Rice agronomic traits and variability induced by mutagenesis

Alejandro Hernandez Soto (alhernandez@itcr.ac.cr)
Instituto Tecnologico de Costa Rica: Tecnologico de Costa Rica

Fabián Echeverría-Beirute
Instituto Tecnologico de Costa Rica: Tecnologico de Costa Rica

Ana Abdelnour-Esquivel
Instituto Tecnologico de Costa Rica: Tecnologico de Costa Rica

Andres Gatica-Arias
Universidad de Costa Rica Escuela de Biología: Universidad de Costa Rica Escuela de Biología

Marta Valdez-Melara
Universidad de Costa Rica Escuela de Biología: Universidad de Costa Rica Escuela de Biología

Research Article

Keywords: Mutagenesis, domestication, CRISPR, gamma radiation, chemical mutagen, biotic stress, abiotic stress

DOI: https://doi.org/10.21203/rs.3.rs-513976/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Understanding agronomic traits at a genetic level enables the leveraging of this knowledge to produce crops that are more productive and resilient, have better quality and are adjusted for consumer preferences. In the last decade, rice has become a model to validate the function of specific genes, resulting in valuable but scattered information. Here, we aimed to identify particular genes in rice related to traits that can be targeted by different mutation techniques in the breeding of crops. We selected gain of function, misfunction, and specific mutations associated with phenotypes of agronomic interest. The review includes specific trait-related genes involved in domestication, stress, herbicide tolerance, pathogen resistance, grain number, quality, weight, plant structure, nitrogen use, and others. The information presented can be used for rice and crops with similar or homologous genes to breed crops that require improvement to achieve more sustainable production in challenging farming conditions.

Introduction

Induced mutagenesis is a valuable tool to support functional genomics studies and the development of new genotypes. Rice serves as an outstanding model not only because of its impact on the worldwide food supply chain but also because of the availability of technological resources to utilize. Rice was the first crop sequenced in 2004 (Matsumoto et al., 2005), biotechnological techniques are available, and the genomic information is available to search for specific target mutations, such as from the Rice Genome Annotation Project and Oryza Genome which can contribute to the precise engineering of the crop (Kawahara et al., 2013; Tanaka et al., 2020; Kajiya-Kanegae et al., 2021). Biological, chemical, and physical agents can induce mutagenesis, such as radiation, first used on vegetables in 1928; ethyl methanesulfonate (EMS), which produces 2-10 mutations per Mb; and specific mutations constructed with new breeding and genetic engineering techniques (Soriano, 1961; Serrat et al., 2014; Romero and Gatica-Arias, 2019; Viana et al., 2019; Yang et al., 2019a). In this review, we present rice traits that have emerged or been validated in the last decade (2010-2021) derived from technological advances in genomics (Benavente and Giménez, 2021). This paper is focused on characteristics that could be targeted by mutagenesis of rice lines and relatives to produce predictable variability in gains or losses of function.

Methods

The methodology applied a consistent search based on PubMed articles based on keywords: rice, traits, stress tolerance, resistance, breeding; selection of papers with agronomic traits linked to specific genes described within 2010-2021. Finally, verification of each gene, trait, and mutation was performed using specialized web servers such as Gramene, EnsemblPlants, Rice Diversity, FunRiceGenes, Rice Genome Annotation Project, Oryza Base, and Rice Information GateWay. The search resulted in a selection of 117 papers out of 500.

| Webserver                        | Link                                                                 |
|----------------------------------|----------------------------------------------------------------------|
| Gramene                          | https://ensembl.gramene.org/genome_browser/index.html                |
| EnsemblPlants                    | http://plants.ensembl.org/index.html                                |
| Rice Diversity                   | http://www.ricediversity.org/data/index.cfm                         |
| FunRiceGenes                     | http://funricegenes.ncpgr.cn/                                       |
| Rice Genome Annotation Project, Michigan State University | http://rice.plantbiology.msu.edu                                    |
| Oryza Base                       | https://shigen.nig.ac.jp/rice/oryzabase/                             |
| Rice Information GateWay         | http://rice.hzau.edu.cn/                                             |

Important For Breeding Rice

Rice, such as many other tropical crops, is susceptible to a large set of biotic (fungi, bacteria, nematodes, insects, and viruses) and abiotic (salinity, drought, heat, and cold) stresses that cause yield and economic losses (Fig. 1). In general, biotic stress cause losses worldwide up to 35% of the total food production (Bainsla and Meena, 2016). As an example, losses in rice due to insects can account for over 40%. Moreover, losses caused by Magnaporthe grisea, Thanatephorus cucumeris, and C. miyabeanus have been estimated worldwide at 35%, 24%, and 16%, respectively (Oerke and Dehne, 2004). On the other hand, abiotic stress represents the primary cause of crop losses worldwide, and yield losses can be as high as 50% of crop production (Ashraf et al., 2008).

In this sense, the generation of rice-resistant varieties to biotic and abiotic conditions represents one of the challenges that breeders face. For decades, breeding strategies include selection, hybridization, mutation induction using chemical and physical agents, and somaclonal variation. More recently, the availability of genome editing technologies, genome sequences, efficient tissue culture, and transformation methodologies could remarkably facilitate the breeding of rice (Fig. 2).

Rice Breeding Systems

Several methods are available for breeding rice with natural or induced mutagenesis; among them, we can mention mutation breeding, tissue culture, and new breeding techniques (CRISPR, base editing, and prime editing) (Fig. 2).

Mutation breeding
The mutation breeding principle is to generate heritable changes in the DNA by external agents. The changes result by exposing plant cells to physical (UV, X-ray, gamma radiation) or chemical (sodium azide and ethyl methanesulfonate) agents (Mba et al., 2010). Induced mutagenesis offers a promising alternative for developing rice varieties resistant to biotic and abiotic stresses since it could accelerate the spontaneous mutation process and increase the pool of genes available for genetic improvement (Gressel and Levy, 2006; Oladosu et al., 2016; Viana et al., 2019).

Tissue culture

Totipotency, a distinguishable characteristic of plant cells, allows each cell to regenerate an entire plant in principle. This process involves the culture on special growth media of tissue fragments or individual cells from a plant enabling the cells to grow and further division (Fehér, 2019). In this sense, tissue culture approaches are helpful to develop biotic or abiotic stress-tolerant plants. Among the techniques available, somaclonal variation enables changes in the DNA causing genetic and phenotypic variation among clonally propagated plants. The somaclonal variants obtained could be detected using in vitro selection by applying selective pressure in culture conditions (Larkin and Scowcroft, 1981; Bairu et al., 2011).

New breeding techniques

CRISPR/Cas9

The clustered regularly interspaced short palindromic repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) system targets a specific genomic sequence using an engineered 20 base pair (bp) RNA guide sequence that binds to its DNA and the Cas9, from S. pyogenes, recognizes the PAM sequence 5¢-NGG-3¢ generating double-stranded breaks in specific genes at desired locations in the genome. This genome editing method allows the insertion, deletion, or modification of DNA with increased specificity and efficiency (Romero and Gatica-Arias, 2019).

CRISPR/Cpf1 system

The nuclease Cas12a requires a small crRNA for inducing double strand breaks with efficiencies similar to those of CRISPR/Cas9. Moreover, this nuclease uses a 22 nt spacer for its maximum efficiency and specificity and identifies a T-rich PAMs located upstream of the guide and generated staggered ends (Schindele et al., 2018).

Base editing

This system allows the conversion of nucleotides without inducing double-stranded DNA breaks or using donor templates. In this sense, it has been used for changing a C-G base pair into T-A, or A-T into G-C (Marx, 2018).

Prime editing

This system uses a catalytically impaired Cas9 endonuclease fused to a reverse transcriptase enzyme, and a prime editing guide RNA (pegRNA). This complex is capable of identifying the target site and replace the target DNA nucleotides without double-stranded DNA breaks or using donor templates (Anzalone et al., 2019; Lin et al., 2020).

Agronomic Traits Of Interest

1. Domestication genes

The Oryza genus is composed of species with a variety of genome structures, including six diploids (n = 12; named AA, BB, CC, ee, ff, gg) and five polyploids (n = 24, named BBCC, CCDD, HHJJ, HHKK, and KKLL) (Kim et al., 2015; Nadir et al., 2017; Wing et al., 2018; Chen et al., 2019). Only two diploid (2n=24) species of rice have been domesticated and used for cultivation: Oryza sativa and African O. glaberrima. Rice domestication emerged because of the selection of specific genes and the loss of function of specific genes. Wild relatives have functional versions of genes such as sh4, waxy, BH4, GW2, aS1, AN1, brown pericarp, PROG1, and OsG1, as described in the following text. The sh4 gene is related to reduced seed shattering (Os04g0670900). The waxy gene controls the amylose content (Os06g0133000). BH4 is related to the hull color of the seeds (Os04g0460200). The gene qSH1 is involved in seed shattering (Os01g0848400). The AN1 gene is related to seeds, morphology, and grain shape (Os04g0350700). RC Brown pericarp is involved in the seed coat (Os07g0211500). PROG1 is related to an erect plant structure (Os07g0135600). OsLG1 is related to a closed-panicle structure (Os04g0565600) (Li et al., 2017). The importance of such genes is critical in understanding how de novo domestication and their further use in plant breeding can be achieved from wild Oryza varieties.

