9 - 10 tillers under transplanted conditions), absence of unproductive tillers, 200 - 250 grains per panicle, dark green, thick, and erect leaves, along with a vigorous and deep root system. Breeding endeavours to develop NPT commenced in 1990 with the aim of producing improved germplasm with 15% - 20% higher yield than existing high-yielding varieties. Subsequently, numerous breeding lines with the desired ideotype were developed and disseminated to national rice improvement programs (Khush, 1995).

### **Molecular Breeding**

Molecular breeding integrates genetic engineering and genomic tools for genetic manipulation at the DNA level to enhance desirable characteristics in plants to augment their economic and practical value for humanity. This integrates various strategies such as genetic engineering, molecular marker-assisted selection, genomic selection, and bioinformatics as an important integral part of molecular breeding. Molecular breeding of rice for better yield, strong environmental adaptability, and efficient grain quality is pivotal for feeding the world’s rapidly growing population. The genetic engineering process of rice crop mainly involves *in vitro* recombination of a desired target gene with the transformation vector and then transferring it into rice for stable integration and expression. At moment, maximizing yield remains the primary goal for rice breeders by aiming to improve the yield-centric traits in rice, which encompass plant height, tillers, grain weight, and panicle structure. Yang *et al.* (2010) and Wang *et al.* (2012b) have reported to enhanced the size of grain and external qualities of the indica variety, named Huajingxian 74, through molecular pyramiding breeding technique. This approach comprised hybridizing a single-segment substitution line characterized by the genetic background of Huajingxian 74, while also harbouring the grain length genes *GS3* and *GW8*, with other advantageous genes.

**Genomics and Bioinformatics :** Recent advances in genomics and bioinformatics present opportunities for expediting the crop improvement for higher yield and enhanced food security. Third-generation sequencing technologies are aiding in overcoming challenges associated with plant genome assembly, particularly concerning polyploidy. This has led to the availability of high-quality crop reference genomes, introducing association mapping to pinpoint breeding targets. Machine learning plays a crucial role in functional genome annotation and real-time phenotyping, both important for

identifying valuable traits. Additionally, crop databases serve as invaluable repositories for genotype-phenotype data, supporting data mining efforts to unearth novel trait-associated genes. An understanding of genome editing emerges as a promising avenue for breeding disease-resistant and stress-tolerant crops with higher yields with a desired quality. Novel resources such as gene activation mutants, EMS mutants and T-DNA-tagged rice mutant populations are powerful genetic resources for functional genomics and crop improvement (Reddy *et al.*, 2020).

Bioinformatics is essential for handling and studying vast genomic datasets, helping scientists understand how plant genomes work (Moore *et al.*, 2010). Third-generation sequencing enables high-quality whole-genome de novo assemblies, especially in complex regions with repetitive sequences, shedding light on structural variants additionally allows precise examination of the exons, splice sites, and alternative splicing through full-length sequenced transcripts, useful in improving genome annotation (Li *et al.*, 2017). Analysing QTLs allows for the identification of genetic regions associated with quantitative phenotypic characters, linking genomics with practical field applications (Mackay *et al.*, 2009). Meta-analysis enhances the precision of predicting QTL locations beyond individual study outcomes, thereby enhancing the utilization of existing resources. Bioinformatics resources are readily accessible to facilitate efficient meta-QTL analysis (Mac and Jordan, 2011). For instance, employing statistical methods and a consensus model, a computational tool like Meta QTL can significantly narrow down the confidence interval of QTL, resulting in precise determination of the exact QTL location and its effect. Recently, a genomic resource based on CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats-associated nuclease 9) genome editing has been developed wherein more than 34,000 genes of rice have been targeted (Lu *et al.*, 2017).

Genome-Wide Association Studies utilize linkage disequilibrium to identify relationship between genotype and phenotype in crop species, yielding superior mapping resolution compared to QTL analysis (Huang and Han, 2014). GWAS has been carried out in various crops such as rice, maize, soybean, wheat. In *Oryza sativa indica*, sequencing 517 landraces revealed 3.6 million single nucleotide polymorphisms (SNPs), and in the GWAS of 14 agronomic traits, explained over 36% of phenotypic variance, aiding in the discovery of trait-linked genes (Huang *et al.*, 2010). In cases where systematic introgression of complex traits is challenging, genomic selection (GS) offers an alternative breeding strategy. In recent genomic analysis, Machine learning (ML) plays a

pivotal role across various domains of genomics research, encompassing tasks such as genome assembly, iterative inference of gene regulatory networks, and accurate identification of single nucleotide polymorphisms (SNPs) in polyploid plants. Ma *et al.* (2014), have compiled a comprehensive list of ML algorithms and associated open-source R packages tailored for data analysis in plant genomics. To accelerate crop improvement, agriculture needs new breeding methods and bioinformatics tools. Methods like meta-QTL analyses and GWAS speed up gene-trait association discovery, while genome editing introduces beneficial mutations for desired traits. Genomic selection enhances selection efficiency, and machine learning automates tasks like genome annotation and by combining these approaches future plant breeding can accelerate crop improvement for food security (Hu *et al.*, 2018). Many high-through put sequencing-based genomic resources for abiotic stress related traits are discussed by Bansal *et al.* (2014). Transcriptomic and micro-RNA-based genomic resources for abiotic stress traits are also available in rice (Bansal *et al.*, 2014; Mangrauthia *et al.*, 2017).

**Molecular marker assisted breeding :** Marker-assisted selection (MAS) is an advanced technology in the field of genetic improvement, enabling the precise identification and selection of plants with specific chromosomal regions tagged with DNA markers linked to desirable traits with economic importance. This technique plays a pivotal role in enhancing the efficiency and accuracy of plant breeding efforts and most importantly the genetic improvement in rice crop. Molecular markers are broadly classified as PCR based and hybridization-based markers (Joshi *et al.* 1999). Various types of molecular markers have been developed such as, Restriction Fragment Length Polymorphism (RFLP), Amplified Length Polymorphism (AFLP), Single Nucleotide Polymorphism (SNPs), Simple Sequence Repeat (SSR), Cleaved Amplified Polymorphic Sequences (CAPS), Sequence Characterized Amplified Regions (SCARS), Expressed Sequence Tags (ESTS) (Akhtar *et al.*, 2010). Important considerations for DNA marker use in MAS include reliability, quantity and quality of DNA, assay procedures, polymorphism levels, and also cost. (Mohler and Singrun, 2004). MAS facilitated the transfer and integration of blast resistance genes/QTLs into KDML105, RD6 and other elite varieties, resultantly, numerous promising lines with grain quality akin to KDML105 and RD6 have emerged (Jairin *et al.*, 2017). Several databases and genomic resources of rice are available in public domain for gene/allele discovery, molecular marker designing and basic studies (Kamboj



