Rice breeding in the new era: comparison of useful agronomic traits

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Rice breeding in the new era: comparison of useful agronomic traits

Here we compiled agronomic traits useful for New Breeding Techniques and conventional mutation of specific genes associated with a known phenotype in rice. We believe that the reviewed information is essential for advancing in precise breeding, de novo domestication, and breeding crops with homolog rice traits. The review includes specific trait-related genes involved in domestication, stress, herbicide tolerance, pathogen resistance, grain number, quality, weight, plant structure, nitrogen use.

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1 **Rice breeding in the new era: comparison of useful agronomic traits**

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1 Rice breeding in the new era: comparison of useful agronomic traits

2 Abstract

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

14 **Keywords.** Mutagenesis, domestication, CRISPR, gamma radiation, chemical mutagen,
 15 biotic stress, abiotic stress

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).

98 *Prime editing*

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

103 **Agronomic traits of interest**

104 **1. Domestication genes**

105 The *Oryza* genus is composed of species with a variety of genome structures, including six
106 diploids (n = 12; named AA, BB, CC, ee, ff, gg) and five polyploids (n = 24, named BBCC,
107 CCDD, HHJJ, HHKK, and KKLL) (Kim et al., 2015; Nadir et al., 2017; Wing et al., 2018;
108 Chen et al., 2019). Only two diploid (2n=24) species of rice have been domesticated and used
109 for cultivation: *Oryza sativa* and African *O. glaberrima*. Rice domestication emerged
110 because of the selection of specific genes and the loss of function of specific genes. Wild
111 relatives have functional versions of genes such as *sh4*, *waxy*, *BH4*, *qSH1*, *AN1*, *brown*
112 *pericarp*, *PROG1*, and *OsG1*, as described in the following text. The *sh4* gene is related to
113 reduced seed shattering (*Os04g0670900*). The *waxy* gene controls the amylose content
114 (*Os06g0133000*). *BH4* is related to the hull color of the seeds (*Os04g0460200*). The gene
115 *qSH1* is involved in seed shattering (*Os01g0848400*). The *AN1* gene is related to seeds,
116 morphology, and grain shape (*Os04g0350700*). *RC Brown pericarp* is involved in the seed
117 coat (*Os07g0211500*). *PROG1* is related to an erect plant structure (*Os07g0153600*). *OsLG1*
118 is related to a closed-panicle structure (*Os04g0656500*) (Li et al., 2017). The importance of
119 such genes is critical in understanding how *de novo* domestication and their further use in
120 plant breeding can be achieved from wild *Oryza* varieties.

121 This concept was demonstrated even in polyploid *O. alta* (CCDD) by (Yu et al., 2021a),
122 targeting *SD1*, *GS3*, *IPA1*, *Ghd7*, *Gn1a*, *Wx*, *Bh4*, *TAC1*, *An-1* homologs, as well as African
123 landraces of *Oryza glaberrima* by disrupting the *HTD1* (*O. sativa* *Os04g0550600*), *GS3* (*O.*
124 *sativa* *Os03g0407400*), *GW2* (*O. sativa* *Os01g0197700*) and *GN1A* (*O. sativa*
125 *Os02g0244100*) genes (Lacchini et al., 2020). In terms of plant breeding, having access to
126 nondomesticated, more genetically diverse rice species that better adapt to stress conditions,
127 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 MI48. In contrast, salt tolerance varieties like IR29, Fatmawati, Pokkali, Cheriviroppu, 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*), *P5CS* (*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; Nuñez-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; Nuñez-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 (Jayabalan et al., 2019). Knocking out an independent but closely related gene, *OsEPFL9* (*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 *OsNramp5* (*Os07g0257200*) and *OsNramp1* (*Os07g0258400*); the plants are able to resist heat stress