This concept was demonstrated even in polyploid O. alta (CCDD) by (Yu et al., 2021a), targeting S01, GS3, IPA1, Ghd7, Gna1a, Wx, Bb4, TAC1, An-1 homologs, as well as African landraces of Oryza glaberrima by disrupting the HTD1 (O. sativa Os04g0550600), GS3 (O. sativa Os03g0407400), GW2 (O. sativa Os01g0197700) and GN1A (O. sativa Os02g0244100) genes (Lacchini et al., 2020). In terms of plant breeding, having access to nondomesticated, more genetically diverse rice species that better adapt to stress conditions, such as African landraces O. glaberrima, O. barthii, O. meridionalis (AA), Australian landraces O. longistaminata (AA), O. australiensis (EE), and Asian landraces O. rufipogon (AA) or Porteresia coarctata (O.coarctata) (KKLL), enables the potential of developing more sustainable rice crops (Reddy et al., 2017; Mammadov et al., 2018).

2. Stress tolerance

Rice susceptibility to salt is evidenced by a yield decrease due to delays in heading and panicle sterility specially in salt-sensitive varieties like M48. In contrast, salt tolerance varieties like IR29, Fatmawati, Pokkali, Chervirippu, FL478, IR651, CSR27, FL30, Fontan, SR86, IR9884-54-3 results from ion exclusion, osmotic and tissue tolerance with multiple genes involved in the process, which confers agronomic stability (Takagi et al., 2015; Reddy et al., 2017; Zhang et
al., 2019a; Qin et al., 2020). The orchestrated stress system is a perfect target for achieving salt tolerance by knocking out key transcription factors, specifically OsRR22 (Os06g0183100), STL1 (Os04g0110600), and zinc finger transcription factor DST (Os03g0786400) (Cui et al., 2015; Zhang et al., 2019a; Liu et al., 2020b; Santosh Kumar et al., 2020; Yuan et al., 2020). Other transcription factors are critical in stress adaptation, which results in stress sensitivity when knocked out. This is the case for MSL37 (Os11g0163500), PSCS (Os05g0455500), which produces proline accumulation out of external ABA application, the transcription factor SNAC2 (Os01g0884300), which is key in root adaptation, and OsNAP (Os03g0327800), which triggers a stress response mediated by ABA (Takasaki et al., 2010; Sripinyowanich et al., 2013; Chen et al., 2014; Lee et al., 2017; Núñez-Muñoz et al., 2021). For details, check Figure 3 and Table 1 below.

Osmoprotection by accumulating molecules such as trehalose is a possible pathway involved in salt tolerance, as proven currently in Arabidopsis (Li et al., 2011b; Núñez-Muñoz et al., 2021). Other individual genes could be of interest, such as the Na+ transporter SKC1 (Os01g0307500) with a V395 that provides salt tolerance (Jayabalani et al., 2019). Knocking out an independent but closely related gene, OsEFL9 (Os01g0824500), results in increased water use efficiency under stress because of the reduced stomatal count(Yin et al., 2017, 2019).

Other stress tolerance pathways have been shown to be effective. Low cadmium accumulation occurs after knocking out the metal transporter genes OsNtamp5 (Os07g0257200) and OsNtamp1 (Os07g0258400); the plants are able to resist heat stress when the gene OsNtL3 (Os01g0261200) is working correctly; tolerance results from knockout of the OsMYB30 (Os02g0624300) gene and more cuticle wax is deposited when the gene DHS (Os02g0682300) is knocked out (Sasaki et al., 2012; Tang et al., 2017; Wang et al., 2018a; Chang et al., 2020; Liu et al., 2020a; Zeng et al., 2020).

### 3. Herbicide resistance monogenic traits

Rice is usually cultivated under two agronomical systems: paddy transplanted rice (PTR) and dry seeded rice (DSR). The first is the conventional method, which requires water flooding and represents a sustainability issue because of water scarcity, methane production and the consumption of nonrenewable energy (Wang et al., 2017). DSR, on the other hand, represents opportunities for efficient water and nitrogen use, and a reduction of both greenhouse gas emissions and labor demand, especially in countries such as China, where 90% of rice is currently produced under PTR (Shekhawat et al., 2020). However, weed management is a challenge in DRS, specifically during the first 41 days after sowing (DAS). Another complication is weedy rice (O. sativa f. spontanea), which can result in yield losses of up to 50% (Nadir et al., 2017). Weedy rice usually involves increased seed longevity, seed shattering and stress tolerance (Durand-Morat et al., 2018). The use of chemical control represents a tool to manage weedy rice, but there are still challenges as described below.

The Herbicide Resistance Action Committee (HRAC) and the Weed Science Society of America (WSSA) classify herbicides into 34 groups and one unknown group based on their 'mode of action' (MoA) at the biochemical level (Forouzesh et al., 2015; Dayan et al., 2019; Gaines et al., 2020). The discovery of a new mode of action has been rare in the last 30 years. A good example is leptospermone, and its analog inhibitors act as hydroxyphenylpyruvate inhibitors of dihydrophenylpyruvate (DPP) (Dayan and Duke, 2020). Different modes of herbicide action, such as rotations, delay the emergence of herbicide-resistant weeds. However, weeds are evolving to resist multiple MoA types of herbicides. For example, Chloris radiata is found in Colombian rice fields with dual resistance to glyphosate (mode of action 9) and the acetolactate synthase (ALS) inhibitor imazamox (mode of action 2) (Hoyos et al., 2021). Herbicide resistance in the USA resulted in 5.7 million tons lost and $457 million in environmental costs between 2002-2014 (Bzour et al., 2018). Mutations to provide herbicide tolerance were introduced into rice 20 years ago based on the Acetohydroxy acid synthase AHAS/ALS (Os02g0510200) gene mutation, providing tolerance to the mode of action 2 (Li et al., 2019). Rice herbicide tolerance varieties are used in the USA (700,000 Ha), Brazil (600,000 Ha), Uruguay (700,000 Ha), Argentina (32,000 Ha), Malaysia (95,000 Ha), and Italy (60,000 Ha), as well as in many Central America countries, such as Costa Rica, Honduras, Panama, and the Dominican Republic (Singh et al., 2017). The incorrect use of this variety allowed introgression and outcrossing of the resistance into red rice, which means that weed herbicide control requires stricter farming practices, such as rotation (Liu et al., 2021). Alternatives such as aryloxypino propionate-resistant rice (mode of action 1), which is the result of mutations in the ACCase2 (Os5g0295300) gene, already exist and will allow for herbicide rotation (de Andrade et al., 2018; Camacho et al., 2019).

According to the literature, at least five target genes have the potential to develop herbicide-resistant rice varieties with a different mode of action. Two of those have already been described previously: ACCase2 on aryloxypino propionates (MoA-1) and AHAS/ALS on ALS (MoA-2). The first ACCase2 includes mutations such as i1781L, S1866F, i1879V, A1884P, W2027C, W2125S, D2176G, and C2186R/P1927F/G2201A/W2125C at exon 32 that provides tolerance at a different rate. The second AHAS/ALS results in ALS MoA-2 resistance when carrying the following mutations: A96V/A122T/P171H/P171S/P197S/C287T and W548L/W574L/W574L/S627I/S653I/G654E. The third OsTuA2 (Os11g0247300) with a mutation in the fourth exon, M268T, providing tolerance to diuron-tolines (MoA-3). Another gene, HPPD (Os02g0280700), provides tolerance to triketones when there is a natural insertion site (GGACACAAAAGAATTAGACAGATATCA) in the fourth exon. Finally, the double mutation known as "TIPS" (T102H/P106S) in the OsEPSPS (Os02g0510200) gene provides tolerance to MoA-9. For details, check Figure 4 and Table 2 below.

Herbicide-resistant weeds to the inhibition of photosynthesis at PSIIL can also provide insights for rice models. The S264G mutation in psbA increases tolerance more than 50-fold in triazine herbicide-tolerant radish (MoA-5). However, it can also compromise fitness because of less efficient photosynthesis (Lu et al., 2019). Other mutations, such as Val219Ile, Asn266Thr, Phe255Ile, and Ala251Val, can also provide tolerance (Gaines et al., 2020). It is important to note that the psbA mutation Val-219-Ile provides tolerance to the amide propanil (MoA-5) on Cyperus difformis (Pedroso et al., 2016). Propanil is widely used in rice cultivation because the crop is naturally capable of degrading the molecule by a putative enzyme located in the mitochondria, and an additional resistance pathway could increase its efficiency (Matsunaka, 1967; Chen and Matsunaka, 1990). The described mutations could also result in herbicide tolerance in rice when targeting the homologous gene AAS4617, encoding protein P0C434, to address an additional MoA.

