Potential of Genome Editing to Capture Diversity From Australian Wild Rice Relatives

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Rice, a staple food worldwide and a model crop, could benefit from the introduction of novel genetics from wild relatives. Wild rice in the AA genome group closely related to domesticated rice is found across the tropical world. Due to their locality outside the range of domesticated rice, Australian wild rice populations are a potential source of unique traits for rice breeding. These rice species provide a diverse gene pool for improvement that could be utilized for desirable traits such as stress resistance, disease tolerance, and nutritional qualities. However, they remain poorly characterized. The CRISPR/Cas system has revolutionized gene editing and has improved our understanding of gene functions. Coupled with the increasing availability of genomic information on the species, genes in Australian wild rice could be modified through genome editing technologies to produce new domesticates. Alternatively, beneficial alleles from these rice species could be incorporated into cultivated rice to improve critical traits. Here, we summarize the beneficial traits in Australian wild rice, the available genomic information and the potential of gene editing to discover and understand the functions of novel alleles. Moreover, we discuss the potential domestication of these wild rice species for health and economic benefits to rice production globally.

Keywords: gene editing, australian wild rice, CRISPR-Cas9, genetic diversity, novel alleles

INTRODUCTION

The CRISPR-Cas system has quickly gained popularity as a strong and widely used tool for genome editing as compared to traditional inefficient and laborious random mutagenesis and screening methods (Ma X. et al., 2015; McCarty et al., 2020). The introduction of genome edits, like substitutions, insertions, and deletions, using the CRISPR-Cas 9 system, can speed up the breeding of plants including rice (Romero and Gatica-Arias, 2019). Australian wild rice represents an untapped source of important alleles that are missing from the rice gene pool (Henry et al., 2010). To ensure rice food security, it is necessary to increase productivity which relies on continuous genetic improvement (Brar and Khush, 2018; Henry, 2019). The wild rice species have higher drought, salinity, lodging, disease, and insect resistance than the most tolerant or resistant rice genotype. Additionally, they have unique traits such as acid soil tolerance, shade tolerance, high micronutrient content and are not only known to tolerate biotic and abiotic stress but also to exhibit extraordinary growth and development traits, such as profuse tillering and the existence of a salt gland that might be transferred to cultivated rice, increasing production and profitability (Henry, 2018; Moner and Henry, 2018).
The primary gene pool of rice comprises the *Oryza* A-genome species that are easily interfertile with rice (Wambugu et al., 2015). Previous research indicates two separate and unique perennial wild populations in tropical Australia (Brozynska et al., 2017; Moner et al., 2020), an *O. rufipogon*-like population, that has been referred to as Taxa-A, and *O. meridionalis*, including both perennial and annual forms and sometimes, referred to as Taxa-B. Genome analysis suggests that the *O. meridionalis* populations diverged from the lineage that became *O. sativa* approximately 3 Mya, while the Australian *O. rufipogon* like populations diverged approximately 1.6 Mya. The phylogenetic relationships between these species have been studied using both chloroplast and nuclear genome sequences (Wambugu et al., 2015; Brozynska et al., 2017). Taxa A (*O. rufipogon*-like taxa) has a chloroplast that is more similar to that of *O. meridionalis* and a nuclear genome that is more similar to that of *O. rufipogon* from Asia (Brozynska et al., 2017). A recent analysis of these taxa has confirmed that there is ongoing reticulate evolution, with rare hybrid plants being found in the wild (Hasan et al., 2022). *O. meridionalis* is the most distant species in the AA genome group that includes domesticated rice making it a significant resource for improving rice and studying rice evolution. In addition to being a source of slowly digestible starch and higher amylose content, its photosynthetic traits and abiotic stress tolerance make it an excellent candidate for use in the rice improvement (Scafaro et al., 2009; Tikapunya et al., 2017). Until recently, Australian wild rice was generally undisturbed by the impact of rice domestication, resulting in the persistence of wild Oryza in vast populations across a large area of northern Australia (Henry et al., 2010). These Australian Oryza may be critical in adapting rice to rapidly changing climate conditions and altering consumer preferences and needs. Moreover, recent data reveals that, even though the rice was first domesticated in Asia, Australian wild rice populations have
provided genes to the domestication of rice (Huang et al., 2012; Fujino et al., 2019).