**Table 5 :** Markers and QTL/genes used in MAS for abiotic and biotic stresses; (Source: Nogoy *et al.*, 2016).

	Marker	QTL/gene	ForwardPrimer (52–32)	Reverse Primer(52–32)
Drought	RM201		CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA
Salinity	Wn11463	<i>SKC1</i>	TCCCTCCTTCTCTCGCAAC	GATCCACTCGTCACAGG
	Wn11466		GCTTCCCAATAAATTCGACCT	CCCACCAATACTAAAGATCCTTG
	Wn13900	<i>SalT</i>	GTACGGGTTCCACATCCTC	ACCCTCTAATAATTCACTACCA
	Wn13902		CACCAGCGTCACTACTCT	CAAAACTGAGTAGGAATACCGTGA
	Wn13903		CTGTATCAACTGCATTOGTGT	GCTTGGTCAAACCTCCGT
	Th32637	<i>DST</i>	TCGTATAGTAGGCTTTCATGGC	TTTACACAGGTGCGAGAGCTT
Th32638		AGAGAAGCCAAAGAAATCGAC	TCCAAAGCTCCACCTACTCC	
Th32639		CTATTTGGCTTCGCAAGGACA	CGCCCCACTTAAATCATATTCCTT	
Cold	RM231	<i>qPSS1-3</i>	CCAGATTAATTCCTGAGGTC	CACITGTCATAGTTGTGCAITG
	RM1377	<i>qPSS1-7</i>	ATAGATAGATGAGGGGGG	GCTGCTGTACGATGTGATCG
	RM24545	<i>qPSS1-9</i>	AGAGGAGAGGAGGGGGAAGG	CGAGCAAAGGGAAGGGGATAAGG
Heat	M4	<i>qHTSF4.1</i>	CAITGTTTTCTTGAGAACTTTAG	TCAAGACTAATTTAATCAATCGTTAC
			CTCTACGTTTCAACTGAGCA	AAGAGATCGCATGTGGAC
	RM229		CACTCACAGGAACGACTGAC	CGCAGGTCTTGTGAAATGT
	RM430		AAACAACGACGTCCTGATC	GTGCCTCGGTGTTATGAAC
	RM210		TCACAITCGGTGGCAITG	CGAGGATGTTGTTCACCTTG
	RM3735		GCGACCGATCAGCTAGCTAG	ATAACTCTCCCTTGCTGCC
		<i>Xa3-Re<sup>o</sup></i>	CCACAATGCCAATGTCAGGTGGCAITCCCCTGCA	AGGTGTTGGAGGATTTGGCAIT
		<i>Xa3-Su<sup>p</sup></i>	CGGAGCGACACAGCTATCAT	CGTGAGGTTCCCTATGGGGATT
		<i>Xa21</i>	AGACGGGAAGGGTGGTCCCCGGA	AGACGGGGTAATCGAAAGATGAAA
		<i>xa13</i>	GGCCATGGCTCAGTGTATTAT	GAGCTCCAGCTCTCCCAAATG
	<i>xa5-Re</i>	AGCTCGCCATTCAAGTCTTGAG	TGACTTGGTCTCCCAAGGCTT	
	<i>xa5-Su</i>	GTCTGGAATTTGCTCGCGITCG	TGGTAAAGTAGATACTTATCAAACCTGGA	
Blast	RM413		GCGGATTCCTTGGATGAAGAG	TCCCCACCAATCTTGCTCTC
	RM1233		GTGTAAATCATGGGCACGTG	AGAITGGCTCCTGAAGAAGG
	RM8225		ATGCGTGTTCAGAAATTAGG	TTGTTGTATACCTCATCGACAG

Table 5 continued...

Table 5 continued...

RM5961		GTATGCTCCTCCTCACCTGC	ACATGCGACGTGATGTGAAC
	<i>Pt9</i>	TTGCTCCATCTCCTCTGTT	ATGGTCCCTTATCTTTAATG
	<i>Pita-2</i>	GAGATGGCCCCCTCCGTGATGG	TGCCCTCAATCGGCCACACCTC
	<i>Pish</i>	AGCTGCAGTAGTCTGTTCCATG	CGTAAGATCATGAGCGAATG
	<i>Pita</i>	CAATGCCGAGTGTGCAAAGG	TCAGGTTGAAGATGCATPAGC
NSb	<i>Pib</i>	ATCAACTCTGCCACAAAATCC	CCCATACACCACTTGTTCCCC
J180	<i>Pii</i>	ATGTAAAGGCCAAAAGCTGAT	TTATGAGATTAGGAGTGTAT
J181		AGCGAAAATCAITTTATCACA	TCATCAAACTCAGTTAAACT
J1113		GGATGATGTGATCTGCAGAG	CTCTTGGTATCTTTGTTC
J1817	<i>Pi5</i>	GATATGGTTGAAAAGCTAATCTCA	ATCAITGTCTTCAATTCAGAGT
YL155/87	<i>Pita-Re</i>	AGCAGGTTATAAGCTAGGCC	CTACCAACAAGTTCATCAAA
YL183/87	<i>Pita-Su</i>	AGCAGGTTATAAGCTAGCTAT	CTACCAACAAGTTCATCAAA
pBA1-4NBS-O/U	<i>Pi9</i>	GCAGTGCATCTTGTCTCTACAAACCCT ACCAATCCCAT	TGGTGCACTCAGAAAAGAACTCAGAACCTGCA AGTCTCG
k6441	<i>Pik-m</i>	TGTAAAATACTTCTATGCCGAGGC	GTTTATGGAGAGATAGTCGCTG
k39575	<i>Pik-p</i>	GGTGTGGGAACTGAAACCCTG	GGTGTGGGAACTGAAACCCTA
l256	<i>Pit</i>	GGATAGCAGAAAGAAGCTTGAGACTA	CATGCTTTCACATAAGAAAGTTCCTC
	<i>qSB-9TQ</i>	ACCTGTACTTGTCTCCTT	CTTTGACCGTTCGTTTAT
RM205	<i>qshb9.2</i>	CTGGTCTCTGTATGGGAGCAG	CTGGCCCTTCACGTTTCAGTG
RM336	<i>qshb7.3</i>	TCCTGTGCTTGTGTCTTTG	AGTCAITTTGGCTCAACAGCC
c3-14	<i>QBph3</i>	GGCAAAATTAGACGGCAGG	GAATATGCAITTTGTTGGAG
IN76-2	<i>Bph14</i>	CTGCTGCTGCTCCTCGTATG	CAGGGAAGCTCCAAAGAACAG
RM261	<i>Bph15, QBph4.1</i>	CTACTTCTCCCTTGTGTG	TGTACCAATCGCCAAATCTCC
g12140-2	<i>Bph15</i>	ACCAAAACACGGTGGATGAGA	AATGGAAAAGAGGAGGACAAACAG
xc4-27	<i>Bph15, QBph4.1</i>	GCAAAAGGCCCTTAGCC	GCTAGTTGCAGGCACGC
20M14	<i>Bph15</i>	ATGCTGACGGTGTAGGAGT	CAGTCCATCCACACAACTTGA
RH7	<i>Bph17</i>	CTTGGCTCCGTAGGAGAAG	TGAGTGTAAACCCGAAAGTGGC
RHC10	<i>Bph17</i>	CAATACGGGAGATTTGGAGT	TTGGGAAGCATAACGAGTGA

Table 5 continued...

Table 5 continued...

INI56	<i>Bph15, Bph17</i>	AGGTGAAGCTGATGCTGTG	CGATACTTATTGCAACACAC
B43	<i>Bph20</i>	ACTCCAATTGGTTCCGTGTGG	TGGACTAAAAGCCGATGAGC
RM119	<i>Bph6</i>	CATCCCCCTGCTGCTGCTGTG	CGCCGGATGTGGGACTAGCG
S00310	<i>Bph25</i>	CAACAAAGATGGACGGCAAGG	TTGGAAGAAAAGCAGGCAC
RM589	<i>Bph3</i>	ATCATGTGCTGGTGGCTTAAC	CAGGTTCCAACCAGACACTG
RM260	<i>Bph10</i>	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG
RM313	<i>Bph10</i>	TGCTACAAAGTGTCTTTCAGGAC	GCTCACCTTTTGTGTCCAC
RM463	<i>Bph2</i>	TTCCCTCCCTTTTATGGTGC	TGTTCTCCTCAGTCACTGCG
RM6869	<i>Bph2</i>	GAGCTCCTGTAGTGACCCG	ATCAGCCTCGCCAGCTTC
RM6217	<i>Bph9</i>	CGCAGATGGAGATTCTTGAAGG	ACAGCAGCAAAGAGCAAAGAAATCC
INI87	<i>Bph18, Bph9</i>	GACCCCTTCGAGTCTAAGAAC	CTTCTTTGAACTCATAGACAG
7312.T4	<i>Bph18</i>	ACGGGGTGTAGCAITGG	TACAGCGAAAAGCATAAAGAGTTC
RM3331	<i>Bph18</i>	CCTCCTCCATGAGCTAATGC	AGGAGGAGCGGATTTCTCTC
RM5479	<i>Bph21, Bph26</i>	AACTCCTGATGCCCTCCTAAG	TCCATAGAAAACAATTTGTGC
BI21	<i>Bph21</i>	CGTCGTACATCTGAAATGGAG	GGACATGGAGATGGTGGAGA
RM11669		AAACCGTTCCAGGGAGACTGACC	TCGTCTGATCCATCCATCCATCC
Yellow	RM241	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTGTG

<sup>2)</sup>Resistant. <sup>3)</sup>Susceptible.