Rice is also known to be resistant to Bentazon (MoA-6), as it is degraded by cytochrome P450 CYP87A6 (Pan et al., 2006). Additionally, the P450 gene CYP72A31 is responsible for conferring tolerance to bispyribac sodium (BS) in Oryza sativa indica, while its absence in japonica rice varieties results in BS-sensitive varieties (Zhang et al., 2002; Saika et al., 2014).
4. Bacteria, fungi and virus resistance

Rice breeding of pathogen resistance is possible by knockout of the Sweet 14,1,1,13 genes named Os11g05088600, Os08g0535200, Os12g0476200, respectively, since they act as a point of access for pathogens causing bacterial blight streaks (BLs), such as Xanthomonas oryzae pv. oryzae (Xoo), and they reduce copper in the xylem (Jiang et al., 2013; Oliva et al., 2019; Varshney et al., 2019). The pathogen emerges by breaking the resistance pressure of varieties planted in approximately 80% of the total crop cultivation area carrying the resistance gene Xa4 on chromosome 11 introduced in the 60s in the variety IR20 (Quibod et al., 2020). Xanthomonas oryzae can also infect wild grasses and become an emergent microorganism that is difficult to control (Lang et al., 2019).

Another outstanding gene to target is the transcription factor IPA1 (Os08g0509600); higher expression levels of IPA1 result in increased yield and immunity when tested against Magnaporthe oryzae. Resistance relies on time- and pathogen-specific phosphorylated activation of the transcription factor at Ser163 and subsequent WRKY45 promoter-resistant gene triggering within 48 hours after infection, while the yield of the nonphosphorylated protein binds to the DEP1 promoter (Jing Wang et al., 2018). A different way to achieve M. oryzae resistance is by knocking out OsERF922 ethylene response factor 922 (Os01g0752500) (Wang et al., 2016). For details, see Table 3 below.

5. Grain number, quality, weight and plant structure

Rice quality traits are essential to achieve a better yield, consumer preference, and growth efficiency. The genes involved in grain number and size, plant density, structure, panicles, and flowering are complex because of their interactions. However, new findings and key mutations provide some insights into their regulatory mechanisms and greater predictability in achieving the desired phenotype, as described next (for details, see Figure 5 and Table 4 below).

Grain size. The GS3 Grain Size3 gene (Os03g0407400) is responsible for negatively controlling the grain length. Its mutation can result in better or worse weight and size that correlates with the composition of its domains: organ size regulation (OSR), a transmembrane necrosis factor receptor/nerve growth factor receptor (TNFR/NFGR), and a von Willebrand factor type C (VWFc) (Takano-Kai et al., 2013; Li et al., 2016; Shen et al., 2017; Yang et al., 2019b; Zeng et al., 2020). The wild type allele contains all of the domains and results in medium grains (Takano-Kai et al., 2013). Loss of function results in long-grain varieties; for example, Minghui 63 has a stop mutation C165A at the second exon, resulting in a loss of function and a long-grain phenotype. In contrast, a mutation or deletion in the fifth exon creates a truncated protein with no VWFc domain and a short seed phenotype (Mao et al., 2010; Takano-Kai et al., 2013). A directed mutation that knocks out the gene results in a larger grain size (Li et al., 2016; Zeng et al., 2020). Size, in general, is also controlled by several genes: higher expression of GW6a (Os06g0650300), and knockout of GW5 (Os05g0187500), GW6 (Os06g0623700), and GW5L (Os01g0190500) results in increased grain size (Shimaru et al., 2013; Song et al., 2015; Tian et al., 2019; Zhang et al., 2020; Ayaad et al., 2021).

Grain number. Malfunction of the gene Os01g01977000 (GNT1a) produces an increment of grain per panicle number and flowering because of a lower degradation of cytokines produced by the corresponding cytokinin oxidation enzyme (Li et al., 2016; Shen et al., 2017; Huang et al., 2018). Another gene that correlates with increased production and downregulates cytokine level regulation is EP3 Erect Panicle 3 (Os02g0260200) (Li et al., 2011a; Shen et al., 2017).

Grain starch. Another essential trait in starch is quality, which depends on the right mixture of amylase and protein. The global starch content relies on the gene ISA1 (Os08g0520900) and the protein content relies on NAC20-26 (Os01g0104500, Os01g0393100) (Shufen et al., 2019; Wang et al., 2020). The waxy gene WX1 (Os06g0133000) controls the grain amylase content (AC). Mutations in this gene correlate with a phenotype that ranges from opaque (8%), semitranslucent (9-12%), and transparent (12% or more) grains (Sano, 1984; Yunyan et al., 2019; Zhang et al., 2019b; Huang et al., 2020; Xu et al., 2021).

Flowering. Flowering and photoperiodic insensitivity results from overexpression of OsMeCP (Os12g06204000 (Qu et al., 2021)) or by knocking out several genes. For example, Se5, Hd2 and Hd1 (Andrés et al., 2009; Gao et al., 2014; Shen et al., 2017; Tanaka et al., 2020). Another critical regulator of heading date and grain weight seems to be HOW, and its homozygous null mutant is embryonic lethal (Li et al., 2012).

Structure. Farmers prefer smaller plants with many panicles and fewer tillering traits. Knockout of the DEP1 (Os09g0441900) gene, as well as the loss of function of the HTD1 (Os04g0550600) gene coming from landraces produces short, dense, erect panicles (Zou et al., 2006; Li et al., 2016; Lacchini et al., 2020).

The transcription factor IPA1 Ideal Plant Architecture1 (Os08g0509600), specific mutations between bases 854 and 876 can increase the expression of transcription factor proteins because they interrupt OsMrR156 transcript cleavage. For example, C874A in the third exon (leucine to isoleucine) generates a rice plant with a reduced tiller number, increased lodging resistance, and an enhanced grain yield (Li et al., 2016; Wang et al., 2018b).

The number of panicles and consequently the yield can be increased by knocking out or indirectly blocking Pin1A and Pin1Gb. The indirect mechanism results in higher expression of DEP1 and LPAT, which interact to suppress PIN1a expression (Huang et al., 2018; Fu et al., 2019; Miao Liu et al., 2020). LPAT is also important in the erect phenotype, and its knockout results in lamina inclination, while BAST seems to be important in stomata closing (Liu et al., 2016; Mao et al., 2018).

6. Other traits

Other rice traits provide value for breeding and for satisfying consumer preferences, such as nitrogen use, fragrancy, oleic acid content, and color. Regarding nitrogen, there is a better efficiency with a higher expression of the nitrate transporter OsNPF6. 7 and the two transcription factors OsNAC42 and OsNLAP4 (Tang et al., 2019; Yu et al., 2021b). Knockout of the FAD2 gene results in an oleic acid increment (Tiwar et al., 2016; Abe et al., 2018). Furthermore, a mutation in the Osor (Os02g0651300) gene results in potential orange-colored rice (Endo et al., 2019), and fragrancy can be increased or decreased by modulating the BAGH2 gene, which prevents the formation of the aromatic compound 2AP (2-acetyl-1-pyrroline) (Shen et al., 2017). For details, check Table 5 below.
Conclusion

Induced mutations targeting specific genes associated with known phenotypes, as described in this review, will allow for advances in more precise rice breeding to improve the varieties that farmers are already using. It can also result in new varieties and de novo domestication from wild relatives and extrapolate the results to other crops with homologous traits. Farmers urgently require advances in this knowledge to respond to the challenges of climate change, consumer demands, water scarcity, nitrogen usage, and sustainable production.

List Of Abbreviations

Ethyl methanesulfonate (EMS)

Puddled Transplanted Rice (PTR)

Dry Seeded Rice (DSR)

Days After Sowing (DAS)

The Herbicide Resistance Action Committee (HRAC)

Weed Science Society of America (WSSA)

Mode of Action (MoA)

Bacterial Blight Streak (BLS)

Amylose Content (AC)

Acetolactate Synthase (ALS)

NUE (nitrogen use efficiency).

Declarations

Ethics approval and consent to participate ‘Not applicable’

Consent for publication ‘Not applicable’

Availability of data and material ‘Not applicable’

Competing interests "The authors declare that they have no competing interests”

Funding This paper was funded by the Project 1510-1022. Research Vice-Rectory of TEC, Costa Rica, and is part of the Doctoral Thesis of the first author, Doctorado en Ciencia Naturales para el Desarrollo (DOCINADE), Instituto Tecnológico de Costa Rica, Universidad Nacional, Universidad Estatal a Distancia, Cartago, Costa Rica.

Authors’ contributions A.H.-S conceived the paper, designed and coordinated the inputs, analyzed the data, and wrote the manuscript; F.E.-B reviewed, discussed the content and edited the paper; A. A-E discussed the results and edited the paper; A.G.-A. wrote, reviewed, discussed the results and edited the paper; M.V.-M. discussed the results and edited the paper. All authors read and approved the final manuscript.

Acknowledgments

Not applicable.

References

Abe, K., E. Araki, Y. Suzuki, S. Toki, and H. Saika. 2018. Production of high oleic/low linoleic rice by genome editing. Plant Physiology and Biochemistry 131(April): 58–62. doi: 10.1016/j.plaphy.2018.04.033.

de Andrade, A., A. Tulmann-Neto, F.A. Tcacenco, R. Marschalek, A. Pereira, et al. 2018. Development of rice (Oryza sativa) lines resistant to aryloxyphenoxypropionate herbicides through induced mutation with gamma rays. Plant Breeding 137(3): 364–369. doi: 10.1111/pbr.12592.

