Rice Genomics and Genetics 2025, Vol.16, No.6, 312-320
http://cropscipublisher.com/index.php/rgg

Research Insight Open Access

Precise Base Editing of Grain Quality Genes in Elite Rice Cultivars

Xinguang Cai, Yaodong Liu
Modern Agricultural Research Center, Cuixi Academy of Biotechnology, Zhuji, 311800, Zhejiang, China
Corresponding email: yaodong.liu@cuixi.org

Rice Genomics and Genetics, 2025, Vol.16, No.6 doi: 10.5376/rgg.2025.16.0028
Received: 30 Sep., 2025
Accepted: 15 Nov., 2025
Published: 30 Nov., 2025

Preferred citation for this article:
Cai X.G., and Liu Y.D., 2025, Precise base editing of grain quality genes in elite rice cultivars, Rice Genomics and Genetics, 16(6): 312-320 (doi: 10.5376/rgg.2025.16.0028)

Abstract Improving the quality traits of rice is an important goal in modern rice breeding, among which the edible taste, appearance quality and nutritional components are all precisely regulated by specific functional genes. Base Editing technology, as a new generation of precision breeding tool, can achieve single-base replacement at specific sites without introducing DNA double-strand breaks, providing an efficient, controllable and safe technical path for the molecular improvement of high-quality rice varieties. This study systematically expounded the experimental process of base editing target design, vector construction and genetic transformation. The functional effect of the editing body was evaluated through molecular detection and quality phenotype determination. The actual case section presented the typical editing results and quality improvement performance of Wx, ALK and OsAAP6. And further discuss the limiting factors of base editing, off-target control and technical optimization directions. This study aims to provide more precise genetic regulatory means for the improvement of rice quality traits, establish an efficient editing system based on the background of superior varieties, lay the foundation for industrial application, and offer a reference path for future targeted editing of "quality design-oriented rice".

Keywords Rice quality traits; Base editing; CRISPR/Cas system; *Wx* gene; Precision breeding

1 Introduction

The quality of rice grains, when described as complex, is indeed not simple. It involves not only appearance, aroma and nutrition, but also the texture, stickiness after steaming and cooking, and even the performance during processing. People have different demands for these traits. Some pay attention to the taste, some value nutrition, and others just want the rice to look good. Behind these characteristics, there are actually multiple genetic factors at play, such as particle shape, chalkiness, amylose content, gel consistency and aroma. The direction of variety improvement is not merely to pursue high yields. More and more breeding goals have elevated quality to an equally important position. Genes and QTL loci such as gs3, GW7TFA, Wxb, and fgr (Gong et al., 2023; Gao et al., 2024; Sharmila et al., 2025) have been proven to be important breakthroughs in regulating grain size, texture and aroma.

Traditional breeding methods have indeed accumulated a lot of experience, but they are not efficient, have too long cycles, and are not precise enough in selection and breeding. At this point, precise gene editing technology becomes a turning point. Especially with the emergence of base editing and CRISPR/Cas systems, it is possible to directly modify target genes without introducing exogenous DNA, and replace those "problem bases" that affect quality. From regulating amylose content to improving flavor and grain shape modification, these editing tools have proven effective in rice, making up for the shortcomings in efficiency and flexibility of traditional breeding and molecular marker techniques (Ren et al., 2023). Furthermore, if these technologies are combined with genomic and phenotypic data, the precise improvement of rice quality will no longer be "dependent on the weather", but "carried out according to the plan".

This study reviewed the genetic basis of grain quality and the latest progress in gene editing, and then elaborated on the specific methods of base editing, the validation of edited strains, and the evaluation of phenotypic results. Finally, the application of this technology in molecular breeding and the prospects for precisely improving the quality of rice grains in the future were discussed. This study aims to modify superior rice varieties by using

precise base editing technology to modify key grain quality genes, thereby improving the cooking quality, taste and aroma of rice without affecting the yield. Its significance lies in verifying the feasibility and effectiveness of base editing technology in precisely regulating complex quality traits in commercial rice varieties.

2 Key Grain Quality Genes in Rice and Their Functions

2.1 Major genes related to eating and cooking quality

Whether rice is sticky or loose, and whether its texture is soft and glutinous or slightly hard, many times it depends on a gene - *Wx*. It regulates the activity of the GBSSI enzyme, which means that the amount of amylose (AC) is basically determined by it. Different Wx alleles, such as Wxmp and Wxb, have varying control over AC levels and also affect indicators like gelatinization and gel consistency, ultimately determining whether the cooked rice is more chewy or soft. Apart from Wx, another one worth mentioning is ALK, which encodes starch synthase IIa. It does not adjust the amount of starch but rather the rate of change inside the rice grains during heating - that is, the alkali diffusion value, which is related to whether the rice is easy to cook and whether it is sticky when eaten. These two genes are currently the key targets for targeted editing to optimize taste and consumer preferences (Yang et al., 2024; Hao et al., 2025).

