Current Plant Biology 27 (2021) 100211

Contents lists available at ScienceDirect

Current Plant Biology

journal homepage: www.elsevier.com/locate/cpb

Review article

# Rice breeding in the new era: Comparison of useful agronomic traits

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ARTICLE INFO

*Keywords:*
Mutagenesis
Domestication
CRISPR
Gamma radiation
Chemical mutagen
Biotic stress
Abiotic stress

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, other cereals, and orphan crops to achieve a superior and sustainable production in challenging farming conditions.

## 1. Introduction

Induced mutagenesis is a valuable tool to support functional genomics studies and the development of new genotypes. Rice serves as an outstanding model because of its impact on the worldwide food supply chain and the availability of genomic and agronomic resources to utilize. Rice was the first crop sequenced in 2004 [1], 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 [2–4]. Biological, chemical, and physical agents can induce mutagenesis. Typical methods are radiation (first used on vegetables in 1928), ethyl methanesulfonate (EMS) (which produces 2–10 mutations per Mb), and new breeding techniques to introduce specific mutations via genetic engineering [5–8]. In this review, we present rice traits that have emerged or have been validated in the last decade (2010–2021) and were derived from technological advances in genomics [9]. This paper is focused on characteristics that could be targeted by mutagenesis of rice lines and related crops to produce predictable changes in gains or losses of function. We present traits that could result from the use of different techniques; remarkably, genome editing represents an exciting opportunity to transfer the information about gene-trait relationships to other crops to improve their traits. Consequently, they represent a challenge from a regulatory point of view for countries that have established a different regulation for genome-edited plants in contrast to other mutagenesis techniques.

## 2. Methods

The methodology applied a search based on PubMed articles and 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, Ensembl-Plants, Rice Diversity, FunRiceGenes, Rice Genome Annotation Project, Oryza Base, and Rice Information GateWay [10]. The search resulted in

*Abbreviations:* EMS, Ethyl methanesulfonate; PTR, Puddled Transplanted Rice; DSR, Direct Seeded Rice; DAS, Days After Sowing; HRAC, The Herbicide Resistance Action Committee; WSSA, Weed Science Society of America; *MoA*, Mode of Action; BLS, Bacterial Blight Streak; AC, Amylose Content; ALS, Acetolactate Synthase; NUE, nitrogen use efficiency.

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https://doi.org/10.1016/j.cpb.2021.100211
Received 11 May 2021; Received in revised form 15 June 2021; Accepted 17 June 2021
Available online 18 June 2021