Seed shattering is a significant drawback affecting yield loss in both taxa of Australian wild rice. Gene editing using CRISPR-Cas to induce loss of function in shattering genes could allow rapid production of potentially new wild rice cultivars (Bohra et al., 2021). Advancement in genome and transcriptome sequencing has been a major contributor to improving gene target identification. The genomes of many wild rice species have been sequenced allowing the discovery of the genes responsible for desirable characteristics. The availability of these genetic resources is highly beneficial in supporting molecular breeding by horizontal transfer of key traits from wild species to cultivated rice.

In this review, we will discuss genome editing and how it has been used to capture diversity in rice (Figure 1). Furthermore, we will discuss how the function of novel alleles have been identified in domesticated rice using CRISPR/cas9 and how these studies can guide the identification of useful alleles in wild rice (especially in the Australian species) with the potential of being used in rice breeding.

TABLE 1 | Summary of gene edited traits in rice.

| Gene Effect of Gene on plant | Genome-editing system | References |
|-----------------------------|-----------------------|------------|
| DST Salinity tolerance, osmotic tolerance | CRISPR-Cas9 | Santosh Kumar et al. (2020) |
| OsFWL4 Grain yield, plant architecture, number of tillers, flag leaf area, grain length | CRISPR-Cas9 | Gao et al. (2020) |
| BADH2 Enhanced fragrance | CRISPR-Cas9 | Ashokkumar et al. (2020) |
| OsSPL16/qGW8 Grain yield, grain weight, grain size | CRISPR-Cas9 | Usman et al. (2020a) |
| OsBADH2 Grain yield, grain size, aroma (2-acetyl-1-pyrroline (2AP) content) | CRISPR-Cas9 | Usman et al. (2020b) |
| OsWaxy Decrease in amylose content (glutinous rice) | CRISPR-Cas9 | Hu et al. (2020) |
| OsMYB30 Cold tolerance | CRISPR-Cas9 | Zeng et al. (2019) |
| OsALS confers herbicide resistance | Base Editor and CRISPR-Cas9 | Li et al. (2019a) |
| OsSPL14 gene for ideal plan architecture | Base Editor | Hua et al. (2019) |
| BBM1 enables embryo formation from a fertilized egg | CRISPR-Cas9 | Wang et al. (2019a) |
| REC8, PAIR, OSD1, and MTL confers resistance to splicing inhibitors | CRISPR-Cas9 | Butt et al. (2019) |
| SF3B1 | CRISPR-Cas9 | Hu et al. (2019) |
| SD1 Grain yield, plant architecture, semi-dwarf plants, resistance to lodging | CRISPR-Cas9 | Shen et al. (2018) |
| Gn1a, GS3 Grain yield, panicle architecture, number of grains per panicle, grain size | CRISPR-Cas9 | Macovei et al. (2018) |
| eF4G Rice tungro spherical virus (RTSV) | CRISPR-Cas9 | Zhao et al. (2018) |
| GS9, DEP1 Slender grain shape, less chalkiness | CRISPR-Cas9 | Li et al. (2018a) |
| OsPDS and OsSBEIIB encode phytoene desaturase and starch branching enzyme | CRISPR-Cas9 | Zong et al. (2018) |
| OSCDC48 regulates senescence and cell death | Base Editor (C-to-T substitution) | Macovei et al. (2018) |
| eF4G candidate rice tungro disease resistance gene | CRISPR-Cas9 | Shen et al. (2018) |
| Gn1a, GS3 grain yield | CRISPR-Cas9 | Shen et al. (2018) |
| Gn1a, DEP1 control plant growth and stress responses | CRISPR-Cas9 | Miao et al. (2018) |
| PYL1, PYL4, PYL6 converts oleic acid into linoleic acid | CRISPR-Cas9 | Abe et al. (2018) |
| OsFA2-1 Grain yield, plant architecture, semi-dwarf plants, reduced, gibberellins and flag leaf length | CRISPR-Cas9 | Shen et al. (2018) |
| OsAnn3 Response to cold tolerance | CRISPR-Cas9 | Shen et al. (2017) |
| OsSAPK2 Reduced drought, salinity, and osmotic stress, tolerance; role of gene in ROS scavenging | CRISPR-Cas9 | Lou et al. (2017) |
| SBE1, SBEIIB control amylose contents | CRISPR-Cas9 | Sun et al. (2017) |
| OsNramp5 metal transporter gene | CRISPR-Cas9 | Lou et al. (2017) |
| SAPK2 functions in ABA-mediated seed dormancy | CRISPR-Cas9 | Lou et al. (2017) |
| GW2, 5 and 6 Grain yield, grain weight | CRISPR-Cas9 | Xu et al. (2016) |
| GW2/GW5/TGW6 Increased grain length and width | CRISPR-Cas9 | Xu et al. (2016) |
| OsERF922 responsible for rice blast resistance | CRISPR-Cas9 | Wang et al. (2016) |
| Badh2 control rice fragrance | CRISPR-Cas9 | Shan et al. (2015) |
| LOXi affect seed storability | TALEN-based genome editing | Ma et al. (2015a) |
| OsSWEET13 bacterial blight susceptibility genes | CRISPR-Cas9 | Zhou et al. (2015) |
| ROC5, SPP, YSA Disruption results in abino phenotype | CRISPR-Cas9 | Feng et al. (2013) |
| OsSWEET14 bacterial blight susceptibility genes | CRISPR-Cas9 | Jiang et al. (2013) |
Recent Advances in Editing Technology

