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*Review Article*

# Rice Mutation Breeding Methods and the Genes Conferring Key Traits for Enhanced Cultivation

**Charli Russell[1], Lilla Irwin[2], Oshini Herath[1], Ella van Wensveen[2], Alison Beavis[2] and Anna Pick Kiong Ling[3*]**
[1]*Faculty of Science, Medicine and Health, University of Wollongong, Australia*
[2]*Faculty of Science, School of Biotechnology & Biomolecular Sciences, University of New South Wales, Australia*
[3]*Division of Applied Biomedical Science & Biotechnology, School of Health Sciences, IMU University, Kuala Lumpur, Malaysia*
[*]For correspondence: anna_ling@imu.edu.my; ORCID: 0000-0003-0930-0619
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## Abstract

Rice (*Oryza sativa* L.) is a staple nutrient source for a significant portion of the world population, and therefore, enhancing its productivity as a major crop is vital in addressing growing global demand. Mutation breeding offers promise in inducing genetic variations from which certain agronomic traits of rice may be improved. This review presents a summary of research on historical and contemporary rice cultivation, including mutation breeding, and the challenges associated with mutagenesis itself, highlighting its feasibility in modern agriculture. This review also explores central aspects of mutagenesis in rice, including a thorough examination of the key traits improved in mutant rice varieties and the genes associated with these traits. The genetic and physiological outcomes of mutagenesis in rice, as reported in both laboratory and field-based reports indicate the success and limitations of this breeding technique. It has been revealed that mutagen breeding has positively contributed to developing more desirable rice varieties with improved traits such as stress tolerance and crop yield. Despite this, challenges such as the imprecision of induced mutation and the potential for subsequent off-target effects further contribute to overarching regulatory concerns that hinder the development of mutagen breeding in crops. Integrating and supplementing mutagenesis with modern genomic tools guide future research into sustainable and resilient rice cultivation practices. By consolidating current knowledge on mutation breeding in rice, we contribute this review as a resource for the design of targeted rice mutagenesis and to inform policymakers in the anticipated development of informed regulatory frameworks.

**Keywords:** Abiotic stress; Bacterial blight; Biotic stress; Mutagenesis; Rice blast

## Introduction

Rice is a dynamic staple in the global agriculture footprint of the world and is central to the daily dietary intake of more than 50% of the world's total population (Wang and Han 2022), with annual consumption reaching approximately 520 million metric tons (Shahbandeh 2024). The high nutritional and energy value of rice has advantageous impacts on individual health and well-being (Burlando and Cornara 2014). As a key source of fibre, energy, minerals, proteins, vitamins and antioxidants, rice plays a crucial role in the enhancement of human health (Burlando and Cornara 2014; Sen *et al.* 2020).

With global demand and nutritional importance in mind, rice faces a variety of complex and multifaceted challenges requiring the evaluation of adaptive strategies to maintain yield under environmental stressors such as climate change, water management, soil health, abiotic factors such as flooding and biotic factors such as disease and pathogen exposure (Rajan 2023). With the ongoing ramifications of climate change progression, studies have shown that the productivity of global agriculture has declined by 21% over 60 years (Schonhardt 2021). Climate change progression is linked with an increase in the incidence of extreme weather conditions such as drought and flooding which in turn is linked to the projection of rice yield to deplete even further (Schonhardt 2021). Adding onto this, rice production is threatened by exposure to pathogens such as rice blast and the impact of disease on rice yield is estimated at 10–30% annual loss (He *et al.* 2023)

With the complex interplay of challenges faced by the rice industry, it was paramount for advancements to be made to mitigate these concerns and enhance the overall adaptability of rice (Rajan 2023). The process of mutation breeding shows high promise in addressing these concerns and allowing for yield and global distribution safety.

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Mutation breeding is an underpinning of genomics studies and aims to evaluate and remodel the evolution and function of a variety of genes to ensure the production of rice can keep up with global demand and adapt to external challenges (Wang and Han 2022). As a critical advancement in genetic and biotechnology research, mutation breeding operates by inducing genetic mutations into a genome; this process involves manipulating and identifying genes to improve rice and build resilience to external factors (Tarakanov 2022).

Rice has shown a plethora of significance concerning advances in functional genomics. It is a highly accessible cereal with a small genome (Moin *et al.* 2017). The Rice Genome project (2002) was a historically significant advancement in the scientific world where the rice genome was successfully mapped and was at the forefront of genome studies and resequencing projects (Guo *et al.* 2014). This review article aims to evaluate the effectiveness of mutation breeding as a tool in agricultural development and understand the challenges and future directions that mutation breeding holds.

## Breeding techniques in rice

Crop breeding development methods have progressed from the more primitive conventions akin to early human agriculture to the highly advanced and precise genomic techniques of recent times (Rao *et al.* 2018). Efforts to improve plant breeding have resulted in the definition of the "crop ideotype" – a plant type under set conditions that combines favourable phenotypic characteristics, working to achieve these model features rather than breed out defective traits (Donald 1968; Fernie and Yan 2019). While the ideal model organism is not static and is rather varied to best suit the environment (Carbajal-Friedrich and Burgess 2024), breeding efforts function to design phenotypic traits to offer the most optimal yields and stress tolerance. In terms of rice, these ideal agronomic traits define a rice ideotype as one combining a panicle density and erectness, tiller number, leaf shape and leaf angle, among other morphologies, that are conducive to increased yield, grain quality, hardiness, etc. This may be achieved in essence by introducing genetic diversity to the rice gene pool and purposefully screening for and combining genes responsible for these characteristics. In practice, this has involved a range of approaches over generations of agricultural practice, including manual selection methods, functional genomic tools and mutagenic breeding.

## Conventional breeding methods

Various plant breeding methods have been historically employed to develop elite cultivars through the observation and manipulation of natural genetic diversity (Khush 1989). Several common methods have remained since the early domestication of crops, including means of cyclical selection and crossing.

**Recurrent selection:** Recurrent selection is the systematic selection of organisms with desirable traits from a population to continue to breed (Morais Júnior *et al.* 2017), effectively advancing the performance of future generations. This process generates the cumulative improvement of a target organism, with favourable alleles increasing in frequency as they are manually selected based on expression (Abdullah 2009). The means of identifying positive trait expression in this breeding technique is based upon the phenotype of an organism and, therefore, allows for a precise pursuit of genetic gains in selected traits (Ahmar *et al.* 2020). However, this approach requires multi-generational intervention. Furthermore, since most critical traits are polygenic and thus recurrent selection is less suitable to address these features as single locus gene frequencies tend to change slowly (White 2004). Upland rice cultivars in Latin America have been successfully developed using recurrent selection that embodies the rice blast resistance of this set "crop ideotype" (Courtois *et al.* 1997; Guimaraes and Correa-Victoria 2000). However, population improvement in rice via recurrent selection has been applied very little in other documentation (Pereira De Castro *et al.* 2023).

**Cross-breeding:** Cross-breeding is the intraspecies mating of two different breeding lines to increase genetic variation; a process that occurs in nature and has been a long-established study in agricultural development (Lowe 1927). The pedigree method is one such crossbreeding method utilized in both self- and cross-pollinated crops that revolve around the recorded ancestry of an individual plant, with superior genotypes being selected, maintained and crossed with other ideal individuals in subsequent generations (Begna 2021). This method is again limited in that much time is required to analyse breeding lines and many crosses are often required to identify useful characteristics due to the random nature of genetic recombination (Institute of Medicine and National Research Council 2004). Further, the rate of progress for yield resulting from the pedigree method rarely exceeds 1% per year (Breseghello and Coelho 2013). Therefore, studies in different growing seasons are required to fully complete the procedure for gathering information on the minor genes required in polygenetic resistances.

**Hybridization:** The crop gene pool may be increased through the hybridization of the target crop with wild varieties via interspecific crossing. While this protocol does not typically occur in nature, it often results in sterile offspring. Diverse hybrid populations can be derived from certain matches of interspecific recombination and successful subsequent progeny (Khush 2013). Hybrid rice plants have been created by the International Rice Research Institute (IRRI) and various governments to adapt this crop to tropical Asian conditions (Virmani 2003) but have displayed limited yield on a large scale. The interspecies cross utilized in this example was with an indica germplasm variety that offers a limited genetic diversity, while still achieving the capacity to thrive in these tropical conditions (Yuan 2001). While

routinely used to develop elite rice varieties, hybridization techniques largely require phenotypic analysis in the form of field testing to gauge performance (Cui *et al.* 2020). This is a labour- and cost-intensive process that limits the application of hybridization technologies.

**Biotechnology approaches**

Modern methods of crop breeding have emerged with the development of molecular biology and biotechnology, namely genome editing. This tool encompasses the insertion, deletion, or substitution of foreign material into a target organism's DNA as well as non-transgenic genome editing (Zhang *et al.* 2017). Upon the successful transformation of a target organism, the newly adjusted sequence may be integrated and expressed by the host genome in the pursuit of the "ideotype".