Andrés, F., D.W. Galbraith, M. Talón, and C. Domingo. 2009. Analysis of Photoperiod Sensitivity5 sheds light on the role of phytochromes in photoperiodic flowering in rice. Plant Physiology 151(2): 681–690. doi: 10.1104/pp.109.139097.

Anzalone, A. v, P.B. Randolph, J.R. Davis, A.A. Sousa, L.W. Koblan, et al. 2019. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. doi: 10.1038/s41586-019-1711-4.
Ashraf, M., H.R. Athar, P.J.C. Harris, and T.R. Kwon. 2008. Some Prospective Strategies for Improving Crop Salt Tolerance. Advances in Agronomy 97(07): 45–110. doi: 10.1016/S0065-2113(07)00002-8.

Ayaad, M., Z. Han, K. Zheng, G. Hu, M. Abo-Yousef, et al. 2021. Bin-based genome-wide association studies reveal superior alleles for improvement of appearance quality using a 4-way MAGIC population in rice. Journal of Advanced Research 28: 183–194. doi: 10.1016/j.jare.2020.08.001.

Bainsla, N.K., and H.P. Meena. 2016. Breeding for Resistance to Biotic Stresses (J. v Yadav P, Kumar S, editor). Daya Publishing House, New Delhi.

Bairu, M.W., A.O. Aremu, and J. van Staden. 2011. Somaclonal variation in plants: Causes and detection methods. Plant Growth Regulation 63(2): 147–173. doi: 10.1007/s10725-010-9554-x.

Benavente, E., and E. Giménez. 2021. Modern Approaches for the Genetic Improvement of Rice, Wheat and Maize for Abiotic Constraints-Related Traits: A Comparative Overview. Agronomy 11(2): 376. doi: 10.3390/agronomy11020376.

Bzour, M.I., F.M. Zuki, and M.S. Mispan. 2018. Introduction of imidazolinone herbicide and Clearfield® rice between weedy rice-control eciency and environmental concerns.

Camacho, J.R., S.D. Linscombe, Y. Sanabria, P.A. Mosquera, and J.H. Oard. 2019. Inheritance of Provisia™ rice resistance to quizalofop-p-ethyl under laboratory and greenhouse environments. Euphytica 215(4). doi: 10.1007/s10681-019-2407-4.

Chang, J.D., Y. Yamaji, S. Zhang, J.F. Ma, et al. 2020. OsNRAMP1 transporter contributes to cadmium and manganese uptake in rice. Plant Cell and Environment 43(10): 2476–2491. doi: 10.1111/pce.13843.

Chen, J.J., and S. Matsunaka. 1990. The propanil hydrolyzing enzyme aryl acylamidase in the wild rices of genus Oryza. Pesticide Biochemistry and Physiology 38(1): 26–33. doi: 10.1016/0048-3575(90)90144-Q.

Chen, X., Y. Wang, B. Lv, L. Luo, et al. 2014. The NAC family transcription factor OsNAP confers abiotic stress response through the ABA pathway. Plant and Cell Physiology 55(3): 604–619. doi: 10.1093/pcp/pct204.

Cui, L.G., J.X. Shan, M. Shi, J.P. Gao, and H.X. Lin. 2015. DCA1 Acts as a Transcriptional Co-activator of DST and Contributes to Drought and Salt Tolerance in Rice. PLoS Genetics 11(10): 1–22. doi: 10.1371/journal.pgen.1005617.

Dayan, F.E., A. Barker, R. Bough, M. Ortiz, H. Takano, et al. 2019. Herbicide mechanisms of action and resistance. Third Edit. Elsevier.

Dayan, F.E., and S.O. Duke. 2020. Discovery for New Herbicide Sites of Action by Quantication of Plant Primary Metabolite and Enzyme Pools. Engineering 6(5): 509–514. doi: 10.1016/j.eng.2020.03.004.

Durand-Morat, A., L.L. Nalley, and G. Thoma. 2018. The implications of red rice on food security. Global Food Security 18(April): 62–75. doi: 10.1016/j.gfs.2018.08.004.

Endo, A., H. Saika, M. Takemura, N. Misawa, and S. Toki. 2019. A novel approach to carotenoid accumulation in rice callus by mimicking the cauliflower Orange mutation via genome editing. Rice 12(1): 1–5. doi: 10.1186/s12284-019-0345-3.

Fehér, A. 2019. Callus, dedifferentiation, totipotency, somatic embryogenesis: What these terms mean in the era of molecular plant biology? Frontiers in Plant Science 10. doi: 10.3389/fpls.2019.00536.

Forouzesh, A., E. Zand, S. Souzadeh, and S. Samadi Foroushani. 2015. Classification of herbicides according to chemical family for weed resistance management strategies-an update. Weed Research 55(4): 334–358. doi: 10.1111/wre.12153.

Fu, X., J. Xu, M. Zhou, M. Chen, L. Shen, et al. 2019. Enhanced expression of QTL qLL9/DEP1 facilitates the improvement of leaf morphology and grain yield in rice. International Journal of Molecular Sciences 20(4). doi: 10.3390/ijms20040866.

Gaines, T.A., S.O. Duke, S. Morran, C.A.G. Rigon, P.J. Tranel, et al. 2020. Mechanisms of evolved herbicide resistance. Journal of Biological Chemistry 295(30): 10307–10330. doi: 10.1074/jbc.REV120.013572.

Gao, H., M. Jin, X.M. Zheng, J. Chen, D. Yuan, et al. 2014. Days to heading 7, a major quantitative locus determining photoperiod sensitivity and regional adaptation in rice. Proceedings of the National Academy of Sciences of the United States of America 111(51): 18399–18400. doi: 10.1073/pnas.1422341112.

Gressel, J., and A.A. Levy. 2006. Agriculture: The selector of improbable mutations. Proceedings of the National Academy of Sciences of the United States of America 103(33): 12215–12216. doi: 10.1073/pnas.0603666103.

Hoyos, V., G. Plaza, J.G. Vázquez-Garcia, C. Palma-Bautista, A.M. Rojano-Delgado, et al. 2021. Confirmation of Multiple Resistant Chloris radiata Population, Harvested in Colombian Rice Fields. Agronomy 11(3): 496. doi: 10.3390/agronomy11030496.

Huang, L., N. Sreenivasulu, and Q. Liu. 2020. Waxy Editing: Old Meets New. Trends in Plant Science 25(10): 963–966. doi: 10.1016/j.tplants.2020.07.009.
Lu, H., Q. Yu, H. Han, M.J. Owen, and S.B. Powles. 2019. A novel psbA mutation (Phe274–Val) confers resistance to PSII herbicides in wild radish (Raphanus raphanistrum). Pest Management Science 75(1): 144–151. doi: 10.1002/ps.5079.

Mammadov, J., R. Buyyarakpu, S.K. Guttikonda, K. Parliament, I.Y. Abdurakhmonov, et al. 2018. Wild Relatives of Maize, Rice, Cotton, and Soybean: Treasure Troves for Tolerance to Biotic and Abiotic Stresses. Frontiers in Plant Science 9(June). doi: 10.3389/fpls.2018.00886.

Mao, H., S. Sun, J. Yao, C. Wang, S. Yu, et al. 2010. Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. Proceedings of the National Academy of Sciences of the United States of America 107(45): 19579–19584. doi: 10.1073/pnas.1014419107.

Mao, X., Y. Zheng, K. Xiao, Y. Wei, Y. Zhu, et al. 2018. OsPRX2 contributes to stomatal closure and improves potassium deficiency tolerance in rice. Biochemical and Biophysical Research Communications 495(1): 461–467. doi: 10.1016/j.bbrc.2017.11.045.

Mao, X., Y. Zheng, K. Xiao, Y. Wei, Y. Zhu, et al. 2018. OsPRX2 contributes to stomatal closure and improves potassium deficiency tolerance in rice. Biochemical and Biophysical Research Communications 495(1): 461–467. doi: 10.1016/j.bbrc.2017.11.045.

Mao, H., S. Sun, J. Yao, C. Wang, S. Yu, et al. 2010. Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. Proceedings of the National Academy of Sciences of the United States of America 107(45): 19579–19584. doi: 10.1073/pnas.1014419107.

Mao, X., Y. Zheng, K. Xiao, Y. Wei, Y. Zhu, et al. 2018. OsPRX2 contributes to stomatal closure and improves potassium deficiency tolerance in rice. Biochemical and Biophysical Research Communications 495(1): 461–467. doi: 10.1016/j.bbrc.2017.11.045.

Marx, V. 2018. Base editing a CRISPR way. Nature Methods 15(10): 767–770. doi: 10.1038/s41592-018-0146-4.

Matsunaka, S. 1967. Propanil Hydrolysis: Inhibition in Rice Plants by Insecticides. Science 160(3834): 1360–1361. doi: 10.1126/science.160.3834.1360.

Mba, C., R. Afza, S. Bado, and S.M. Jain. 2010. Induced Mutagenesis in Plants Using Physical and Chemical Agents. Plant Cell Culture: Essential Methods (March): 111–130. doi: 10.1002/9780470686522.ch7.

Miao Liu, J., Q. Mei, C. Yun Xue, Z. Yuan Wang, D. Pin Li, et al. 2020. Mutation of G-protein γ subunit DEP1 increases planting density and resistance to sheath blight disease in rice. Plant Biotechnology Journal: 0–2. doi: 10.1111/pbi.13500.

