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Case Study Open Access

CRISPR Applications in Rice Breeding: Case Studies of Yield and Stress Tolerance

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Abstract This study explored the application of CRISPR/Cas9 technology in rice breeding, focusing on the analysis of cases for improving yield and stress resistance. CRISPR/Cas9 technology achieves targeted modification of specific genes through precise genome editing, thereby enhancing agronomic traits of rice, such as yield, drought resistance, cold tolerance, and salinity tolerance. Studies have shown that using CRISPR/Cas9 to edit multiple genes simultaneously can effectively improve rice's ability to adapt to environmental stress and increase productivity. In addition, future research should focus on expanding the range of target genes, improving editing efficiency and specificity, and combining CRISPR technology with other breeding methods to accelerate the development of rice varieties with complex traits. CRISPR/Cas9 technology is of great significance in sustainable agriculture, helping to achieve food security and reduce dependence on chemical inputs by developing more resilient and high-yielding rice varieties.
Keywords CRISPR/Cas9; Rice Breeding; Stress Tolerance; Gene Editing; Yield Improvement

1 Introduction

Rice (*Oryza sativa* L.) is definitely one of the most important food crops in the world-after all, more than half of the world's population relies on it to feed themselves. Although it is indeed highly adaptable and can grow in a variety of environments (which is why it is so important), growing rice is not that simple. Pests and diseases are a headache, and bacteria and fungi are not quiet, not to mention various viruses and pests that come to cause trouble. The weather is also not worrying. Saline-alkali land, drought, extreme temperatures, and other abiotic stresses are also troublesome (Farhat et al., 2019; Romero and Gatica-Arias, 2019; Zeng et al., 2020). Not only does the yield drop, but even the quality and nutrition of rice are affected. Of course, people have tried to use traditional breeding to solve the problem and cultivate varieties with strong resistance and high yield, but to be honest, these old methods are not very efficient, time-consuming, labor-intensive, and not necessarily accurate (Nazir et al., 2022; Park et al., 2022).

Agricultural breeding has made a big breakthrough in recent years-the emergence of CRISPR/Cas9 technology has made genome editing like "precision guidance". You know, traditional breeding can take ten or eight years, but now? You can directly target the key genes that control stress resistance and high yield to start the transformation (Ricroch et al., 2017; Romero and Gatica-Arias, 2019; Tang et al., 2023). The most powerful thing about this technology is its flexibility. If you want to "shut up" the gene, just knock it out, and if you want to enhance the expression, just overexpress it. It is simply a universal toolbox. Take rice as an example. Scientists have tinkered with genes such as *OsPIN5b* and *GS3*, and have really come up with new varieties that are both high-yielding and cold-resistant (Zeng et al., 2020). What’s even more amazing is that rice is no longer afraid of saline-alkali land. After genes such as *OsRR22* are edited, rice can actually survive well in a high-salt environment (Zhang et al., 2019; Wang, 2024)-in the past, it was impossible to grow decent crops in such land.

The main purpose of this study is to see how much change CRISPR technology can bring to rice breeding-to put it bluntly, it is to focus on those successful cases that can increase yields and enhance stress resistance. There are too many troubles in growing rice now, either pests and diseases or extreme weather, and CRISPR/Cas9 technology comes at the right time. For example, some teams have really created more drought-resistant and salt-tolerant varieties by editing specific genes, which is much faster than traditional breeding. We broke down these actual cases and studied them in detail, just to prove that this technology can really solve the urgent needs of rice planting. After all, the global pressure to eat is getting greater and greater. If we can cultivate rice varieties that are both high-yielding and hardy, it will really help a lot.

2 CRISPR Applications for Enhancing Rice Yield

2.1 Gene editing to increase grain number

When it comes to increasing rice yields, CRISPR/Cas9 technology has really made a lot of progress recently. Take the case of rice ears, for example. Scientists have found that by modifying the *GS3* gene, rice ears can be significantly larger-this is not a minor change, but a real increase in yield (Zeng et al., 2020). What's more interesting is that when researchers edited *GS3* and several other key genes together, the effect was even more amazing. Not only did the rice ears become larger, but the number of rice ears also increased (Chennakesavulu et al., 2021). The most powerful thing about this technology is that it can accurately locate specific genes such as *GS3* like a scalpel, and change whatever you want. In the past, breeders had to rely on luck to increase the number of rice ears. Now with CRISPR, they can directly target the target gene, and the efficiency is unknown.

Scientists are getting better and better at CRISPR/Cas9, especially in the "killing multiple birds with one stone" multi-gene editing. Look at the genes they have been tinkering with recently-*OsPIN5b*, *GS3* and *OsMYB30*. This combination of punches has an amazing effect: not only do rice ears become longer and bigger, but also their cold resistance is improved (Khush, 2013). The best thing about this trick is that it not only solves the yield problem (the number of rice ears is rising), but also makes rice harder and not afraid of bad weather (Zeng et al., 2020). To be honest, who would have thought that they could improve several traits so accurately at the same time? Now with CRISPR technology, breeders have found a new magic weapon. They can edit any gene they want for any characteristic, and the efficiency is very high.