**Transgenic technology:** The use of transgenes is referred to as genetic modification (GM) and is a means of artificially introducing foreign genetic material into an unrelated organism to produce a genetically modified organism (GMO). Such insertional transfer DNA (T-DNA) is delivered to host cells by a microbial vector at a high frequency to introduce variation into the gene pool and produce a stable phenotype (Schouten *et al.* 2017). *Agrobacterium tumefaciens* is a soil microbe that can infect a host and transfer a portion of its DNA into the host cell, with this naturally occurring mechanism commonly utilized as a microbial vector for commercial plant genetic engineering (Institute of Medicine and National Research Council 2004). If this transferred DNA is stably integrated into the host plant, it may then be read and expressed in the form of phenotypic traits (Kathuria *et al.* 2007). A system of transgenic rice has been developed in indica rice through this *A. tumefaciens* gene transfer in which morphologically normal and fertile transgenic organisms were generated, with the successful integration of select foreign genes in the host genome determined by Southern blot analysis (Rashid *et al.* 1996). Further, transposons similarly create a mutation in that these small mobile DNA sequences within the genome may occasionally break and fuse DNA strands in a "cut and paste" fashion to intracellularly transfer DNA (Chen *et al.* 2017). The effect of the movement of transposable elements is similar to that of genetic effects via microbial vector insertion and thus presents a similar level of risk when considered in a constant genetic context (Schnell *et al.* 2015). Despite the overall efficacy of this breeding technique, the use of transgenic technology in the modification of food crops, such as rice, has presented regulatory issues that have discouraged its widespread adoption (Pixley *et al.* 2022) and necessitated the development of crop breeding via transgene-free genome editing.

**New breeding techniques (NBTs):** The NBTs offer technical advantages in generating genetic variation, targeted mutagenesis, and introduction of new genes in crop breeding programs with the final product uniquely remaining free of transgenes, unlike GM organisms (Seyran and Craig 2018). The most common NBT is genome editing, a more precise alternative to conventional breeding and GM. Through this, genetic features can be input into a cell and expressed without the integration of transgenes into the host genome. One technology capable of genome editing in regulating crop varieties is clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR-associated (Cas) proteins, known as CRISPR/Cas9 (Chen *et al.* 2019). The CRISPR/Cas9 unit is a highly effective contemporary genomics system that introduces double-stranded breaks (DSBs) to a target DNA sequence related to a particular trait to activate gene expression in transformation, drug delivery, and mutation knockout applications (Bortesi *et al.* 2016). Using genome editing technologies, it is possible to obtain stable homozygous crop plant phenotypes in under a year (Ahmar *et al.* 2020), with this method decreasing the generation time that typically limits the development of a GMO crop. Despite the impressive effectiveness of this cutting-edge technology, the disproportionate availability of this equipment and the associated cost limit the applicability of genome editing in crop breeding (Pixley *et al.* 2022). Although functionally suitable, CRISPR/Cas9 and similar advanced genome editing tools are not widely accessed by rice smallholders and farmers, meaning alternate breeding methods will be preferred.

Rice yield is characterised by the number of panicles per plant, the number of grains per panicle and the grain weight (Xing and Zhang 2010). Single-stranded nucleases can include zinc finger nucleases (ZFNs), Transcription Activator-like Effector Nuclease (TALENs) and Clustered Regularly Interspaced Short Palindrome Repeats (CRISPR) and can result in double-stranded breaks in the target DNA (Miglani 2017). The CRISPR/Cas9 systems, which can be used for rice, include FnCPf1, LbCpf1 and AsCpf1 acting as a form of genome editing (Hu *et al.* 2017; Tang *et al.* 2017; Xu *et al.* 2017). TALENs will disrupt the *Oryza sativa* betaine aldehyde dehydrogenase 2 (OsBADH2) gene which will increase the presence of a scent in the rice (Shan *et al.* 2015). Certain CRISPR/Cas9 systems such as the Prevotella and Francisella 1 systems have proven to have an efficient mutation effect with a range from 13.6 to 43.1% increase in yield in rice mutants (Xu *et al.* 2017). The LbCpf1 gene is expressed with a Pol-II promoter, which is then used for mutagenesis in rice and seems to be effective in expressing crRNAs (Tang *et al.* 2017). DNA Ligase 4 (Lig4) genes found within the cNHEJ pathway cause the knockout of the DNA ligase 4 and will increase the frequency of TALEN-mediated mutagenesis in rice crops (Nishizawa-Yokoi *et al.* 2016). nCas9-PBE genes can be used in base editing with target point mutation as it is used to fuse with cytidine deaminase enzyme (Li *et al.* 2017). There was an increase in amylose content when CRISPR/Cas9 was used due to the specific starch branching enzymes SBE1 and SBEIIb (Sun *et al.* 2017). This shows the effect of the use of the CRISPR/Cas9 technology on rice. From the use of genetic

technology including CRISPR/Cas9, the rice grain varieties can tolerate different abiotic stresses such as drought.

## Mutation breeding methods

Among all crop breeding methods, mutations have acted as a natural source of genetic variability. Any change to a DNA sequence is defined as a mutation and may either occur spontaneously or can be a result of exposure to a chemical, physical, or biological agent known as a mutagen (Roychowdhury and Tah 2013).

**Mechanism:** Mutation breeding differs from conventional and biotechnological approaches to crop breeding in that it involves the purposeful exposure of plants or seeds to a mutagen to induce these random changes in the target DNA sequence (Mba 2013). From this exposure, stable mutants are screened for desirable traits and incorporated into further breeding programs. Considering the random nature of mutation, the majority of mutations achieved via a mutagenic agent are deleterious and therefore without any tangible use for crop breeding. Over 2,300 different crop varieties have been developed using this method of induced mutagenesis according to the International Atomic Energy Agency and Agriculture Organization of the United Nations (IAEA/FAO) Mutant Variety Database (Institute of Medicine and National Research Council 2004), including examples of mutation-bred rice varieties.

Spontaneous mutations are by definition not induced, so breeders employing conventional methods of selection often process many generations of a crop before useful DNA variation can be observed (Mba 2013; Roychowdhury and Tah 2013). The induction of mutation in mutation breeding increases the frequency of this occurrence (Maluszynski *et al.* 2000) and therefore enables more effective studies of functional genomics and subsequent development of new genotypes in the interest of crop improvement. The efficiency of this method is perpetuated by the totipotency of vegetatively propagated plants in which *In vitro* cultured plant tissues may initiate the induction of optimal mutations (Mba *et al.* 2010).

**Practical application:** Mutagen selection and exposure are the only means of control in the process of mutation breeding, however, this still does not offer the precision to target particular genes or relevant traits (Parry *et al.* 2009). A foundational understanding of the genetic basis of the target traits to be improved is required to apply this technique. For example, like many of the breeding techniques previously mentioned, polygenic traits have a significantly reduced chance of modification with induced mutations (Shu 2009).

Mutation breeding in rice is often used in tandem with conventional breeding since this technique is historically effective for amplifying improved major traits. Targeted mutagenesis is particularly advantageous in rice due to its small genome (Wang *et al.* 2013), with promising results having been reported in several countries. This includes the release of an RD6 rice variety by the Thai Department of Agriculture; a radiation-induced glutinous mutant of a popular non-glutinous rice variety (Miah *et al.* 2013).

**Mutagens:** As mentioned, mutation is a heritable change that drives evolution and speciation (Nei 2007) that may be manipulated to achieve an ideotype in plant agriculture. Further, the continual generation of genetic diversity is also relevant to the process of domestication in that novel traits may be triggered by only a single mutation (Wu *et al.* 2017). To achieve such subtle DNA mutation as opposed to more risk-prone gross alterations, the precise selection and application of a mutagen is critical. Mutation breeding is classed by two types of mutagenesis: physical and chemical (Oladosu *et al.* 2016). Several mutagen examples specific to the mutation breeding of rice are summarised in Table 1.

Early discoveries of radiation lent to interest in its mutagenic effects, including the exposure of crops to radiation in the 1920s (Stadler 1930), with such agents of mutation known as physical mutagens. These agents induce mutagenesis by causing specific damage, including both DNA aberrations and gross chromosomal breakages/rearrangement, with an intensity that is somewhat prone to defective progeny in crops. Ionizing radiations are among the most common physical mutagens for crop mutation breeding (Mba *et al.* 2012), including the cosmic, gamma (γ) and X-rays within the electromagnetic spectrum. The γ rays, as included in Table 1, have been widely used in the mutation of rice, with studies indicating γ-rays generate genetic variability in a range of rice genotypes as an effective tool for improvement (Harding *et al.* 2012).