MAS: marker-assisted selection, QTL: quantitative trait locus, BLB: bacterial leaf blight, BPH: brown planthopper, WBPH: white-backed plant hopper.

*et al.*, 2020). These resources have facilitated the QTL discovery and gene cloning for marker-assisted breeding programs and transgenic research.

Abiotic and biotic stresses affect growth and yield of rice significantly. DNA-based markers offer immense potential to augment breeding efficiency through marker-assisted selection (MAS). Numerous quantitative trait loci (QTLs) mapping studies have yielded a wealth of DNA marker-trait associations for rice. Marker-assisted selection and introgression have been used for developing biotic and abiotic stress-tolerant rice genotypes (Das *et al.*, 2017). Three major bacterial blight resistance genes (Xa21, xa13 and xa5) were introduced through marker-assisted breeding to produce a bacterial blight resistant rice cultivar, Improved Samba Mahsuri (Sundaram *et al.*, 2008). Nogoy *et al.* (2016) in their review highlighted the latest markers and QTL for key abiotic and biotic stresses in rice breeding programs (Table 5) and emphasized that the adoption of molecular marker-assisted breeding presents a crucial challenge for agricultural scientists aiming to significantly impact crop improvement in the coming decades. Through QTL mapping and marker validation, Marker Assisted Selection enhances breeding process by facilitating genotype-based selection for desired traits. DNA markers possess great potential in advancing Molecular Assisted Breeding (MAB) for stress and disease resistance, serving as a valuable resource for rice breeders.

### Genetic Engineering and Gene Editing

Genome editing technologies, notably CRISPR-Cas systems have revolutionized the rice breeding process, offering both precision and efficiency (Wang *et al.*, 2017b). Rice, being an excellent model system for functional

**Table 6 :** Genetic engineering approaches for developing abiotic stress tolerance in rice.

Gene	Gene description	Gene source	Phenotype	Reference
HVAI	LEA (Late Embryogenesis Abundant) protein	<i>Hordeum vulgare</i>	Salinity and drought tolerance	Xu <i>et al.</i> (1996)
OsLEA3-2	LEA protein	<i>Oryza sativa</i>	Salinity and drought tolerance	Duan and Cai (2012)
OsPIPI	Aquaporin (plasma membrane intrinsic protein)	<i>Oryza sativa</i>	Salinity tolerance	Liu <i>et al.</i> (2013)
OsTSPI	Trehalose-6-phosphate synthase	<i>Oryza sativa</i>	Salinity, drought, and cold tolerance	Fan <i>et al.</i> (2012)
HSP70	Heat shock protein	<i>Citrus tristeza virus</i>	Salinity tolerance	Hoang <i>et al.</i> (2015)
sHSP18.6	Heat shock protein	<i>Oryza sativa</i>	Heat, drought, salt and cold tolerance	Wang <i>et al.</i> (2015b)
pdcl	Pyruvate Decarboxylase	<i>Oryza sativa</i>	Submergence tolerance	Quimio <i>et al.</i> (2000)
PYL10	ABA receptor	<i>Oryza sativa</i> (Nagina22)	Drought and cold tolerance	Verma <i>et al.</i> (2019)
Rab7	ABA pathway protein	<i>Oryza sativa</i>	Drought and heat tolerance	El-Esawi <i>et al.</i> (2019)
OsMYB6	Transcription factor	<i>Oryza sativa</i>	Drought and salinity tolerance	Tang <i>et al.</i> (2019)
OsmiR156k	Regulatory non-coding small RNA	<i>Oryza sativa</i>	Cold tolerance	Cui <i>et al.</i> (2015)
miR390	Regulatory non-coding small RNA	<i>Oryza sativa</i>	Cadmium tolerance	Ding <i>et al.</i> (2016)
miR319	Regulatory non-coding small RNA	<i>Oryza sativa</i>	Cold tolerance	Yang <i>et al.</i> (2013)
miR159	Regulatory non-coding small RNA		Drought tolerance	Zhao <i>et al.</i> (2017a, 2017b)
miR393	Regulatory non-coding small RNA	<i>Oryza sativa</i>	Sensitivity to salinity and alkalinity	Gao <i>et al.</i> (2011)
miR164b	Regulatory non-coding small RNA	<i>Oryza sativa</i>	Drought and salt tolerance	Jiang <i>et al.</i> (2019b)
dst	DST protein	<i>Oryza sativa</i>	Drought and salinity tolerance	Kumar <i>et al.</i> (2020b)
OsRR22	Transcription factor	<i>Oryza sativa</i>	Salinity tolerance	Zeng <i>et al.</i> (2020)
OsMYB30	Transcription factor	<i>Oryza sativa</i>	Cold tolerance	Zeng <i>et al.</i> (2020)
OsTPS1	Trehalose-6-phosphate synthase	<i>Oryza sativa</i>	Drought and cold tolerance	
OsERF922	Ethylene response factor	<i>Oryza sativa</i>	Drought tolerance	
OsSAPK4	Serine/threonine-protein kinase	<i>Oryza sativa</i>	Salt and drought tolerance	
OsVPE2	Vacuolar processing enzyme	<i>Oryza sativa</i>	Salt and drought tolerance	
OsAMT1.1	Ammonium transporter	<i>Oryza sativa</i>	Ammonium uptake and tolerance	

studies, has seen significant advancements in genetic improvement due to the development of new genome-editing tools. CRISPR-Cas9, known for its cost-effectiveness, ease of use and high accuracy has emerged as a prominent technology for plant genome editing, particularly in rice (Zafar *et al.*, 2020). It has been

successfully applied to target specific genes in rice, resulting in desired phenotypic changes (Feng *et al.*, 2013; Abe *et al.*, 2018). For instance, CRISPR-Cas9-mediated mutagenesis has led to the development of knockout rice plants with improved fatty acid composition, positively impacting rice bran oil quality (Abe *et al.*, 2018).

Additionally, CRISPR/Cas9 technology has been utilized to enhance rice cold tolerance, addressing the challenge of salinity stress (Zeng *et al.*, 2020; Farhat *et al.*, 2019). These advancements offer precise modification of rice genotypes, potentially combating various environmental stresses. Moreover, recent developments in genome editing, such as Cas12a and Cas13a, offer new avenues for targeted modifications in rice, including the targeting of single-stranded DNA and RNA, which could aid in combating plant viruses (Zafar *et al.*, 2020). However, the efficiency of genome editing can vary among different plant species, necessitating improved delivery methods and regulatory clarity for field cultivation (Ali *et al.*, 2015; Zaidi and Mansoor, 2017).

Furthermore, several genes associated with heat and cold stress tolerance in rice have been identified, offering valuable targets for genetic improvement. Genes such as ERECTA (ER) and the  $\alpha 2$  subunit of the 26S proteasome have been implicated in heat stress tolerance (Shen *et al.*, 2015). Similarly, genes like OsDPB3-2 and CBF/DREB1 play crucial roles in conferring heat and cold tolerance, respectively (Sato *et al.*, 2016). Transcription factors like OsMYB4, MYBS3, OsbHLH002 and OsMAPK3 have also been identified as positive regulators of cold stress tolerance in rice (Su *et al.*, 2010). Furthermore, genetic studies have revealed the importance of specific genes, such as qLTG3-1 and COLD1, in low-temperature germinability and chilling tolerance, respectively (Ma *et al.*, 2015). Table 6 provides a more comprehensive list of genes involved in enhancing various stress tolerances in rice through over expression, RNA interference, and genome editing techniques.