2.2 Modifying plant structure for higher yield

Scientists can now even precisely adjust the "body shape" of rice-after editing the *OsPIN5b* gene with CRISPR/Cas9, the rice panicles became significantly longer. This is not a simple cosmetic surgery, but a real secret to increasing yields. Think about it, the longer the panicle, the more rice grains can be produced, which directly increases the yield (Zeng et al., 2020). Interestingly, this precise control of plant structure allows us to design rice plant types like building blocks for the first time. In the past, breeders could only rely on natural mutations to take their chances, but now they can directly manipulate key genes, changing the panicle length if they want to, and adjusting the plant height if they want to, turning rice into a programmable "living machine". This groundbreaking method may allow rice yields to reach new highs in the future.

In order to increase rice yield, scientists have even arranged for "height management". Using CRISPR/Cas9 to tinker with genes that control plant height and tillering, the effect is immediate-the height that should be high is high, the tillering that should be tillering, the yield immediately increases (Biswal et al., 2019). The most amazing thing about this technology is that the modified rice is not only pleasing to the eye, but also very economical in using fertilizers and sunlight. For example, some improved varieties have just the right plant height, which will not fall over and can fully photosynthesize; the number of tillers is also controlled just right, which is not too dense to affect growth, and can maximize the use of land (Romero and Gatica-Arias, 2019). To put it bluntly, now breeders are like playing high-end customization, and they can edit any plant type you want, which is much more reliable than the previous breeding method that relied on nature.

2.3 Challenges in the implementation process

But then again, although CRISPR/Cas9 technology is powerful, it is not perfect. The most troublesome thing is the "off-target effect"-simply put, it is the wrong place that is accidentally modified during editing. Take the previous multi-gene editing experiment of *OsPIN5b*, *GS3* and *OsMYB30* as an example. Some plants did have unplanned genetic mutations (Table 1) (Zeng et al., 2020). This kind of accident is like injuring other organs during surgery. At the least, it will affect the expected effect, and at the worst, it may produce some strange new traits. So now researchers are racking their brains to find ways to improve the accuracy of editing. After all, to truly apply this technology to breeding practice, we must first ensure that it is reliable enough.

Table 1 The putative off-target event in the *OsPIN5b, GS3, and OsMYB30* triple mutant lines (Adopted from Zeng et al., 2020)

Target Site	Putative off-target site	The sequence of putative off-target site	No.of plants	No.of plants with mutations	Mutation rate (%)
OsPIN5b-Site1	Chr3:9566074-9566052	CCTCGAGCTCTGCAAGGCTTTGG	8	0	0
	Chr1:19390027-19390049	TTTCGAGCTGCGCAAGGCGCGGG	8	0	0
OsPIN5b-Site2	Chr7:8747238-8747260	CTCGTGTGCGCCAACGTTCTCGG	8	0	0
	Chr3:24251589-24251567	AACGTGCTCGTCAGCGTCCTCGG	8	0	0
GS3-Site1	Chr8:2495427-2495449	GACGCGCTCCACCGCGCGCTCGG	8	0	0
	Chr7:14816697-14816719	AACTCGCTCAACCGCGAGAGGGG	8	0	0
GS3-Site2	Chr9:1832101-1832123	TCGCGCCACCCCACACGAGGTGG	8	0	0
	Chr7:27472889-27472911	TAACCCCGCACCGCATGAGGCGG	8	0	0
OsMYB30-Site1	Chr3:13108929-13108951	GTGGCGGAGAGCGACTGCACGGG	8	6	75
	Chr5:1475321-1475343	CTGCGCGAGAGCGACTGCGCCGG	8	0	0
OsMYB30-Site2	Chr4:24538627-24538649	TGACAAATGCTGCATGCCATGGG	8	0	0
	Chr2:28909447-28909469	CGTCAAATTCTGCATGACAGTGG	8	0	0

Table caption: The green font represented the PAM motif (NGG);the red font represented the mismatch bases (Adopted from Zeng et al., 2020)

To be honest, the biggest obstacle that CRISPR technology encounters in rice breeding may not be the technology itself, but the regulatory regulations. The attitudes of different countries towards gene-edited crops are very different-some countries give the green light, while others set up many barriers (Jaganathan et al., 2018). These regulations are no joke. Safety tests need to be done for several years at any time. By the time the approval process is completed, the new varieties may have missed the best promotion period. What is more troublesome is the acceptance of the general public. Although scientists think that gene editing and genetic modification are not the same thing, ordinary consumers don't care about these professional differences (Ansari et al., 2020). I remember that a few years ago, a team developed a new rice variety that was disease-resistant and high-yield, but it ended up rotting in the laboratory due to public resistance. So, no matter how good the technology is, it must pass the social recognition stage. It is not enough to succeed in the laboratory.