Similar to physical agents, chemical mutagens have also been extensively shown to cause mutations and modify DNA in cells (Auerbach 1957; Mba *et al.* 2010). These changes manifest in strand breaks, deamination, transitions and insertions, interrupted transcription and replication, as described in Table 1. Recently, high-throughput screening methods have allowed the detection of single nucleotide mutations resulting from chemical-induced mutagenesis in a gene of interest, including that of Targeting Induced Local Lesions in Genomes (TILLING) (Taheri *et al.* 2017), for the detection and exploitation of genetic variation. Chemical mutagens offer the capacity to improve traits while largely avoiding mass undesirable changes (Jeng *et al.* 2011) when compared to physical mutagens.

## Improved traits of mutant rice varieties and its key genes

**Impact on rice crop yield:** Rice yield is known to be affected by the characteristics of panicle morphology, tiller traits, leaf shape and angle. It can also be positively impacted by the number of grains per panicle and the 1000-grain weight measurements (Zhong *et al.* 2023).

**Table 1:** Examples of common physical and chemical mutagens used in the mutation breeding of rice

| Mutagen Type | Example | Description | References |
|---|---|---|---|
| Physical | X-rays | Highly penetrative, small rate of linear electron transfer | Mba *et al.* 2012 |
| | Gamma (γ) rays | Most commonly used, frequently cause small deletions | Viana *et al.* 2019 |
| | Ion beam radiation (IBR) | High rate of linear electron transfer causes large deletions, point mutations and intergenic rearrangements | Tanaka *et al.* 2010 |
| Chemical | Ethyl Methane Sulfonate (EMS) | Induces a high frequency of point mutations via alkylation of guanine bases and subsequent mismatch with thymine | Talebi *et al.* 2012 |
| | N-Methyl-N-Nitrosourea (MNU) | Alkylating agent, highly reactive with oxygen, adds alkyl groups to nitrogenous bases | Suzuki *et al.* 2008 |
| | Sodium azide | Organic azide metabolite interacts with DNA, creates point mutations | Gruszka *et al.* 2012 |

**Genes affecting tillering:** Interacting Protein 1 (IPI1) is a type of nuclear gene localised in the E3 ligase. Wang and Wang (2017) explain in their article the effect which the IPI1 gene has on tillers. When this gene loses its function, it increases the number of tillers and size of the panicles. As a result, the yield of rice crops increases with an increase in tiller number. Nitrogen-mediated Tiller Growth Response 5 or NGR5 is a type of nitrogen-sensitive gene that plays a role in the positive regulation of both rice growth and development whilst harming tillering which then results in inhibitory gene expression (Wu *et al.* 2020). The NGR5 protein associated with the NGR5 gene has been shown to interact with a Gibberellin Insensitive Dwarf 1 (GID1) gene which causes inhibitory gene expression in rice growth and development. TN1 or Tiller Number 1 gene will encode for a protein that contains both a bromo-adjacent homology (BAH) and RNA domain which will negatively affect rice tillering (Zhang *et al.* 2023). The TN1 interaction factor (TIF1) will counteract the effects of the gene by being knocked out and thus increasing the tillering in crops. Dwarf 53 (D53) is a gene that negatively regulates the Strigolactone (SL) pathway and is degraded through the proteasome pathway. Song *et al.* (2017) determined through their research that this D53 gene would interact with the IPA1 gene to regulate the amount of rice tiller. Tillering I and Dwarf I (HTD1) genes can encode for the CCD7 which is the carotenoid cleavage dioxygenase 7 playing a vital role in the negative regulation of rice tillers alongside upregulation of the transcription process (Zou *et al.* 2006). PAY1 is the Plant and Architecture and Yield 1 gene which is pleiotropic meaning it has several functions. This is attributable to overexpression, which results in smaller tiller angles, greater height, thicker stalks, and a greater crop yield (Zhao *et al.* 2015).

**Genes affecting the angle of tillering:** The TAC1 or Tiller Angle Control 1 gene affects the stem and leaf angle with the upregulation of the gene increasing the angle whilst its downregulation results in a decrease of the leaf angle (Yu *et al.* 2007). The Tiller Inclined Growth 1 (TIG1) gene also affects the plant's angle like the TAC1 gene. It also has a role in the positive regulation of cell elongation (Zhang *et al.* 2019). The LAZY2 or LA2 gene codes for a novel chloroplast protein, which contains starch stored within the gravity-sensitive cell that controls the tiller angle (Huang *et al.* 2021). LA1 is another gene that controls plants' tiller angle through gravitropism and auxin Field regulation (Li *et al.* 2007). The Heat Stress Transcription Factor2D (HSFA2D) gene codes for a heat shock transcription factor which is a positive regulator accounting for an imbalance in auxin levels of crops (Zhang *et al.* 2018). The auxin used in these processes, such as tiller angle control, is achievable by promoting the genetic expression of the specific genes Wuschel Related HomeoboX6 (WOX6) and Wuschel Related HomeoboX11 (WOX11).

**Genes affecting the angle of the stem and leaf:** The grouping of genes Oryza sativa receptor-like cytoplasmic kinases includes OsRLCK57, OsRLCK107, OsRLCK118, and OsRLCK176 amongst others. These can encode cytoplasmic kinases, which play a role in the leaf inclination (Zhou *et al.* 2016). Many other genes can affect inclination including LC3 and LC2 with LC2 specifically having a positive regulation on this trait. This also relates to the quality of the rice grain, which is further expanded in the next section. This is because of the effect that the angle of the stem and leaf has on the quality of rice grain through its crop production. Table 2 summarises the key genes that affect tillering, tiller angles and the angle of the leaf.

## Impact on rice grain characteristics

**Rice grain yield:** The Grain Size 2 (GS2) protein is found localised in the nucleus for the regulation of the grain's length and width through the processes of cell division and expansion. The Grain Length 2 (GL2) gene can increase the grain size, and the number of tillers and grains and thus have a positive effect on the overall yield per plant (Hu *et al.* 2015). The Grain Size 3 (GS3) gene combines with glycoprotein B to reduce the grain length but when combined with G Protein Gamma Subunit 2 (GGC2) and Dense Panicle 1 (DEP1), it will increase the length of the grain. The DEP1 gene promotes cell division which is what allows the increase in grain length and increases the yield (Sun *et al.* 2018). The Grain Width 2 (GW2) gene influences the width of the grain along with the weight through the increase of cell numbers (Song *et al.* 2007). Wide Grain 1 (WG1) gene can encode glutaredoxin and control the size of seeds through cell proliferation (Hao *et al.* 2021). OsPPKLI1 has a role in the negative regulation of rice grain length through the dephosphorylation of Oryza Sativa Glycogen Synthase Kinase 3 (OsGSK3) and the inhibition of the BR signalling (Zhang *et al.* 2012; Gao *et al.* 2019). GLW7 genes bind to the Small and Round Seed 5 (SRS5) gene to control the grain length thus producing longer grains, decreasing its chalkiness, and improving the transparency (Wang *et al.* 2012).

**Table 2:** Genes accounting for rice yield and quantity

| Genes | Mechanism of Action | Functional Roles | Method of mutation/desired phenotype | References |
|---|---|---|---|---|
| *D53* | Tillering | Interacts with IPA1 to inhibit transcriptional activation activity | Suppresses *IPA1* transcriptional activation | Song *et al.* 2017 |
| *GID1* | Tillering | A gibberellin receptor functioning to reduce tiller numbers | Inhibition of nitrogen promoted tillering | Wu *et al.* 2020 |
| *HSFA2D* | Tiller Angle | Heat Stress Transcription Factor 2D induces the transcription of LA1 for auxin distribution. | Upstream positive regulator of LA-ZY1 mediated asymmetric auxin distribution pathway | Zhang *et al.* 2018 |
| *HTD1* | Tillering | A defect in the gene results in high-tillering and dwarf phenotypes with the htd1 mutant. | Defect in gene | Zou *et al.* 2006 |
| *IPI1* | Tillering | Ring-finger E3 ligase which is also an IPA1 interacting protein for panicle degradation. | | Wang and Wang 2017 |
| *LA1* | Tiller Angle | Grass-specific gene which is a negative regulator for polar auxin transport resulting in reduced gravitropism. | Alters endogenous Indole-3-acetic acid distribution in shoots | Li *et al.* 2007 |
| *LA2/LAZY2* | Tiller Angle | LAZY2 is a mutant gene and LA2 is the causative gene for regulating rice shoot gravitropism and rice tiller angle. | Acts upstream of *LA1* to mediate lateral auxin-transport | Huang *et al.* 2021 |
| *LC2* | Angle of Leaf | The positive regulation of stem and leaf angle | Encodes Vernalisation-insensitive 3-like protein due to influence of plant hormones | Zhao *et al.* 2010 |
| *LC3* | Angle of Leaf | Encodes a transcriptional suppressor for IAA homeostasis and signal transduction | SPOC domain-containing transcription suppressor interacts with *LIP1* | Chen *et al.* 2018 |
| *NGR5* | Tillering | NGR5 mutation causes the enhancement of nitrogen-induced tillering. | | Wu *et al.* 2020 |
| *OsRCLK57, OsRCLK107, OsRCLK118, OsRCLK176* | Angle of Leaf | Function in the negative regulation of BL-mediated signalling which produces larger leaf angles. | | Zhou *et al.* 2016 |
| *PAY1* | Tillering | PAY1 mutants reduced tillering and produced a smaller tiller angle when expressed. | Impacting polar auxin transport activity. Altering endogenous indole-3-acetic acid distribution | Zhao *et al.* 2015 |
| *TAC1* | Tiller Angle | Major quantitative trait locus which controls tiller angle | Mutation in the 3 prime-splicing site of this 1.5-kb intron decreases tiller angle | Yu *et al.* 2007 |
| *TIG 1* | Tiller Angle | TIG1 alleles at the mRNA level will regulate tiller angle with the H1 haplotype resulting in a lesser tiller angle than H2-8. | Positively regulates expression of EXPA3, EXPB5, and SAUR39 promoting cell elongation increasing tiller angle | Zhang *et al.* 2019 |
| *TN1* | Tillering | Negatively regulating tiller formation. | Encodes for a protein with mutants such as tn1-2 and tn1-3 | Zhang *et al.* 2023 |
| *WOX 6 and WOX 11* | Tiller Angle | Two types of transcription factors for the regulatory pathway of rice tiller angle. | Connect gravitropism response via Auxin | Zhang *et al.* 2018 |