**Advancements in Transgenic Rice for Improved Nutrition: A Focus on Golden Rice :** Malnutrition disorders remain a pressing global concern, contributing to approximately 24,000 deaths each day. Among the various strategies aimed at addressing malnutrition, genetic engineering offers promising avenues for enhancing the nutritional content of staple crops. In this context, “Golden Rice” represents a pioneering genetic engineering concept designed to combat nutrient deficiencies prevalent in populations reliant on rice as a primary food source (Fraiture *et al.*, 2016). Rice is a staple food for millions worldwide, particularly in developing countries where malnutrition is widespread. However, rice lacks essential nutrients such as  $\beta$ -carotene (provitamin A) and other C40 carotenoid precursors in its endosperm, rendering it deficient in vitamin A and its precursors. This deficiency contributes significantly to vitamin A deficiency disorders among rice-consuming populations. To address this nutritional gap, researchers

have developed transgenic rice lines, commonly known as Golden Rice, with the aim of fortifying rice with provitamin A. This approach involves the genetic engineering of the provitamin A biosynthetic pathway into the rice endosperm, thereby enriching the nutritional content of the grain (Aglawe *et al.*, 2018). Key genes involved in this pathway include phytoene synthase (psy), phytoene desaturase (crt1) and lycopene cyclase (lcy).

In a seminal study by Ye *et al.* (2000), transgenic rice expressing the provitamin A biosynthetic pathway was successfully generated using *Agrobacterium*-mediated transformation. High-performance liquid chromatography (HPLC) analysis confirmed the presence of  $\beta$ -carotene in the seeds of the transgenic rice. Notably, the transformed rice cultivar Taipei 309, while no longer cultivated, served as a proof of concept for the feasibility of engineering provitamin A-rich rice varieties. Subsequent efforts have focused on transferring the provitamin A genes into widely grown rice varieties, such as IR 64, through conventional breeding methods and transformation techniques (Aglawe *et al.*, 2018). In addition to addressing vitamin A deficiency, researchers have explored strategies for enhancing the iron content of rice, another crucial micronutrient. Iron deficiency is a significant public health concern, particularly in regions where rice consumption is high. In a groundbreaking study of Goto *et al.* (1999), the soybean ferritin gene was introduced into rice to increase its iron content. The ferritin gene was strategically regulated by the rice seed storage protein glutelin promoter (GluB-1) to ensure specific accumulation in the endosperm. Western blot analysis confirmed the synthesis of soybean ferritin protein in the transformed rice seeds, with iron content up to three times higher than that of untransformed seeds.

These advancements underscore the potential of transgenic rice as a viable solution for addressing malnutrition and enhancing food security. However, challenges remain, including regulatory hurdles, public acceptance and intellectual property rights issues. Transgenic rice, exemplified by Golden Rice, holds immense promise for addressing widespread malnutrition by fortifying staple crops with essential nutrients. Nonetheless, ongoing research efforts continue to explore innovative approaches for improving the nutritional quality of rice and other staple crops, thereby contributing to global efforts to combat malnutrition and improve public health outcomes

### **Tissue Culture Applications**

Tissue culture have immense importance in plant breeding, including rice breeding, due to its ability to rapidly

propagate plant material, preserve valuable germplasm, and also facilitate genetic transformation. In rice breeding specifically, tissue culture enables the production of large numbers of genetically uniform plants from limited propagating material. The term tissue culture denotes all type of cultures including meristem culture, ovule culture, anther culture and embryo culture (Debergh and Read, 1991). Moreover, tissue culture techniques allow for the regeneration of plants from single cells or tissues, bypassing the lengthy process of propagation and accelerating the breeding cycle. Rising demand for rice production and the urgent need to address malnutrition, the application of biotechnological tools becomes paramount in the development of robust rice varieties. Thanh Xa and Thi Lang (2011), utilized anther culture technique, yielding 22 promising Doubled Haploid Lines (DHLs) with enhanced yield, uniformity, grain quality. Successful tissue culture of rice plants originated from the culturing of rice roots (Kawata and Inshihara, 1968), seeds (Nishi *et al.*, 1968) and embryos (Tamura, 1968). Mishra and Rao (2016) reviewed double haploid techniques in rice improvement by using anther culture/ in vitro androgenesis method, highlighting success in isolating high-yield, superior grain quality doubled haploid indica hybrid lines. They suggest selecting responsive rice genotypes and manipulating culture conditions to enhance anther culture ability. Various rice genotypes have been developed through anther culture technique in China, and several anther-derived lines have been reported in India, Japan, South Korea, Hungary and the USA (Siddique, 2015). Popielarska (2005) investigated in vitro self-pollination of isolated sunflower ovules by culturing them with pollen on modified MS culture media. The study successfully obtained significant seedlings in culture, laying a foundation for future research. Modification of the medium and semi *in vivo* techniques could further enhance pollen germination and tube growth in sunflower, leading to seedling production following in vitro pollination of isolated ovules. Intergeneric hybridization has been attempted in cereals, with somatic hybrids being generated between rice (*O. sativa*) and barley, *Hordeum vulgare* (Kisaka *et al.*, 1998) rice with *Zizania latifolia* (Liu *et al.*, 1999).

### Mutation Breeding

The utilization of induced mutations from various sources emerged as a prevalent strategy for introducing genetic diversity tailored to specific traits in rice during the 1980s. Today, this technique has become an integral component of the breeder's arsenal, facilitating the enhancement of desirable rice characteristics in adapted varieties. While this section does not aim to

comprehensively address all facets of mutation breeding, it seeks to underscore notable successes and underscore the significance of mutations in rice improvement. According to Wang (1992), between 1966 and 1990, China released 78 rice varieties derived from mutation breeding. Subsequently, from 1991 to 2004, a comparable number of new releases (77) originated from mutation applications (Chen *et al.*, 2006). Gamma rays remain the predominant mutagen, inducing alterations responsible for agronomic traits such as pest resistance and grain quality phenotypes. In Indonesia, the inaugural mutant variety, Atomita 1, was released in 1982, with a total of 14 officially released varieties to date, 13 of which were enhanced for biotic stress resilience, notably against the brown plant hopper, all through gamma ray mutagenesis (Ismachin and Sobrizal, 2006).

Vietnam, a key rice-producing nation has seen significant developments as well. Reports from Tran *et al.* (2006) indicated that the Agricultural Genetic Institute released 10 varieties between 1990 and 2002, primarily characterized by improved grain quality alongside other agronomic traits, with gamma rays again serving as the primary mutagen. Notably, major improved traits in rice included early maturity, altered plant height, and enhanced disease resistance. Noteworthy is the *sd1* gene, a celebrated mutant trait. The Food and Agriculture Organization of the United Nations (FAO)/International Atomic Energy Agency (IAEA) Mutant Varieties Database, as of March 2007, recorded a total of 2,541 releases. Cereals constituted the largest proportion (1,212), with rice leading the count (525), followed by barley (303) and wheat (200), signifying the profound impact of mutation breeding on rice and other crops alike.

### Conclusion and Prospects

The advent of Green Revolution technology, centred around the cultivation of high-yielding, disease- and insect-resistant rice varieties, has profoundly transformed rice production since the late 1960s. Across the rice belt of Asia, numerous nations, previously reliant on rice imports, have achieved self-sufficiency and even generated surpluses for exportation. Enhancing the yield potential of rice stands as the primary strategy for augmenting global rice output. Diverse approaches have been employed to bolster this potential, encompassing conventional hybridization, ideotype breeding, heterosis breeding, male sterility, wide hybridization, genetic engineering, and molecular marker-assisted breeding. Conventional breeding remains a prevalent method for cultivating crop varieties with heightened yield potential. The fusion of molecular biology, genomic research,

transgenic breeding, and molecular marker applications with traditional plant breeding methodologies has laid the groundwork for molecular plant breeding, promising to expedite rice improvement initiatives worldwide, including in India. Molecular-assisted selection (MAS) has emerged as a pivotal component in germplasm enhancement, with numerous genes linked to various traits identified through molecular markers, facilitating MAS for trait refinement. The advent of map-based cloning has furthered this progress, enabling the isolation of genes conferring resistance to both biotic and abiotic stresses, as well as those associated with yield-related traits, thereby opening avenues for applying MAS to enhance yields. The advent of recombinant DNA technology has enabled the creation of transgenic rice harboring novel genetic traits and resistance to biotic and abiotic stresses. Innovative breeding methodologies, alongside the burgeoning field of biotechnology, serve to complement conventional breeding techniques in realizing future rice breeding objectives.