3 CRISPR Applications for Improving Stress Tolerance in Rice

3.1 Case studies on drought tolerance gene editing

Recently, CRISPR technology has made new breakthroughs in rice drought resistance research, but the results are quite surprising. After scientists edited the *OsSAP* gene related to aging, they found that these rice plants were more vulnerable under drought conditions-their ability to remove harmful reactive oxygen species became worse, and their overall growth was not as good as that of ordinary rice (Park et al., 2022). As you can see from the data in Figure 1, all indicators have declined across the board, from survival rate to plant height, from root development to final yield. This proves that the *OsSAP* gene is like a "drought resistance switch" and plays a key role in resisting drought. Although this experiment seems to have failed, it actually helped us understand the drought resistance mechanism, and we will know which direction to work hard in the future when we want to cultivate drought-resistant varieties. This "reverse verification" method can be regarded as an unexpected gain brought by CRISPR technology.

This time, scientists have set their sights on the *OsPYL9* gene, which controls a "drought-resistant hormone" called ABA in rice. After the gene was modified using CRISPR technology, something magical happened: during drought, the ABA hormone in these rice plants rose sharply, with their own "antioxidant buff", and their survival rate was significantly improved (Usman et al., 2020). What's even more surprising is that through proteomic analysis, it was found that these mutants not only had upgraded drought resistance, but also optimized their biological clocks and stress resistance mechanisms. The most practical thing is that regardless of drought or flooding, the yield is much higher than that of ordinary rice (Jiang et al., 2023). This is like installing an intelligent regulator for rice, which automatically activates drought resistance mode during drought and maintains high yield at other times. It seems that the key to solving the problem of food security lies in these smart genes that can "assess the situation".

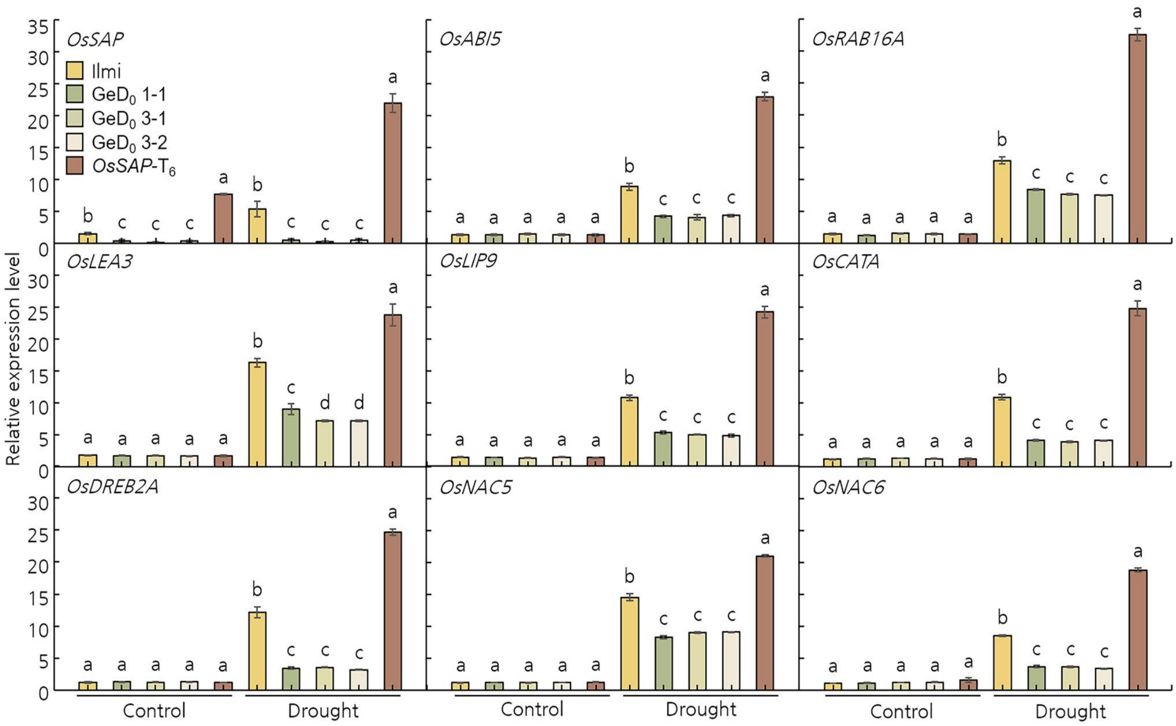

Figure 1 *Oryza sativa* Senescence-associated protein is involved in the expression levels of several critical stress-related transcription factors under drought conditions (Adopted from Park et al., 2022)