when the gene *OsNTL3* (*Os01g0261200*) is working correctly cold 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 DSR, 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 dioxygenase (HPPD) (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 imazomox (mode of action 2) (Hoyos et al., 2021). Weedy rice infestation 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 (70,000 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, Panamá, 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 aryloxyphenoxy propionate-resistant rice (mode of action 1), which is the result of mutations in the *ACCcase2* (*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: *ACCcase2* on aryloxyphenoxy propionates (*MoA-1*) and *AHAS/ALS* on ALS (*MoA-2*). The first *ACCcase2* 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/S627I/S653I/S653N/G654E. The third *OsTubA2* (*Os11g0247300*) with a mutation in the fourth exon, M268T, providing tolerance to dinitroanilines (*MoA-3*). Another gene, *HPPD* (*Os02g0280700*), provides tolerance to triketones when there is a natural insertion site (GGAACCAAAAGAATTAGAGACGATATCA) in the fourth exon. Finally, the double mutation known as “TIPS” (T102I+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 PSII 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 AAS46167, 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 *CYP81A6* (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 14,11,13* genes named *Os11g0508600*, *Os08g0535200*, *Os12g0476200*, respectively, since they act as a point of access for pathogens causing bacterial blight streaks (BLSs), 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 *IPAI* (*Os08g0509600*); higher expression levels of *IPAI* 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/NGFR), 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 (Ishimaru 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 *Os01g0197700* (*GN1a*) 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 grains is starch quality, which depends on the right mixture of amylose 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 amylose content (AC). Mutations in this gene correlate with a phenotype that ranges from opaque (8%), semitranslucent (8-12%), 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* (*Os12g0620400*) (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 *HGW*, 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 OsmiR156 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 *Pin15b*. The indirect mechanism results in higher expression of *DEP1* and *LPA1*, which interact to suppress *PIN1a* expression (Huang et al., 2018; Fu et al., 2019; Miao Liu et al., 2020). *LPA1* is also important in the erect phenotype, and its knockout results in lamina inclination, while *BASI* 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, fragranc y, oleic acid content, and color. Regarding nitrogen, there is a better efficiency with a higher expression of the nitrate transporter *OsNPF6.1* and the two transcription factors *OsNAC42* and *OsNLP4* (Tang et al., 2019; Yu et al., 2021b). Knockout of the *FAD2* gene results in an oleic acid increment (Tiwari 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 fragranc y can be increased or decreased by modulating the *BADH2* 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 requires 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)

331 Amylose Content (AC)

332 Acetolactate Synthase (ALS)

333 NUE (nitrogen use efficiency).

334 **Declarations**

335 All manuscripts must contain the following sections under the heading 'Declarations':

336 **Ethics approval and consent to participate** 'Not applicable'

337 **Consent for publication** 'Not applicable'

338 **Availability of data and material** 'Not applicable'

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1 Rice agronomic traits and variability induced by mutagenesis

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3 **Figure 1.** Representation of biotic and abiotic stress factors that affect rice production.

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7 **Figure 2.** Schematic representation of different systems used for breeding rice: natural
 8 variability, mutation breeding, tissue culture mutation, and new breeding techniques. Created
 9 with BioRender.com

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

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22 **Figure 4.** Representation of five rice genes and the corresponding mutation that results in
 23 herbicide tolerance. The genes are shown organized by their Mode of Action (MoA). Note
 24 the name of the gene in orange circles, the exons in blue filled boxes and the corresponding
 25 untranscribed exon regions in the blue empty boxes. Created with BioRender.com.

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27 **Figure 5.** Representation of traits such as grain number, quality, weight and plant structure
 28 and gene relationships in rice. Note that heading and flowering are positively influenced by
 29 *Se5*, *Hd2*, and *Hd1* knockout; structure by *DEP1*, *HTD1*, *IPA1*, *LPA1*, *Pin1a*, and *Pin15b*;
 30 grain size by *Gn1a*, and *Ep3*; grain size by *GS3*, *GW6a*, *GW5*, and *GW5L*; and grain starch
 31 by *ISA1*, *NAC20-26*, and *WX1*. Created with BioRender.com.

1 Rice agronomic traits and variability induced by mutagenesis

2 **Table 1.** Rice genes and mutations involved in stress tolerance or sensitivity traits.