## References

- Abe, K., Araki E., Suzuki Y., Toki S. and Saika H. (2018). Production of high oleic/low linoleic rice by genome editing. *Plant Physiol. Biochem.*, **131**, 58-62.
- Aglawe, S.B., Barbadikar K.M., Mangrauthia S.K. and Madhav M.S. (2018). New breeding technique “genome editing” for crop improvement: applications, potentials and challenges. *3 Biotech*, **8**, 1-20.
- Agrama, H.A., Yan W., Jia M., Fjellstrom R. and McClung A.M. (2010). Genetic structure associated with diversity and geographic distribution in the USDA rice world collection. *Nat. Sci.*, **2(04)**, 247.
- Akhtar, S., Bhat M.A., Wani S.A., Bhat K.A., Chalkoo S., Mir M.R. and Wani S.A. (2010). Marker assisted selection in rice. *J. Phytol.*, **2(10)**.
- Ali, Z., Abul-Faraj A., Li L., Ghosh N., Piatek M., Mahjoub A., Aouida M., Piatek A., Baltés N.J., Voytas D.F. and Dinesh-Kumar S. (2015). Efficient virus-mediated genome editing in plants using the CRISPR/Cas9 system. *Molecular Plant*, **8(8)**, 1288-1291.
- Bansal, K.C., Lenka S.K. and Mondal T.K. (2014). Genomic resources for breeding crops with enhanced abiotic stress tolerance. *Plant Breeding*, **133(1)**, 1-11.
- Bernier, J., Kumar A., Ramaiah V., Spaner D. and Atlin G (2007). A large effect QTL for grain yield under reproductive stage drought stress in upland rice. *Crop Sci.*, **47(2)**, 507-516.
- Bhogireddy, S., Babu M.S., Swamy K.N., Vishnukiran T., Subrahmanyam D., Sarla N., Voleti S.R., Rao P.R. and Mangrauthia S.K. (2021). Expression dynamics of genes and micrnas at different growth stages and heat treatments in contrasting high temperature responsive rice genotypes. *J. Plant Growth Regulation*, 1-18.
- Biswal, A.K., Mangrauthia S.K., Reddy M.R. and Yugandhar P. (2019, December). CRISPR mediated genome engineering to develop climate smart rice: Challenges and opportunities. In : *Seminars in Cell & Developmental Biology* (Vol. **96**, pp. 100-106). Academic Press.
- Brar, D.S. and Khush G.S. (1997). Wide hybridization for rice improvements: alien gene transfer and molecular characterization of introgression. *Interspecific hybridization: progress and Prospect* WARDA, 21-29.
- Chauhan, B.S. and Johnson D.E. (2011). Ecological studies on *Echinochloa crus-galli* and the implications for weed management in direct-seeded rice. *Crop Protection*, **30(11)**, 1385-1391.
- Chen, X., Liu X., Wu D. and Shu Q.Y. (2006). Recent progress of rice mutation breeding and germplasm enhancement in China.
- Cuevas Pérez, F.E., Guimarães E.P., Berrío L. and González D.I. (1992). Genetic base of irrigated rice in Latin America and the Caribbean, 1971 to 1989. *Crop Sci.*, **32(4)**, 1054-1059.
- Cui, N., Sun X., Sun M., Jia B., Duanmu H., Lv D. and Zhu Y. (2015). Overexpression of OsmiR156k leads to reduced tolerance to cold stress in rice (*Oryza sativa*). *Molecular Breeding*, **35**, 1-11.
- Das, G., Patra J.K. and Baek K.H. (2017). Insight into MAS: a molecular tool for development of stress resistant and quality of rice through gene stacking. *Front. Plant Sci.*, **8**, 233392.
- Debergh, P.C. and Read P.E. (1991). Micropropagation. In: *Micropropagation: technology and application* (pp. 1-13). Dordrecht: Springer Netherlands.
- Ding, Y., Ye Y., Jiang Z., Wang Y. and Zhu C. (2016). MicroRNA390 is involved in cadmium tolerance and accumulation in rice. *Front. Plant Sci.*, **7**, 235.
- Duan, J. and Cai W. (2012). OsLEA3-2, an abiotic stress induced gene of rice plays a key role in salt and drought tolerance.
- Dudley, J.W. and Lambert R.J. (2004). 100 generations of selection for oil and protein in corn. *Plant Breed. Rev.*, **24(1)**, 79-110.
- El-Esawi, M.A. and Alayafi A.A. (2019). Overexpression of rice Rab7 gene improves drought and heat tolerance and increases grain yield in rice (*Oryza sativa* L.). *Genes*, **10(1)**, 56.
- Fan, W., Zhang M., Zhang H. and Zhang P. (2012). Improved tolerance to various abiotic stresses in transgenic sweet potato (*Ipomoea batatas*) expressing spinach betaine aldehyde dehydrogenase. *PLoS One*, **7(5)**, e37344.
- Farhat, S., Jain N., Singh N., Sreevathsa R., Dash P.K., Rai R., Yadav S., Kumar P., Sarkar A.K., Jain A. and Singh N.K. (2019, December). CRISPR-Cas9 directed genome engineering for enhancing salt stress tolerance in rice. In : *Seminars in cell & developmental biology* (Vol. **96**, pp. 91-99). Academic Press.
- Feng, Z., Zhang B., Ding W., Liu X., Yang D. L., Wei P. and