Image caption: Under normal conditions OsABI5, OsRAB16A, OsLEA3, OsLIP9, OsCATA, OsDREB2A, OsNAC5, and OsNAC6 expression levels were the same. However, overexpression of OsSAP under drought conditions improved the expression levels of the evaluated stress-related transcription factors. Bars represent mean ± SE. Means denoted by the same letter are not significantly different ($p < 0.05$) as evaluated by Duncan's multiple range test. Different letters on columns represent significant ($p < 0.05$) difference between rice lines based on Duncan's test (Adopted from Park et al., 2022)

3.2 Research on salinity and heat tolerance

Planting rice in saline-alkali land? This was something that was unthinkable before. But now scientists have used CRISPR technology to tinker with the *OsRR22* gene, and the new varieties they have cultivated can withstand high-salt environments at the seedling stage (Zhang et al., 2019). The best part is that these "salt-resistant experts" are no different from ordinary rice in other aspects-they grow as tall as they should and produce as much rice as they should. This shows that we can give rice the ability to resist salt without sacrificing yield. You know, there are nearly 1 billion hectares of saline-alkali land in the world. If all of them can be planted with this improved rice, how many people will it feed! But then again, these varieties are still in the laboratory stage. If they are really to be promoted on a large scale, they still have to go through many trials and tribulations.

Global warming is getting worse, and high temperatures can easily "cook" rice and reduce yields. However, scientists have begun using CRISPR technology to deal with this problem-they specifically target genes that control "heat resistance", such as genes that control heat shock proteins (Zafar et al., 2020). Although there are not many public success stories, considering the breakthroughs this technology has made in drought and salt resistance, it should be a matter of time before it can handle high temperatures (Kumar et al., 2023). In fact, rice itself has a mechanism to cope with high temperatures, but it is not strong enough. Now that we have gene editing technology, it is equivalent to upgrading this system. When we really cultivate a variety that is not afraid of heat and has high yields, we will not have to worry about having no food to eat even in extreme weather.

3.3 Balancing stress tolerance and yield

Indeed, the biggest fear of developing stress-resistant varieties is to lose sight of one thing while focusing on another-resistance is improved, but yield drops. But this time, CRISPR technology really showed us its hand. Take the *OsPYL9* gene for example. After editing, it was like a cheat: not only did the drought resistance soar, but even the yield increased (Usman et al., 2020). This is like installing an intelligent regulator for rice, which automatically starts water-saving mode in the dry season and can produce at full capacity in normal times. Scientists are now increasingly finding these key genes that "kill two birds with one stone", for example, some genes control both the opening and closing of stomata and the filling of grains. This ability of precise regulation is much stronger than the traditional breeding method of taking chances. If this trend continues, maybe we can really cultivate super rice that can "guarantee yield in drought and flood" in the future.

When it comes to rice breeding, CRISPR/Cas9 technology has come up with new tricks. It can not only modify genes directly, but also operate on regulatory switches (cis-regulatory sequences). Interestingly, this can lead to new stress resistance QTLs, and most importantly, it will not reduce yield (Romero and Gatica-Arias, 2019). In fact, in the early years, people were worried that stress resistance and yield could not be achieved at the same time, but now researchers have found a trick: by fine-tuning the expression levels of those stress response genes, rice can withstand harsh environments and maintain high yields (Zafar et al., 2020). This fine-tuning ability is much smarter than the drastic transformation methods of traditional breeding.

4 Technical and Ethical Considerations

4.1 Precision of CRISPR editing

The most powerful thing about CRISPR/Cas9 is its ability to precisely edit anything. Take rice, for example. Scientists have successfully modified several key genes with it. For example, by changing the *OsPIN5b* gene, the rice ears become significantly longer; by changing the *GS3* gene, the rice ears become larger; by adjusting the *OsMYB30* gene, the cold tolerance is immediately improved (Zeng et al., 2020). Although the editing efficiency of different genes varies (approximately between 42% and 66%), this ability to precisely target specific genes is a revolutionary breakthrough in the breeding industry (Sami et al., 2021). In the past, improving rice traits depended on luck. Now with this system, you can change any gene you want to change, and the efficiency is many times higher.

To make the CRISPR/Cas9 "gene scissors" cut accurately, the key lies in whether the guide RNA (gRNA) is well designed. It's like equipping the scissors with a navigator-the more accurate the gRNA, the less likely it is to cut the wrong place during editing. Interestingly, researchers found that the edited mutant traits were particularly "obedient" in the offspring of rice, and were transmitted completely according to Mendel's laws of inheritance (Zhang et al., 2014). This shows that as long as the gRNA is designed in place, a successful edit can be stably inherited. But to be honest, designing gRNA is not an easy job, and it requires repeated testing and verification. After all, no one wants to see new varieties that have been cultivated with great effort have unexpected traits due to inaccurate editing. Fortunately, the technology is becoming more and more mature, and the success rate of precise editing is also constantly improving.