2.2 Candidate genes associated with appearance traits (chalkiness, milling quality)

Not all appearance issues are related to the growing environment; the influence of genes is also considerable. Products like GL7/GW7/SLG7, GS3 and OsSPL14 are all closely related to the appearance of the rice grains. GL7 can adjust the grain shape, reduce the chalkiness, make the grain shape more slender and the rate of whole polished rice is also higher. OsSPL14 is even more versatile. Besides regulating appearance, it can also activate genes related to starch and protein processing, such as *Wx* and *PDIL1-1*. What should be done if appearance defects are prone to occur at high temperatures? Studies have identified genes such as *LOC_Os05g06920* and *LOC_Os11g28104*, which may have the ability to maintain good morphology of grains under heat stress by regulating development and stress response (Chen et al., 2025; Li et al., 2025; Xia et al., 2025).

2.3 Expression and regulation of genes involved in protein and starch composition

The ratio, structure and distribution of protein and starch largely shape the quality perception of rice. But behind this, it is not as simple as "one gene determines one trait". Transcription factors like OsSPL14 and NF-YB1-YC12-bHLH144 have the ability to synergically regulate starch synthesis and protein folding - for instance, directly activating Wx expression to smooth the starch synthesis pathway. Some other genes, such as OsANK3, focus more on regulating cell cyclin-related factors and starch metabolism enzymes, and the results will also be reflected in amylose content, gel consistency, etc. Fluctuations in gene expression during seed development, even a little bit, can cause subsequent changes in texture, appearance and even nutritional aspects (Bello et al., 2019; Li et al., 2022; Zhao et al., 2025).

3 Overview of Precise Base Editing Technologies

3.1 Principles of base editors (BEs): CBE and ABE systems

Not all gene editing requires cutting DNA strands. The Base editor (BE) takes a different approach - it can directly modify a single base, eliminating the traditional double-strand break step. There are two common types. One is CBE, which is used to replace C with T. Another type is ABE, where A is replaced with G. Their principles are actually quite similar: on the basis of a "paralyzed version" of the Cas9 protein, a deaminase is carried to quietly rewrite the target base. APOBEC1 is commonly used in CBE, while the evolved TadA variant is often employed in ABE (Rees and Liu, 2018; Hua et al., 2018; Huang et al., 2021). Without double-strand breaks, the "self-repair" of DNA will not be triggered, thus avoiding the chaos of additional insertions or deletions. For research aimed at creating point mutations, this approach is more direct and cleaner.

3.2 Advantages and application scopes compared with conventional CRISPR-Cas9

If it is in those cells with active division, the traditional CRISPR system works relatively well. However, once cell division slows down or almost stops, the repair efficiency cannot keep up, and unexpected mutations increase. At this point, the advantages of the base editor become obvious - it does not require homologous repair of DNA, does

not break the chain, has less accidental damage, and is more stable. This also makes it more applicable in functional gene validation, treatment of single-base mutation diseases and crop breeding (Rees and Liu, 2018; Newby and Liu, 2021; Porto et al., 2020). Although traditional CRISPR still has its uses, such as deleting large fragments or inserting new ones, the BE system is clearly superior in the fine operations of "stopping at the right point".

3.3 Current progress of base editing tools successfully applied in rice

In rice, base editing is no longer a "new technology" in the laboratory. CBE tools like BE3 can already stably change C·G pairs to T·A, and they basically have no side effects. For instance, if one wants to improve the quality of grains or enhance agronomic traits, it is possible to achieve this by editing specific loci of the target gene. Over the years, the editing tools themselves have been constantly "evolving" - various improvements have been made to deaminases, fusion proteins, and Cas9 variants, making the effects more stable and the target range wider. The addition of adenine-based editors has made the editing choices much more flexible (Hua et al., 2018; Yang et al., 2019; Chen et al., 2023). For breeding, these new tools have made "customized rice" truly operational.

4 Target Design and Vector Construction for Precise Editing

4.1 Principles for selecting editing sites and sequence conservation analysis

How to select a target is not a matter that can be dealt with in a one-size-fits-all manner. Some functional areas are too conservative, and even a slight change may affect other key processes. But if you are too casual, you may not be able to edit it or its effect may be limited. Therefore, when selecting these key grain quality genes, it is usually necessary to first analyze the sequence conservation of different varieties to see which regions change less in different genotypes and are close enough to the nucleotide sites that need to be altered. In addition, whether the editing window just covers the target base and whether the PAM sites are available enough are also unavoidable considerations (Cermak et al., 2017). Between precision and efficiency, a balance often has to be struck.