- Zhu J.K. (2013). Efficient genome editing in plants using a CRISPR/Cas system. *Cell Research*, **23**(10), 1229-1232.
- Fraiture, M.A., Roosens N.H., Taverniers I., De Loose M., Deforce D. and Herman P. (2016). Biotech rice: current developments and future detection challenges in food and feed chain. *Trends Food Sci. Technol.*, **52**, 66-79.
- Frey, K.J. (1984). Breeding approaches for increasing cereal crop yields. *Cereal production. Proc. 2nd Intl. Summer School in Agriculture held by the Royal Dublin Society in cooperation with WK Kellogg Found*, 47-68.
- Fujimaki, H. (1979). Recurrent selection by using genetic male sterility for rice improvement. *JARQ (Tsukuba)*, **13**(3), 153-156.
- Gao, P., Bai X., Yang L., Lv D., Pan X., Li Y., Cai H., Ji W., Chen Q. and Zhu Y. (2011). osa-MIR393: a salinity-and alkaline stress-related microRNA gene. *Mole. Biol. Rep.*, **38**, 237-242.
- Garris, A.J., Tai T.H., Coburn J., Kresovich S. and McCouch S. (2005). Genetic structure and diversity in *Oryza sativa* L. *Genetics*.
- Ge, S., Sang T., Lu B.R. and Hong D.Y. (1999). Phylogeny of rice genomes with emphasis on origins of allotetraploid species. *Proc. Nat. Acad. Sci.*, **96**(25), 14400-14405.
- Gerardeaux, E., Giner M., Ramanantsoanirina A. and Dusserre J. (2012). Positive effects of climate change on rice in Madagascar. *Agron. Sust. Develop.*, **32**, 619-627.
- Glaszmann, J.C. (1986). Rice plant type variation: Japonica-Javanica relationships. *Rice Genet. Newslett.*, **3**, 41-43.
- Glaszmann, J.C. (1987). Isozymes and classification of Asian rice varieties. *Theoret. Appl. Gen.*, **74**, 21-30.
- Goto, F., Yoshihara T., Shigemoto N., Toki S. and Takaiwa F. (1999). Iron fortification of rice seed by the soybean ferritin gene. *Nat. Biotechnol.*, **17**(3), 282-286.
- He, Y., Li X., Zhang J., Jiang G, Liu S., Chen S., Tu J., Xu C. and Zhang Q. (2004). Gene pyramiding to improve hybrid rice by molecular marker techniques. *New Directions for a Diverse Planet: Proc. 4th Intern. Crop Sci. Cong.*
- Hoang, T.M., Moghaddam L., Williams B., Khanna H., Dale J. and Mundree S.G. (2015). Development of salinity tolerance in rice by constitutive-overexpression of genes involved in the regulation of programmed cell death. *Front. Plant Sci.*, **6**, 111733.
- Hu, H., Scheben A. and Edwards D. (2018). Advances in integrating genomics and bioinformatics in the plant breeding pipeline. *Agriculture*, **8**(6), 75.
- Huang, X. and Han B. (2014). Natural variations and genome-wide association studies in crop plants. *Annu. Rev. Plant Biol.*, **65**, 531-551.
- Huang, X., Wei X., Sang T., Zhao Q., Feng Q., Zhao Y., Li C., Zhu C., Lu T., Zhang Z. and Li M. (2010). Genome-wide association studies of 14 agronomic traits in rice landraces. *Nature Genetics*, **42**(11), 961-967.
- Huang, X., Zhao Y., Wei X., Li C., Wang A., Zhao Q., Li W., Guo Y., Deng L., Zhu C. and Fan D. (2012). Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nature Genetics*, **44**(1), 32-39.
- Ismachin, M. and Sobrizal S. (2006). A significant contribution of mutation techniques to rice breeding in Indonesia.
- Jairin, J., Kotchasatit U., Saleeto S., Jearakongman S., Srivilai K., Chamarek V., Kothcharek J., Pattawatang P., Korinsak S., Wongsaprom C. and Toojinda T. (2017). Application of marker-assisted breeding to improve biotic stress resistance for rainfed lowland rice in Northeastern Thailand.
- Jiang, Y., Carrijo D., Huang S., Chen J.I., Balaine N., Zhang W., van Groenigen K.J. and Linnquist B. (2019). Water management to mitigate the global warming potential of rice systems: A global meta-analysis. *Field Crops Res.*, **234**, 47-54.
- Jin, J., Huang W., Gao J.P., Yang J., Shi M., Zhu M.Z., Luo D. and Lin H.X. (2008). Genetic control of rice plant architecture under domestication. *Nature Genetics*, **40**(11), 1365-1369.
- Joshi, S.P., Ranjekar P.K. and Gupta V.S. (1999). Molecular markers in plant genome analysis. *Curr. Sci.*, 230-240.
- Kamboj, R., Singh B., Mondal T.K. and Bisht D.S. (2020). Current status of genomic resources on wild relatives of rice. *Breeding Science*, **70**(2), 135-144.
- KAwATA, S.I. and ISHIHARA A. (1968). The regeneration of rice plant, *Oryza sativa* L., in the callus derived from the seminal root. *Proc. Japan Acad.*, **44**(6), 549-553.
- Khush, G.S. (1995). Breaking the yield frontier of rice. *GeoJournal*, **35**, 329-332.
- Khush, G.S. (1999). Green revolution: preparing for the 21st century. *Genome*, **42**(4), 646-655.
- Khush, G.S. (1994). Increasing the genetic yield potential of rice, prospects and approaches. *Int. Rice Comm. Newslett.*, **43**, 1-8.
- Kilasi, N.L., Singh J., Vallejos C.E., Ye C., Jagadish S.K., Kusolwa P. and Rathinasabapathi B. (2018). Heat stress tolerance in rice (*Oryza sativa* L.): identification of quantitative trait loci and candidate genes for seedling growth under heat stress. *Front. Plant Sci.*, **9**, 1578.
- Kisaka, H., Kisaka M., Kanno A. and Kameya T. (1998). Intergeneric somatic hybridization of rice (*Oryza sativa* L.) and barley (*Hordeum vulgare* L.) by protoplast fusion. *Plant Cell Reports*, **17**, 362-367.
- Konishi, S., Izawa T., Lin S.Y., Ebana K., Fukuta Y., Sasaki T. and Yano M. (2006). An SNP caused loss of seed shattering during rice domestication. *Science*, **312**(5778), 1392-1396.
- Santosh Kumar, V.V., Verma R.K., Yadav S. K., Yadav P., Watts A., Rao M.V. and Chinnusamy V. (2020). CRISPR-Cas9 mediated genome editing of drought and salt tolerance (OsDST) gene in indica mega rice cultivar MTU1010. *Physiol. Mole. Biol. Plants*, **26**, 1099-1110.
- Kumar, N., Chhokar R.S., Meena R.P., Kharub A.S., Gill S.C.,