**Table 3:** Genes affecting the rice grains characteristics

| Genes | Mechanism of Action | Functional Roles | Method of mutation/desired phenotype | References |
|---|---|---|---|---|
| *AH2* | Rice Grain Quality | A mutation in this gene results in smaller grains and reduces rice grain quality by decreasing amylose content and increasing protein content. | CRISPR-Cas9 | Ren *et al.* 2019 |
| *FZP* | Number of Grains per Panicle | Frizzy Pannicle gene repression results in an approximately 15% increase in grain yield. | Insertion of *SGDP7* which is identical to *FZP* resulting in tandem duplication in Chuan-7 repressing expression | Bai *et al.* 2017 |
| *GGC2* | Grain Length and Width | A G*γ* protein involved in the positive regulation of grain size | Complex with Gβ | Sun *et al.* 2018 |
| *GL7* | Rice Grain Quality | Regulation of rice grain appearance quality through cell elongation affecting the length-to-width ratio. | Tandem duplication of 17.1kb segment at *GL7* subsequently leads to upregulation | Wang *et al.* 2015a, b |
| *GNP1* | Number of Grains per Panicle | Increase in cytokinin activity will increase the grains per panicle in rice. | Encodes for Ga20ox1to allow for processes to occur | Wu *et al.* 2016 |
| *GS2/GL2* | Grain Length and Width | Encodes OsGRF4 acting as a transcription activator. A mutation of this gene affects the binding site and as a result, increases expression causing larger cells to increase grain width. | Binding site impact through mutation | Hu *et al.* 2015 |
| *GS3* | Grain Length and Width | A subunit of a G*γ*-protein in the signalling pathway blocks the interaction of DEP1 and GGC2 reducing grain size. | Competes with Gβ | Sun *et al.* 2018 |
| *GW2* | Grain Length and Width | The breeding of an isogenic line increased grain length and width. | Encodes a RING protein with E3 ubiquitin ligase activity which targets degradation. | Song *et al.* 2007 |
| *GW8* | Rice Grain Quality | With high expression, there is positive cell proliferation to promote cell division and grain filling for rice grain yield. | Increased expression leads to positive regulation | Wang *et al.* 2012 |
| *NAL1* | Number of Grains per Panicle | A mutant that promotes lower grain numbers, narrow leaves, reduced height and longer grains. | Interacts with *FZP* to downregulate expression and upregulation own expression | Huang *et al.* 2018 |
| *NOG1* | Number of Grains per Panicle | This gene encodes an enoyl-CoA hydratase/isomerase to increase rice grain yield by 25.8% through increasing grain weight. | Encodes an enoyl-CoA hydratase/isomerase | Huo *et al.* 2017 |
| *WG1* | Grain Length and Width | The Wide Grain 1 (WG1) gene encodes for a glutaredoxin for the positive regulation of grain width and is associated with ZIP transcription factors to repress activity. | Encodes for a glutaredoxin protein | Hao *et al.* 2021 |

**Number of grains per panicle:** The Grain Number per Panicle 1 (GNP1) gene can encode GA which can increase the grain number which then increases the rice yield (Wu *et al.* 2016). Similarly, the Number of Grains 1 (NOG1) will increase the grains per panicle to also increase rice yield by 25.8% (Huo *et al.* 2017). The Frizzy Panicle (FZP) gene will increase the number of grains per panicle but produce a smaller grain than average. When overexpressed, the gene will grow larger grains but have a lesser quantity per panicle (Bai *et al.* 2017). The Narrow Leaf 1 (NAL1) gene will interact with the FZP protein and with its degradation, it also increases the grains per panicle and overall rice yield. Table 3 shows different characteristics of rice grains.

**Genes affecting rice grain quality:** Expanding on Table 3 above, Grain Length 7 (GL7) gene controls the shape and quality of rice grains whilst regulating the longitudinal elongation of cells (Wang *et al.* 2015a, b). Grain Width 8 (GW8) gene is found in high-quality Pakistani Basmati rice and is responsible for the elongation and rice quality but produces a 14% yield loss (Wang *et al.* 2012). The GW8

**Table 4:** Genes affecting grain-specific characteristics

| Genes | Mechanism of Action | Functional Roles | Method of mutation/desired phenotype | References |
|---|---|---|---|---|
| *BG1* | Grain Size | When the gene is overexpressed, it results in an increase of grain size through regulating auxin transport. | Increased expression of the gene through insertion into the genome | Liu *et al.* 2015; Milner *et al.* 2021 |
| *BG3* | Grain Size | Encodes a purine permease for plant morphology to regulate grain size, number and leaf elongation through the modulation of cytokinin. | Increased expression of the gene through insertion into the genome | Xiao *et al.* 2019 |
| *BR* | Grain Size | Growth promoting hormone with positive regulation in rice grain size | Regulation of division, elongation and division of cells | Li *et al.*, 2022; Manghwar *et al.* 2009 |
| *DEP1* | Grain Size | Phosphatidylethanolamine-binding protein-like domain protein to increase the grains per panicle and yield. | Gain-of-function mutation | Huang *et al.* 2009; Zhou *et al.* 2009 |
| *GS5* | Grain Size | Positive regulator of grain width, length and size. It achieves this through the encoding of a putative serine carboxypeptidase-like (SCLP) protein. | Increased expression of the gene through insertion into the genome | Xu *et al.* 2015 |
| *GSK2* | Grain Size | Regulation of phosphorylation, BR signaling protein accumulation | Negative regulator of BR signaling | Tong *et al.* 2012 |
| *GW6a* | Grain Weight | Encodes a histone acetyltransferase for the positive regulation of grain size and yield. | Increased expression of the gene through insertion into the genome | Gao *et al.* 2021 |
| *GLW7* | Grain Length | Binds to the SRS5 promoter for the positive regulation of grain length through gene expression. | Increased expression of the gene through insertion into the genome | Segami *et al.* 2017 |
| *OsPPKL1* | Grain Length | Contains a qgl3 allele increasing grain yield and produces a long-grain phenotype that is expressed. | qgl3 allele | Gao *et al.* 2019; Zhang *et al.* 2012 |
| *qTGW6* | Grain Weight | A genetic region of TGW6 which increases carbohydrate concentration in rice to improve rice grain quality and weight. | Loss of function of gene | Ishimaru *et al.* 2013 |
| *SMOS1* | Grain Size | A rice mutant which decreases grain size as cell size also decreases. Its expression is induced by exogenous auxin treatment. | Loss of function mutation | Hirano *et al.* 2017 |
| *SMOS2/DLT* | Grain Size | A positive regulator of BR signaling. A loss-of-function mutant in this gene causes abnormal cell arrangement and increase in cell numbers. | Positive regulation of BR signaling | Hirano *et al.* 2017 |

gene will bind to the Grain Width 7 (GW7) gene promoter to improve the quality of the rice whilst not affecting the yield negatively (Wang *et al.* 2012). Abnormal Hull 2 (AH2) encodes for the MYB domain protein, which is responsible for glume and grain development with smaller grains due to a decrease in amylose, gel consistency and increased protein (Ren *et al.* 2019).