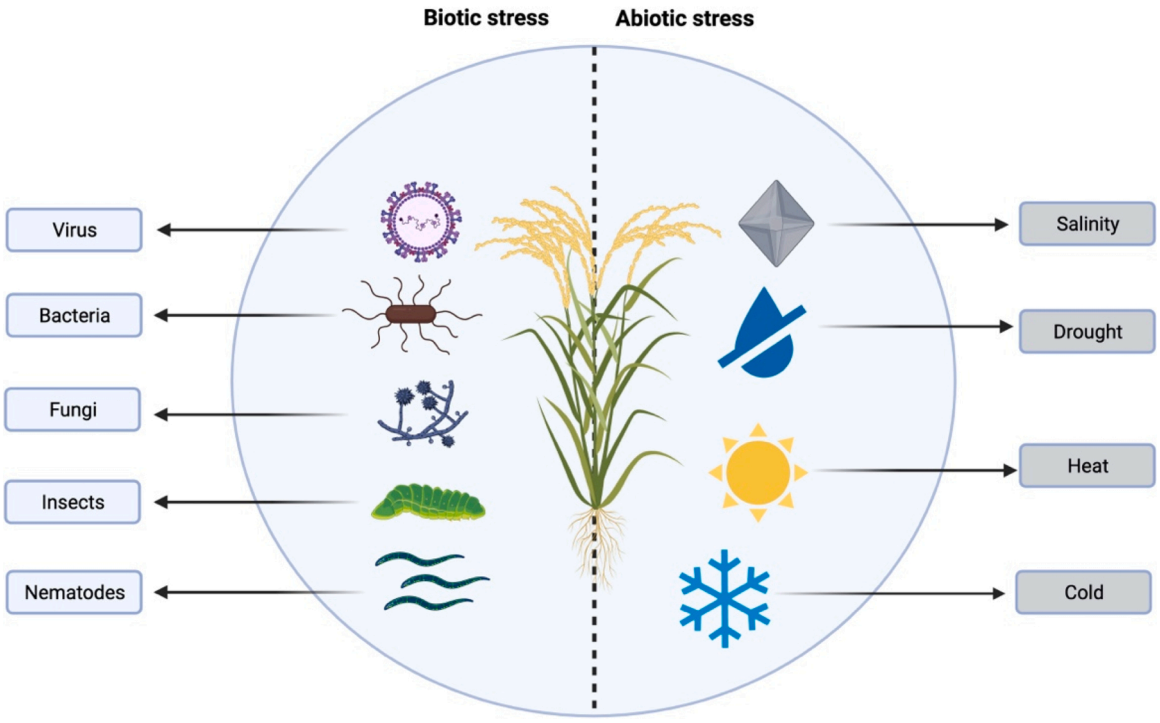


**Fig. 1.** Representation of biotic and abiotic stress factors that affect rice production. Created with BioRender.com.

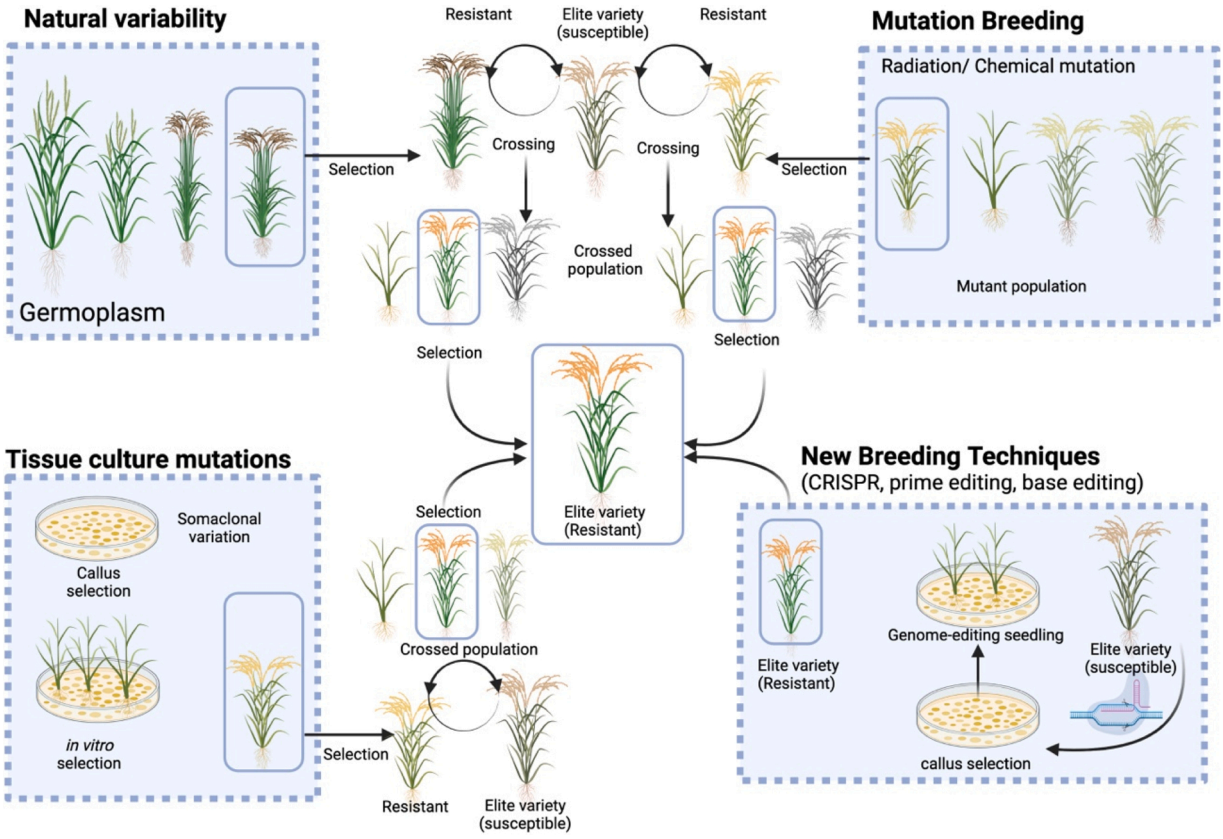


**Fig. 2.** Schematic representation of different systems used for breeding rice: natural variability, mutation breeding, tissue culture mutation, and new breeding techniques. Created with BioRender.com.

a selection of 117 papers out of 500.

(*continued on next column*)

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| Webserver | Link |
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| 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/ |

## 3. Importance 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 [11]. As an example, losses in rice due to insects can account for over 40 %. Moreover, losses caused by the fungal pathogens *Magnaporthe grisea*, *Thanatephorus cucumeris*, and *C. miyabeanus* have been estimated worldwide at 35 %, 24 %, and 16 %, respectively [12]. 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 [128].

In this regard, the generation of rice-resistant varieties to biotic and abiotic conditions represents one of the major 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).

## 4. 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 mutagenesis, base editing, and prime editing) (Fig. 2).

### 4.1. 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 [13]. 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 allelic variants available for genetic improvement [14,15,8].

### 4.2. Tissue culture

Totipotency, a distinguishable characteristic of plant cells, in principle allows each cell to regenerate an entire plant. This process involves the culture of plant tissue fragments or individual cells on special growth media enabling the cells to grow, divide, and differentiate into organs [16]. Among the techniques available, somaclonal variation are spontaneous changes in the DNA leading to genetic and phenotypic variations among clonally propagated plants. The somaclonal variants obtained could be detected using in vitro selection by applying selective pressure in culture conditions [17,18].

### 4.3. New breeding techniques

#### 4.3.1. CRISPR/Cas9

The clustered regularly interspaced short palindromic repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) system from *Streptococcus pyogenes* targets a specific genomic sequence using an engineered 20 base pair (bp) RNA guide sequence that binds to matching DNA and the Cas9 protein, upon recognition of an additional 3' localized PAM sequence 5′-NGG-3′, generates a double-strand break at a desired location in the genome. This genome editing method allows the insertion, deletion, or modification of DNA with high specificity and efficiency [5].

#### 4.3.2. 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 18–23 nt spacer for its maximum efficiency and specificity and identifies a T-rich PAM located 5' upstream of the guide and generates staggered ends with 5' overhangs [19].

#### 4.3.3. Base editing

This system allows the conversion of nucleotides without inducing double-stranded DNA breaks or using donor templates. It is based on Cas9 nickase fusions to a nucleotide deaminase domain and has been used for changing a C–G base pairs into T-A (cytidine deaminase base editor), or A–T into G-C (adenosine deaminase base editor) [20].

#### 4.3.4. 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 a target site and replace the target DNA nucleotides without double-stranded DNA breaks or using donor templates [21,22].

## 5. Agronomic traits of interest

### 5.1. 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) [23–26]. Only two diploid (2n = 24) species of rice have been domesticated and used for cultivation: *Oryza sativa* and African *O. glaberrima.* Rice domestication favored the selection of specific loss of function alleles. Wild relatives typically have functional versions of these genes such as *sh4*, *waxy*, *BH4*, *qSH1*, *AN1*, *brown pericarp, PROG1*, and *OsG1*. 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 (*Os07g0153600*). *OsLG1* is related to a closed-panicle structure (*Os04g0656500*) [27]. The importance of such genes and their domesticated alleles is critical in understanding how *de novo* domestication can be achieved from wild *Oryza* varieties and how such genes can be further used for breeding of rice and other crops.