Nadır, S., H.B. Xiong, Q. Zhu, X.L. Zhang, H.Y. Xu, et al. 2017. Weedy rice in sustainable rice production. A review. Agronomy for Sustainable Development 37(5). doi: 10.1007/s13593-017-0456-4.

Nuñez-Muñoz, L., B. Vargas-Hernández, J. Hinojosa-Moya, R. Ruiz-Medrano, and B. Xoconostle-Cázares. 2021. Plant drought tolerance provided through genome editing of the trehalase gene. Plant Signaling and Behavior 16(4). doi: 10.1080/15592324.2021.1877005.

Oerke, E.C., and H.W. Dehne. 2004. Safeguarding production - Losses in major crops and the role of crop protection. Crop Protection 23(4): 275–285. doi: 10.1016/j.cropro.2003.10.001.

Oladosu, Y., M.Y. Rafii, N. Abdulláh, G. Hussin, A. Ramli, et al. 2016. Principle and application of plant mutagenesis in crop improvement: A review. Biotechnology and Biotechnological Equipment 30(1): 1–16. doi: 10.1080/13102818.2015.1087333.

Oliva, R., C. Ji, G. Atienza-Grande, J.C. Huguet-Tapia, A. Perez-Quintero, et al. 2019. Broad-spectrum resistance to bacterial blight in rice using genome editing. Nature Biotechnology 37(11): 1344–1350. doi: 10.1038/s41587-019-0267-z.

Pan, G., X. Zhang, K. Liu, J. Zhang, X. Wu, et al. 2006. Map-based cloning of a novel rice cytochrome P450 gene CYP81A6 that confers resistance to two different classes of herbicides. Plant Molecular Biology 61(6): 933–943. doi: 10.1007/s11103-006-0058-z.

Pedroso, R.M., K. Al-Khatib, R. Alarcón-Reverte, and A.J. Fischer. 2016. A psbA mutation (Val219 to Ile) causes resistance to propanil and increased susceptibility to bentazon in Cyperus difformis. Pest Management Science 72(9): 1673–1680. doi: 10.1002/ps.4267.

Qin, H., Y. Li, and R. Huang. 2020. Advances and challenges in the breeding of salt-tolerant rice. International Journal of Molecular Sciences 21(21): 1–15. doi: 10.3390/ijms21218385.

Qu, M., Z. Zhang, T. Liang, P. Niu, M. Wu, et al. 2021. Overexpression of a methyl-CpG-binding protein gene OsMBD707 leads to larger tiller angles and reduced photoperiod sensitivity in rice. BMC Plant Biology 21(1): 1–14. doi: 10.1186/s12870-021-02880-3.

Quibod, I.L., G. Atieza-Grande, E.G. Oreiro, D. Palmos, M.H. Nguyen, et al. 2020. The Green Revolution shaped the population structure of the rice pathogen Xanthomonas oryzae pv. oryzae. ISME Journal 14(2): 492–505. doi: 10.1038/s41396-019-0545-2.

Reddy, I.N.B.L., B.K. Kim, I.S. Yoon, K.H. Kim, and T.R. Kwon. 2017. Salt Tolerance in Rice: Focus on Mechanisms and Approaches. Rice Science 24(3): 123–144. doi: 10.1016/j.rsci.2016.09.004.

Romero, F.M., and A. Gatica-Arias. 2019. CRISPR/Cas9: Development and Application in Rice Breeding. Rice Science 26(5): 265–281. doi: 10.1016/j.rsci.2019.08.001.

Saika, H., J. Horita, F. Taguchi-Shiobara, S. Nonaka, A. Nishizawa-Yokoi, et al. 2014. A novel rice cytochrome P450 gene, CYP72A31, confers tolerance to acetylacetate synthase-inhibiting herbicides in rice and arabidopsis. Plant Physiology 166(3): 1232–1240. doi: 10.1104/pp.113.231266.

Sano, Y. 1984. Differential regulation of waxy gene expression in rice endosperm. Theoretical and Applied Genetics 68(5): 467–473. doi: 10.1007/BF00254822.
Santosh Kumar, V. v., R.K. Verma, S.K. Yadav, P. Yadav, A. Watts, et al. 2020. CRISPR-Cas9 mediated genome editing of drought and salt tolerance (OsDST) gene in indica mega rice cultivar MTU1010. Physiology and Molecular Biology of Plants 26(6): 1099–1110. doi: 10.1007/s12298-020-00819-w.

Sasaki, A., N. Yamaji, K. Yokoshio, and J.F. Ma. 2012. Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. Plant Cell 24(5): 2155–2167. doi: 10.1105/tocr.112.096925.

Schindele, P., F. Wolter, and H. Puchta. 2018. Transforming plant biology and breeding with CRISPR/Cas9, Cas12 and Cas13. FEBS Letters 592(12): 1954–1967. doi: 10.1002/1873-3468.13073.

Serrat, X., R. Esteban, N. Guibourt, L. Moysset, S. Nogués, et al. 2014. EMS mutagenesis in mature seed-derived rice calli as a new method for rapidly obtaining TILLING mutant populations. Plant Methods 10(1). doi: 10.1186/s11147-017-9008-8.

Shekhawat, K., S.S. Rathore, and B.S. Chauhan. 2020. Weed management in dry direct-seeded rice: A review on challenges and opportunities for sustainable rice production. Agronomy 10(9): 1–19. doi: 10.3390/agronomy10091264.

Shen, L., Y. Hua, Y. Fu, J. Li, Q. Liu, et al. 2017. Rapid generation of genetic diversity by multiplex CRISPR/Cas9 genome editing in rice. Science China Life Sciences 60(5): 506–515. doi: 10.1007/s11427-017-9008-8.

Shufen, C., C. Yicong, F. Baobing, J. Guiai, S. Zhonghua, et al. 2019. Editing of Rice Isoamylase Gene ISA1 Provides Insights into Its Function in Starch Formation. Rice Science 26(2): 77–87. doi: 10.1016/j.rsci.2018.07.001.

Shekhawat, K., S.S. Rathore, and B.S. Chauhan. 2020. Weed management in dry direct-seeded rice: A review on challenges and opportunities for sustainable rice production. Agronomy 10(9): 1–19. doi: 10.3390/agronomy10091264.

Shukla, S., S. Jain, S.K. Yadav, P. Yadav, A. Watts, et al. 2020. CRISPR-Cas9 mediated genome editing of drought and salt tolerance (OsDST) gene in indica mega rice cultivar MTU1010. Physiology and Molecular Biology of Plants 26(6): 1099–1110. doi: 10.1007/s12298-020-00819-w.

Sasaki, A., N. Yamaji, K. Yokoshio, and J.F. Ma. 2012. Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. Plant Cell 24(5): 2155–2167. doi: 10.1105/tocr.112.096925.

Schindele, P., F. Wolter, and H. Puchta. 2018. Transforming plant biology and breeding with CRISPR/Cas9, Cas12 and Cas13. FEBS Letters 592(12): 1954–1967. doi: 10.1002/1873-3468.13073.

Serrat, X., R. Esteban, N. Guibourt, L. Moysset, S. Nogués, et al. 2014. EMS mutagenesis in mature seed-derived rice calli as a new method for rapidly obtaining TILLING mutant populations. Plant Methods 10(1). doi: 10.1186/s11147-017-9008-8.

Shekhawat, K., S.S. Rathore, and B.S. Chauhan. 2020. Weed management in dry direct-seeded rice: A review on challenges and opportunities for sustainable rice production. Agronomy 10(9): 1–19. doi: 10.3390/agronomy10091264.

Shen, L., Y. Hua, Y. Fu, J. Li, Q. Liu, et al. 2017. Rapid generation of genetic diversity by multiplex CRISPR/Cas9 genome editing in rice. Science China Life Sciences 60(5): 506–515. doi: 10.1007/s11427-017-9008-8.

Scaffold, C., C. Yicong, F. Baobing, J. Guiai, S. Zhonghua, et al. 2019. Editing of Rice Isoamylase Gene ISA1 Provides Insights into Its Function in Starch Formation. Rice Science 26(2): 77–87. doi: 10.1016/j.rsci.2018.07.001.

Shukla, S., S. Jain, S.K. Yadav, P. Yadav, A. Watts, et al. 2020. CRISPR-Cas9 mediated genome editing of drought and salt tolerance (OsDST) gene in indica mega rice cultivar MTU1010. Physiology and Molecular Biology of Plants 26(6): 1099–1110. doi: 10.1007/s12298-020-00819-w.

Sasaki, A., N. Yamaji, K. Yokoshio, and J.F. Ma. 2012. Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. Plant Cell 24(5): 2155–2167. doi: 10.1105/tocr.112.096925.

Schindele, P., F. Wolter, and H. Puchta. 2018. Transforming plant biology and breeding with CRISPR/Cas9, Cas12 and Cas13. FEBS Letters 592(12): 1954–1967. doi: 10.1002/1873-3468.13073.

Serrat, X., R. Esteban, N. Guibourt, L. Moysset, S. Nogués, et al. 2014. EMS mutagenesis in mature seed-derived rice calli as a new method for rapidly obtaining TILLING mutant populations. Plant Methods 10(1). doi: 10.1186/s11147-017-9008-8.