Gene	Position	Protein	Obtained mutation	Method	Trait details	Reference
<i>OsRR22</i> <i>Os06g0183100</i>	Chr 6	Q5SML5	Knockout	CRISPR/Cas9	Two-component response regulator ORR22. Salt tolerance 0.75% NaCl.	(Zhang et al., 2019a)
<i>STL1</i> <i>Os04g0110600</i> <i>Salt tolerance Level 1, Stress repressive zinc finger protein 4</i>	Chr 4	Q7XXF2	SNP	None	hap1 tolerance 0.9% salt, the gene is the homolog of Arabidopsis salt tolerance gene SRP1 (Stress associated RNA-binding protein 1, AT2G17975). Knock-out mutation in the srp1 allele reduced sensitivity to ABA and salt stress.	(Yuan et al., 2020)
<i>MSL37</i> <i>Os11g0163500</i> <i>OsGTgamma-2</i>	Chr 11	Q53PP7	Natural variability- Knockout	Spontaneous mutation- CRISPR/Cas9	Knock-out results in salt sensitivity.	(Liu et al., 2020d)
<i>Os03g0786400</i> <i>OsDST, DLN102, OsDLN102, Negative regulation of response to salt stress</i>	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)
<i>P5C</i> <i>Os05g0455500</i>	Chr 5	O04226	Natural: cultivar LPT123 is salt-susceptible versus salt-tolerant line LPT123-TC171	None	The enzyme increases the proline accumulation and salt resistance mediated by ABA application.	(Sripinyowanich et al., 2013)
<i>SKC1</i> <i>Os01g0307500</i> <i>OsHKT1;5, OsHKT8</i>	Chr 1	Q0JNB6	Wild relatives	None	Variant V395 (is salt tolerant), while L395 is sensitive.	(Jayabalan et al., 2019)
<i>Os10g0521000</i> <i>Based on Z.mays GRMZM2G162690 and A. thaliana AT4G24040</i>	Chr 10	Q9FWC1	Substitution S163T	CRISPR/Cas9	Mutation of domain WDS to replicate <i>Selaginella moellendoffii</i> WDT. The enzyme may be less efficient in allowing the accumulation of trehalose.	(Nuñez-Muñoz et al., 2021)
<i>OsEPFL9</i> <i>Os01g0824500</i> <i>Epidermal Patterning Factor Like-9</i>	Chr 1	Q5JN76	Knockout	CRISPR/Cpf1	Increased water use efficiency under stress because of reduced stomatal count	(Yin et al., 2017, 2019)
<i>DHS</i> <i>Os02g0682300</i> <i>Drought hypersensitive</i>	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)
<i>RCS1</i> <i>Os12g0625000</i>	Chr 12	Q9XEA6	S189N	EMS	Tolerates 20 μ M As (III). The mutation increases As tolerance/decreased	(Sun et al., 2021)

O-acetylserine (thiol) lyase, Cysteine synthase. <i>arsenite tolerant 1</i>					accumulation in the grain/increase Se accumulation in the grain.	
<i>OsNramp5</i> <i>Os07g0257200</i> Manganese and Cadmium transporter, Mn and Cd uptake,	Chr 7	Q8H4H5 I7GYG6	Knockout	CRISPR/Cas9	Low Cd accumulation	(Sasaki et al., 2012; Tang et al., 2017; Chang et al., 2020)
<i>OsNramp1</i> <i>Os07g0258400</i> Metal transporter	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)
<i>OsNTL3</i> <i>Os01g0261200</i> Thermotolerance	Chr 1	Q7GCL7	Natural variability-	None	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)
<i>OsMYB30</i> <i>Os02g0624300</i> , Cold tolerance gene	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)

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5 **Table 2.** Rice genes and mutations in herbicide resistance traits.

Gene (*)	Position	Protein	Obtained mutation	Method	Trait details	References
<i>OsTubA2</i> <i>Os11g0247300</i>	Chr 11	Q53M51	M268T	CRISPR/Cas9-Base editor	<i>In vitro</i> trifluralin 4 mg/, pendimethaline 6.6 mg/L	L. Liu et al., 2021)
<i>ACCase2</i> <i>Os05g0295300</i>	Chr 5	B9FK36	W2027C	Seeds-Gamma Rays 280Gy	quizalofop-p-ethyl = 75 g/ha; haloxyfop-p-methyl = 62.35 g/ha	(de Andrade et al., 2018)
			I1879V W2125S	CRISPR/Cas9-Base editor	haloxyfop-R-methyl, 1 and 2 μ M <i>in vitro</i> .	(Liu et al., 2020b; c)
			I1781L	Tissue culture mutation	quizalofop-p-ethyl=235 g ai ha-1	(Camacho et al., 2019)
			D2176G G2201A	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)
<i>psbA</i> <i>AAS46167</i> (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)
<i>HPPD</i> <i>Os02g0280700</i> 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)
<i>AHAS, ALS</i> <i>Os02g0510200</i> Acetohydroxy acid synthase	Chr 2	Q6K2E8	W548L P171S	CRISPR-Prime Editing	Herbicide tolerance	(Lin et al., 2020; Xu et al., 2020)
			A96V (C287T)	CRISPR/Cas9 Base editor	Imazamox (quantity not reported)	(Shimatani et al., 2017)
			G654E	Chemical mutation	Clearfield 121 Clearfield 141 IRGA422	(Singh et al., 2017; Bzour et al., 2018)
			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 E549	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		P171H/W548L W574L P197S S653I	Recombinant	100 mM IQ 100 mM CS/BM/IQ/IP/PS 100 mM BM 100 mM IP	(Kawai et al., 2008)
<i>OsEPSPS</i> <i>Os06g0133900</i>	Chr 6	A0A0N7KLH2	T169I A170V P173S	CRISPR-Prime Editing	NA	(Li et al., 2020a)
			T102I + P106S	CRISPR	<i>In vitro</i> resistance 1 mg l-1 glyphosate, 400x dilution Greenhouse.	(Li et al., 2016b)