- Tripathi S.C., Gupta O.P., Mangrauthia S.K., Sundaram R.M., Sawant C.P. and Gupta A. (2021). Challenges and opportunities in productivity and sustainability of rice cultivation system: a critical review in Indian perspective. *Cereal Res. Commun.*, 1-29.
- Latha, R., Thiyagarajan K. and Senthilvel S. (2004). Genetics, fertility behaviour and molecular marker analysis of a new TGMS line, TS6, in rice. *Plant Breeding*, **123**(3), 235-240.
- Lawrence, P.K. and Frey K.J. (1975). Backcross variability for grain yield in oat species crosses (*Avena sativa* L. x *A. sterilis* L.). *Euphytica*, **24**(1), 77-85.
- Li, C., Lin F., An D., Wang W. and Huang R. (2017). Genome sequencing and assembly by long reads in plants. *Genes*, **9**(1), 6.
- Li, C., Zhou A. and Sang T. (2006). Rice domestication by reducing shattering. *Science*, **311**(5769), 1936-1939.
- Liu, C., Fukumoto T., Matsumoto T., Gena P., Frascaria D., Kaneko T., Katsuhara M., Zhong S., Sun X., Zhu Y. and Iwasaki I. (2013). Aquaporin OsPIP1; 1 promotes rice salt resistance and seed germination. *Plant Physiol. Biochem.*, **63**, 151-158.
- Liu, B., Liu Z.L. and Li X.W. (1999). Production of a highly asymmetric somatic hybrid between rice and *Zizania latifolia* (Griseb): Evidence for inter-genomic exchange. *Theoret. Appl. Gene.*, **98**, 1099-1103.
- Lu, Y., Ye X., Guo R., Huang J., Wang W., Tang J., Tan L., Zhu J.K., Chu C. and Qian Y. (2017). Genome-wide targeted mutagenesis in rice using the CRISPR/Cas9 system. *Molecular Plant*, **10**(9), 1242-1245.
- Ma, Y., Dai X., Xu Y., Luo W., Zheng X., Zeng D., Pan Y., Lin X., Liu H., Zhang D. and Xiao J. (2015). *COLD1* confers chilling tolerance in rice. *Cell*, **160**(6), 1209-1221.
- Ma, C., Zhang H.H. and Wang X. (2014). Machine learning for big data analytics in plants. *Trends Plant Sci.*, **19**(12), 798-808.
- Mace, E.S. and Jordan D.R. (2011). Integrating sorghum whole genome sequence information with a compendium of sorghum QTL studies reveals uneven distribution of QTL and of gene-rich regions with significant implications for crop improvement. *Theoret. Appl. Gene.*, **123**(1), 169-191.
- Mackay, T.F., Stone E.A. and Ayroles J.F. (2009). The genetics of quantitative traits: challenges and prospects. *Nat. Rev. Gene.*, **10**(8), 565-577.
- Makkar, G.S., Bhatia D., Suri K.S. and Kaur S. (2019). Insect resistance in Rice (*Oryza sativa* L.): overview on current breeding interventions. *Int. J. Trop. Insect Sci.*, **39**(4), 259-272.
- Mangrauthia, S.K., Bhogireddy S., Agarwal S., Prasanth V.V., Voleti S.R., Neelamraju S. and Subrahmanyam D. (2017). Genome-wide changes in microRNA expression during short and prolonged heat stress and recovery in contrasting rice cultivars. *J. Exp. Bot.*, **68**(9), 2399-2412.
- McNally, K.L., Childs K.L., Bohnert R., Davidson R.M., Zhao K., Ulat V.J., Zeller G., Clark R.M., Hoen D.R., Bureau T.E. and Stokowski R. (2009). Genomewide SNP variation reveals relationships among landraces and modern varieties of rice. *Proc. Nat. Acad. Sci.*, **106**(30), 12273-12278.
- Mishra, R. and Rao G.J.N. (2016). *In-vitro* androgenesis in rice: advantages, constraints and future prospects. *Rice Science*, **23**(2), 57-68.
- Mohler, V. and Singrün C. (2004). General considerations: marker-assisted selection. In: *Molecular marker systems in plant breeding and crop improvement* (pp. 305-317). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Montalván, R., Destro D., Silva E.D. and Montano J.C. (1998). Genetic base of Brazilian upland rice cultivars.
- Moore, J.H., Asselbergs F.W. and Williams S.M. (2010). Bioinformatics challenges for genome-wide association studies. *Bioinformatics*, **26**(4), 445-455.
- Morinaga, T. (1954). Classification of rice varieties on the basis of affinity. In: *International Rice Commission. Working Party on Rice Breeding Rep.* 5th Meeting.
- Multani, D.S., Jena K.K., Brar D.S., de los Reyes B.G., Angeles E.R. and Khush G.S. (1994). Development of monosomic alien addition lines and introgression of genes from *Oryza australiensis* Domin. to cultivated rice *O. sativa* L. *Theoret. Appl. Gene.*, **88**, 102-109.
- Nakano, M.A. (1992). Phylogenetic study of cultivated rice and its wild relatives by RFLP. *Rice Genet. Newslett.*, **9**, 132-134.
- Nishi, T., Yamada Y. and Takahashi E. (1968). Organ redifferentiation and plant restoration in rice callus. *Nature*, **219**(5153), 508-509.
- Nogoy, F.M., Song J.Y., Ouk S., Rahimi S., Kwon S.W., Kang K.K. and Cho Y.G. (2016). Current applicable DNA markers for marker assisted breeding in abiotic and biotic stress tolerance in rice (*Oryza sativa* L.). *Plant Breed. Biotechnol.*, **4**(3), 271-284.
- Ntanos, D.A. and Roupakias D.G. (2001). Comparative efficiency of two breeding methods for yield and quality in rice. *Crop Science*, **41**(2), 345-350.
- Oka, H.I. (1958). Intervarietal variation and classification of cultivated rice. *Indian J. Gene. Plant Breed.*, **18**, 79-89.
- Paterson, A.H., Bowers J.E. and Chapman B. (2004). Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. *Proc. Nat. Acad. Sci.*, **101**(26), 9903-9908.
- Popielarska-Konieczna, M. (2005). *In vitro* pollination of isolated ovules of sunflower (*Helianthus annuus* L.). *Acta Biologica Cracoviensia. Series Botanica*, **47**(1).
- Quimio, C.A., Torrizo L.B., Setter T.L., Ellis M., Grover A., Abrigo E.M., Oliva N.P., Ella E.S., Carpena A.L., Ito O. and Peacock W.J. (2000). Enhancement of submergence tolerance in transgenic rice overproducing pyruvate decarboxylase. *J. Plant Physiol.*, **156**(4), 516-521.
- Reddy, M.R., Mangrauthia S.K., Reddy S.V., Manimaran P., Yugandhar P., Babu P.N., Vishnukiran T., Subrahmanyam

- D., Sundaram R.M. and Balachandran S.M. (2021). PAP90, a novel rice protein plays a critical role in regulation of D1 protein stability of PSII. *J. Adv. Res.*, **30**, 197-211.
- Rout, D., Jena D., Singh V., Kumar M., Arsode P., Singh P., Katara J.L., Samantaray S. and Verma R.L. (2020). *Hybrid rice research: Current status and prospects* (Vol. 2020). London, United Kingdom: IntechOpen.
- Sailaja, B., Subrahmanyam D., Neelamraju S., Vishnukiran T., Rao Y.V., Vijayalakshmi P., Voleti S.R., Bhadana V.P. and Mangrauthia S.K. (2015). Integrated physiological, biochemical, and molecular analysis identifies important traits and mechanisms associated with differential response of rice genotypes to elevated temperature. *Front. Plant Sci.*, **6**, 1044.
- Salse, J., Bolot S., Throude M., Jouffe V., Piegu B., Quraishi U.M., Calcagno T., Cooke R., Delseny M. and Feuillet C. (2008). Identification and characterization of shared duplications between rice and wheat provide new insight into grass genome evolution. *The Plant Cell*, **20**(1), 11-24.
- Sato, H., Todaka D., Kudo M., Mizoi J., Kidokoro S., Zhao Y., Shinozaki K. and Yamaguchi Shinozaki K. (2016). The Arabidopsis transcriptional regulator DPB 3 1 enhances heat stress tolerance without growth retardation in rice. *Plant Biotechnol. J.*, **14**(8), 1756-1767.
- Septiningsih, E.M., Pamplona A.M., Sanchez D.L., Neeraja C.N., Vergara G.V., Heuer S., Ismail A.M. and Mackill D.J. (2009). Development of submergence-tolerant rice cultivars: the Sub1 locus and beyond. *Annals Bot.*, **103**(2), 151-160.
- Shen, H., Zhong X., Zhao F., Wang Y., Yan B., Li Q. and He Z. (2015). Overexpression of receptor-like kinase ERECTA improves thermotolerance in rice and tomato. *Nat. Biotechnol.*, **33**(9), 996-1003.
- Shih-Cheng, L. and Loung Ping Yuan (1980). Hybrid rice breeding in China. In: *Innovative approaches to rice breeding: Selected papers from the 1979 International Rice Research Conference. International Rice Research Institute, Los Banos, Philippines* (pp. 35-51).
- Siddique, R. (2015). Impact of different media and genotypes in improving anther culture response in rice (*Oryza sativa*) in Bangladesh. *Europ. Scientif. J.*, **11**(6).
- Singh, B., Singh N., Mishra S., Tripathi K., Singh B.P., Rai V., Singh A.K. and Singh N.K. (2018). Morphological and molecular data reveal three distinct populations of Indian wild rice *Oryza rufipogon* Griff. species complex. *Front. Plant Sci.*, **9**, 272865.
- Singh, M.K., Singh R.P., Singh P., Singh R.K. and Srivastava R.P. (2018). Reciprocal crosses in early maturing x high yielding rice (*Oryza sativa* L.) cultivars. *J. Pharmacog. Phytochem.*, **7**(5S), 50-55.
- Singh, R.J. and Ikehashi H. (1981). Monogenic male sterility in rice: induction, identification and inheritance 1. *Crop Science*, **21**(2), 286-289.
- Singh, V.K., Singh A., Singh S.P., Ellur R.K., Choudhary V., Sarkel S., Singh D., Krishnan S.G., Nagarajan M., Vinod K.K. and Singh U.D. (2012). Incorporation of blast resistance into "PRR78", an elite Basmati rice restorer line, through marker assisted backcross breeding. *Field Crops Res.*, **128**, 8-16.
- Su, C.F., Wang Y.C., Hsieh T.H., Lu C.A., Tseng T.H. and Yu S.M. (2010). A novel MYBS3-dependent pathway confers cold tolerance in rice. *Plant Physiology*, **153**(1), 145-158.
- Sun, C.Q., Wang X.K., Li Z.C., Yoshimura A. and Iwata N. (2001). Comparison of the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers. *Theoret. Appl. Gene.*, **102**, 157-162.
- Sundaram, R.M., Vishnupriya M.R., Biradar S.K., Laha G.S., Reddy G.A., Rani N.S., Sarma N.P. and Sonti R.V. (2008). Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica*, **160**, 411-422.
- Sweeney, M.T., Thomson M.J., Pfeil B.E. and McCouch S. (2006). Caught red-handed: Rc encodes a basic helix-loop-helix protein conditioning red pericarp in rice. *The Plant Cell*, **18**(2), 283-294.
- Takahashi, Y., Teshima K.M., Yokoi S., Innan H. and Shimamoto K. (2009). Variations in Hd1 proteins, Hd3a promoters, and Ehd1 expression levels contribute to diversity of flowering time in cultivated rice. *Proc. Nat. Acad. Sci.*, **106**(11), 4555-4560.
- TAMURA, S. (1968). Shoot formation in calli originated from rice embryo. *Proc. Japan Acad.*, **44**(6), 544-548.
- Tan, L., Li X., Liu F., Sun X., Li C., Zhu Z., Fu Y., Cai H., Wang X., Xie D. and Sun C. (2008). Control of a key transition from prostrate to erect growth in rice domestication. *Nature Genetics*, **40**(11), 1360-1364.
- Tan, X.L., Vanavichit A., Amornsilpa S. and Trangoonrung S. (1998). Genetic analysis of rice CMS-WA fertility restoration based on QTL mapping. *Theoret. Appl. Gene.*, **97**, 994-999.
- Tang, Y., Bao X., Zhi Y., Wu Q., Guo Y., Yin X., Zeng L., Li J., Zhang J., He W. and Liu W. (2019). Overexpression of a MYB family gene, OsMYB6, increases drought and salinity stress tolerance in transgenic rice. *Front. Plant Sci.*, **10**, 168.
- Xa, T.T. and Lang N.T. (2011). Rice breeding for high grain quality through anther culture. *Omonrice*, **18**, 68-72.
- Tran, D.Q., Dao T.T., Nguyen H.D., Lam Q.D., Bui H.T., Nguyen V.B., Nguyen V.X., Le V.N., Do H.A. and Phan P. (2006). Rice mutation breeding in Institute of Agricultural Genetics, Viet Nam.
- Verma, R.K., Santosh Kumar V.V., Yadav S.K., Pushkar S., Rao M.V. and Chinnusamy V. (2019). Overexpression of ABA receptor PYL10 gene confers drought and cold tolerance to indica rice. *Front. Plant Sci.*, **10**, 1488.
- Wang, S., Wu K., Yuan Q., Liu X., Liu Z., Lin X. and Fu X. (2012). Control of grain size, shape and quality by OsSPL16 in rice. *Nature Genetics*, **44**(8), 950-954.