Shekhawat, K., S.S. Rathore, and B.S. Chauhan. 2020. Weed management in dry direct-seeded rice: A review on challenges and opportunities for sustainable rice production. Agronomy 10(9): 1–19. doi: 10.3390/agronomy10091264.

Shen, L., Y. Hua, Y. Fu, J. Li, Q. Liu, et al. 2017. Rapid generation of genetic diversity by multiplex CRISPR/Cas9 genome editing in rice. Science China Life Sciences 60(5): 506–515. doi: 10.1007/s11427-017-9008-8.

Scaffold, C., C. Yicong, F. Baobing, J. Guiai, S. Zhonghua, et al. 2019. Editing of Rice Isoamylase Gene ISA1 Provides Insights into Its Function in Starch Formation. Rice Science 26(2): 77–87. doi: 10.1016/j.rsci.2018.07.001.

Shukla, S., S. Jain, S.K. Yadav, P. Yadav, A. Watts, et al. 2020. CRISPR-Cas9 mediated genome editing of drought and salt tolerance (OsDST) gene in indica mega rice cultivar MTU1010. Physiology and Molecular Biology of Plants 26(6): 1099–1110. doi: 10.1007/s12298-020-00819-w.

Sasaki, A., N. Yamaji, K. Yokoshio, and J.F. Ma. 2012. Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. Plant Cell 24(5): 2155–2167. doi: 10.1105/tocr.112.096925.

Schindele, P., F. Wolter, and H. Puchta. 2018. Transforming plant biology and breeding with CRISPR/Cas9, Cas12 and Cas13. FEBS Letters 592(12): 1954–1967. doi: 10.1002/1873-3468.13073.

Serrat, X., R. Esteban, N. Guibourt, L. Moysset, S. Nogués, et al. 2014. EMS mutagenesis in mature seed-derived rice calli as a new method for rapidly obtaining TILLING mutant populations. Plant Methods 10(1). doi: 10.1186/s11147-017-9008-8.
Wang, W., S. Peng, H. Liu, Y. Tao, J. Huang, et al. 2017. The possibility of replacing puddled transplanted flooded rice with dry seeded rice in central China: A review. Field Crops Research 214(August): 310–320. doi: 10.1016/j.fcr.2017.09.028.

Wang, Z., X. Tian, Q. Zhao, Z. Liu, X. Li, et al. 2018a. The E3 ligase drought hypersensitive negatively regulates cuticular wax biosynthesis by promoting the degradation of transcription factor ROC4 in rice. Plant Cell 30(1): 228–244. doi: 10.1105/tpc.17.00823.

Wang, F., C. Wang, P. Liu, C. Lei, W. Hao, et al. 2016. Enhanced rice blast resistance by CRISPR/ Cas9-Targeted mutagenesis of the ERF transcription factor gene OsERF922. PLoS ONE 11(4): 1–18. doi: 10.1371/journal.pone.0154027.

Wang, J., L. Zhou, H. Shi, M. Chen, H. Yu, et al. 2018b. A single transcription factor promotes both yield and immunity in rice. Science 361(6406): 1026–1028. doi: 10.1126/science.aat7675.

Wing, R.A., M.D. Purugganan, and Q. Zhang. 2018. The rice genome revolution: From an ancient grain to Green Super Rice. Nature Reviews Genetics 19(8): 505–517. doi: 10.1038/s41576-018-0024-z.

Xu, Y., Q. Lin, X. Li, F. Wang, Z. Chen, et al. 2021. Fine-tuning the amylose content of rice by precise base editing of the Wx gene. Plant Biotechnology Journal 19(1): 11–13. doi: 10.1111/pbi.13433.

Zeng, Y., J. Wen, W. Zhao, Q. Wang, and W. Huang. 2020. Rational Improvement of Rice Yield and Cold Tolerance by Editing the Three Genes OsPIN5b, GS3, and OsMYB30 With the CRISPR–Cas9 System. Frontiers in Plant Science 10(January): 1–13. doi: 10.3389/fpls.2019.01663.

Table 1. Rice genes and mutations involved in stress tolerance or sensitivity traits.
| Gene         | Position | Protein        | Obtained mutation | Method                  | Trait details                                                                 | Reference                                      |
|-------------|----------|----------------|-------------------|-------------------------|--------------------------------------------------------------------------------|-----------------------------------------------|
| OsRR22      | Chr 6    | Q5SML5         | Knockout          | CRISPR/Cas9             | Two-component response regulator ORR22. Salt tolerance 0.75% NaCl.             | (Zhang et al., 2019a)                         |
| Os06g0183100|          |                |                   |                         |                                                                                 |                                               |
| STL1        | Chr 4    | Q7XXF2         | SNP               | None                    | hap1 tolerance 0.9% salt, the gene is the homolog of Arabidopsis salt tolerance gene SRP1. Knock-out mutation in the srp1 allele reduced sensitivity to ABA and salt stress. | (Yuan et al., 2020)                           |
| Os04g0110600|          |                |                   |                         |                                                                                 |                                               |
| Salt tolerance Level 1, Stress repressive zinc finger protein 4 |          |                |                   |                         |                                                                                 |                                               |
| MSL.37      | Chr 11   | Q53PP7         | Natural variability-Knockout | Spontaneous mutation-Knockout-CRISPR/Cas9 | Knock-out results in salt sensitivity.                                        | (Liu et al., 2020d)                           |
| Os11g0163500|          |                |                   |                         |                                                                                 |                                               |
| Os03g0786400 OsDST, DLN102, OsDLN102, Negative regulation of response to salt stress | Chr 3    | Q10CE2          | Knockdown Mutant/CRISPR/Cas9 | Knockdown improved the tolerance to stress, as also observed in the dst mutant. C2H2 zinc finger transcription factor, drought and salt tolerance, stomatal aperture control | (Cui et al., 2015; Santosh Kumar et al., 2020) |
| P5C         | Chr 5    | O04226         | Natural: cultivar LPT123 is salt-susceptible versus salt-tolerant line LPT123-TG171 | None | The enzyme increases the proline accumulation and salt resistance mediated by ABA application. | (Sripinyowanich et al., 2013)                 |
| Os05g0455500|          |                |                   |                         |                                                                                 |                                               |
| SKC1        | Chr 1    | Q0JNB6         | Wild relatives    | None                    | Variant V395 (is salt tolerant), while L395 is sensitive.                       | (Jayabal et al., 2019)                        |
| Os01g0307500|          |                |                   |                         |                                                                                 |                                               |
| OsHKT1,5, OsHKT8 |          |                |                   |                         |                                                                                 |                                               |
| Os10g0521000| Chr 10   | Q9FWC1         | Substitution S163T | CRISPR/Cas9             | Mutation of domain WDS to replicate Selaginella moellendorfii WDT. The enzyme may be less efficient in allowing the accumulation of trehalose. | (Nuñez-Muñoz et al., 2021)                    |
| Based on Z mays GRMZM2G162690 and A. thaliana AT4G24040 |          |                |                   |                         |                                                                                 |                                               |
| OsEPFL9     | Chr 1    | Q5JN76         | Knockout          | CRISPR/Cpf1             | Increased water use efficiency under stress because of reduced stomatal count | (Yin et al., 2017, 2019)                      |
| Os01g0824500|          |                |                   |                         |                                                                                 |                                               |
| Drought hypersensitive |          |                |                   |                         |                                                                                 |                                               |
| DHS         | Chr 2    | Q6EU38         | Knockout-Overexpression | CRISPR/Cas9-gene transfer | Knockout results in more cuticular wax. Overexpression (DHS OE) plantlets grew more slowly. The enzyme is a ubiquitin that degrades ROC4 that positively regulates cuticular wax biosynthesis | (Wang et al., 2018a)                          |
| Os02g0682300|          |                |                   |                         |                                                                                 |                                               |
| RCS1        | Chr 12   | Q9XE6A         | S189N             | EMS                     | Tolerates 20 µM As (III). The mutation increases As tolerance/decreased accumulation in the grain/increase Se accumulation in the grain. | (Sun et al., 2021)                            |
| Os12g0625000|          |                |                   |                         |                                                                                 |                                               |
| O-acetylserine (thiol) lyase, Cysteine synthase, arsenite tolerant 1 |          |                |                   |                         |                                                                                 |                                               |
| OsNramp5    | Chr 7    | Q8H4H5         | Knockout          | CRISPR/Cas9             | Low Cd accumulation                                                            | (Sasaki et al., 2012; Tang et al., 2017; Chang et al., 2020) |
| Os07g0257200|          |                |                   |                         |                                                                                 |                                               |
| Gene        | Chromosome | ID   | Mutagenesis   | Method          | Function                                                                                   | Source                        |
|------------|------------|------|---------------|-----------------|--------------------------------------------------------------------------------------------|-------------------------------|
| OsNram1    | Chr 7      | Q0D7E4 | Knockout      | CRISPR/Cas9     | Low Cd accumulation. It works as a plasma membrane-localized transporter/uptake for Mn and Cd; it is complementary to OsNRAMP5 in the uptake of Mn and Cd. | (Chang et al., 2020)          |
| Os07g0258400 | OsNTL3     | Chr 1 | Q7GCL7        | Natural variability- | OsNTL3 is required for heat stress tolerance in rice. Loss-of-function mutation of OsNTL3 confers heat sensitivity. It regulates the expression of genes involved in ER protein folding. | (Liu et al., 2020a)          |
| OsNTL3     | Chr 2      | Q6K1S6 | Knockout      | CRISPR/Cas9     | The protein OsMYB30 is a nuclear protein that acts as a negative regulator of cold tolerance. Mutant shows increased cold tolerance. | (Zeng et al., 2020)          |