Rice grain size is determined by traits of length, width, thickness, and ratio of length to width affecting appearance quality (AQ), milling quality (MQ) and eating and cooking quality (ECQ) (Li *et al.* 2022). Rice endosperm has starch, storage proteins, lipids, minerals, and trace elements. Rice AQ is related to its size, chalkiness, and transparency. Rice MQ is negatively correlated with grain length but positively with width and thickness. The chalkiness of rice has a negative correlation with rice quality. As a result, the higher the chalkiness, the poorer AQ, MQ and ECQ of rice are (Cheng *et al.* 2005; Yamakawa *et al.* 2007). Table 4 summarises the different genes that can affect rice grain quality through size, weight and length.

**Genes affecting drain size:** The DEP1/qPE9 encodes for the rice panicle architecture and the size of the grain (Huang *et al.* 2009; Zhou *et al.* 2009). Also, it can regulate the prolonging of starch for grain filling thus affecting its size. Glycogen Synthase Kinase 2 (GSK 2) is expressed to increase the grain size and the leaf angles (Tong *et al.* 2012). Brassinosteroid (BR) is a growth-promoting hormone for regulating grain size. An increase in the expression of Grain Size 5 (GS5) will inhibit endocytosis, increase the BR signalling, and increase the grain size (Xu *et al.* 2015). Big Grain 1 (BG1) is an auxin primary response gene that encodes for auxin transport and affects grain size through cell division and elongation (Liu *et al.* 2015). The Big Grain 3 (BG3) gene encodes for purine permease and the OsPUP4 gene regulates the grain size (Xiao *et al.* 2019). Small Organ Size 1 (SMOS1) genes will interact with the SMOS2/DLT genes to form a complex involved in rice's growth and development, including its grain size (Hirano *et al.* 2017). When a point mutation occurs in the ninth intron of the Supernumerary Bract (SNB), it alters mRNA splicing which reduces the rice shattering and increases the grain size (Jiang *et al.* 2019).

**Genes affecting grain weight:** Grain Width 6a (GW6a) is a type of histone acetyltransferase and when it is overexpressed, it results in the grain's increased weight and yield. When analysed, the expression of GW6a upon interacting with the Homolog of DA1 on Rice Chromosome 3 (HDR3) results in a 6.8 and 10.3% increase in rice grain length and width (Gao *et al.* 2021). This is achieved through increasing cell numbers and accelerating grain filling (Gao *et al.* 2021). qTGW6 controls the rice weight per grain. A loss-of-function allele will promote grain length and weight (Ishimaru *et al.* 2013).

## Abiotic stress tolerance

The rice industry has evaluated the impacts of abiotic stressors on the overall yield and quality of grain being cultivated and produced for mass consumption around the world. Abiotic factors such as drought, flooding and soil salinity constantly interfere with the physiological, molecular, biochemical, and morphological response of rice (Sarma *et al.* 2023) which consequently leads to significant loss in crop production. Through copious amounts of genetic research and interval mapping, important genes have been identified enabling scientists to understand genes responsible for abiotic stress tolerance in rice and enhance the growth under suboptimal abiotic conditions (Radha *et al.* 2023).

## Tolerance to flooding

Flooding is a common yet destructive natural disaster that

**Table 5:** Genes associated with flooding resistance in rice

| Genes | Mechanism of action | Functional roles | Method of mutation/desired phenotype | References |
|---|---|---|---|---|
| *CIPK15* | Allows rice to grow without access to oxygen supplies | Regulation of energy and stress sensors. Regulation of energy and sugar production allowing for rice growth under floodwater | Regulation of SnRK1A which is the key energy and stress sensor | Lee *et al.* 2009 |
| *LGF1* | Determines wax composition of leaves allowing for gas film retention | Regulation of primary alcohol synthesis which enhances ability of underwater photosynthesis | Insertion into genome via varying mutagenesis methods | Kurokawa *et al.* 2018 |
| *OsHIGD2* | Hypoxia signalling | Reduction of respiratory damage in the mitochondria due to hypoxia-induced by flooding | Mitochondrial protein present in Hypoxia induced gene domain | Hwang and Choi 2016 |
| *SNORKEL1 (SK1)* | Stem elongation in response to submergence | Ethylene response factor encoding resulting in internode elongation by plant hormones – Gibberellins | Ethylene accumulation leading to gene expression | Hattori *et al.* 2009 |
| *SNORKEL2 (SK2)* | Stem elongation in response to submergence | Ethylene response factor encoding resulting in internode elongation by plant hormones – Gibberellins | Ethylene accumulation leading to gene expression | Hattori *et al.* 2009 |
| *SUB1A-1* | Inhibition of growth during submergence | Reduction of carbohydrate consumption inhibiting stem elongation allowing for regrowth once water recedes "quiescence strategy" | Upregulated by Ethylene and exposure to submergence | Fukao *et al.*, 2006; Perata 2018 |

significantly reduces the overall production of rice and consequently the economic footprint of agricultural rice production. Currently, over 35% of rice fields are subjected to flooding (Panda *et al.* 2021). Flooding is expected to significantly increase over the coming years due to the ongoing impact associated with climate change and its unpredictability in terms of the length of natural events or its impact on wider agriculture farming. Due to this, significant efforts have been undertaken to breed strains with flooding tolerance and through extensive genomic research there has been a significant breakthrough in the identification of *SUB1* which shows significant enhancement in rice tolerance in flooding-prone areas (Panda *et al.* 2021). Through the application of random-amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) Xu and Mackill (1996) were able to map the submergence tolerance in rice species IR40931-26 (tolerant species) and P1543851(susceptible species), respectively. Tolerant species when scaled from 1-9 (tolerant to susceptible) scored 1.5 and markers produced from 624 RAPD were subsequently mapped and associated with a region on the 9$^{th}$ chromosome, through RFLP this submergence tolerance quantitative trait locus was then deemed *SUB1* (Xu and Mackill 1996). More research conducted 10 years later further revealed that *SUB1* could be broken down further as a cluster of genes that made up the qualitative trait locus, the most crucial of those being *SUB1A-1*, which has allowed for the submergence tolerance to be bred into rice varieties through marker-assisted backcrossing (Perata 2018). The mechanism of *SUB1A-1* enables rice varieties to stop growth once submerged under flooding conditions and subsequently continue regrowth upon water recession due to the preservation of carbohydrate reserves (Perata 2018). Beyond this, studies have shown a variety of genes that have been closely linked to flood tolerance in rice varieties, these genes are summarised in Table 5 below.

**Drought tolerance**

Similar to that of flooding, drought is projected to increase significantly over the coming years due to the impact of climate change (Zaveri *et al.* 2023). Drought can significantly alter the growth of a variety of plants and formulation of resistance to drought is critical to ensure the security of rice. Drought substantially impacts the flowering stage of rice which leads to an exponential decline in photosynthesis rates, stomatal conductance, transpiration rate, water potential and air-leaf temperature gap (Sarma *et al.* 2023).

A method of adjustment to drought in rice results in the activation of abscisic acid (ABA) dependent and ABA-independent pathways. ABA is responsible for stomatal movement and can allow for the transpiration rate to be decreased in conditions of drought (Chen *et al.* 2021). *OsPYL/RCAR5* has been linked with regulatory mechanisms such as the closure of stomata and when overexpressed the *OsPYL/RCAR5* gene has been linked with the fability of rice to withstand drought conditions (Sarma *et al.* 2023).

Increasing the expression and regulation of reactive oxygen species (ROS) scavenging genes through the activation of genes such as *SNAC3* can increase the tolerance of rice to drought due to the regulation of ROS homeostasis (Chen *et al.* 2023). The production of ROS is enhanced significantly in plants under the stress of drought leading to oxidative stress (Cruz de Carvalho 2008). Overexpression of *SNAC3* has been linked with increased tolerance to drought and suppression of this gene has been linked with increased levels of sensitivity to drought stresses, species with overexpression of *SNAC3* displayed significantly lower levels of ROS such as hydrogen peroxide and malondialdehyde, which are byproducts of lipid peroxidation – a process initiated by ROS (Fang *et al.* 2015). PCR experimentation showed that plants overexpressed with *SNAC3* had a significantly higher expression of ROS scavenging genes compared to species with repressed *SNAC3* genes (Fang *et al.* 2015), which is suggestive that mutation breeding with *SNAC3* could be crucial in ensuring rice species are bred with drought stress tolerance. Table 6 summarises the mechanisms of genes associated with drought resistance in rice varieties.