4.2 Safety measures and control of off-target effects

CRISPR/Cas9, this "gene scissors", is indeed precise, but it can occasionally cut the wrong place-this is the troublesome off-target effect. Imagine that you originally wanted to modify gene *A*, but accidentally modified the similar-looking gene B as well. This accidental editing can affect the expected effect at best, or even produce some harmful traits at worst. To be honest, although this situation is rare, it would be troublesome if it really happened in breeding. Therefore, researchers are now very cautious. They must repeatedly confirm the uniqueness of gRNA before editing, and they must do a whole genome scan after editing to check for omissions. After all, when using CRISPR technology to cultivate new varieties, safety and reliability are the first priority.

Scientists now have ways to deal with off-target effects. The first is to start with the "scissors" themselves-using the modified high-fidelity Cas9 protein, which is like adding a locator to the scissors, and the probability of error is much lower. However, the most reliable way is to do a "full body checkup" after editing, such as a large-scale screening such as whole genome resequencing. Zhang's team did such an experiment in 2014, checking the edited rice inside and out, and found no obvious off-target problems (Zhang et al., 2014). This is like a comprehensive review after surgery. Although it takes some time, it can ensure that there is no risk of error. If you ask me, gene editing must be so rigorous, after all, these new varieties will eventually be served on the tables of ordinary people.

4.3 Public acceptance and regulatory context

Ordinary people actually have a very contradictory attitude towards using CRISPR technology to grow rice. On the one hand, this technology is indeed much more accurate and efficient than the old method-just like using a scalpel instead of a machete, you can change any gene you want (Zeng et al., 2020). But on the other hand, many people are nervous when they hear the word "gene editing" and always feel uneasy. In fact, the key is to make it clear: for example, isn't the drought-resistant rice we are developing now just to cope with the increasingly frequent extreme weather? If these tangible benefits are explained clearly, everyone will naturally be more receptive (Elena et al., 2021). In the final analysis, the promotion of new technologies cannot be hidden, and the pros and cons must be laid out in words that ordinary people can understand.

The attitudes of different countries towards CRISPR-edited rice are very different-the EU is very strict and treats gene-edited crops and genetically modified crops equally; but the United States and Japan are much more flexible, and they pay more attention to whether the product itself is safe, rather than how it is cultivated (Ansari et al., 2020). This difference in supervision has caused headaches for researchers. The same variety can be promoted in one country, but it may be illegal in another country. To be honest, to promote the commercialization of gene-edited rice, it is not enough to pass the technical test, but also to find out the policy red lines of various countries. Now some teams have begun to "tailor" and develop varieties that comply with local regulations for different markets. This is a very smart move.

5 Case Study Analysis and Comparison

5.1 Differences between yield enhancement and stress tolerance editing

When it comes to using CRISPR technology to improve rice, increasing yield and stress resistance are actually two different approaches. In terms of increasing yield, scientists mainly focus on genes that control "appearance"-for example, modifying the *GS3* gene can make rice grains larger, and adjusting the *OsPIN5b* gene can make rice ears longer (Zeng et al., 2020). To put it bluntly, in a good year with good weather and good harvests, rice can maximize its yield potential. But what's interesting is that these changes often only focus on the "external beauty" of rice, just like performing plastic surgery on rice. In contrast, editing for stress resistance is more like training rice's "survival skills" so that they can survive in harsh environments. Both ideas are actually important, and the key is where to plant-choose high-yield varieties for good fields and good land, and naturally choose stress-resistant varieties for saline-alkali land and arid areas.

Editing stress tolerance is another matter entirely-it's about creating a "survival skill package" for rice. For example, by tinkering with the *OsMYB30* gene, rice suddenly becomes less afraid of cold; by tinkering with the *OsSAP* gene, drought resistance is directly improved to a higher level (Zeng et al., 2020). Scientists have discovered that these genes are like "panic buttons" for rice, which can activate various defense mechanisms when encountering harsh environments: some can quickly remove harmful substances, and some can maintain normal cell function (Park et al., 2022). The most amazing thing is that complex characteristics such as salt tolerance can actually be achieved by editing specific genes (Nazir et al., 2022). To put it bluntly, this is to install a "stress tolerance program" for rice at the molecular level, so that they will not easily die when they encounter drought, salinity and other troubles.