This concept was demonstrated in polyploid *O. alta* (CCDD) by Yu et al. [28], targeting *SD1, GS3, IPA1, Ghd7, Gn1a, Wx, Bh4, 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 [29]. For plant breeding, the use of non-domesticated, more genetically diverse rice species that better adapt to stress conditions, such as African landraces *O. glaberrima*, *O. barthii*, *O. meridionalis* (AA),

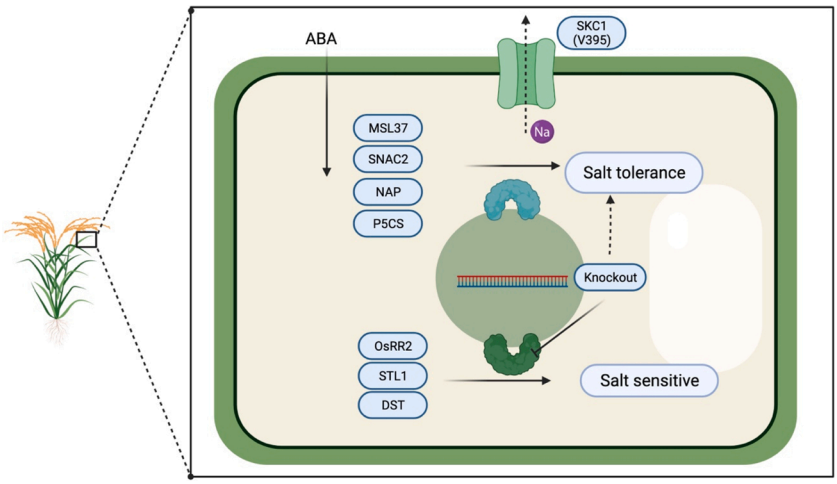


**Fig. 3.** Representation of salt tolerance traits mediated by three different methods: 1) overexpression, 2) knockout of specific genes, and 3) particular sodium channels. Note that the first corresponds to transcription factors that trigger adaptive responses labeled *MSL37*, *NAC2*, *NAP*, and *P5CS*. 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.

Australian landraces *O. longistaminata* (AA), *O. australiensis* (EE), and Asian landraces *O. rufipogon* (AA) or *Porteresia coarct*ata (*O.coarctata*) (KKLL), harbors a valuable potential of developing more sustainable rice crops [30,31].

### 5.2. Stress tolerance

Rice susceptibility to salt is evidenced by a yield decrease due to delays in heading and panicle sterility especially in salt-sensitive varieties like MI48, IR29 [31–33]. In contrast, salt tolerance in varieties like 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 of this trait [31–40]. The orchestrated stress system can be targeted for achieving salt tolerance by mutating genes encoding key transcription factors, specifically *OsRR22* (*Os06g0183100*), *STL1* (*Os04g0110600*), and the zinc finger transcription factor encoded by *DST* (*Os03g0786400*) [38,41–44]. Other transcription factors are critical in stress adaptation, which results in stress sensitivity when inactivated. This is the case for *MSL37* (*Os11g0163500*) which encodes a positive salt stress transcription factor response by regulating ion transporters, *P5CS* (*Os05g0455500*), which causes accumulation of the osmoprotectant proline, the transcription factor *SNAC2* (*Os01g0884300*), which is key in root adaptation, and *OsNAP* (*Os03g0327800*), which triggers a stress response mediated by ABA [45–49]. For details, see Fig. 3 and Table 1.

Osmoprotection by accumulating molecules such as trehalose or proline is a possible pathway leading to salt tolerance, as proven currently in plants like rice and *Arabidopsis* [59,47,60,61]. Other individual genes can confer osmoprotection, such as the Na + transporter *SKC1* (*Os01g0307500*) with a V395 L that provides salt tolerance [50]. Knocking out an independent gene, *OsEPFL9* (*Os01g0824500*), results in increased water use efficiency under stress because of the reduced stomatal count [51,52].

Other stress tolerance pathways can be modified by specific alleles, too. Low cadmium accumulation occurs after mutating the metal transporter genes *OsNramp5* (*LC196140;* japonica homologue: *Os07g0257200*) and *OsNramp1* (*LC196122*; japonica homologue: *Os07g0258400*). Plants are able to resist heat stress only when the gene *OsNTL3* (*Os01g0261200*) is functioning correctly, whereas cold tolerance can result from mutation of the *OsMYB30* (*Os02g0624300*) gene. Finally, more cuticle wax is deposited when the gene *DHS* (*Os02g0682300*) is mutated [54–58,62]

### 5.3. Herbicide resistance monogenic traits

Rice is usually cultivated under two agronomical systems: paddy-transplanted-rice (PTR) and direct-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 [63]. 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 [64]. However, weed management is a challenge in DSR, specifically during the first 41 days after sowing (DAS). This includes complication by weedy rice (*O. sativa f. spontanea)*, which is a variety of rice that is morphologically similar to cultivated rice, but grows as a weed. It produces far fewer grains than cultivated rice, and can result in rice yield losses of up to 50 % [25]. Weedy rice usually has increased seed longevity, seed shattering and stress tolerance which makes it difficult to control [65]. The use of chemical control represents a tool to manage weeds including weedy rice, but poses additional challenges.

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 [66–68]. The discovery of a new mode of action has been rare in the last 30 years. A good example is leptospermone, and its analogous inhibitors that act as hydroxyphenylpyruvate inhibitors of dioxygenase (HPPD) [69]. Different modes of herbicide use, 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) [70]. Weedy rice infestation in the USA resulted in 5.7 million tons of harvest lost and $457 million in environmental costs between 2002–2014 [71]. To control weedy rice, herbicide tolerance was introduced into cultivated rice 20 years ago based on an *acetohydroxy acid synthase AHAS/ALS* (*Os02g0510200*) gene mutation, providing tolerance to the mode of action 2 [72]. Currently, rice herbicide tolerant 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 [73]. The incorrect use of this variety allowed introgression and outcrossing of the resistance into weedy rice, which means that weed herbicide control requires stricter farming practices, such as herbicide rotation [74]. Alternatives such as aryloxphenoxy 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 [75,76].