A key driver of drought is the increase in environmental temperatures (Heat Waves and Climate Change 2024). Recent studies conducted have shown that the increasing rise in overall atmospheric temperature is associated with overall decline in product yield of rice across the world (Zhang *et al.* 2017) with a current estimation that overall rice yield could decline by a dramatic 41% by the end of the 21$^{st}$ century with rising heat levels

**Table 6:** Genes associated with drought resistance in rice

| Genes | Mechanism of action | Functional roles | Method of mutation/desired phenotype | References |
|---|---|---|---|---|
| *DRO1* | Allows access of water from deeper level in the soil when water reserves are sparse | Increase in root growth angle which subsequently increases deep rooting of the rice | Backcrossing for introduction into rice varieties. Increased expression of the gene through insertion into genome | Uga *et al.* 2013 |
| *HVA1* | Stabilise enzymes and protect cellular processes | Increase in the leaf relative water content, stability of the plasma membrane | Transformation with DNA-Blot hybridisation of rice and immunoblot analysis of *HVA1* | Chandra Babu *et al.* 2004 |
| *OsbZIP72* | Abscisic acid signalling | Increased sensitivity to abscisic acid - vital phytohormone to allow stress tolerance in rice | Increased expression of the gene through insertion into genome | Lu *et al.* 2009 |
| *OsIAA6* | Optimise water use and preserve vital water supplies | Regulation of auxin biosynthesis genes | Increased expression of the gene through insertion into genome | Jung *et al.* 2015 |
| *OsPYL/RCAR5* | Closure of stomata and enhanced root growth | Regulation of Abscisic acid synthesis | Increased expression of the gene through insertion into genome | Kim *et al.* 2020; Sarma *et al.* 2023 |
| *SNAC3* | Improved water retention and reduced water loss | Regulation of reactive oxygen species homeostasis | Increased expression of the gene through insertion into genome | Fang *et al.* 2015; Chen *et al.* 2023 |

**Table 7:** Genes associated with soil salinity resistance in rice

| Genes | Mechanism of action | Functional Roles | Method of mutation/desired phenotype | References |
|---|---|---|---|---|
| *OsHAK5* | Sensitive to potassium levels, acting as a transporter. No reaction to levels of sodium | Contributes to ion homeostasis by increasing accumulation of K+ over $Na^+$ | Breeding of OsHAK5 to express during salt stress in root epidermis, cortex and shoots | Horie *et al.* 2011 |
| *OsJRL40* | Regulates the genes which encode Potassium and sodium transportes | $Na^+/K^+$ homeostasis regulation and antioxidant enzyme activation | Increase expression of gene through insertion into genome | Gao *et al.* 2023 |
| *OsKAT1* | Maintenance of cytosolic cation homeostasis | Increase in $K^+$ uptake which decreases the accumulation of $Na^+$ | Increase expression of gene through insertion into genome | Obata *et al.* 2007 |
| *OsMAPK44* | Greater ratio of $K^+/Na^+$ (favourable iron balance) | Ion homeostasis in high salinity environments | Upregulated by exogenous ABA and $H_2O_2$ | Jeong *et al.* 2006 |
| *OsMYB91* | Osmolyte accumulation for osmoregulation | Proline levels increase and active oxygen scavenging is increased. | Increase expression of gene through insertion into genome | Zhu *et al.* 2015 |
| *OsZFP213* | Enhanced expression of antioxidant enzyme processes | Promotion of ROS scavenger genes to limit the impact of oxidative stress | Increase expression of gene through insertion into genome | Zhang *et al.* 2018 |

(Xu *et al*. 2021). Heat has an impact on rice in each growth stage with varying impacts associated with the developmental stage, in the vegetative stage high levels of excessive heat exposure has been linked with a reduction in the thermostability of the plasma membrane (Shrestha *et al*. 2022) and therefore overall germination and seed vigor (Reed *et al*. 2022). Exposure to heat greater than 35°C is linked with a decrease in mature seedling sizes and temperatures above 39°C has been linked with endosperm collapse (Shrestha *et al*. 2022).

Heat stress can result in the overproduction of ROS which as aforementioned plays a critical role in the regulation of stomatal closure in rice species. The expression of *OsHTAS* within leaf blades of rice species has been linked with modulatory action of stomatal closure induced by hydrogen peroxide preventing excess water loss associated with heat exposure (Liu *et al*. 2023).

## Salinity tolerance

Toxic ion levels in soil are projected to rise as time progresses which will significantly impact the growth of crops and may lead to widespread crop death if not evaluated and treated (Ullah *et al.* 2021). Nearly 90% of the world's rice crop is produced in Southeast Asia and by 2050 it is expected that at least half of rice fields will be impacted by high salt concentration within the soil (Kumar *et al.* 2021). High salt concentration is responsible for poor crop yield as rice is a highly salinity-sensitive crop where high salt concentration can lead to oxidative stress, metabolic process disruption and membrane disorganisation if not addressed (Kumar *et al.* 2021). High salt levels in soil have been linked with $Na^+$ poisoning which if unaddressed leads to physiological altering in rice crops which can result in widespread cell death (Hussain *et al.* 2017).

Tolerance to salt in rice crops occurs via two mechanisms; ion exclusion which focuses on increased sodium and chloride transport in roots and inhibits the accumulation of these ions within the leaves and osmotic tolerance which is attributed to drought aspects of salinity and allows for leaves to maintain shape and stomatal conductance (Munns and Tester 2008). Through molecular analysis, it was discovered that the jacalin-related lectin gene *OsJRL40* regulates the homeostasis pathway of $Na^+/K^+$ through controlled expression of ion transporters and enhances the activity of critical antioxidant enzymes (Gao *et al.* 2023). It has been reported that *OsZFP213* promoted the activity of ROS scavenger genes to diminish the impact caused by oxidative stress of high salt concentrations (Zhang *et al.* 2018). Table 7 summarises key genes associated with salinity tolerance in rice.

## Biotic stress tolerance

Biotic resistance among plants is necessary to prevent the serious spread of disease in agricultural industries. As a result of monocultural and modern farming practices, crop species are becoming less genetically diverse, resulting in increased susceptibility to disease (Zhu *et al.* 2000). Among bacterial and fungal pathogens, bacterial leaf blight caused by *Xanthomonas oryzae* and rice blast *Magnaporthe oryzae* posed a substantial threat to ongoing rice breeding practice.

**Table 8:** Relevant genes associated with biotic resistance in rice

| Gene | Mechanism of action | Functional Roles | References |
|---|---|---|---|
| *Rmg 2-3* | Nucleotide-binding site and leucine-rich-repeat (NBS-LRR) | Major temperature-sensitive resistance genes against *M. oryzae*, expressed in seedlings | Zhan *et al.* 2008; Phuke *et al.* 2022 |
| *Rmg 7-8* | NBS-LRR resistance genes | Resistance genes expressed in both seedlings and mature crops against *M. oryzae.* | Inoue *et al.* 2021; Guo *et al.* 2021 |
| *Pi-d2* | S-receptor-like serine/-protein kinase | Involved in production of Ubiquitin ligase for cellular signalling against *M. oryzae* | Xie *et al.* 2022 |
| *Pi-ta* | Blast resistance protein | Protease inhibitor responsible for hyper-sensitive response, to restrict *M. oryzae* replication | Meng *et al.* 2020 |
| *Pi54* | Blast resistance protein | Resistance gene against *M. oryzae*, which activates enzymes and transcription factors. | Singh *et al.* 2020 |
| *OsSWEET11-14* | Bacterial blight susceptibility genes | Restriction limits bi-directional sugar transport to reduce persistence of *X. oryzae.* | Antony *et al.* 2010; Oliva *et al.* 2019 |
| *Bsr-k1* | Regulatory gene for Os-PAL transcription | Increases mRNA turnover for broad-spectrum resistance. | Guo *et al.* 2021 |
| *Os-PAL1-4* | Broad-spectrum resistance gene | Responsible for lignin and salicylic acid production. | Yang *et al.* 2022b |
| *Xa4* | Resistance against *X. oryzae* | Accelerates cellulose production to reduce cell wall porosity | Yang *et al.* 2022c |
| *Xa13* | Recessive resistance allele | Plasma membrane protein which inhibits induces transcription within infected cells | Yuan *et al.* 2009; Antony *et al.* 2010 |
| *Xa21* and *Xa26* | Receptor-like kinase | Broad spectrum resistance through pattern recognition against *X. oryzae* | White and Yang 2009 |

This has resulted in 20–40% of rice yields lost to bacterial blight and an average of 50% due to rice blast, resulting in increased concerns for food insecurity and poverty (Singh *et al.* 2020). Currently, 41 resistance genes against bacterial blight have been identified in rice varieties (Jiang *et al.* 2020), with a range of these summarised in Table 8.

Species of pathogen and rice varieties have co-evolved alongside each other, exchanging and integrating genetic material (Arora *et al*. 2012). Consequently, rice species can recognize characteristic signals and patterns enabling the use of a targeted defence system against pathogenic factors. Similarly, pathogens are continuously adapting and thus able to overcome plant defences through the production of specialised proteins to bypass detection and persist in the host cell. As a result, mutagenic breeding techniques in rice are necessary to ensure diversity is maintained, improving the frequency of resistance genes. Mutagenic breeding methods aim to identify genes that are associated with resistance to specific pathogens to produce new variations and improve gene expression (Mba *et al*. 2012).