(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, pyriithiobac-sodium; BS, bispyribac-sodium.

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

Gene	Position	Protein	Obtained Mutation	Method	Trait details	Reference
<i>Os11g0508600</i> <i>Sweet 14</i>	Chr 11	Q2R3P9	Knockout	CRISPR-Cas9	<i>Xanthomonas oryzae</i> pv. <i>Oryzae</i> 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)
<i>Os08g0535200</i> <i>Sweet 11</i>	Chr 8	Q6YZF3	Knockout	CRISPR-Cas9	<i>Xanthomonas oryzae</i> pv. <i>Oryzae</i> 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)
<i>Os12g0476200</i> <i>Sweet 13</i>	Chr 12	Q2QR07	Knockout	CRISPR-Cas9	<i>Xanthomonas oryzae</i> pv. <i>Oryzae</i> 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)
<i>Os07g0555200</i> <i>translation initiation factor 4 gamma gene (eIF4G)</i>	Chr 7	B9FXV5	Knockout and mutations on SVLFPNLAGKS	CRISPR-Cas9	Resistance to rice tungro spherical virus (RTSV)	(Macovei et al., 2018)
<i>Os01g0752500</i> , ethylene response factor 922 <i>OsERF922</i> , <i>LOC_Os01g54890.1</i>	Chr 1	Q5JMX7	Knockout	CRISPR	<i>Magnaporthe oryzae</i> , Blast resistance	(Wang et al., 2016)

13 **Table 4.** Rice genes and mutations involved in grain quality, quantity, weight, and plant
14 structural traits.

Gene	Position	Protein	Obtained mutation	Method	Trait details	Reference
<i>OsDEP1</i> <i>Os09g0441900</i>	Chr 2	Q67UU9	Mutation, promoter	Spontaneous mutation - CRISPR /Cas9	More expression, yield increase 15%. The interaction between DEP1 and LPA1 suppresses <i>PIN1a</i> 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)
<i>Gn1a</i> <i>Os01g0197700</i> <i>OsCKX2</i>	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)
<i>GS3</i> <i>Os03g0407400</i>	Chr 3	C6L686	Knockout	CRISPR/Cas9 -Spontaneous mutation	δ 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)
<i>IPA1</i> <i>Os08g0509600</i> Transcription factor Ideal Plant Architecture 1	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)
<i>WX1</i> <i>Os06g0133000</i> granule-bound starch synthase 1 <i>GBSSI</i> , <i>OsGBSSI</i> , <i>waxy</i>	Chr 6	Q0DEV5	Knockout, mutations	CRISPR/Cas9 P124F, R125W, T178I, T178S, R158H, Y191H, R158H, G159A, D161N, 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/semitranslucent (8–12%)/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	(Sano, 1984; Yunyan et al., 2019; Zhang et al., 2019b; Huang et al., 2020; Xu et al., 2021)