To put it bluntly, the difference between these two editing strategies is like the difference between "adding icing on the cake" and "providing timely assistance". Yield-increasing editing focuses on genes that control "high yields"-for example, making rice ears longer and grains fuller. To put it bluntly, it allows rice to produce high yields while eating well and living well. Stress-resistance editing is much more pragmatic, specifically strengthening those "life-saving genes" so that rice can at least survive when encountering drought, salinity and other troubles, and not suffer a total crop failure. Interestingly, some studies are now beginning to try to combine the two, making rice grow strong and equipping it with various "survival skills". This is a true all-round player.

5.2 Key results and success metrics

To measure the effect of CRISPR technology in rice breeding, we have to look at several hard indicators. Let's talk about the increase in yield first-the most intuitive is to look at the changes in rice ears and rice grains. For example, the rice grains of rice with the *GS3* gene modified are significantly larger; the rice ears of rice with the *OsPIN5b* gene modified are longer (Zeng et al., 2020). These are all real yield-increasing characteristics, and farmers can see them at a glance in the field. However, it is not enough to just look at the performance of the season. The key is to see whether these improved traits can be stably inherited. The experimental data tracked to the third and fourth generations are quite optimistic, indicating that these edits are indeed reliable. Of course, in the end, it still depends on the per-acre yield figures. After all, the end point of breeding improvement is always yield.

To judge whether the stress resistance editing is successful, it mainly depends on the performance of rice when it is "abused". For example, those with modified *OsMYB30* genes are still energetic in the ice and snow; those with modified *OsSAP* genes can also survive years of drought-specifically, it depends on hard indicators such as survival rate and plant height, as well as whether the cells are oxidatively damaged (Zeng et al., 2020). Park and his team's 2022 study found that when drought-resistant rice is short of water, the damage markers in the leaves are indeed much less (Park et al., 2022). However, the most reassuring thing is that these stress resistance characteristics can be steadily passed on to the next generation, and they are well maintained in the T2 generation (Zeng et al., 2020). To put it bluntly, a good variety must be both effective at the moment and reliable for future generations. This is a truly valuable improvement.

5.3 Potential for practical application

Faced with the increasingly severe challenges of global food security, CRISPR technology has demonstrated unique application value. The most attractive thing about this technology is that it can achieve simultaneous improvements in yield and stress resistance-for example, it can not only cultivate varieties with large panicles and more grains, but also enable these high-yield rice to cope with harsh environments such as drought and salinity (Romero and Gatica-Arias, 2019). Studies have shown that in areas where extreme weather is frequent due to climate change, such gene-edited rice varieties show significant advantages (Zeng et al., 2020). Especially in agriculturally vulnerable areas such as Southeast Asia and Africa, field trial data have confirmed the practical application value of these varieties (Nazir et al., 2022). However, in order to truly maximize the benefits of this technology, it is currently urgent to solve the problem of connection from laboratory research to commercial promotion.

When it comes to breeding speed, CRISPR technology is much faster than traditional methods-this is particularly useful in adapting rice to climate change and market changes. Take drought resistance as an example. The new varieties produced by editing the *OsSAP* gene are a timely rain for rain-fed agricultural areas in Asia and South America that rely on the weather for food (Park et al., 2022). Although traditional breeding can also produce stress-resistant varieties, it often takes ten to eight years. Salt-tolerant varieties are also a good example. Varieties improved through gene editing can allow rice to be grown in saline-alkali land, which is equivalent to a lot of arable land out of thin air (Nazir et al., 2022). But then again, if these new varieties are to be truly promoted, they still have to pass the level of acceptance by farmers.

Now, regulatory authorities in various countries have clearly relaxed their attitude towards CRISPR technology, especially for those edited varieties that do not introduce foreign DNA-in other words, rice that only changes its own genes and does not touch foreign genes. This "clean" editing method is indeed easier to pass approval (Zegeze et al., 2022). Farmers and consumers are also much more accepting, after all, it sounds more reliable than genetic modification. But then again, although the policy is being relaxed, it still has to go through many hurdles to promote it on a large scale. But in any case, this brings the new high-yield and stress-resistant rice varieties one step closer to the field, which is definitely a good thing for solving food security problems.

6 Future Prospects

6.1 Emerging CRISPR technologies

Gene editing technology has made great progress in the past two years. The CRISPR system alone has been upgraded to several versions. For example, the new Cas12 is more accurate than the old Cas9, and it is more stable when cutting genes, and basically does not accidentally damage other parts (Mishra et al., 2018). Rice breeding experts have already used this new tool and have achieved good results in increasing yields and resisting stress (Tabassum et al., 2021). Even more powerful is the base editing technology that emerged later. It does not even need to cut the double strands of DNA, but directly modifies a single base like a fine-tuning screw-this not only avoids accidental mutations, but also allows excellent traits to be stably inherited. But then again, although new technologies are emerging in an endless stream, it ultimately depends on the actual performance in the field.

