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

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

Gene	Position	Protein	Obtained mutation	Method	Trait details	Reference
OsRR22 *Os06g0183100*	Chr 6	Q5SML5	Knockout	CRISPR/Cas9	Two-component response regulator ORR22. Salt tolerance 0.75% NaCl.	(Zhang et al., 2019a)
STL1 *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.	(Yuan et al., 2020)
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.	(Liu et al., 2020d)
Os03g0786400 *OsDST, DLN102, OsDLN102, Negative regulation of response to salt stress*	Chr 3	Q10CE2	Knockdown	Mutant/ CRISPR/Cas9	Knockdown improved the tolerance to stress, as also observed in the dst mutant. C2H2 zinc finger transcription factor, drought and salt tolerance, stomatal aperture control	(Cui et al., 2015; Santosh Kumar et al., 2020)
P5C *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.	(Sripinyowanich et al., 2013)
SKC1 *Os01g0307500* *OsHKT1;5, OsHKT8*	Chr 1	Q0JNB6	Wild relatives	None	Variant V395 (is salt tolerant), while L395 is sensitive.	(Jayabalan et al., 2019)
Os10g0521000 *Based on Z.mays GRMZM2G162690 and A. thaliana AT4G24040*	Chr 10	Q9FWC1	Substitution S163T	CRISPR/Cas9	Mutation of domain WDS to replicate *Selaginella moellendoffii* WDT. The enzyme may be less efficient in allowing the accumulation of trehalose.	(Nuñez-Muñoz et al., 2021)
OsEPFL9 *Os01g0824500* Epidermal Patterning Factor Like-9	Chr 1	Q5JN76	Knockout	CRISPR/Cpf1	Increased water use efficiency under stress because of reduced stomatal count	(Yin et al., 2017, 2019)
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	(Wang et al., 2018a)
RCS1 *Os12g0625000*	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.	(Sun et al., 2021)

O-acetylserine (thiol) lyase, Cysteine synthase. *arsenite tolerant 1*						
OsNramp5 Os07g0257200 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)
OsNramp1 Os07g0258400 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)
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.	(Liu et al., 2020a)
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.	(Zeng et al., 2020)

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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	L. Liu et al., 2021)
ACCase2 *Os05g0295300*	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 µM *in vitro*.	(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)
psbA *AAS46167* (Photosystem II protein D1, psbA)	Chloroplast	P0C434	S264G	Wild radish, Spontaneous mutation-	Atrazine > 50-fold (4000(187 g a.i. ha-1 atrazine), (S) Bromoxynil	(Lu et al., 2019)
HPPD *Os02g0280700* Inhibitor Sensitive 1	Chr 2	Fe(II)/2-oxoglutarate–dependent oxygenase	28-bp deletion allele (his1).	wild Nipponbare lacked deletion (HIS1)	b-Triketone herbicides, HIS1detoxifies b-triketone herbicides by hydroxylation.	(Maeda et al., 2019)
AHAS, ALS *Os02g0510200* 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)
OsEPSPS *Os06g0133900*	Chr 6	A0A0N7KLH2	T169I A170V P173S	CRISPR-Prime Editing	NA	(Li et al., 2020a)
			T102I + P106S	CRISPR	*In vitro* 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, 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	(Oliva et al., 2019; Varshney et al., 2019)
Os08g0535200 Sweet11	Chr 8	Q6YZF3	promoter edited	CRISPR-Cas9	*Xanthomonas oryzae* pv. *oryzae* resistance, probably by avoiding sugar access for the pathogen growth	(Oliva et al., 2019; Varshney et al., 2019)
Os12g0476200 Sweet13	Chr 12	Q2QR07	promoter edited	CRISPR-Cas9	*Xanthomonas oryzae* pv. *oryzae* resistance, probably by avoiding sugar access for the pathogen growth	(Oliva et al., 2019; Varshney et al., 2019)
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)	(Macovei et al., 2018)
Os01g0752500, ethylene response factor 922 OsERF922, LOC_Os01g54890.1	Chr 1	Q5JMX7	Knockout	CRISPR	*Magnaporthe oryzae*, Blast resistance	(Wang et al., 2016)

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

Gene	Position	Protein	Obtained mutation	Method	Trait details	Reference
OsDEP1 *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.	(Huang et al., 2018; Fu et al., 2019; Miao Liu et al., 2020)
Gn1a *Os01g0197700* *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.	(Li et al., 2016a; Shen et al., 2017; Huang et al., 2018)
GS3 *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	(Takano-Kai et al., 2013; Shen et al., 2017; Yang et al., 2019b)
IPA1 *Os08g0509600* 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)
WX1 *Os06g0133000* granule-bound *starch synthase I GBSSI, OsGBSS1, waxy*	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.	
ISA1 *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.	(Shufen et al., 2019)
OsNAC20 *Os01g0104500* *OsNAC26* *Os01g0393100*	Chr 1	Q9FTY0 (*OsNAC20*) 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.	(Wang et al., 2020)
GW5 *Os05g0187500* Grain Size on Chromosome 5 , qSW5/GW5, GSE5	Chr 5	Q75KY5 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.	(Zhang et al., 2020)
GW5L *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.	(Tian et al., 2019)
GW6a *Os06g0650300* 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)
GW6 *Os06g0623700* *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.	(Ishimaru et al., 2013)
OsPIN5b *Os08g0529000* *a panicle length gene*	Chr 8	Q6ZIB5	Knockout	CRISPR	Increased panicle length in the mutant.	(Zeng et al., 2020)
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.	(Shen et al., 2017; Tanaka et al., 2020)

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	(Zou et al., 2006; Lacchini et al., 2020)
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.	(Liu et al., 2016a)
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.	(Qu et al., 2021)
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	(Gao et al., 2014)
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	(Li et al., 2011a; Shen et al., 2017)
Se5 *Os06g0603000* *Photosensitivity*5	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)
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.	(Li et al., 2012)

Table 5. Rice genes and mutations in traits such as oleic acid, color, fragrancy, and nitrogen use.

Gene	Position	Protein	Obtained Mutation	Method	Trait details	Reference
FAD2 Os02g0716500 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)
Osor Os02g0651300	Chr 2	Q6H3Y3	Knockout	CRISPR/Cas9	β-carotene accumulation resulting in orange-colored calli.	(Endo et al., 2019)
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.	(Shen et al., 2017)
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.	(Tang et al., 2019)
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, 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)
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 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)

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 WTO International Statement on Agricultural Applications of Precision Biotechnology	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
WTO- G/SPS/GEN/1699 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