The CRISPR-Cas9 system is mainly confined to genome editing at canonical NGG protospecator adjacent motif (PAM) sites. These sites are extremely important for nuclease identification, cleavage and efficient editing. Cas9 orthologs with changed PAM specificities have been discovered such as SaCas9 (Staphylococcus aureus) and Cas9-VQR (D1135V/R1335Q/T1337R) (Kleinistiver et al., 2015; Hu et al., 2016). Cas9-VQR has been designed to cleave the sites containing a NGA PAM, however its editing efficiency was found to be insufficient in rice. To boost the VQR variant’s editing efficiency, the sgRNA structure was changed and significantly increased the editing efficiency (Hu et al., 2018). The CRISPR-SaCas9 toolkit was recently refined in rice by adding three important mutations (E782K/N968K/R105H) to improve the editing efficiency (Qin et al., 2019; Zafar et al., 2020). The editing efficiency of SaCas9 in the PDS and DL genes was determined via Agrobacterium-mediated transformation of Japonica rice. After mutagenesis, 34 out of 53 lines (64.2%) and 28 out of 36 (77.8%) lines had targeted mutations in the PDS/T1 and DL/T1 areas, respectively (Qin et al., 2019).

Cas9 with extended PAM SpCas9 (xCas9) and Cas9-NG (Cas9-NG) have also been tried in rice (Zhong et al., 2019) with xCas9 technology showing a better outcome in the rice genome editing (Wang J. et al., 2019; Endo et al., 2019). These enzymes can detect NG and GAA PAMs. The Cas9-NG also detects non-canonical PAM sites such as NCGGA and NG in addition to NCG (Ren et al., 2019; Zhong et al., 2019). These findings have broadened the breadth of rice genome editing.

Base editing is a novel approach to genome editing that enables irreversible base alterations at target loci without the use of double-stranded breaks or homology guided repair (Hua et al., 2019). The combination of Cas9 nickase and cytidine deaminase enzymes allows for the creation of C to T or G to A substitutions anywhere in the genome (Komor et al., 2016; Mishra et al., 2018). For instance, the substitution of C to T in the OsALS gene resulted in an amino acid change at position 96 from alanine to valine conferred herbicide tolerance in Oryza sativa L (cv. Nipponbare) (Sun et al., 2016; Shimatani et al., 2017) (Table 1).