According to the literature, at least five target genes have the

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

| Gene | Position | Protein | Obtained mutation | Method | Trait details | Reference |
|---|---|---|---|---|---|---|
| *OsRR22* *Os06g0183100* *STL1* | Chr 6 | Q5SML5 | Knockout | CRISPR/Cas9 | Two-component response regulator ORR22. Salt tolerance 0.75 % NaCl. | [38] |
| *Os04g0110600* *Salt tolerance Level 1, Stress repressive zinc finger protein 4* | 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. | [44] |
| *MSL37* *Os11g0163500* *OsGTgamma-2, OsGTγ-2* | Chr 11 | Q53PP7 | Natural variability-Knockout | Spontaneous mutation-CRISPR/Cas9 | Knock-out results in salt sensitivity. The transcription factor is a positive salt stress regulator, and binds to promoters of OsHKT2; 1, OsNHX1 and OsHKT1. | [42] |
| *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 | [41,43] |
| *P5C* *Os05g0455500* | 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. | [48] |
| *SKC1* *Os01g0307500* *OsHKT1;5,* *OsHKT8* | Chr 1 | Q0JNB6 | Wild relatives | None | Variant V395 (is salt tolerant), while L395 is sensitive. | [50] |
| *Os10g0521000* *Based on Z.mays GRMZM2G162690 and A. thaliana AT4G24040* | Chr 10 | Q9FWC1 | Substitutio S163T | CRISPR/Cas9 | Mutation of domain WDS to replicate *Selaginella moellendoffii* WDT. The enzyme may be less efficient in allowing the accumulation of trehalose. | [47] |
| *OsEPFL9* *Os01g0824500* Epidermal Patterning Factor Like-9 | Chr 1 | Q5JN76 | Knockout | CRISPR/Cpf1 | Increased water use efficiency under stress because of reduced stomatal count | [51,52] |
| *DHS* *Os02g0682300* Drought hypersensitive | 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 | [53] |
| *RCS1* *Os12g0625000* O-acetylserine (thiol) lyase, Cysteine synthase. *arsenite tolerant 1* | Chr 12 | Q9XEA6 | S189N | EMS | Tolerates 20 μM As (III). The mutation increases As tolerance/decreased accumulation in the grain/increase Se accumulation in the grain. | [141] |
| *OsNramp5* *LC196140 (indica rice)* *Os07g0257200 (japonica rice)* *Manganese and Cadmium transporter, Mn and Cd uptake,* | Chr 7 | B8B4U0 (*indica rice*) Q8H4H5 I7GYG6 (*japonica rice*) | Knockout | CRISPR/Cas9 | Low Cd accumulation | [54,55, 56] |
| *OsNramp1* *LC196122 (indica rice)* *Os07g0258400* Metal transporter *(japonica rice)* | Chr 7 | A2YK11 (*indica rice*) Q0D7E4 (*japonica rice*) | 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. | [56] |
| *OsNTL3* *Os01g0261200* *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. | [57] |
| *OsMYB30* *Os02g0624300,* 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. | [58] |

potential to develop herbicide-resistant rice varieties with a different mode of action. Two of those have already been described above: *ACCase2* on aryloxphenoxy propionates (*MoA-1*) and *AHAS/ALS* on ALS (*MoA-2*). For *ACCase2*, mutations such as I1781 L, S1866 F, I1879 V, A1884 P, W2027C, W2125S, D2176 G, and C2186R/P1927 F/G2201A/W2125C in exon 32 provide herbicide tolerance at a different level. *AHAS/ALS* alleles cause ALS (*MoA-2*) resistance when carrying the following mutations: A96 V/A122 T/P171 H/P171S/P197S/C287 T and W548 L/W574 L/S627I/S653I/S653 N/G654E. *OsTubA2* (*Os11g0247300*) provides tolerance to dinitroanilines (*MoA-3*) with a mutation in the fourth exon, M268 T. *HPPD* (*Os02g0280700*), provides tolerance to triketones with a natural insertion (GGAACCAAAAGAATTAGAGACGATATCA) in the fourth exon. Finally, the double mutation known as "TIPS" (T102I + P106S) in the *OsEPSPS* (*Os02g0510200*) gene provides tolerance to *MoA-9* (glyphosate). For details, see Fig. 4 and Table 2.

Weeds that are tolerant to the inhibition of photosynthesis at PSII by herbicides can also provide insights to generate herbicide tolerant crops. The S264 G mutation in *psbA* increases tolerance more than 50-fold to triazine in herbicide-tolerant radish (*MoA-5*). However, it can also compromise fitness because of less efficient photosynthesis [77]. Other mutations, such as Val219Ile, Asn266Thr, Phe255Ile, and Ala251Val, can also provide tolerance [68]. It is important to note that the *psbA* mutation Val-219-Ile provides tolerance to the amide propanil *MoA-5* on