					waxy or glutenous starch. S415P changes phosphorylation, resulting in moderate enzyme activity and a content of amylose.	
<i>ISA1</i> <i>Os08g0520900</i> isoamylase 1	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., 2019)
<i>OsNAC20</i> <i>Os01g0104500</i> <i>OsNAC26</i> <i>Os01g0393100</i>	Chr 1	Q9FTY0 (<i>OsNAC20</i>) Q5VVK1 (<i>OsNAC26</i>)	Knockout	CRISPR/Cas9	Double knockout <i>osnac20/26</i> 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)
<i>GW5</i> <i>Os05g0187500</i> Grain Size on Chromosome 5 , qSW5/GW5, GSE5	Chr 5	Q75KY5 A0A1D8GZC0	Knockout	Spontaneous mutation	<i>GW5</i> could function as a key regulator to coordinate the performance of the other grain size genes. <i>gw5</i> contributes to an increased grain width and weight. Positive regulator of brassinosteroid signaling.	(Zhang et al., 2020)
<i>GW5L</i> <i>Os01g0190500</i> GW5L homologue of GW5	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)
<i>GW6a</i> <i>Os06g0650300</i> OsglHAT1, Grain weight on chromosome 6	Chr 6	Q67UR2	Over expression	Spontaneous mutation	Histone H4 acetyltransferase, regulation of grain weight, yield, and plant biomass. Elevated OsglHAT1 expression enhances the grain weight and yield. Increases global acetylation levels of histone H4.	(Song et al., 2015; Ayaad et al., 2021)
<i>GW6</i> <i>Os06g0623700</i> <i>TOTAL GRAIN WEIGHT6</i> , <i>total grain weight6</i> ,	Chr 6	Q69U01	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)
<i>OsPIN5b</i> <i>Os08g0529000</i> <i>a panicle length gene</i>	Chr 8	Q6ZIB5	Knockout	CRISPR	Increased panicle length in the mutant.	(Zeng et al., 2020)
<i>Hd1/SE1</i> <i>Os06g0275000</i>	Chr 6	Q9FDX8	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)

<i>HTD1</i> <i>Os04g0550600</i> High-Tillering Dwarf 1	Chr 4	Q7XU29	Loss of function	Spontaneous mutation/ CRISPR	Landraces contain HTD1, while domesticated rice have <i>htd1</i> . The defect in HTD1 is responsible for both high-tillering and dwarf phenotypes in the <i>htd1</i> mutant. Auxin induces HTD1 expression. The protein negatively regulates the outgrowth of axillary buds and is related to strigolactones biosynthesis	(Zou et al., 2006; Lacchini et al., 2020)
<i>LPA1</i> <i>Os03g0237250</i> Loose Plant Architecture 1	Chr 3	L7PBL4	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 (<i>lpa1</i>). <i>lpa1</i> mutants might exhibit less efficient auxin flux.	(Liu et al., 2016a)
<i>OsMeCP</i> <i>Os12g0620400</i> methyl-CpG binding domain protein, Methyl-CpG binding domain containing protein	Chr 12	Q0ILV0	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)
<i>Hd2</i> <i>Os07g0695100</i> Heading date 2	Chr 7	Q0D3B6	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)
<i>Ep3</i> <i>Os02g0260200</i> ERECT PANICLE 3,	Chr 2	G3CKN6	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)
<i>Se5</i> <i>Os06g0603000</i> <i>Photosensitivity5</i>	Chr 6	Q69XJ4	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)
<i>BASI</i> <i>Os02g0537700</i>	Chr 2	Q6ER94	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)

<i>HGW</i> <i>Os06g0160400</i> <i>heading and grain weight, heading date- and grain weight-related protein</i>	Chr 6	B6TN35	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)
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17 **Table 5.** Rice genes and mutations in traits such as oleic acid, color, fragranc, and nitrogen
18 use.

Gene	Position	Protein	Obtained Mutation	Method	Trait details	Reference
<i>FAD2</i> <i>Os02g0716500</i> fatty acid desaturase 2	Chr 2	Q6ZGW6	Knockout	CRISPR/Cas9-RNAi	Increased oleic acid (twice) and decreased linoleic acid content.	(Tiwari et al., 2016; Abe et al., 2018)
<i>Osor</i> <i>Os02g0651300</i>	Chr 2	Q6H3Y3	Knockout	CRISPR/Cas9	β -carotene accumulation resulting in orange-colored calli.	(Endo et al., 2019)
<i>BADH2</i> <i>Os08g0424500</i>	Chr 8	A0A0P0XG36	Knockout	CRISPR/Cas9	Betaine aldehyde dehydrogenase 2, prevents the formation of 2-acetyl-1-pyrroline (2AP), which gives fragrant rice its aromatic properties. Change in fragrance.	(Shen et al., 2017)
<i>OsNPF6.1</i> <i>Os01g0103100</i> Nitrate transporter	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.	(Tang et al., 2019)
<i>OsNAC42</i> <i>Os09g0493700</i> NUE (nitrogen use efficiency)-related transcription factor	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 transporter OsNPF6.1. Loss of function decreased expression of nitrate transporter OsNPF6.1	(Tang et al., 2019)
<i>OsNLP4</i> <i>Os09g0549450</i> transcriptional factor, Promotion of nitrogen use efficiency (NUE)	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 OsNiR that encodes a critical nitrite reductase in nitrogen assimilation.	(Yu et al., 2021b)

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Figures

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.