Applications of Gene Editing to Wild Rice Relatives

CRISPR-Cas technology allows for rapid de novo domestication of wild plant relatives. Traditional domestication requires considerable cross-breeding and selection of naturally occurring genetic alterations. Groundcherry (Physalis pruinosa) and wild tomato were recently de novo domesticated by utilizing genome editing (Li T. et al., 2018; Lemmon et al., 2018; Zhu and Zhu, 2021). Yu et al., 2021 outlined a de novo domestication strategy for Oryza alta, an allotetraploid rice with high biomass that is widely adapted to the environment (Yu et al., 2021). Yu et al., 2021 knocked out genes associated with seed shattering and awn length (qSH1 and An-1 orthologues), resulting in a considerably lower seed shattering rate and shorter awn length. To improve additional traits, they edited several orthologues of rice genes semi-dwarf stature (SD1), grain

environment. The small size of the rice genome, high efficiency of transformation, abundance of genetic resources, and genomic synteny with other cereals provides an excellent model system for the study of functional genomics. In recent years, rice has been used to evaluate the efficacy of several genome editing methods, as well as to explore gene functions and their potential in the rice improvement (Li et al., 2012; Feng et al., 2013; Zafar et al., 2020) as briefly discussed below and highlighted in Table 1. CRISPR/Cas9-mediated editing of the bsr-k1 gene produced higher-yielding rice plants resistant to leaf blast and bacterial leaf blight (Zhou et al., 2018). When Bsr-d1, Pi21, and ERP922 were mutated using CRISPER/Cas9 in all single and triple mutants of TGMS rice line (Indica thermosensitive genic male sterile) and longke638S (LK638S) were more resistant to rice blast than the wild type (Zhou et al., 2021). To find new sources of RTD (rice tungro disease) resistance, a CRISPR/Cas9 system was used to create mutations in the elf4G gene in the RTSV-susceptible variety IR64, which is grown all over tropical Asia. elf4G alleles with mutations in the SVFLPNLAGKS (mostly NL) close to the YVV residues were the only ones that were identified resistant (Macovei et al., 2018). Overexpression of OsAAP3 in transgenic plants resulted in reduced bud outgrowth and rice tillering while OsAAP3 RNAi slightly reduced the transport of amino acids, with lower concentrations of Arg, Lys, Asp, and Thr, but increased the number of bud outgrowth, tillers, grain production, and nitrogen usage efficiency (NUE). OsAAP3 promoter sequences differed in Japonica and Indica rice, and expression was higher in Japonica, which had fewer tillers. CRISPR technology was used to create OsAAP3 knockout lines in Japonica ZH11 and KY131 resulting in an increased grain yield (Lu et al., 2018).
length and size (GS3), heading date (Ghd7, DTH7), and ideal plant architecture (IPA1) in O. alta. This remarkable study introduced a new era of rapid domestication of crops with desired traits by applying precise genome editing technologies. To domesticate a wild crop relative, it must have a well-sequenced genome and be amenable to tissue culture and transformation. The capacity to induce callus and regenerate plantlets is frequently a bottleneck to build a plant genetic transformation system. Only a few plant species, including a few Oryza sativa cultivars, have adequate and robust transformation procedures, several hurdles remain in applying genome editing to rice wild relatives.