Table 2. Rice genes and mutations in herbicide resistance traits.
| Gene (*) | Position | Protein | Obtained mutation | Method | Trait details | References |
|----------|----------|---------|-------------------|--------|---------------|------------|
| OsTubA2  | Chr 11   | Q53M51  | M268T             | CRISPR/Cas9-Base editor | In vitro trifuralin 4 mg/L, pendimethaline 6.6 mg/L | L. Liu et al., 2021 |

**ACCase2**

**Os05g0295300**

| Chr 5 | B9FK36 | W2027C | I1879V/W212SS | Tissue culture mutation | CRISPR/Cas9-Base editor | Haloxyfop-R-methyl, 1 and 2 μM in vitro. | (Liu et al., 2020b; c) |
|-------|--------|--------|---------------|-------------------------|-------------------------|---------------------------------|-----------------------------|
|       |        |        |               | I1781L                  | CRISPR-Prime Editing    | Herbicide resistance            | (Xu et al., 2020) |
|       |        |        |               | C2186R                  | CRISPR-Base editor      | Herbicide resistance            | (Li et al., 2018; Liu et al., 2020c) |
|       |        |        |               | P1927F, W2125C, S1866F and A1884P | CRISPR-Base editor | Herbicide resistance 34g/Ha. High tolerance 
|       |        |        |               |                         |                         | P1927F, W2125C versus low tolerance S1866F and A1884P | (Li et al., 2020b; Liu et al., 2020c) |

**psbA AAS46167**

(Photosystem II protein D1, psbA)

| Chloroplast | P0C434 | S264G | Wild radish, Spontaneous mutation | Atrazine > 50-fold (4000(187 g a.i. ha-1 atrazine), (S) Bromoxynil | (Lu et al., 2019) |

**HPPD**

**Os02g0280700** Inhibitor Sensitive 1

| Chr 2 | Fe(II)/2-oxoglutarate–dependent oxygenase | 28-bp deletion allele (his1). wild Nipponbare lacked deletion (HIS1) | b-Triketone herbicides, HIS1 detoxifies b-triketone herbicides by hydroxylation. | (Maeda et al., 2019) |

**AHAS, ALS**

**Os02g0510200** Acetohydroxy acid synthase

| Chr 2 | Q6K2E8 | W548L P171S | A96V (C287T) | Chemical mutation | Clearfield 121, Clearfield 141 IRGA422 | (Singh et al., 2017; Bzour et al., 2018) |
|-------|--------|-------------|-------------|-----------------|----------------------------------|---------------------------------|
|       |        | W548 | G654E | Chemical mutation | Clearfield 121 | Clearfield 141 IRGA422 | (Singh et al., 2017; Bzour et al., 2018) |
|       |        | E549 | S653N | Chemical mutation | Named CL161 and CLXL8 increased herbicide tolerance | (Singh et al., 2017) |
|       |        |       | W548L or P171S | Recombinant protein | Herbicide tolerance | (Kawai et al., 2008) |
|       |        |       | W548 | CRISPR-Prime Editing | Herbicide tolerance | (Xu et al., 2020) |
|       |        |       | A122T | Sodium Azide | IMINTA1, IMINTA4 | (Sagare et al., 2020) |
|       |        |       | W548L S627I | CRISPR | Herbicide tolerance | (Sun et al., 2016) |

**Arabidopsis Modeling**

| Arabidopsis | P171H/W548L | W574L P197S S653I | P171H/W548L | Recombinant | 100 mM IQ 100 mM CS/BM/IQ/IP/PS 100 mM BM 100 mM IP | (Kawai et al., 2008) |
|-------------|-------------|------------------|-------------|-------------|-------------------------------------------------|-----------------------------|

**OsEPSPS**

| Chr 6 | A0A0N7KLH2 | T169I | CRISPR-Prime | NA | (Li et al., 2018) |

Page 14/23
(S) = Susceptible, genomic

(*) Additional information at The Rice Annotation Project (RAP). (Jiang et al., 2013; Oliva et al., 2019; Varshney et al., 2019).

CS, chlorsulfuron; BM, bensulfuron-methyl; IQ, imazaquin; IP, imazapyr; PM, pyriminobac; PS, pyrithiobac-sodium; BS, bispyribac-sodium.

**Table 3.** Rice genes and mutations with pathogen-resistant traits.

| Gene                                              | Position | Protein | Obtained Mutation | Method      | Trait details                                                                 | Reference                                                                 |
|---------------------------------------------------|----------|---------|-------------------|-------------|-------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Os11g0508600 Sweet 14                              | Chr 11   | Q2R3P9  | Knockout          | CRISPR-Cas9 | *Xanthomonas oryzae* pv. *Oryzae* resistance, probably by avoiding sugar access for the pathogen growth, or by not reducing copper in the xylem | (Jiang et al., 2013; Oliva et al., 2019; Varshney et al., 2019)            |
| Os08g0535200 Sweet11                               | Chr 8    | Q6YZF3  | Knockout          | CRISPR-Cas9 | *Xanthomonas oryzae* pv. *Oryzae* resistance, probably by avoiding sugar access for the pathogen growth, or by not reducing copper in the xylem | (Oliva et al., 2019; Varshney et al., 2019)                                |
| Os12g0476200 Sweet13                               | Chr 12   | Q2QR07  | Knockout          | CRISPR-Cas9 | *Xanthomonas oryzae* pv. *Oryzae* resistance, probably by avoiding sugar access for the pathogen growth, or by not reducing copper in the xylem | (Oliva et al., 2019; Varshney et al., 2019)                                |
| Os07g0555200 translation initiation factor 4 gamma gene (elf4G) | Chr 7    | B9FXV5  | Knockout and mutations on SVLFPNLAGKS | CRISPR-Cas9 | Resistance to rice tungro spherical virus (RTSV)                                 | (Macovei et al., 2018)                                                  |
| Os01g0752500, ethylene response factor 922 OsERF922, LOC_Os01g54890.1 | Chr 1    | Q5JMX7  | Knockout          | CRISPR      | *Magnaporthe oryzae*, Blast resistance                                         | (Wang et al., 2016)                                                     |