4.2 gRNA design and fusion strategies for deaminase-based editing modules

An ill-designed gRNA may cause the entire editor to "fall through". Therefore, from the very beginning, it is necessary to screen out those guide Rnas that are both specific and less likely to deviate, while also ensuring that they match the working region of the deaminase. It's not just a matter of the target sequence; the editing efficiency is also related to the fusion mode between the Cas9 variant and the deaminase. For instance, if the length or direction of the connector is incorrect, it will affect the result. To modify multiple genes simultaneously, a common practice nowadays is to tandem multiple grnas, such as using tRNA-gRNA modules or Csy4 processing systems. These structures can make multiple edits more controllable (Cermak et al., 2017; Moon and Jung, 2023).

4.3 Construction of expression vectors and optimization of rice transformation systems

The construction of an expression carrier is not difficult, but whether it is done well or not directly affects the editing effect. A common approach is to use a strong promoter to drive the expression of Cas9-deaminase fusion protein and gRNA, aiming to enhance their activity as much as possible in rice cells. With modular assembly systems, such as Golden Gate, it is possible to build multiple gRNA editing carriers more quickly (Fu, 2025). If the editing effect is not satisfactory, it might also be due to the poor performance of the conversion system, especially for crops like rice that are difficult to convert. Recently, some studies have attempted to use twin virus replicators to increase the target insertion rate, which seems to indeed enhance the editing frequency. Especially when one wants to obtain stable editing strains quickly, such systems may be more practical (Cermak et al., 2017; Ohtsuki et al., 2021).

5 Screening and Phenotypic Validation of Edited Lines

5.1 Molecular verification methods (sequencing, off-target analysis)

The first step in verifying base-edited rice usually cannot bypass sequencing. For quality-related genes like *Wx* or *GS3*, whether the expected base substitution has really occurred can only be confirmed by directly reading the sequence (Naing, 2025). But things are often not that simple. Even if the target is hit, sometimes one has to be on guard against "accidental damage" - especially for systems like CRISPR/Cas9, when encountering regions with

similar sequences, they might accidentally move areas that should not be touched. Therefore, off-target analysis becomes the key link, which requires a comprehensive screening of the whole genome to determine whether these editing tools are "clean" (Li et al., 2016). Moreover, if it is to be truly applied to breeding or commercial promotion, it is not only necessary to see whether the editing is successful, but also to check whether the exogenous fragments have been isolated. The non-genetically modified edited bodies that remain in the end are more in line with the standards for practical use.

5.2 Phenotypic evaluation of grain quality traits in edited lines

After verifying at the molecular level, it is still necessary to look at the appearance - that is, the phenotype. Such as amylose content (AC), grain size, appearance, weight, etc., these are the real indicators that consumers and farmers care about. Studies have found that some new Wx alleles, after editing, can flexibly adjust AC at different levels, not only improving the taste of cooking, but also sacrificing the original yield or resistance (Huang et al., 2021). Another example is GS3. When it is moved, the grains of many strains become longer, heavier, and appear more uniform and plump (Huang et al., 2022). Moreover, these improvements are also tenable under field conditions - including details such as translucency and chalkiness, all of which are supported by actual measurements (Wang et al., 2024). From the laboratory to the field, the editing department has indeed demonstrated the expected improvement effect (Figure 1).