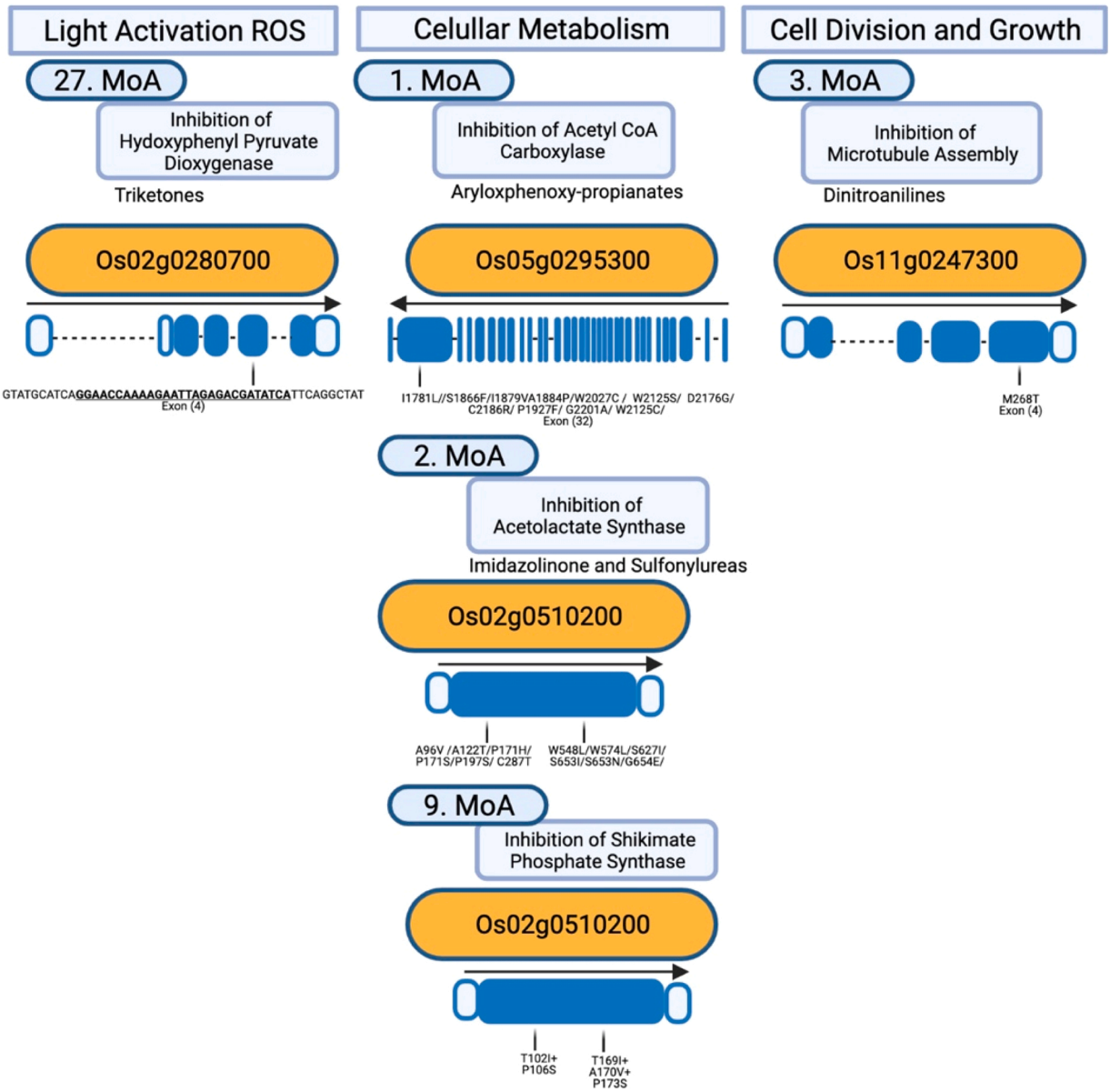


**Fig. 4.** Representation of five rice genes and the corresponding mutation that results in herbicide tolerance. The genes are shown organized by their Mode of Action (MoA). Note the name of the gene in orange circles, the exons in blue filled boxes and the corresponding untranslated exon regions in the blue empty boxes. Created with BioRender.com (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

*Cyperus difformis* [79]. 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 pathway could increase its tolerance [80,81]. 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* [82]. Additionally, the P450 gene *CYP72A31* is responsible for conferring tolerance to bispyribac sodium (BS) in *Oryza sativa* cv. *indica*, while its absence in japonica rice varieties results in BS-sensitivity [83,84].

### 5.4. Bacteria, fungi and virus resistance

Rice breeding of pathogen resistance is possible by mutation of specific promoter regions of the *Sweet 14,11,13* genes (*Os11g0508600*, *Os08g0535200*, *Os12g0476200*), respectively, since they are required for infection by bacterial *Xanthomonas oryzae* pv. *oryzae* pathogens causing bacterial leaf blight (BLB) [85–87]. The pathogen emerges by breaking the resistance of varieties planted in approximately 80 % of the total crop cultivation area carrying the resistance gene *Xa4* on chromosome 11 introduced in the 60 s [88]. Some *Xanthomonas oryzae* pathovars can also infect wild grasses and could become an emergent pathogen that is difficult to control [89].

A gene to target for fungal resistance is the transcription factor *IPA1* (*Os08g0509600)*; higher expression levels of IPA1 result in increased yield and immunity when tested against the fungal pathogen *Magnaporthe oryzae*. Resistance relies on time- and pathogen-specific phosphorylation and activation of the transcription factor at Ser163. Subsequently, phosphorylated IPA1 activates the WRKY45 promoter and following basal resistant gene expression within 48 h after infection, while the nonphosphorylated IPA1 protein binds to the DEP1 promoter related to yield (Jing [53]). A different way to achieve *M. oryzae* resistance is by mutation of *OsERF922 ethylene response factor 922* (*Os01g0752500*) [90]. Another important trait is tungro spherical virus

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

| Gene (*) | Position | Protein | Obtained mutation | Method | Trait details | References |
|---|---|---|---|---|---|---|
| *OsTubA2* *Os11g0247300* | Chr 11 | Q53M51 | M268T | CRISPR/Cas9-Base editor | *In vitro* trifluralin 4 mg/, pendimethaline 6.6 mg/L | [140] |
| *ACCase2* | | | W2027C | Seeds-Gamma Rays 280Gy | quizalofop-p-ethyl =75 g/ha; haloxyfop-p-methyl =62.35 g/ha | [76] |
| | | | I1879V<br>W2125S | CRISPR/Cas9-Base editor | haloxyfop-R-methyl, 1 and 2 μM *in vitro*. | [142,42] |
| | Chr 5 | B9FK36 | I1781L | Tissue culture mutation | quizalofop-p-ethyl = 235 g ai ha-1 | [75] |
| *Os05g0295300* | | | D2176G<br>G2201A | CRISPR-Prime Editing | Herbicide resistance | [139] |
| | | | C2186R | CRISPR-Base editor | Herbicide resistance | [134,42] |
| | | | P1927 F, W2125C, S1866 F and A1884 P | CRISPR-Base editor | Herbicide resistance 34 g/Ha. High tolerance P1927 F, W2125C versus low tolerance S1866 F and A1884P | [133,42] |
| *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 | [77] |
| *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, HIS1detoxifies b-triketone herbicides by hydroxylation. | [135] |
| *AHAS, ALS* | | | W548L<br>P171S | CRISPR-Prime Editing | Herbicide tolerance | [22,139] |
| *Os02g0510200* | | | A96V (C287 T) | CRISPR/Cas9 Base editor | Imazamox (quantity not reported) | [137] |
| | | | G654E | Chemical mutation | Clearfield 121<br>Clearfield 141<br>IRGA422 | [73,71] |
| | Chr 2 | | S653N | Chemical mutation | Named CL161 and CLXL8 increased herbicide tolerance | [73] |
| | | Q6K2E8 | W548 L or P171S | Recombinant protein | Herbicide tolerance | [131] |
| Acetohydroxy acid synthase | | | W548<br>E549 | CRISPR-Prime Editing | Herbicide tolerance | [139] |
| | | | A122T | Sodium Azide | IMINTA1, IMINTA4 | [136] |
| | | | W548L<br>S627I | CRISPR | Herbicide tolerance | [138] |
| | Arabidopsis | | P171 H/W548 L | Recombinant | 100 mM IQ | [131] |
| | Modeling | | W574 L<br>P197S<br>S653I | | 100 mM CS/BM/IQ/IP/PS<br>100 mM BM<br>100 mM IP | |
| *OsEPSPS* | Chr 6 | A0A0N7KLH2 | T169I<br>A170 V P173S | CRISPR-Prime Editing | NA | [132] |
| *Os06g0133900* | | | T102I + P106S | CRISPR | *In vitro* resistance 1 mg l–1 glyphosate, 400x dilution Greenhouse. | [78] |