## Rice blast

Research efforts into rice blast resistance have led to the discovery of over 100 known resistance genes (R-genes) involved in rice defences (Srivastava *et al.* 2017). R-genes are involved in effector-triggered immunity to mount defensive responses against invading pathogens. Thus, strategies to mutagenically enhance R genes are under trial for biotic resistance (Pandolfi *et al.* 2017). In particular, recently mutated genes (Rmg's) including Rmg8 have been researched for resistance against *M. oryzae* under standard and high temperatures (Guo *et al.* 2021). However, it has since been found that the fungal effector gene PWT4 can suppress the Rmg8 resistance gene (Inoue *et al.* 2021); indicating the necessity for a wider range of resistance genes as well as diversity to ensure species survival. Similarly, Rmg 2 and Rmg 3 genes are temperature sensitive and can provide disease resistance in seedlings at higher temperatures, which is a beneficial feature, particularly in tropical climates (Phuke *et al.* 2022).

Other possible means of inhibiting the entry and growth of pathogens include Protease inhibitor (Pi) genes to restrict *M. oryzae* (Han *et al.* 2004). In particular, the Pi54 resistance gene is responsible for the activation of defence enzymes including laccase, callose and peroxidase as well as transcription factors (Singh *et al.* 2020). Consequently, the production of the enzyme laccase enhances the lignification of cell walls to provide structural defences against the pathogen, (Dwivedi *et al.* 2011), whereas callose slows pathogenic spread by forming deposits between the cell wall and plasma membrane (Wang *et al.* 2021). Further, peroxidase increases cell wall rigidity to protect healthy cells (Almagro 2008). Consequently, among Pi genes, over-expression of the Pi54 gene has been shown to provide broad-spectrum resistance and a hyper-sensitive response against *M. oryzae* strains (Singh *et al.* 2020). Mutational breeding of these genes will assist with improving resistance capabilities in rice varieties, to maintain diversity and reduce crop losses to rice blast disease.

## Bacterial blight

The bacterial pathogen *X. oryzae* poses a substantial threat to the quality and yield of rice, infecting crops with bacterial blight; a disease that causes yellowing and wilting to leaves (White and Yang 2009). Under moderate conditions, 10–20% of yield is lost to bacterial blight in Southeast Asian countries (Baltes 2017). At least 44 resistance genes to bacterial blight have been discovered and are classified into receptor-like kinase genes (RLK), executor genes (E) and sugar-will-eventually-be-exported-transporter (SWEET) genes (Jiang *et al.* 2020). RLKs are involved in pattern recognition of invading pathogens, including the Xa4, Xa21 and Xa26 genes which recognise characteristic proteins via nucleotide binding (Ji *et al.* 2022). In particular, the Xa4 and Xa26 genes are responsible for producing a cell-wall kinase; a protein that connects the cell wall with the plasma membrane to assist with extra-cellular signalling, thus providing a defensive pathway against *X. oryzae*. (Jiang *et al.* 2020) Contrastingly, executor genes produce proteins to initiate programmed cell death of infected plant cells. E genes Xa7, Xa10 and Xa23 are shown to induce cellular apoptosis in the presence of pathogenic effector proteins. Studies of the Xa23 gene, in particular, indicate extremely broad resistance to the pathogen, suggesting this gene contributes to the virulence of *X. oryzae* during infection

and growth (Xu *et al.* 2022).

Susceptibility genes, a class of R-genes are also considered to be an integral part of research to reduce infection among rice species. Unlike NBS-LRR genes, S genes are involved with allowing the establishment of pathogens within the host cell. SWEET genes are a susceptibility gene type that assists with transporting sugars; an energy source utilized by fungal pathogens to sustain themselves (Oliva *et al.* 2019). Thus, such genes are often targeted by pathogens through the secretion of transcription activator-like effectors (TALEs) to promote expression, to allow pathogens to persist in the plant cell (Gupta *et al.* 2021). In rice varieties, 21 SWEET genes have been discovered (Hu *et al.* 2021), with OsSWEET11, OsSWEET13 and OsSWEET14 genes among the most targeted by *X. oryzae,* with OsSWEET11 providing broad-spectrum resistance. Furthermore, studies show the Xa7 anti-virulence effector binds to the OsSWEET14 promoter to enable expression (Antony *et al.* 2010), which suggests the possibility of implementing a mutational breeding method to deactivate this gene during periods of biotic stress to further reduce pathogenic development.

Further genes have been researched with the function of promoting cellular processes. For example, genotypes containing a mutated inactivated allele of the Bsr-k1 gene – a broad-spectrum resistance gene - have provided resistance to both rice blast and bacterial blight (Guo *et al.* 2021). This is due to the function of the Bsr-K1 gene as a regulator of mRNA turnover of the Os-PAL genes; used in lignin and salicylic acid production (Yang *et al.* 2022a). As a result, this study found that over-expression of the Os-PAL gene provides broad-spectrum resistance against a variety of diseases, by strengthening cellular defences (Zhou *et al.* 2018). Thus, identification of genes can improve gene expression of select R-genes to improve variety and gene expression among rice varieties.

**Challenges associated with the application of mutation breeding techniques in rice**

Although mutation breeding provides an effective means of inducing desired characteristics in rice varieties, numerous considerations and challenges impact its usefulness in agricultural industries. Despite substantial research into gene expression in plants, an incomplete understanding of plant responses, particularly to environmental conditions, restricts the development of effective resistance pathways in rice species (Miller *et al.* 2017). Mutation breeding is a time-consuming process, with the additional challenge of inducing non-specific changes that may yield only a fraction of useful mutations (Zakir 2018). Furthermore, mutational breeding requires extensive screening and breeding methods following mutation therapy, to secure mutants. Finally, mutagenesis of the organism is unpredictable and may have environmental consequences if managed incorrectly.

**Incomplete understanding of gene expression**

Methods to improve resistance in plant species due to biotic and abiotic stress are hindered due to an incomplete understanding of gene expression. An improved understanding of targeted plant defence pathways against pathogens could provide effective pathways toward resistance in crop species. Co-evolution between pathogens and plants has resulted in specialised pathways for targeted interactions, with pathogens producing virulent proteins to manipulate cellular processes and plant species counteracting invasion using anti-virulence factors and signalling molecules (Miller *et al.* 2017). This complex interplay of plant and pathogen interactions needs to be further understood for effective methods of resistance to be established to initiate a cascade of defence mechanisms. Similarly, plant responses and signalling mechanisms during drought-like conditions can be further studied to determine means of preserving normal function whilst increasing tolerance to harsher environmental conditions. Thus, increasing genetic diversity is important for disease and environmental management, providing opportunities for further development of species alongside changing environments (Hajjar *et al.* 2008).

**Disease resistance**

Disease resistance is vital among rice species to persist alongside pathogenic species such as *X. oryzae* and *M. oryzae*, responsible for bacterial blight and rice blast respectively. In the context of mutation breeding, a significant challenge is maintaining the quality and yield characteristics demanded while producing resistance to pathogens (Soares *et al.* 2019). Unintended outcomes may arise from the deactivation of a susceptibility gene that provides broad-spectrum resistance, yet in consequence, may impair other vital functions. For example, inhibiting certain sugar transporter genes, prevents fungal microbes from hijacking cellular metabolism processes, thus reducing pathogenic virulence in the host cell. In some instances, this has resulted in reduced plant yield, due to restricted metabolic and growth processes (Oliva *et al.* 2019).

Furthermore, point mutations conducted on the S gene, Pi-21 have produced non-specific resistance to rice blast, however, this has also resulted in poor consumption quality (Guo *et al.* 2021). As a result, this mutant variety is not suitable for agricultural use. Thus, the challenge in producing resistance arises from identifying and modifying resistance genes that are capable of preventing pathogenic invasion without the consequences of reduced growth, yield and quality. Only a select few R genes have exhibited very few or no side effects impacting plant development, indicating the need for additional trials and research to be conducted on the pathways for gene expression in rice varieties (Guo *et al.* 2021).

**Drought resistance**

Terminal drought is a lack or decrease in water availability for plants, which causes drought stress and when experienced for prolonged periods results in the death of the organism (Polania *et al.* 2017). Intermittent drought is not typically lethal and instead results in a lack of plant growth due to periodical rainfall and irrigation (Polania *et al.* 2017). Physiological and morphological adaptations in plants provide survival advantages against drought-like conditions, improving the organism's ability to persist in a changing environment (Bhandari *et al.* 2023). Physiological adaptations include a higher stomatal density and conductance, reduction in transpiration rates, better production, and biomass yield partitioning (Sahebi *et al.* 2018) whereas morphological adaptations referring to structural features in plants include increases in root thickness and length, leaf coverings, smaller epithelial cells and decreased weight and size ratio in leaves (Sahebi *et al.* 2018).