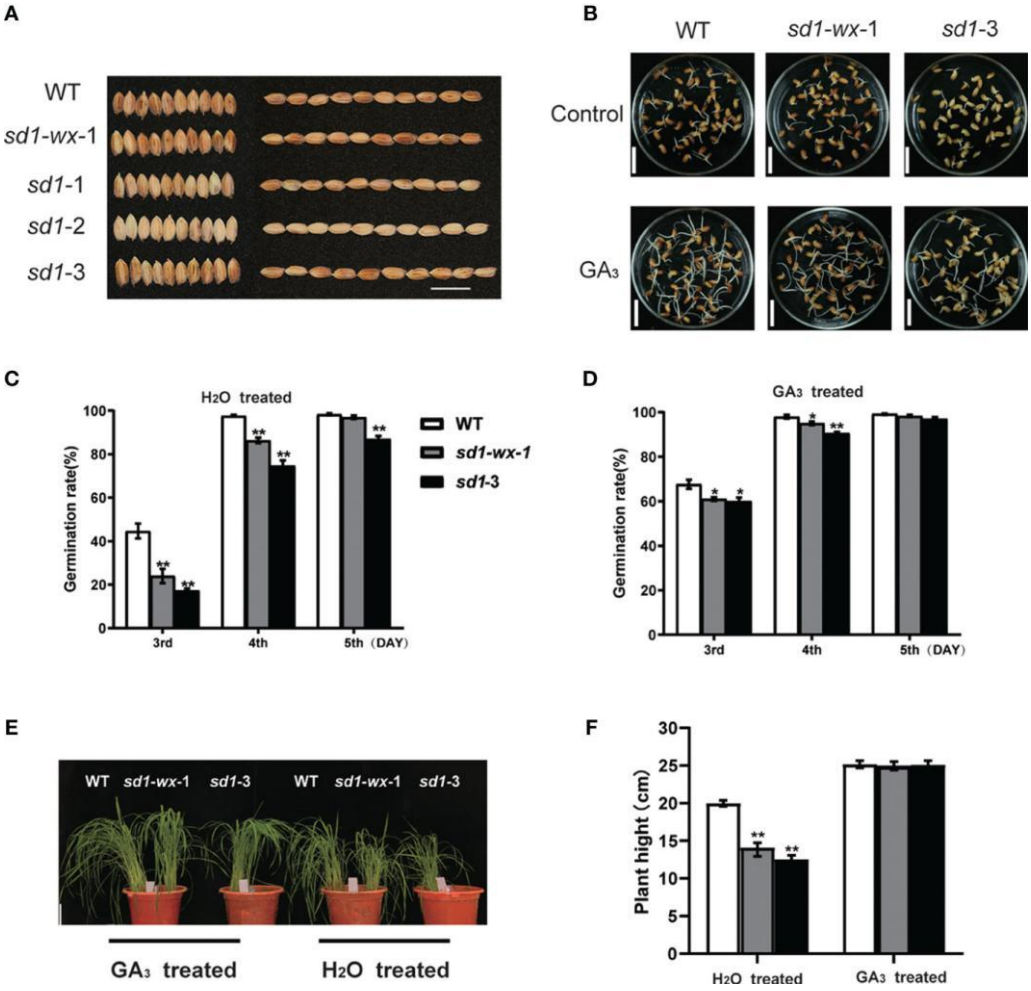

Figure 1 Seed germination and effect of exogenous GA_3 in mutant lines and WT (Adopted from Wang et al., 2024)

5.3 Segregation and assessment of transgenic vs. transgene-free edited plants

Not all editing styles are suitable for promotion. Whether one can "extricate oneself" without carrying exogenous DNA is an unavoidable hurdle. Many breeding projects now prefer to use non-genetically modified materials because they are easier to pass regulation and more readily accepted by farmers. Therefore, after editing is

completed, it is often necessary to screen out the offspring with carrier fragments through self-crossing or backcrossing, and then use molecular detection and multi-representational validation to confirm that the remaining plants without exogenous DNA but retaining the improvement effect of quality and yield are the truly ideal target materials (Zhang et al., 2018). These non-transgenic editing bodies are both stable and uncontroversial, making them a very realistic choice in current precision breeding.

6 Case Study: Precision Editing of Specific Grain Quality Genes

6.1 Editing *Wx* gene loci to fine-tune amylose content

Not all methods to improve the quality of rice require radical transformation. Sometimes, just changing a few bases can yield obvious results. Take the *Wx* gene for example. It is closely related to the content of amylose (AC). Altering this site can change the texture of rice. Researchers modified specific sites in the middle region of Wx using adenine or cytosine editors, resulting in different AC levels ranging from 0.3% to 29.43%. Some alleles, such as waxy abe2 type, not only have a softer texture and a more attractive appearance, but also do not bring adverse agronomic traits (Huang et al., 2021). It is worth noting that these adjustments are not confined to the N-terminal, which is often the focus of traditional research, but are targeted at other structural regions in the Wx protein. This approach not only enhances accuracy but also leaves much room for imagination in breeding, especially for those materials after the exogenous fragments have been removed, which seem to have great breeding prospects.

6.2 Modifying *ALK* gene to optimize gelatinization temperature and cooking traits

Many people have heard of the connection between the *ALK* gene and gelatinization temperature, but there are not many reports of directly modifying it with base editing at present. However, its significance in quality control is beyond doubt. Some studies have begun to use it as an editing target, with the aim of making rice easier to cook and softer in texture (Figure 2) (Ren et al., 2023). If Wx is regulating amylose, then ALK is adjusting the "state of cooked rice". The combination of the two is more likely to achieve the goal of "comfortable to eat and beautiful to look at". Although the technical route is still under exploration, the thinking of the editor ALK is actually quite similar to that of Wx - both are seeking more detailed and precise ways to adjust traits.