(S)= Susceptible, genomic.
(*) Additional information at The Rice Annotation Project (RAP). [85–87].
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 | promoter edited | TALEN / CRISPR-Cas9 | *Xanthomonas oryzae* pv. *oryzae* resistance, probably by avoiding sugar access for the pathogen growth | [86,87] |
| *Os08g0535200* *Sweet11* | Chr 8 | Q6YZF3 | promoter edited | CRISPR-Cas9 | *Xanthomonas oryzae* pv. *oryzae* resistance, probably by avoiding sugar access for the pathogen growth | [86,87] |
| *Os12g0476200* *Sweet13* | Chr 12 | Q2QR07 | promoter edited | CRISPR-Cas9 | *Xanthomonas oryzae* pv. *oryzae* resistance, probably by avoiding sugar access for the pathogen growth | [86,87] |
| Os07g0555200 *translation initiation factor 4 gamma gene (eIF4G)* | Chr 7 | B9FXV5 | Knockout and mutations on SVLFPNLAGKS | CRISPR-Cas9 | Resistance to rice tungro spherical virus (RTSV) | [91] |
| Os01g0752500, ethylene response factor 922 OsERF922, LOC_Os01g54890.1 | Chr 1 | Q5JMX7 | Knockout | CRISPR | *Magnaporthe oryzae*, Blast resistance | [90] |

resistance which results by mutation of gene eIF4G (Os07g0555200) coding a translational factor that is key in the initiation of the virus mRNA [91]. For details, see Table 3.

### 5.5. 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

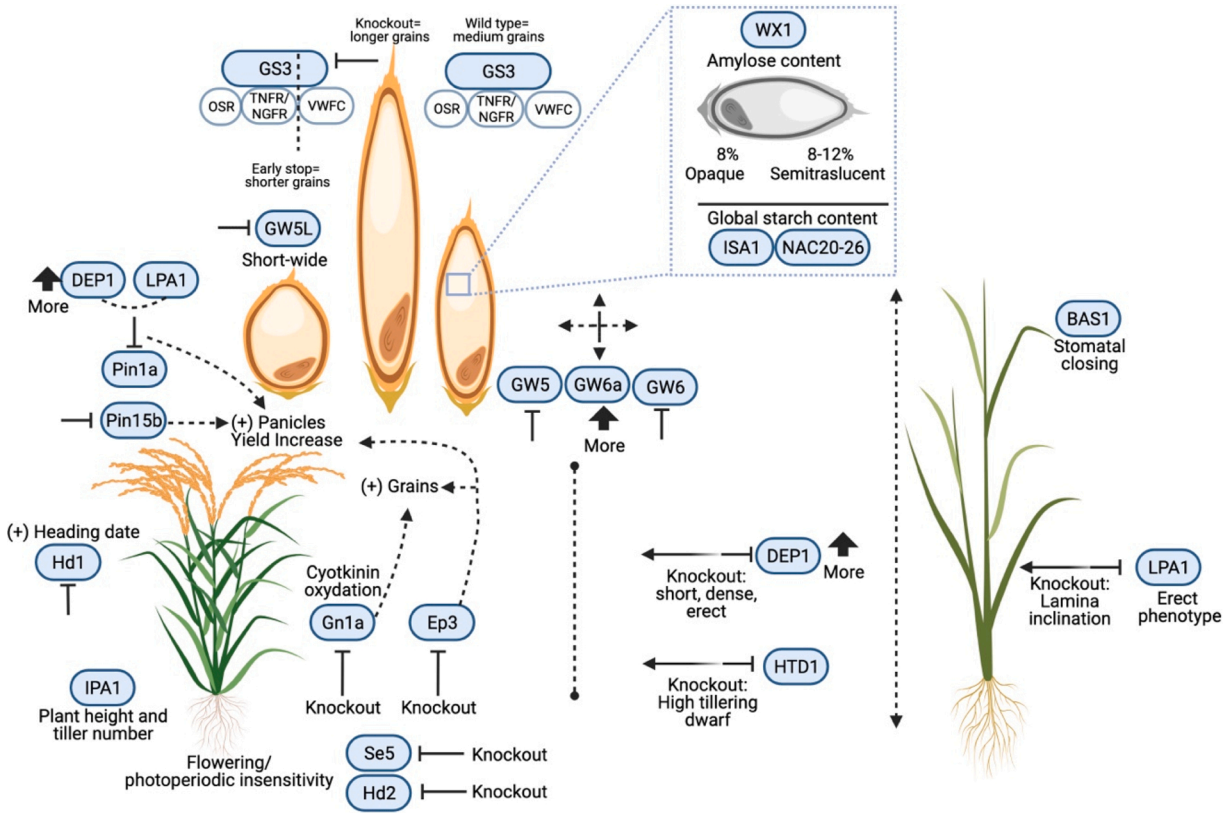


**Fig. 5.** Representation of traits such as grain number, quality, weight and plant structure and gene relationships in rice. Note that heading and flowering are positively influenced by *Se5, Hd2,* and *Hd1* knockout; structure by *DEP1, HTD1, IPA1, LPA1, Pin1a,* and *Pin15b*; grain size by *Gn1a,*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

and size, plant density, structure, panicles, and flowering have complex interactions. However, recent findings and key mutations now provide insight into their regulatory mechanisms and greater predictability in achieving the desired phenotype (for details, see Fig. 5 and Table 4).

### 5.5.1. 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) (Meiru [78,94–96,58]). The wild type allele contains all of the domains and results in medium grains [95]. 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 (Meiru [78,58]). In contrast, a mutation or deletion in the fifth exon creates a truncated protein with no VWFC domain and a short seed phenotype [95,114]. Grain size, in general, is controlled by several additional genes: higher expression of *GW6a* (*Os06g0650300*), and knockout of *GW5* (*Os05g0187500*), *GW6* (*Os06g0623700*), and *GW5L* (*Os01g0190500*) results in increased grain size [104–107,103].

### 5.5.2. 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 [92,78,94]. Another gene that correlates with increased production and downregulates cytokine level regulation is *EP3 Erect Panicle 3* (*Os02g0260200*) [94,115].

### 5.5.3. Grain starch

Grain starch quality is an essential trait, which depends on the relative content of amylose and protein. The global starch content relies on the gene *ISA1* (*Os08g0520900*) and the protein content relies on NAC20−26 (*Os01g0104500*, *Os01g0393100*) [101,102]. 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 [97–100,39].