- Wang, W., Mauleon R., Hu Z., Chebotarov D., Tai S., Wu Z., Li M., Zheng T., Fuentes R.R., Zhang F. and Mansueto L. (2018). Genomic variation in 3,010 diverse accessions of Asian cultivated rice. *Nature*, **557(7703)**, 43-49.
- Wang, Y., Li Y., Li Y., Liu F., Liu X., Gong D., Ma Q., Li W. and Wu J. (2015). Intensive rice agriculture deteriorates the quality of shallow groundwater in a typical agricultural catchment in subtropical central China. *Environ. Sci. Poll. Res.*, **22**, 13278-13290.
- Wang, L.Q. (1992). Advances in plant mutation breeding in China: a full analysis. *Bull. Nucl. Agric. Sci.*, **13**, 282-295.
- Wang, M., Wang S., Liang Z., Shi W., Gao C. and Xia G. (2018). From genetic stock to genome editing: gene exploitation in wheat. *Trends Biotechnol.*, **36(2)**, 160-172.
- Wing, R.A., Ammiraju J.S., Luo M., Kim H., Yu Y., Kudrna D., Goicoechea J.L., Wang W., Nelson W., Rao K. and Brar D. (2005). The *Oryza* map alignment project: the golden path to unlocking the genetic potential of wild rice species. *Plant Mole. Biol.*, **59**, 53-62.
- Wu, W., Zheng X.M., Lu G., Zhong Z., Gao H., Chen L., Wu C., Wang H.J., Wang Q., Zhou K. and Wang J.L. (2013). Association of functional nucleotide polymorphisms at DTH2 with the northward expansion of rice cultivation in Asia. *Proc. Nat. Acad. Sci.*, **110(8)**, 2775-2780.
- Xiao, J., Grandillo S., Ahn S.N., McCouch S.R., Tanksley S.D., Li J. and Yuan L. (1996). Genes from wild rice improve yield. *Nature (London)*, **384(6606)**, 223-224.
- Xie, W., Wang G., Yuan M., Yao W., Lyu K., Zhao H., Yang M., Li P., Zhang X., Yuan J. and Wang Q. (2015). Breeding signatures of rice improvement revealed by a genomic variation map from a large germplasm collection. *Proc. Nat. Acad. Sci.*, **112(39)**, E5411-E5419.
- Xu, D., Duan X., Wang B., Hong B., Ho T.H.D. and Wu R. (1996). Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. *Plant Physiology*, **110(1)**, 249-257.
- Yang, C., Li D., Mao D., Liu X.U.E., Ji C., Li X., Zhao X., Cheng Z., Chen C. and Zhu L. (2013). Overexpression of micro RNA 319 impacts leaf morphogenesis and leads to enhanced cold tolerance in rice (*Oryza sativa* L.). *Plant, Cell & Environment*, **36(12)**, 2207-2218.
- Yang, T., Zeng R., Zhu H., Chen L., Zhang Z., Ding X., Li W. and Zhang G. (2010). Effect of grain length gene GS3 in pyramiding breeding of rice. *Mole. Plant Breed.*, **8(1)**, 59-66.
- Ye, X., Al-Babili S., Klott A., Zhang J., Lucca P., Beyer P. and Potrykus I. (2000). Engineering the provitamin A ( $\beta$ -carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science*, **287(5451)**, 303-305.
- Yuan, L.P. (2003). The second generation of hybrid rice in China. In: Sustainable rice production for food security. *Proceedings of the 20th session of the International Rice Commission*. Bangkok, Thailand, 23-26 July 2002, pp. 117-120.
- Zafar, K., Sedeek K.E., Rao G.S., Khan M.Z., Amin I., Kamel R., Mukhtar Z., Zafar M., Mansoor S. and Mahfouz M.M. (2020). Genome editing technologies for rice improvement: progress, prospects and safety concerns. *Frontiers in Genome Editing*, **2**, 5.
- Zaidi, S.S.E.A. and Mansoor S. (2017). Viral vectors for plant genome engineering. *Front. Plant Sci.*, **8**, 260724.
- Zeng, Y., Wen J., Zhao W., Wang Q. and Huang W. (2020). Rational improvement of rice yield and cold tolerance by editing the three genes OsPIN5b, GS3 and OsMYB30 with the CRISPR-Cas9 system. *Front. Plant Sci.*, **10**, 1663.
- Zhang, L.B., Zhu Q., Wu Z.Q., Ross Ibarra J., Gaut B.S., Ge S. and Sang T. (2009). Selection on grain shattering genes and rates of rice domestication. *New Phytologist*, **184(3)**, 708-720.
- Zhao, C., Liu B., Piao S., Wang X., Lobell D.B., Huang Y., Huang M., Yao Y., Bassu S., Ciaia P. and Durand J.L. (2017). Temperature increase reduces global yields of major crops in four independent estimates. *Proc. Nat. Acad. Sci.*, **114(35)**, 9326-9331.
- Zhao, J., Zhang S., Dong J., Yang T., Mao X., Liu Q., Wang X. and Liu B. (2017). A novel functional gene associated with cold tolerance at the seedling stage in rice. *Plant Biotechnol. J.*, **15(9)**, 1141-1148.
- Zhao, Y., Chan Z., Gao J., Xing L., Cao M., Yu C., Hu Y., You J., Shi H., Zhu Y. and Gong Y. (2016). ABA receptor PYL9 promotes drought resistance and leaf senescence. *Proc. Nat. Acad. Sci.*, **113(7)**, 1949-1954.
- Zhao, K., Tung C.W., Eizenga G.C., Wright M.H., Ali M.L., Price A.H., Norton G.J., Islam M.R., Reynolds A., Mezey J. and McClung A.M. (2011). Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. *Nat. Commun.*, **2(1)**, 467.