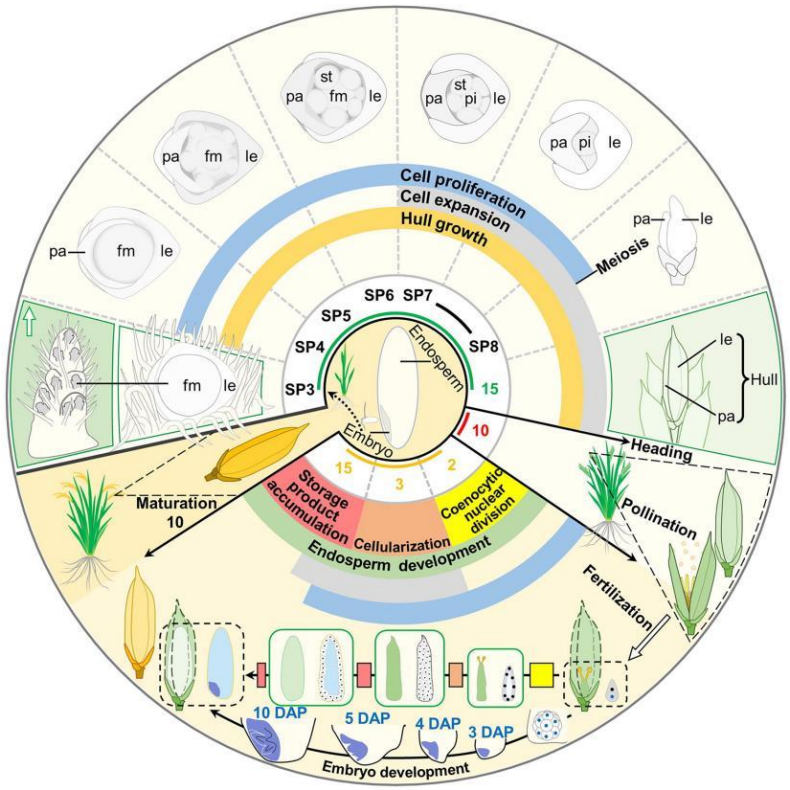

Figure 2 Rice spikelet hull, endosperm, and embryo development (Adopted from Ren et al., 2023)

6.3 Editing OsAAP6 to enhance glutelin content and nutritional quality

As for OsAAP6, its focus is somewhat different from the previous two, mainly related to the protein content of rice. Some consumers not only care about the taste but are also increasingly concerned about nutrition. OsAAP6 has been found to be a key factor in regulating gluten accumulation, and thus is regarded as a potential target for enhancing nutritional quality. At present, there are no very detailed reports on exactly how to manipulate this gene using the base editor, but existing studies have confirmed that it is feasible to increase the protein content through gene editing (Ren et al., 2023). From an application perspective, the modification of OsAAP6 and its combined use with genes such as *Wx* and *ALK* may be more meaningful. It not only enhances nutrition but also simultaneously improves appearance and taste.

To sum up, the thinking reflected in these cases is quite clear: it is not about making major changes, but about creating varieties that better meet consumers' demands through "adjusting precision and modifying details". Whether it is Wx adjusting AC, ALK adjusting gelatinization temperature, or OsAAP6 enhancing protein, their precise editing has laid the foundation for breeding rice varieties that balance nutrition, taste and processability. In the future, combining these precise strategies might be the mainstream direction of customized rice breeding.

7 Challenges and Optimization Strategies in Base Editing

7.1 Control of editing efficiency, window range, and off-target effects

When it comes to base editing in rice, many people's first reaction is "Can it be precise?" But in fact, what really troubles researchers is often how to achieve both accuracy and speed. Editing efficiency is not always stable. It is related to the target site, the editing tools used, and even the variety background of the rice itself. That is to say, even if the same editor is used, the effects obtained on different materials may vary greatly. Then there is the issue with the editing window. The window is too large, which can easily cause changes in non-target bases. If it's too small, it might miss the ideal conversion position. Similar problems also occur in off-target effects. Although CRISPR/Cas9 emphasizes "precision", it may mistakenly damage fragments that are very similar to the target sequence when encountering them. This kind of unexpected editing may seem insignificant, but once it reaches a crucial area, it can cause trouble. So nowadays, many teams conduct whole-genome off-target analyses before and after experiments to eliminate potential safety hazards (Li et al., 2016; Huang et al., 2021). The commonly used optimization methods at present include upgrading the base editor version, switching to a more precise variant, or adding a filtering step when designing GRnas to reduce unnecessary errors.