### 5.5.4. Flowering

Flowering and photoperiodic insensitivity results from overexpression of *OsMeCP (Os12g0620400* [110] or by knocking out several genes. For example, *Se5*, *Hd2* and *Hd1* [4,94,111,112]. Another critical regulator of heading date and grain weight seems to be *HGW*, but its homozygous null mutant is embryonic lethal [113].

### 5.5.5. 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 introgressed from landraces produces short, dense, erect panicles [29,78,108].

The transcription factor *IPA1 Ideal Plant Architecture1* (*Os08g0509600*), is related to fungal resistance and yield as mention previously, and its specific mutations between bases 854 and 876 can increase the production of the transcription factor protein because they interrupt transcript cleavage due to the micro RNA OsmiR156. 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 [78.53].

The number of panicles and consequently the yield can be increased by mutating the genes *Pin1A* and *Pin15b* or indirectly blocking their

**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*<br>*Os09g0441900* | 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. | [92,93, 116] |
| *Gn1a*<br>*Os01g0197700*<br>*OsCKX2* | 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. | [78,92,94] |
| *GS3*<br>*Os03g0407400* | 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 | [94,95,96] b |
| *IPA1*<br>*Os08g0509600*<br>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. | [78,62] |
| *WX1*<br>*Os06g0133000* granule-bound *starch synthase I GBSSI, OsGBSS1, waxy* | Chr 6 | Q0DEV5 | Knockout, mutations | CRISPR/Cas9<br>P124 F, R125W<br>T178I, T178S, R158H, Y191H, R158H, G159A, D161 N, G159 K, G159A, G159E, V160 F, S415 P | 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 waxy or glutenous starch. S415 P changes phosphorylation, resulting in moderate enzyme activity and a content of amylose. | [39,97,98, 99,100] |
| *ISA1*<br>*Os08g0520900* 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. | [101] |
| *OsNAC20*<br>*Os01g0104500*<br>*OsNAC26*<br>*Os01g0393100* | Chr 1 | Q9FTY0 (*OsNAC20*)<br>Q5VNK1 *(OsNAC26)* | 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. | [102] |
| *GW5*<br>*Os05g0187500*<br>Grain Size on Chromosome 5, qSW5/GW5, GSE5 | Chr 5 | Q75KY5<br>A0A1D8GZC0 | 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. | [103] |
| *GW5L*<br>*Os01g0190500* 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. | [104] |
| *GW6a*<br>*Os06g0650300*<br>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. | [105,106] |
| *GW6*<br>*Os06g0623700*<br>*TOTAL GRAIN WEIGHT6, total grain weight6,* | 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. | [107] |
| *OsPIN5b* | Chr 8 | Q6ZIB5 | Knockout | CRISPR | Increased panicle length in the mutant. | [58] |

(*continued on next page*)

**Table 4** (*continued*)

| Gene | Position | Protein | Obtained mutation | Method | Trait details | Reference |
|---|---|---|---|---|---|---|
| *Os08g0529000* *a panicle length gene* *Hd1/ SE1* *Os06g0275000* | 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. | [94,4] |
| *HTD1* *Os04g0550600* High-Tillering Dwarf 1 | Chr 4 | Q7XU29 | Loss of function | Spontaneous mutation/ CRISPR | Landraces contain HTD1, while domesticated rice have *htd1*. The defect in HTD1 is responsible for both high-tillering and dwarf phenotypes in the htd1 mutant. Auxin induces HTD1 expression. The protein negatively regulates the outgrowth of axillary buds and is related to strigolactones biosynthesis | [108,29] |
| *LPA1* *Os03g0237250* Loose Plant Architecture1 | 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 (lpa1). lpa1 mutants might exhibit less efficient auxin flux. | [109] |
| *OsMeCP* *Os12g0620400* 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. | [110] |
| *Hd2* *Os07g0695100* 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 | [111] |
| *Ep3* *Os02g0260200* ERECT PANICLE 3, | Chr 2 | G3CKN6 | Mutation (knockout, recesive) | 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 | [60,94] |
| *Se5* *Os06g0603000* *Photosensitivity*5 | Chr 6 | Q69XJ4 | Gamma rays | *s*73 mutant | Identified in a gamma-irradiated Bahia collection, displays early flowering and photoperiodic insensitivity due to a null mutation. | [112] |
| *HGW* *Os06g0160400 heading and grain weight, heading date- and grain weight-related protein* | 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. | [113] |

expression. The indirect mechanism results in higher expression of *DEP1* and *LPA1*, which interact to suppress *PIN1a* expression [93,92,116]. *LPA1* is also important in the erect phenotype, and its mutation results in lamina inclination [109,117].

### 5.6. Other traits

Other rice traits provide value for breeding and for satisfying consumer preferences, such as nitrogen use, fragrance, oleic acid content, and color. Regarding nitrogen provision, there is a better efficiency with a higher expression of the nitrate transporter *OsNPF6.1* and the two transcription factors *OsNAC42* and *OsNLP4* [118,119]. Mutation of the *FAD2* gene results in an oleic acid increment [120,121]. Furthermore, a mutation in the *Osor* (*Os02g0651300*) gene results in potential orange-colored rice [122], and the fragrance can be increased or decreased by modulating the *BADH2* gene, which prevents the formation of the aromatic compound 2AP (2-acetyl-1-pyrroline) [94]. For details, check Table 5.

## 6. Regulatory approaches

The traits presented in this article can result from the application of conventional or new breeding techniques, such as genome editing. It is important to note that the advance in sequencing technologies allow for a detection of mutations; however, it is unfeasible to identify the specific technique or natural cause that resulted in a mutation like a single nucleotide polymorphism or a few nucleotide variations [123,129,130]. Trying to create a legal system that differentiates between genome editing and other mutagenesis approaches or natural variations represents a challenge, given that detection is not achievable under realistic circumstances [123]. It is a challenge to regulate a product that cannot be practically distinguished once in the market, but that falls under a norm that requests such a differentiation. Such a legal norm is currently applied in Europe. A supreme court resolution on case C-528/16 enforced that the genetically modified organism (GMO) norm (Directive 2001/18/EC) is applied on genome-edited plants [124]. A recent study of the European Commission delivered to the Council of the European Union in April 2021 has collected opinions from different stakeholders and concluded that "*similar products with similar risk profiles can be obtained with conventional breeding techniques, certain genome editing*