AUSTRALIAN WILD RICE

Henry et al., 2010 reported four Australian wild relatives Oryza rufipogon like population (Taxa-A), Oryza meridionalis like population (Taxa-B), Oryza officinalis, and Oryza australiensis (Henry et al., 2010; Brozynska et al., 2017). The characterization of unique wild rice species in Australia, via genetic and morphological investigation, has led to the discovery of novel Oryza gene pools (Waters et al., 2012; Sotowa et al., 2013; Brozynska et al., 2014). The AA genome species of most interest have been described above but the much more divergent O. australiensis is also of potential value in rice improvement. Oryza australiensis, the only known member of the E genome in the genus Oryza has unique characteristics such as an underground rhizome that a prospective source of novel genes for rice development because it allows plants to survive during the dry season (Henry et al., 2010). The relationships of Oryza australiensis with other species in the Oryza genus suggested that it may be useful in understanding the evolution of the Oryza genus. Oryza australiensis has a large and poorly characterised, with a high proportion of repeated sequences, making it challenging to study (Henry, 2018). In addition, the species shows outstanding grain properties, which suggests that it might potentially be used as a crop if domesticated (Tikapunya et al., 2016).

Genomic sequencing of these novel Australian wild rice species has been reported (Brozynska et al., 2017) but improved genome sequences are required to facilitate genome editing of rice to transfer their desirable traits.

Potential Applications to the Domestication of Australian Wild Rice

Population growth and climate change threaten global agriculture productivity. To feed 10 billion people by 2050 is a massive challenge. To meet the world's food needs and increase crop yields quickly, existing methods of domesticating crops are insufficient. Together with a deeper understanding of domestication's genetic foundation, provided by pangenomes, recent advancements in gene editing technologies open the intriguing probability of developing novel crops by modifying few genes in wild species. Using a new platform for domestication, it may be possible to convert crop wild relatives quickly and precisely into economically desirable crops while keeping some of the beneficial resilience and nutritional properties that have been lost during domestication and breeding.

Australian wild rice has many unique and novel traits that can feed the future population. Australian wild rice domestication can potentially be achieved by following and optimizing the de novo route highlighted by Li's group; the development of a high-performance transformation system, putting together and annotating a high-quality reference genome, and editing several genes that are important for domestication, e.g., shattering, awn length, panicle architecture and nutritional benefits to improve a variety of features. In this way, genome engineering might be used to generate nutritionally and climate-smart crops from the start in a wide range of crops currently used for human consumption, food production, animal feed, or biofuel.

FUTURE PROSPECTS

Traditionally, domestication of wild plants into commercial crops took hundreds or even thousands of years, but newly emerging genome editing technologies enable this to be accomplished in a few generations (Van Tassel et al., 2020). As a result, effective genome editing techniques are critical for accelerating the speed of domestication. Only the O. sativa subspecies japonica and indica have been successfully transformed using Agrobacterium-mediated transformation systems (Hiei et al., 1994). To determine the most promising starting material, priority must
be given to callus induction and regeneration capacities with suitable biomass traits and stress tolerance etc. During the domestication, traits that were good for farming instead of natural growth were chosen and improved, such as grain size, hull colour, erect growth, shattering, pericarp colour and awn etc (Chen et al., 2019). Many traits of Australian wild rice species are similar to those of the wild ancestors of the present cultivars because they are closely related. Identifying the wild rice homologs of the domestication-related genes from domesticated rice is the first step, for example qSH1 gene homolog for seed shattering, Bh4 homolog gene for hull colour, An-1 and An-2 for awn length, Rc for pericarp colour, OsLG1 for panicle shape, and GW5 for grain width. Editing these homologs genes by utilising a CRISPR/Cas9-mutagenesis technique may genuinely achieve quick domestication of Australian wild rice.

Most crop improvements have involved targeted editing and transformation, which require the efficient transformation and precise large-scale genome editing system. For example, RNA viral vectors, may infect plants and deliver gene-editing reagents to the germline, inducing hundreds to thousands of different mutations. Using developmental regulators, altered somatic cells can generate meristems that produce seed-bearing branches, boosting productivity and minimizing timeframes (Nasti and Voytas, 2021). These and other techniques will allow faster breeding, domestication of Australian wild rice, and metabolic reengineering than previously conceivable. So, developing an efficient transformation and genome editing system for Australian wild rice is very important.

Furthermore, Australian wild rices have beneficial traits including biotic and abiotic stress tolerance that can be used in breeding programs for improved yield. Studies on the loss or gain of function of the genes associated with these traits need to be conducted to definitively understand their mechanisms and potentially edit them into cultivated rice varieties.