Now there is another powerful character in the gene editing toolbox-the CRISPR/Cpf1 system. Unlike the common Cas9 scissors, this new tool can stagger the cuts when cutting DNA, just like cutting cloth with serrated scissors, which makes it easier to insert and delete genes (Mishra et al., 2018). Researchers have found that it is more efficient than the old Cas9 when dealing with certain specific genes. As these technologies continue to upgrade, rice breeding is undergoing a revolutionary change (Zegeye et al., 2022). To put it bluntly, gene editing is now not only more precise, but also more diverse in the types of genes that can be modified, which opens up new horizons for the cultivation of various "superpower" rice-such as new varieties with stronger disease resistance and higher yields. However, what farmers are most concerned about is when these breakthroughs in the laboratory can really turn into harvests in the fields.

6.2 Combined editing for multiple traits

Nowadays, rice breeding is more and more like playing a combination game-the most powerful thing about CRISPR technology is that it can modify several genes at the same time. For example, editing the three genes *OsPIN5b*, *GS3* and *OsMYB30* together will result in larger rice ears and improved cold resistance (Zeng et al., 2020). This trick can solve the long-standing problem in the breeding industry: in the past, if you want high yield, you have to sacrifice stress resistance, and if you want strong stress resistance, your yield may be discounted. But with the simultaneous operation of multiple genes like this, you can suddenly have the best of both worlds (Chen et al., 2024). To put it bluntly, this technology allows breeders to combine various excellent traits like building blocks to cultivate "all-round" rice that is both high-yielding and stress-resistant. However, in actual operation, it takes a lot of effort to find the right gene combination.

The most convenient thing about using CRISPR technology for rice breeding now is that you can "do it all at once"-modify several genes at once. Park and his team tried this trick in 2022, editing several drought-resistant genes in a package, and the new variety was both drought-resistant and high-yielding (Park et al., 2022). This operation is much faster than modifying each gene one by one, saving time and effort. Tang's team also verified this last year. Through simultaneous editing of multiple genes, rice that can adapt to different environments can be cultivated quickly (Tang et al., 2023). To put it bluntly, this is like giving rice a "package upgrade", and you don't have to slowly add up individual traits as before. The most practical thing for farmers is that these new varieties can produce stable yields no matter what the weather is, so they are much more assured to grow them.

6.3 Impact on global food security

The global food problem is becoming more and more of a headache-after all, rice feeds more than half of the world's population. CRISPR technology comes at the right time. This thing can make rice both high-yielding and resistant. Think about it, climate change is now either drought or flooding, and there are rampant pests and diseases everywhere. How can traditional rice withstand this? But scientists have used gene editing to create a number of new varieties that are drought-resistant and salt-tolerant (Ricroch et al., 2017). Romero's 2019 research proved that these improved varieties perform particularly well in harsh environments (Romero and Gatica-Arias, 2019). To put it bluntly, to ensure that everyone has rice in their bowls, it is definitely not enough to rely on the old varieties alone, and these "enhanced" rice must be used.

Now, countries have clearly relaxed their attitudes towards CRISPR technology, especially those edited varieties that do not introduce foreign DNA-in other words, rice that only changes its own genes and does not touch foreign genes. This "clean" editing method is indeed easier to pass approval (Zegeze et al., 2022). Farmers and consumers are also much more accepting, after all, it sounds more reliable than genetic modification. But then again, although the policy is being relaxed, it still has to go through many hurdles to promote it on a large scale. But in any case, this brings the new high-yield and stress-resistant rice varieties one step closer to the field, which is definitely a good thing for solving food security problems.

7 Challenges and Solutions

7.1 Improving editing efficiency

Currently, CRISPR technology has encountered a headache in rice breeding-the editing efficiency is sometimes high and sometimes low, and it is not very stable. Take *OsPIN5b* and *GS3*, the popular target genes, for example. The editing success rate can jump from 42% to 66% in different experiments, like a roller coaster ride (Zeng et al., 2020). If the efficiency cannot be improved, it will not only be time-consuming and labor-intensive, but also costly. Researchers have found that this matter is affected by many factors: it is important to choose the right target site, the gRNA design must also be particular, and even the method of delivering CRISPR components must be particular. To put it bluntly, if you want to improve the success rate, you have to adjust the parameters of each link to the best state like a tuner. Fortunately, with the accumulation of experience, everyone is slowly getting the hang of it, and the editing efficiency is also steadily improving.

Researchers now have many tricks to deal with the problem of editing efficiency. The most direct one is "multi-pronged approach"-designing several different gRNAs for the same gene, there is always one that can hit the bull's eye. The development of bioinformatics has also helped a lot. Now it is much more accurate to use computers to predict target sites, unlike before when you have to rely on guessing (Zegeye et al., 2022). What is even more exciting is the new generation of editing tools, such as "upgraded scissors" such as Cas12 and base editors, which not only cut more accurately, but also more efficiently (Tabassum et al., 2021). For example, some teams have found that when using Cas12 to process certain genes, the success rate can be about 20% higher than that of Cas9. These technological advances combined have given rice breeders more and more confidence in producing stable and reliable improved varieties in the field.