7.2 Reproducibility of editing across different genetic backgrounds in rice

The base editing technique, which seems like a standard process, often yields different results when applied to different rice varieties. For instance, some varieties have a high editing rate and distinct traits, while others show almost no response. This kind of difference is often related to genotype background. Varieties like J809, L237 and CNXJ show differences under the same editing conditions (Zhou et al., 2018). So before doing it, it's best not to treat all varieties as the same "template". For each variety, it is often the details that determine success or failure to adjust the conversion conditions and carrier design. In addition, even if the editing is successful, it is necessary to see whether it can be stably expressed under different genetic backgrounds without affecting other agronomic traits. Such cross-background verifications are indispensable (Li et al., 2016).

7.3 Multi-locus editing and development of marker-free transformation systems

After all, the improvement that can be brought about by modifying just one gene is limited. Nowadays, more and more research is beginning to attempt a "one-size-fits-all" editing strategy, especially for those gene combinations related to both quality and yield. Typical targets such as GS3, GW2, and Gn1a have been simultaneously edited by many studies using the multi-cis-trans tRNA-gRNA (PTG) CRISPR system, and the results are quite good, with single mutations, double mutations, and even triple mutations produced (Zhou et al., 2018; Waqas et al., 2025). These superimposed traits also behave more stably in the field. Not only that, considering the future review process, whether the traces of genetic modification can be removed has also become a focus. Nowadays, many teams have adopted the methods of isolated expression or transient transformation to produce "label-free" plants

without exogenous DNA (Huang et al., 2021). Removing the carrier without changing the editing result, although this step is not complicated, is directly related to whether it can truly be promoted to commercial breeding. Therefore, multiple editing combined with a label-free system precisely makes up for the shortcomings of the traditional editing route and also opens up a channel for the rapid cultivation of high-quality rice.

8 Conclusion and Future Perspectives

In some studies on sorghum and corn, site-specific base modification was once regarded as a "laboratory concept" that was difficult to apply on a large scale, but the progress of such technologies in rice has been surprisingly rapid. Key genes that control quality, such as *Wx*, *ALK*, and *OsAAP6*, can now be precisely edited to regulate amylose content, gelatinization temperature, and even protein composition. Especially, base editing, which does not introduce large fragments of variation and leaves no traces of genetic modification, has been adopted by multiple research teams to design new strains that better meet the requirements of edible quality, and even to improve nutritional characteristics without sacrificing particle shape and yield. Some edited strains even have better cooking palatability and industrial processing characteristics. Of course, all of this is attributed to the increasingly mature multi-gene editing strategy (*Wx*, *ALK*, *OsAAP6*) behind it, as well as the subsequent de-labeling method that completely removes the edited fragments.

However, when it comes to practical application, the outlook is not so optimistic. Although gene editing is precise, its efficiency is not necessarily stable. The expression differences under different genetic backgrounds often make people "do it more than once". In addition, the overly narrow editing window and the difficulty in controlling the probability of missing the target remain unavoidable issues in engineering optimization. Especially in some varieties where grain traits are jointly determined by multiple regulatory levels, merely editing one gene often fails to fundamentally change the situation. In this case, multiple editing becomes particularly important, but the unlabeled system has not yet been fully operational, and the conversion efficiency is not ideal either.

How will the future unfold? Base editing is certainly not a solo effort. When combined with gene superposition, genomic selection, and even AI-driven intelligent design platforms, it might be the key to accelerating breeding. Only through the coordinated regulation of multiple genes, precise control of regional expression, and the support of precise breeding data, can "customized rice" with stronger adaptability and in line with consumer preferences be rapidly selected and bred in different ecological zones. Of course, the prerequisite is the specificity of the editor, the efficiency of the delivery system, and a more refined control over the editing window. From this perspective, base editing is not merely a tool; it is more like a bridge connecting traditional breeding and future algorithmic breeding.