### AUTHOR CONTRIBUTIONS

MA write the draft, PO read carefully and give suggestions, AF technically helps and give the suggestions, RH give the outlines and idea of this review and technically improved with many revisions.

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### TABLE 2 | List of the key biotic stress resistance genes and QTLs identified within wild rice species.

| Genes/QTLs | Marker | Inheritance | Wild species | References |
|------------|--------|-------------|--------------|------------|
| Bacterial blight | xa45(t) | LOC_Os08g42410 (STS) | Recessive | O. glaberrima | Neelam et al. (2020) |
| | x32 | RM6293 and RM5926 | Recessive | O. australiensis | Zheng et al. (2009) |
| | Xa27 | M964 and M1197 | Dominant and cloned | O. minuta | Gu et al. (2004) |
| | Xa30 | RM1341, V88, G 189, 03STS | Dominant | O. rufipogon | Xuwei et al. (2007) |
| | qBBRS | RM7081–RM3616 5 | Dominant | O. meyeriana | Chen et al. (2012) |
| Rice blast | P169(t) | STS69-15-STS69-7 and RM20676 | Dominant | O. glaberrima | Dong et al. (2020) |
| | qShB6 | RM3431 | Dominant | O. nivara | Eizenga et al. (2013) |
| | qSh7 | RM27892 and RM28093 | Dominant | O. longistaminata | Xu et al. (2015) |
| | qBLAST8 | RM1148–RM210 | Dominant | O. nivara | Eizenga et al. (2013) |
| | P164rh | P164rh Specific primer 625 bp | Dominant and cloned | O. rhizomatis | Das et al. (2012) |
| | P168 | SNMP5 and RM14738 | Dominant and cloned | O. glumaepatula | Devi et al. (2020) |
| Brown Planthopper (BPH) | Bph18 | BIM3-BN162 | Dominant and cloned | O. australiensis | Ji et al. (2018) |
| | qBph1-2 | RM291-XO4-27 | Dominant and cloned | O. australiensis | Hu et al. (2015) |
| | Bph14 | SM1-G1318 | Dominant and cloned | O. officinalis | Du et al. (2009) |
| | Wbph9 | R288-S11182 | Dominant | O. officinalis | Tan et al. (2004) |
| | bph20(t) | BYL7-BYL8 | Recessive | O. rufipogon | Yang et al. (2011) |
| | Bph21(t) | RM222-RM244 | Dominant | O. rufipogon | Yang et al. (2011) |
| | bph22(t) | RM8212-RM261 | Recessive | O. rufipogon | Hou et al. (2011) |
| | bph23(t) | RM2655-RM3572 | Recessive | O. rufipogon | Hou et al. (2011) |
| | Bph27 | RM1848-RM18853 | Dominant | O. rufipogon | Huang et al. (2013) |
| | Bph36 | RM18485-RM18502 | Dominant | O. rufipogon | Li et al. (2019b) |
| | Bph38 | RM16563-RM16763 | Dominant | O. rufipogon | Yang et al. (2020) |

Italic value for scientific name and genes.
Abdullah et al. Australia Wild Rice Genome Editing

Tang, L., Mao, B., Li, Y., Lv, Q., Zhang, L., Chen, C., et al. (2017). Knockout of OsNrm5p Using the CRISPR/Cas9 System Produces Low Cd-Accumulating Indica rice without Compromising Yield. Sci. Rep. 7 (1), 14438. doi:10.1038/s41598-017-14832-9

Tikapunya, T., Fox, G., Furtado, A., and Henry, R. (2016). Grain Physical Characteristic of the Australian Wild Rices. Plant Genet. Resour. 15 (5), 409–420. doi:10.1111/pjgr.12115

Usman, B., Nawaz, G., Zhao, N., Liao, S., Qin, B., Liu, F., et al. (2020a). OsPIN5b, GS3, and OsMYB30 with the CRISPR-Cas9 System. Front. Plant Sci. 11, 711. doi:10.3389/fpls.2020.00709