**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 | Reference |
|---|---|---|---|---|---|---|
| *FAD2* *Os02g0716500* fatty acid desaturase 2 | Chr 2 | Q6ZGW6 | Knockout | CRISPR/Cas9- RNAi | Increased oleic acid (twice) and decreased linoleic acid content. | [120, 121] |
| *Osor* *Os02g0651300* | Chr 2 | Q6H3Y3 | Knockout | CRISPR/Cas9 | β-carotene accumulation resulting in orange-colored calli. | [122] |
| *BADH2* *Os08g0424500* | 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. | [94] |
| *OsNPF6.1* *Os01g0103100* 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. | [118] |
| *OsNAC42* *Os09g0493700* 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, P51 L) | Transcription factor OsNAC42 related to the expression of the nitrate transporter OsNPF6.1. Loss of function decreased expression of nitrate transporter OsNPF6.1 | [118] |
| *OsNLP4* *Os09g0549450* transcriptional factor, Promotion of nitrogen use efficiency (NUE) | Chr 9 | A0A0P0XQL5 | Natural | Natural, HapB distributed in South China, India and South-East Asia 131 T (UTR), 181 T (UTR), 614A, 842 T, 2889C, 4662 T (UTR), 4674 T(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. | [119] |

*techniques and cisgenesis. It may not be justified to apply different levels of regulatory oversight to similar products with similar levels of risk*" [125]. An adjustment of the GMO norms should be endorsed to correspond with such a conclusion.

The legal status of a genome editing product depends on norms established at a country level based on a discriminate process to determine whether the final product is a Living Modified Organism (LMO) or not. For countries like Argentina, Australia, Colombia, Brazil, and the United States, a variety is equivalent to conventional in the absence of a foreign DNA [126,127]. For details, see Table 6.

The legal frameworks dealing with genome editing plants currently are country-specific. Still, there is some common background in the international definitions of a Living Modified Organism (LMO) given in the Cartagena Protocol on Biosafety as "*any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology*". The keyword in defining such differentiation is "*novel combination of genetic material*," which is usually explained in legal terms as the presence of foreign DNA, as described previously.

There is an international Central America norm RT 65.06.01:18 approved by Resolution 60−2019 that provides a legally binding definition on Article 4.6 for "novel combination of genetic material," currently applied in Honduras and Guatemala. The definition states in simple words, that a new combination of genetic material means a stable insertion of DNA that could not be obtained by conventional breeding or available in nature. The procedures and information requested in both countries are aligned with the international definition and are available in decree CD-008-SENASA-2019 for Honduras and 271-MAGA chapter VI for Guatemala. This legal antecedent provides a background for comparative laws within countries with norms still in discussion. The latter is interesting because the Supreme Court of Guatemala endorsed the international standard in Case Resolution 6767−2019. For details, see Table 6.

## 7. 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 varieties that farmers are currently using. It can also result in new varieties and *de novo* domestication from wild relatives and the results can be extrapolated to other crops with homologous traits. Farmers urgently require advanced breeding to respond to the challenges of climate change, consumer demands, water scarcity, nitrogen usage, and sustainable production.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data and material

Not applicable.

## Funding

This study was financed by the Project "Producción de mutantes de arroz (*Oryza sativa*) tolerantes a herbicidas utilizando rayos gamma para contribuir con el manejo sostenible del cultivo" (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; J.B. reviewed, discussed the content and edited the paper. All authors read and approved the final manuscript.

**Table 6**
Genome Editing related norms and links.

| Norm | Country | Link (Visited on May, 2021) |
|---|---|---|
| Court of Justice's judgment in Case C-528/16. | The EU | https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX:62016CJ0528 |
| EC study on new genomic techniques | The EU | https://ec.europa.eu/food/plant/gmo/modern_biotech/new-genomic-techniques_en |
| Food Hygiene Handling Procedures for Food and Additives Derived from Genome Editing Technology | Japan | https://www.mhlw.go.jp/content/000550824.pdf |
| RESOL-2021-21-APN-SABYDR#MAGYP | Argentine | https://www.boletinoficial.gob.ar/detalleAviso/primera/240529/20210208 |
| Resolution 00029299 | Colombia | https://www.ica.gov.co/getattachment/2d02cc52-d1c5-4123-8a5a-aea9ad2ce926/2018R29299.aspx |
| Secure | USA | https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/biotech-rule-revision/secure-rule/secure-about/ |
| Resolution CTNBio-No 16 | Brasil | http://ctnbio.mctic.gov.br/en/resolucoes-normativas/-/asset_publisher/OgW431Rs9dQ6/content/resolucao-normativa-nº-16-de-15-de-janeiro-de-2018 |
| Applicability of Resolution N° 1.523/2001 | Chile | http://www.sag.cl/ambitos-de-accion/aplicabilidad-de-resolucion-ndeg-15232001-en-material-de-propagacion-desarrollado-por-nuevas-tecnicas-de-fitomejoramiento |
| Resolution 20565-2019 | Paraguay | https://conbio.mag.gov.py/media/ckfinder/files/Resolucion%20565%20de%202019.pdf |
| Resolution 60-2019, approving RT 65.06.01:18 | Centralamerica GT-HN | https://www.sieca.int/index.php/download/resolucion-no-60-2019-aprueba-rt-65-06-0118-bioseguridad-de-organismos-vivos-para-uso-agropecuario/ |
| 271-MAGA | Guatemala | https://visar.maga.gob.gt/visar/2019/20/MANPROCT.pdf |
| CD-SENASA-008-2019 | Honduras | http://senasa.gob.hn/images/ACD/2019/ACUERDO-CD-SENASA-008-2019%20GACETA%2035047.PDF |
| Supreme Court Resolution 6767-2019 | Guatemala | http://138.94.255.164/Sentencias/846825.6767-2019.pdf |
| WTO- G/SPS/GEN/1658/Rev.3<br>WTO International Statement on Agricultural Applications of Precision Biotechnology<br>WTO- G/SPS/GEN/1699 | Argentina, Australia, Brazil, Canada, the Dominican Republic, Guatemala, Honduras, Paraguay, the United States of America and Uruguay. | https://docs.wto.org/dol2fe/Pages/SS/directdoc.aspx?filename=q:/G/SPS/GEN1658R3.pdf |
| South America Ministries of Agriculture | Argentina, Brazil, Chile, Paraguay and Uruguay | https://docs.wto.org/dol2fe/Pages/SS/directdoc.aspx?filename=q:/G/SPS/GEN1699.pdf |

## Declaration of Competing Interest

The authors report no declarations of interest.

## Acknowledgments

Not applicable.

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