Acknowledgments

We would like to express our gratitude to the reviewers for their valuable feedback, which helped improve the manuscript.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Bello B., Hou Y., Zhao J., Jiao G., Wu Y., Li Z., Wang Y., Tong X., Wang W., Yuan W., Wei X., and Zhang J., 2019, NF‐YB1‐YC12‐bHLH144 complex directly activates Wx to regulate grain quality in rice (*Oryza sativa* L.), Plant Biotechnology Journal, 17: 1222-1235. https://doi.org/10.1111/pbi.13048

Čermák T., Curtin S., Gil‐Humanes J., Čegan R., Kono T., Konecna E., Belanto J., Starker C., Mathre J., Greenstein R., and Voytas D., 2017, A multipurpose toolkit to enable advanced genome engineering in plants, Plant Cell, 29: 1196-1217. https://doi.org/10.1105/tpc.16.00922

Chen L., Chen W., Li J., Wei Y., Qing D., Huang J., Yang X., Tang M., Zhang Z., Yu J., Deng G., Dai G., Chen C., Liang T., and Pan Y., 2025, Identifying heat adaptability QTLs and candidate genes for grain appearance quality at the flowering stage in rice, Rice, 18: 13. https://doi.org/10.1186/s12284-025-00770-y

Chen L., Hong M., Luan C., Gao H., Ru G., Guo X., Zhang D., Zhang S., Li C., Wu J., Randolph P., Sousa A., Qu C., Zhu Y., Guan Y., Wang L., Liu M., Feng B., Song G., Liu D., and Li D., 2023, Adenine transversion editors enable precise efficient A•T-to-C•G base editing in mammalian cells and embryos, Nature Biotechnology, 42: 638-650.

Fu Z.G., 2025, Enhancing nitrogen use efficiency in rice for sustainable agriculture, Molecular Soil Biology, 16(4): 162-174.
https://doi.org/10.5376/msb.2024.15.0016

Gao J., Gao L., Chen W., Huang J., Qing D., Pan Y., Ma C., Wu H., Zhou W., Li J., Yang X., Dai G., and Deng G., 2024, Genetic effects of grain quality enhancement in indica hybrid rice: insights for molecular design breeding, Rice, 17: 23.
https://doi.org/10.1186/s12284-024-00719-7

Gong D., Zhang X., He F., Chen Y., Li R., Yao J., Zhang M., Zheng W., and Yu G., 2023, Genetic improvements in rice grain quality: a review of elite genes and their applications in molecular breeding, Agronomy, 13(5): 1315-1334.
https://doi.org/10.3390/agronomy13051375

Hao Y., Xu J., Wu M., Zhu Y., Song J., Han Y., Li C., and Huang F., 2025, The Wx/SSIIa and GS3/GW7 alleles both individually and in combination can significantly distinguish rice germplasm quality, International Journal of Molecular Sciences, 26(14): 6726.
https://doi.org/10.3390/ijms26146726

Hua K., Tao X., Yuan F., Wang D., and Zhu J., 2018, Precise A·T to G·C base editing in the rice genome, Molecular Plant, 11(4): 627-630.
https://doi.org/10.1016/j.molp.2018.02.007

Huang J., Gao L., Luo S., Liu K., Qing D., Pan Y., Dai G., Deng G., and Zhu C., 2022, The genetic editing of GS3 via CRISPR/Cas9 accelerates the breeding of three-line hybrid rice with superior yield and grain quality, Molecular Breeding : New Strategies in Plant Improvement, 42: 22.
https://doi.org/10.1007/s11032-022-01290-z

Huang T., Newby G., and Liu D., 2021, Precision genome editing using cytosine and adenine base editors in mammalian cells, Nature Protocols, 16: 1089-1128.
https://doi.org/10.1038/s41596-020-00450-9

Li M., Li X., Zhou Z., Wu P., Fang M., Pan X., Lin Q., Luo W., Wu G., and Li H., 2016, Reassessment of the four yield-related genes *Gn1a DEP1 GS3* and *IPA1* in rice using a CRISPR/Cas9 system, Frontiers in Plant Science, 7: 377.
https://doi.org/10.3389/fpls.2016.00377

Li P., Chen Y., Lu J., Zhang C., Liu Q., and Li Q., 2022, Genes and their molecular functions determining seed structure components and quality of rice, Rice, 15: 18.
https://doi.org/10.1186/s12284-022-00562-8

Li Y., Guo X., Song J., Xu K., Qin T., Zhang X., Song Z., He Y., Zhang B., Zhang H., Li H., and Yuan W., 2025, OsSPL14 and OsNF-YB9/YC8-12 subunits cooperate to enhance grain appearance quality by promoting Waxy and PDIL1-1 expression in rice, Plant Communications, 6: 101348.
https://doi.org/10.1016/j.xplc.2025.101348

Moon S., and Jung K., 2023, A speed cloning method for editing multiple targets, Journal of Plant Biology, 66: 311-316.
https://doi.org/10.1007/s12374-023-09400-w