**Table 4.** Rice genes and mutations involved in grain quality, quantity, weight, and plant structural traits.
| Gene     | Position | Protein         | Obtained mutation                  | Method                  | Trait details                                                                                                                                                                                                 | Reference                                      |
|----------|----------|-----------------|------------------------------------|-------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|
| **OsDEP1** | Chr 2    | Q67UU9          | Mutation, promoter                 | Spontaneous mutation - CRISPR/Cas9 | More expression, yield increase 15%. The interaction between DEP1 and LPA1 suppresses PIn1a expression, leading to an increase in planting density. The panicle number per plant was the main contributor to the increase in grains per rice plant in the DEP1 mutants. | (Huang et al., 2018; Fu et al., 2019; Miao Liu et al., 2020) |
| **Gn1a**  | Chr 1    | Q4ADV8          | Knockout                           | CRISPR/Cas9             | Catalyzes the oxidation of cytokinin, enhanced the grain yield by increasing the grain number per panicle. Twice flowering relative to the wild type.                                                            | (Li et al., 2016a; Shen et al., 2017; Huang et al., 2018) |
| **GS3**   | Chr 3    | C6L686          | Knockout                           | CRISPR/Cas9 - Spontaneous mutation | $\delta$ subunit of G protein. Regulator of grain size and organ size. Produces a longer grain length. Knockout and deletions produce short seeds, such as 320 bp and 13 bp deletions in the fifth exon of GS3 that occurred in a japonica-like ancestor. The 4 bp and 1 + 3 bp deletions occurred in an indica-like ancestor. Farmers and early breeders imposed artificial selection favoring short seeds. | (Takano-Kai et al., 2013; Shen et al., 2017; Yang et al., 2019b) |
| **IPA1**  | Chr 8    | Q7EXZ2          | Knockout                           | CRISPR/Cas9             | Squamosa promoter-binding-like protein 14. Specific mutations between bases 854 to 876 result in more protein and produce less tillering, more grains and a higher frequency of seed set. It reduces unproductive tillers and increases the number of grains per panicle, while higher IPA1 levels enhance immunity. | (Li et al., 2016a; Wang et al., 2018b) |
| **WX1**   | Chr 6    | Q0DEV5          | Knockout, mutations                | CRISPR/Cas9 P124F, R125W T178I, T178S, R158H, Y191H, R158H, G159A, DT61N, G159K, G159A, G159E, V160F, S415P | Modulate the synthesis of amylose in the endosperm. Amylose contents change the appearance of the rice endosperm >12% results in transparent endosperm (6–12%)/or opaque (<8%). Favorable rice palatability usually requires low to intermediate AC (10–20%). The null wax results in an absence of amylose, resulting in starch granules with 100% amylopectin production, referred to as waxy or glutenous starch. S415P changes phosphorylation, resulting in moderate enzyme activity and a content of amylose. | (Sano, 1984; Yunyan et al., 2019; Zhang et al., 2019b; Xu et al., 2021) |
| **ISA1**  | Chr 8    | D0TZF0          | Knockout                           | CRISPR/Cas9             | Decreased endosperm contents of total starch, amylose and amylopectin. Increased soluble sugar content and starch gel consistency.                                                                            | (Shufen et al., 1999) |
| **OsNAC20** | Chr 1    | Q9FTY0 (OsNAC20) | Knockout                           | CRISPR/Cas9             | Double knockout osnac20/26 displayed a floury grain caused by decreased starch and storage protein content. Both proteins transactivate the expression of SSI, Pul, GluA1, GluB4/5, α-globulin and 16 kD prolamin and indirectly influence DPE1 expression to regulate starch and storage protein synthesis. | (Wang et al., 2020) |
| **GW5**   | Chr 5    | Q7SKY5          | Knockout                           | Spontaneous mutation    | GW5 could function as a key regulator to coordinate the performance of the other grain size genes. gw5 contributes to an increased grain width and weight. Positive regulator of brassinosteroid signaling. | (Zhang et al., 2020) |
| **GW5L**  | Chr 1    | B8ADP5          | Knockout                           | Spontaneous mutation    | Knockout results in shorter and wider grains. Overexpression could confer salt stress resistance through an association with calmodulin protein OsCaM1-1.                                                            | (Tian et al., 2019) |
| **GW6a**  | Chr 6    | Q67UR2          | Over expression                    | Spontaneous mutation    | Histone H4 acetyltransferase, regulation of grain weight, yield, and plant biomass. Elevated OsglHAT1                                                                                                                                                      | (Song et al., 2015; |
| **OsglHAT1**, Grain weight on chromosome 6 | expression enhances the grain weight and yield. Increases global acetylation levels of histone H4. (Ayaad et al., 2021) |
| **GW6** | Loss of function | Spontaneous mutation | Loss of function of the Kasalath allele enhances the grain weight through pleiotropic effects on source organs and leads to significant yield increases. Encodes a protein with indole-3-acetic acid (IAA)-glucose hydrolase activity. (Ishimaru et al., 2013) |
| **OsPIN5b** | Knockout | CRISPR | Increased panicle length in the mutant. (Zeng et al., 2020) |
| **Hd1/SE1** | Knockout | CRISPR-Cas9/Spontaneous mutation | Zinc finger protein, Heading date. Under long day conditions suppresses HD3A/FT expression, causing the suppression of flowering. (Shen et al., 2017; Tanaka et al., 2020) |
| **HTD1** | Loss of function | Spontaneous mutation | Landraces contain HTD1, while domesticated rice have htd1. The defect in HTD1 is responsible for heading date early/low photosensitivity. Plants can be planted at any time of year (Gao et al., 2014) |
| **LPA1** | Overexpression/ Knockout | Spontaneous mutation | Plant architecture. Related to lamina inclination by suppressing auxin signaling. LPA1 is an active transcriptional repressor. Negatively controls the tiller and lamina joint angle in an expression level-dependent manner. LPA1 overexpressors contain higher levels of IAA, increases planting density and resistance to sheath blight disease via activation of PIN-FORMED 1a. Exaggerated lamina angles observed in knockout mutants (lpa1). Ipa1 mutants might exhibit less efficient auxin flux. (Liu et al., 2016a) |
| **OsMeCP** | Overexpression/ RNAi/ CRISPR Knockout | CRISPR/Cas9 knockout, Gene transfer overexpression and RNAi | Overexpression of OsMBD707 results in larger tiller angles and reduced photoperiod sensitivity. (Qu et al., 2021) |
| **Hd2** | 2-8bp deletion in Hd2 | Hap_3 and Hap_6 mutants | Early flowering/low photosensitivity. Plants can be planted at any time of year (Gao et al., 2014) |
| **Ep3** | Mutation (knockout, recessive) | 60Co Irradiated japonica cultivar Zhonghua 11, CRISPR/Cas9 knockout | Increased panicle size. Mutants modulate cytokinin level in plant tissues by down regulating cytokinin oxidase/dehydrogenase (Li et al., 2011a; Shen et al., 2017) |
| **Se5** | Gamma rays | s73 mutant | Identified in a gamma-irradiated Bahia collection, displays early flowering and photoperiodic insensitivity due to a null mutation. (Andrés et al., 2009) |
| **BAS1** | Knockout/ overexpression gene transfer | CRISPR/Cas9 gene transfer | Overexpression causes stomatal closing and increased K+ deficiency tolerance. Knockout results in defects in the leaves and the stomata openings. The protein is localized in the chloroplast, reducing hydrogen peroxide and organic hydroperoxides to water and alcohols. (Mao et al., 2018) |
| **HGW** | Natural | Spontaneous mutation | Is a key regulator of heading date and grain weight. Encodes a protein with a UBA domain. Homozygous null mutant is embryonic lethal. (Li et al., 2012) |
Table 5. Rice genes and mutations in traits such as oleic acid, color, fragrancy, and nitrogen use.

| Gene      | Position | Protein                  | Obtained Mutation | Method           | Trait details                                           |
|-----------|----------|--------------------------|-------------------|------------------|--------------------------------------------------------|
| FAD2      | Chr 2    | Q6ZGW6                   | Knockout          | CRISPR/Cas9-RNAi | Increased oleic acid (twice) and decreased linoleic acid content. |
| Os02g0716500 fatty acid desaturase 2 |          |                          |                   |                  |                                                        |
| Osor      | Chr 2    | Q6H3Y3                   | Knockout          | CRISPR/Cas9      | β-carotene accumulation resulting in orange-colored calli. |
| Os02g0651300 |        |                          |                   |                  |                                                        |
| BADH2     | Chr 8    | A0A0P0XG36               | Knockout          | CRISPR/Cas9      | Betaine aldehyde dehydrogenase 2, prevents the formation of 2-acetyl-1-pyrro which gives fragrant rice its aromatic properties. Change in fragrance. |
| Os08g0424500 |        |                          |                   |                  |                                                        |
| OsNPF6.1  | Chr 1    | Q9FTZ3                   | HapB, 160 Gly to Asp and two additional CACG motifs at the promoter -0.5Kb and -1Kb | Natural, validation with CRISPR/CAS9 Knockout-Gene transfer | Nitrate transporter OsNPF6.1 is more efficient and has increased expression. |
| Os01g0103100 Nitrate transporter |          |                          |                   |                  |                                                        |
| OsNAC42   | Chr 9    | Q0J0L8                   | Natural-Knockout  | Natural, validation with CRISPR/CAS9 Knockout-lost-of-function SNP mutation (Pro51 changed to Leu, P51L) | Transcription factor OsNAC42 related to the expression of the nitrate transport Loss of function decreased expression of nitrate transporter OsNPF6.1 |
| Os09g0493700 NUE (nitrogen use efficiency)-related transcription factor |          |                          |                   |                  |                                                        |
| OsNLP4    | Chr 9    | A0A0P0XQL5               | Natural           | Natural, HapB distributed in South China, India and South-East Asia 131T (UTR), 181T (UTR), 614A, 842T, 2889C, 4662T(UTR), 4674T(UTR), 4888C (UTR) | The gene is upregulated by nitrogen starvation. OsNLP4 binds to the NRE motif and promotes the expression of OsNr that encodes a critical nitrite nitrogen assimilation. |
| Os09g0549450 transcriptional factor, Promotion of nitrogen use efficiency (NUE) |          |                          |                   |                  |                                                        |
Figure 1

Representation of biotic and abiotic stress factors that affect rice production. Created with BioRender.com
Figure 2

Schematic representation of different systems used for breeding rice: natural variability, mutation breeding, tissue culture mutation, and new breeding techniques. Created with BioRender.com
Figure 3

Representation of salt tolerance traits mediated by three different methods: 1) overexpression, 2) knockout of specific genes, and 3) particular sodium channels. Note that the first corresponds to transcription factors that trigger adaptive responses labeled MSL37, NAC2, NAP, and P5CS. The second is a knockout of those that result in salt sensitivity: OsRR2, STL1, DST; and the sodium channel SKC1 in rice. The third is the sodium channel SKC1 containing amino acid V395. Created with BioRender.com.
Figure 4

Representation of five rice genes and the corresponding mutation that results in herbicide tolerance. The genes are shown organized by their Mode of Action (MoA). Note the name of the gene in orange circles, the exons in blue filled boxes and the corresponding untranslated exon regions in the blue empty boxes. Created with BioRender.com.
Figure 5

Representation of traits such as grain number, quality, weight and plant structure and gene relationships in rice. Note that heading and flowering are positively influenced by Se5, Hd2, and Hd1 knockout; structure by DEP1, HTD1, IPA1, LPA1, Pin1a, and Pin15b; grain size by Gn1a, and Ep3; grain size by GS3, GW6a, GW5, and GW5L; and grain starch by ISA1, NAC20-26, and WX1. Created with BioRender.com.