Wang, C., Liu, Q., Shen, Y., Hua, Y., Wang, J., Lin, J., et al. (2019a). Clonal Seeds of Enhance Rice. Proc. Natl. Acad. Sci. U.S.A. 116 (1), 1027–1036. doi:10.1073/pnas.1614877115

Zhu, Y., Xu, H., Xue, X., Shi, J., Wang, X., et al. (2019b). OsAs9 Expands the Scope of Genome Editing with Reduced Efficiency in rice. Plant Biotechnol. J. 17 (4), 709–711. doi:10.1111/plj.13053

Waters, D. L. E., Nock, C. J., Ishikawa, R., Rice, N., and Henry, R. J. (2012). Chloroplast Genome Sequence Confirms Distinctiveness of Australian and Asian Wild rice. Ecol. Evol. 2 (1), 211–217. doi:10.1002/ece3.66

Xu, P., Dong, L., Zhou, J., Li, J., Zhang, Y., Hu, F., et al. (2015). Identification and Mapping of a Novel Blast Resistance Gene P35(t) in OsPm27. Mol. Genet. Genomics 289 (3), 329–337. doi:10.1007/s00438-014-3995-7

Xu, R., Yang, Y., Qin, R., Li, H., Qiu, C., Li, L., et al. (2016). Rapid Improvement of Grain Weight via Highly Efficient CRISPR/Cas9-mediated Multiplex Genome Editing in rice. J. Genet. Genomics 43 (8), 529–532. doi:10.1016/j.jgg.2016.07.003

Xuwei, J., Chunlian, W., and Qing, Y. (2007). Breeding of Near-isogenic Line 9534. China J. Crop Breed. 28 (4), 217–223. doi:10.1007/s11800-007-1025-8

Yang, L., Li, R., Li, Y., Huang, F. K., Chen, Y. Z., Huang, S. S., et al. (2011). Genetic Mapping of Bph20(t) and Bph21(t) Loci Conferring Brown Planthopper Resistance to Nilaparvata Lugens Stål in rice (Oryza Sativa L.). Euphytica 183 (2), 161–171. doi:10.1007/s10681-011-0437-7

Zafar, K., Seedek, K. E. M., Rao, G. S., Khan, M. Z., Amin, I., Kamel, R., et al. (2020). Genome Editing Technologies for Rice Improvement: Progress, Prospects, and Safety Concerns. Front. Genom. Ed. 2, 5. doi:10.3389/fgede.2020.00005

Zeng, Y., Wen, J., Zhao, W., Wang, Q., and Huang, W. (2019). Rational Improvement of Rice Yield and Cold Tolerance by Editing the Three Genes OsPnIN5b, GS3, and OsMYB30 with the CRISPR-Cas9 System. Front. Plant Sci. 10, 1663. doi:10.3389/fpls.2019.01663

Zhao, D.-S., Li, Q.-F., Zhang, C.-Q., Zhang, C., Yang, Q.-Q., Pan, L.-X., et al. (2018). Cas9 as a Transcriptional Activator to Regulate rice Grain Shape and Appearance Quality. Nat. Commun. 9 (1), 1240. doi:10.1038/s41467-018-0243-1

Zheng, C.-K., Wang, C.-L., Yu, Y.-J., Liang, Y.-T., and Zhao, K.-J. (2009). Identification and Molecular Mapping of Xa32(t), a Novel Resistance Gene for Bacterial Blight (Xanthomonas Oryzae Pv. Oryzae) in rice. Acta Agronomica Sinica 35 (7), 1173–1180. doi:10.1007/s11785-008-0898-9

Zhong, Z., Sretenovic, S., Ren, Q., Yang, L., Bao, Y., Qi, C., et al. (2019). Improving Plant Genome Editing with High-Fidelity xCas9 and Non-canonical PAM-Targeting Cas9-NG. Mol. Plant 12 (7), 1027–1036. doi:10.1093/mp/yzz080