Naing N.N.Z.N., Wang C.L., Zhou X.L., Zhang C., Li J.J., Li J., Zhu Q., Lee D.S., and Chen L.J., 2025, Molecular mechanisms of rice drought resistance genes and their prospects in breeding, Molecular Plant Breeding, 16(3): 165-179.
https://doi.org/10.5376/mpb.2025.16.0017

Newby G., and Liu D., 2021, In vivo somatic cell base editing and prime editing, Molecular Therapy : the Journal of the American Society of Gene Therapy, 29(11): 3107-3124.
https://doi.org/10.1016/j.ymthe.2021.09.002

Ohtsuki N., Kizawa K., Mori A., Nishizawa-Yokoi A., Komatsuda T., Yoshida H., Hayakawa K., Toki S., and Saika H., 2021, Precise genome editing in miRNA target site via gene targeting and subsequent single-strand-annealing-mediated excision of the marker gene in plants, Frontiers in Genome Editing, 2: 617713.
https://doi.org/10.3389/fgeed.2020.617713

Porto E., Komor A., Slaymaker I., and Yeo G., 2020, Base editing: advances and therapeutic opportunities, Nature Reviews Drug Discovery, 19: 839-859.
https://doi.org/10.1038/s41573-020-0084-6

Rees H., and Liu D., 2018, Base editing: precision chemistry on the genome and transcriptome of living cells, Nature Reviews Genetics, 19: 770-788.
https://doi.org/10.1038/s41576-018-0059-1

Ren D., Ding C., and Qian Q., 2023, Molecular bases of rice grain size and quality for optimized productivity, Science Bulletin, 68(3): 314-350.
https://doi.org/10.1016/j.scib.2023.01.026

Sharmila K., Gauraha D., Sao A., Indumathi S., and Nagaraju D., 2025, Exploring genetic variability and diversity in advanced breeding lines of rice for yield-attributing and grain quality traits, Journal of Advances in Biology and Biotechnology, 28(4): 42-56.
https://doi.org/10.9734/jabb/2025/v28i42167

Wang Q., Gao H., Liu K., Wang H., Zhang F., Wei L., Lu K., Li M., Shi Y., Zhao J., Zhou W., Peng B., and Yuan H., 2024, CRISPR/Cas9-mediated enhancement of semi-dwarf glutinous traits in elite Xiangdaowan rice (*Oryza sativa* L.): targeting *SD1* and *Wx* genes for yield and quality improvement, Frontiers in Plant Science, 15: 1333191.
https://doi.org/10.3389/fpls.2024.1333191

Waqas M., Awan M., Amin I., Arif M., Mukhtar Z., and Mansoor S., 2025, Engineering high yield basmati rice by editing multiple negative regulators of yield, Molecular Biology Reports, 52(1): 545.
https://doi.org/10.1007/s11033-025-10660-7

Xia D., Xu P., Zheng S., Xue L., Yin Y., Yao Z., Ding S., Lai Y., Wang Y.X., Wu X., and Zhou H., 2025, Haplotype analysis of the *GL7*/*GW7*/*SLG7* gene and its application in improving the grain quality of three-line hybrid rice, Rice, 18: 14.
https://doi.org/10.1186/s12284-025-00786-4

Yang B., Yang L., and Chen J., 2019, Development and application of base editors, The CRISPR Journal, 2: 91-104.
https://doi.org/10.1089/crispr.2019.0001

Yang Y., Zhou L., Feng L., Jiang J., Huang L., Liu Q., Zhang Y., Zhang C., and Liu Q., 2024, Deciphering the role of waxy gene mutations in enhancing rice grain quality, Foods, 13(11): 1624.
https://doi.org/10.3390/foods13111624

Zhang J., Zhang H., Botella J., and Zhu J., 2018, Generation of new glutinous rice by CRISPR/Cas9-targeted mutagenesis of the *Waxy* gene in elite rice varieties, Journal of Integrative Plant Biology, 60(5): 369-375.
https://doi.org/10.1111/jipb.12620

Zhao J., Xin Y., Cui W., Li P., Su J., Zhao L., and Wang Q., 2025, The ankyrin repeat-containing protein OsANK3 affects grain size and quality in rice, Planta, 262(1): 21.
https://doi.org/10.1007/s00425-025-04734-w

Zhou J., Xin X., He Y., Chen H., Li Q., Tang X., Zhong Z., Deng K., Zheng X., Akher S., Cai G., Qi Y., and Zhang Y., 2018, Multiplex QTL editing of grain-related genes improves yield in elite rice varieties, Plant Cell Reports, 38: 475-485.
https://doi.org/10.1007/s00299-018-2340-3