Xia *et al.* (2019) compared upland rice with lowland rice to compare the drought resistance. Upland rice is an adaptation in the rice that has been modified to improve drought resistance in drought-prone mountainous areas (Gupta and O'Toole 1986). Lowland rice has a lower risk of drought encounters with better quality and production. The study also showed upland rice having a high ratio of deep rooting (RDR), low water loss rate (RWL), high relative water content (RWC), high relative fecundity (RF) and high relative grain weight (RGW) (Xia *et al.* 2019). In comparison, the lowland rice had superior GDP, yield, and panicles.

VP64-TF fusion type is an activated form that characterises the features of upland rice as advantageous in comparison to lowland rice. Some of these features include a greater height, wide leaves, poor productivity, and fewer tillers (Zhang *et al.* 2009). Upland rice which does not contain standing water has genotypes that acquire the challenges of drought more than lowland rice (Zhang *et al.* 2009). Researchers have found that high latitudes present fewer variations, smaller populations and decreased genetic diversity (Khush 1997). Having a lower-quality rice variety causes complications in rice production as there is a lack of genetic diversity and adaptations. The upland rice lacks key genes outlined in the review, which result in the reduction of weight and height. This makes it a challenge as the rice production rate decreases causing a reduction in yield.

**Random and non-specific process**

In comparison to other genetic engineering techniques, the primary objective of mutational breeding methods is to improve diversity, with the possible added benefit of producing a new resistance allele. As a result, mutational breeding techniques can produce broad variations, which may not be suitable for exacting a specific genetic alteration (Kozjak and Meglič 2012). Mutationally induced disease resistance among plant varieties is challenging as it can unpredictably alter DNA. Another restriction is that most mutations are deleterious, which impair protein function. Therefore, these common mutations do not assist with producing characteristics favourable to the agricultural industry (Institute of Medicine and National Research Council 2004). Furthermore, mutational frequency is very low, ranging from $7.5\times10^{-6}$ to $9.8\times10^{-6}$ per locai -in dry seeds by γ radiation (Viana *et al.* 2019), of which only a select few mutations are beneficial. As a result, the unpredictable nature of mutation breeding can present challenges in developing resistant, high-quality rice varieties.

**Length of screening process and mutant stability**

Mutation breeding techniques involve subjecting a large volume of plant crops to mutagens, to increase the probability of an observed advantageous mutation (Tarakanov 2022). Consequently, this process requires extensive screening and breeding strategies to be successful, which may be challenging to establish and coordinate. Screening protocols for disease, salt and drought tolerance typically involve subjecting organisms to relevant environmental conditions to assess their resistance under controlled conditions (International Atomic Energy Agency 2016). This process can be time-consuming, as it requires the organism to initiate signalling pathways for gene expression, and monitoring for numerous weeks. Often mutation treatment is applied to rice seeds, thus requiring further time for propagation and development to occur (Jankowicz-Cieslak *et al.* 2017). Following the development of a successful mutant, further breeding processes are conducted to secure a mutant gene. Screening occurs in the second generation to determine whether the allele is a germline mutation. Selective breeding continues to occur for a total of seven generations, to secure the gene for large-scale agricultural use (Viana *et al.* 2019). As a result, screening and post-breeding processes are time-consuming, which hinders the immediate usefulness of mutation breeding.

**Biotechnology limitations**

Biotechnological approaches to mutation breeding carry additional financial and technical limitations which restrict the effectiveness with which genetic diversity can be re-established in rice species. Transgenic technology usage in agriculture is widely controversial, posing the concern of mutagenesis among consumers as well as ecological risks (Bawa and Anilakumar 2013). Furthermore, there has been little evidence of transgenic technology usage to broaden genetic diversity, rather to produce favourable ideotypes (Garland and Curry 2022). Regulatory issues have also discouraged the widespread use of GM crops (Pixley *et al.* 2022), thus posing additional limitations on its usage. Modern genome editing techniques, including the use of CRISPR/Cas9 complexes have become increasingly acceptable for use in the agricultural industry, by-passing

regulatory limitations and becoming welcomed by society particularly in its usage in improving nutritional value (Carroll and Charo 2015). However, in the context of rice breeding, such technologies are financially inaccessible to smallholders and rice farmers. As such, the procurement of these technologies should become more affordable for widespread usage. Thus, despite the impressive effectiveness of modern biotechnologies, the financial barrier of usage costs, as well as societal controversy and strict regulatory limitations, limit the applicability and large-scale efficacy of biotechnology use in rice breeding (Pixley *et al.* 2022).

**Mutagenesis and environmental considerations**

Mutations drive evolution and can result in advantageous changes favouring survival or disadvantageous ones that impact the organism's survival capabilities (Durland and Ahmadian-Moghadam 2022). Combinations of genes and alterations of the entire genome through mutation breeding techniques have the potential to introduce unexpected mutations, which in turn can result in decreased yield, economic loss and adverse health consequences in rice varieties (Institute of Medicine and National Research Council 2004).

Radiation mutation breeding is a common technique utilized in agriculture and has a significant impact on increasing genetic variation and overall crop yield in rice varieties. Radiation breeding techniques have shown significant promise, however there is still a lack of substantial research into the overall mechanism of action and the stability of mutagenic plants (Ma *et al.* 2021). To further understand the genomic alterations that occur in radiation breeding and understand the unpredictable nature of this, further research is critical in understanding the future of mutation breeding with radiation to limit the impact of negative mutagenesis (Ma *et al.* 2021).

Chemical mutation breeding is a widespread tool for the creation of mutations within plant genetic systems (Vivian *et al.* 2004). The introduction of chemical mutagens into plant genomes has been linked with base pairing mutations which leads to the formation of non-watson pairing of DNA (Dixit and Kumar 2018). Chemical mutagens such as nitrous acid and sodium azide can lead to deamination which modifies the regular base pairing in DNA (Dixit and Kumar 2018), although this is the aim in some cases it can also be an off-target impact of mutagenesis.

Intervention into plant systems can harm the biodiversity and dynamics of ecosystems when mutagenesis is employed. Mutationally altered varieties have the potential of transferring genetic material into genomes of other species which could cause a variety of unforeseen consequences (Champer *et al.* 2018).

## Conclusion

With increasing pressures on food security, mutation breeding methods to strengthen rice species become ever more critical. Mutation breeding as a practice to increase genetic variety, remains a highly beneficial method to increase the frequency of desired traits within a species. While it is shown to be less effective in producing a specific genomic alteration compared to other methods of genetic engineering, its benefits are observed in its ability to accelerate evolutionary processes and induce genome-wide mutations (Kozjak and Meglič 2012; Bado *et al.* 2015). This is particularly advantageous in contexts where there is limited understanding of particular gene sequences and their functions, as it does not require such data and instead can be used to experimentally observe changes in gene expression resulting from mutation (Tarakanov 2022). Despite lengthy screening process and need for further breeding strategies to stabilise the mutant, mutational breeding can induce mutations that would otherwise require an extensive time to arise naturally. Thus, mutation breeding provides a valuable means of attaining useful characteristics to better respond to rapidly changing climatic conditions and food insecurity.

Numerous challenges continue to impact the effectiveness and efficiency of mutational breeding, requiring further research and development. Directions for future research should aim to continue identifying relevant genes to improve crop quality and provide resistance against biotic and abiotic factors. This will contribute toward furthering scientific understanding of plant-pathogen interactions, as well as plant stress in various climatic conditions. In combination with other genetic engineering methods including transgenic engineering, new signalling and response pathways could be induced, to offer new means of resistance in plants (Vo *et al.* 2023). Further benefits of combining genomic methods could also result in faster screening and stabilisation processes for mutations. Gene mapping of mutant varieties can be used to identify regions of genetic variance, for comparison with databases such as MutMap (Fekih *et al.* 2013). Finally, gene stacking provides another means of increasing plant responses to environmental stress, particularly against diseases such as rice blast. This involves the use of genetic engineering to insert foreign genes into plant genomes, which are expressed in tandem with pre-existing resistance genes, upon activation of defence pathways (Das and Rao 2015).

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## Author Contributions

CR, LI, OH and EVW contributed equally to the work on conception and design, collection and assembly of research, analysis and interpretation of research, preparation and

proofread the manuscript. AB prepared and proofread the manuscript. APKL involved in the conception and design, preparation, editing and proofread of the manuscript.

## Conflicts of Interest

The authors declare that there is no conflict of interests.

## Data Availability

Not applicable

## Ethics Approval

Not applicable

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