7.2 Reducing off-target effects

The most troublesome thing about CRISPR editing is the "accidental injury" problem-originally wanted to modify gene A, but accidentally moved gene B as well. For example, this situation occurred when editing the *OsMYB30* gene before, and the entire breeding experiment was almost wasted (Zeng et al., 2020). This off-target effect is like injuring other organs during surgery. At the least, it will affect the experimental results, and at worst, it may cause various problems in the plants. Therefore, the gene editing teams are now very careful. Some use computer simulations to predict possible off-target locations, and some have developed new Cas proteins to reduce accidental injuries. After all, if you want to cultivate a truly reliable new rice variety, you cannot fail to meet the accuracy standards.

Scientists now have several tricks to deal with off-target problems. The first is to upgrade the Cas9 protein to a "high-precision version", just like adding an anti-shake function to scissors, which can cut accurately and not easily slip (Zegeye et al., 2022). Designing gRNA is also more sophisticated. Now we use intelligent algorithms to screen the most specific targets, which is completely different from the past decision-making (Romero and Gatica-Arias, 2019).

Another smart way is to use a "time-limited tool"-let the editing component disappear automatically after staying in the cell for a while, so that even if you want to cut randomly, you won't have the chance (Wang et al., 2022). These methods can be used together to basically minimize the risk of off-target. But to be honest, no matter how good the technology is, it must be repeatedly verified, so now everyone will do a whole genome scan after editing rice to ensure that there is no risk before they dare to plant it in the field.

7.3 Enhancing social acceptance

Getting the public to accept CRISPR-edited rice is more difficult than technical breakthroughs. Although scientists think the technology is accurate and efficient, ordinary consumers always wonder if there will be any problems after eating it (Ansari et al., 2020). To be honest, this worry is understandable. After all, the word "gene" is easy to make people nervous. The most important thing now is to make things clear: for example, explain in a simple and easy-to-understand way that gene editing and genetic modification are not the same thing; for example, make field trial data public so that everyone can see that the new varieties are both safe and increase yields. Some teams are quite smart and directly invite farmers to participate in trial planting, using real harvests to speak. In the final analysis, the promotion of new technologies cannot rely solely on laboratory data. Ordinary people must be able to see, eat, and buy them with confidence.

In order to make the people really accept CRISPR rice, it is not enough to just tinker in the laboratory, but we need to bring in all parties to talk. Farmers are most concerned about whether the yield can be increased, consumers are worried about safety, and policymakers have to consider how to formulate regulations-to address the concerns of these people (Zafar et al., 2020). Now some places are doing well, such as organizing some popular science activities, using plain language to explain how gene editing can make rice both high-yield and less pesticide (Sampath et al., 2023). There have also been improvements in policies. Countries like the United States and Japan have learned to distinguish between traditional genetically modified and CRISPR-edited crops, and the approval process has been significantly simplified (Thomson, 2019). In the final analysis, no matter how advanced the technology is, it must be grounded. Only when those who grow crops, eat, and manage things are assured, can CRISPR rice really be served on the tables of ordinary people.

8 Concluding Remarks

Speaking of the technological revolution in rice breeding, CRISPR/Cas9 is definitely a "game changer". The most powerful thing about this technology is that it can edit genes as accurately as a scalpel-you can change whatever you want, and the efficiency is very high. For example, in the matter of drought resistance, scientists tinkered with the *OsSAP* gene, and as a result, rice not only survived better under drought conditions, but also grew stronger. However, it is not enough to solve drought resistance alone.

Now even the yield problem can be solved together: after genes such as *OsPIN5b* and *GS3* are edited, the new varieties not only have higher yields, but also have improved cold resistance. What's more, this technology can also play a "combination punch" and change several genes at one time. The rice cultivated in this way is simply an "all-round player" that can withstand harsh environments and has high yields. Although traditional breeding methods have not been completely eliminated, CRISPR technology has indeed opened up new horizons for rice breeding.

How can CRISPR technology make breakthroughs in rice breeding next? Scientists have several key research directions. First of all, we have to expand the "gene target library" and can't always focus on those old genes. The new tools such as CRISPR-Cpf1 and base editors are quite promising. They are more accurate than the old Cas9 and can perform more complex editing operations.

However, the most troublesome problem now is the off-target problem-just like shooting off the target, we have to find a way to increase the hit rate. In addition, it is also critical whether the excellent traits after editing can be stably inherited. We can't let good traits disappear after being passed on. Interestingly, some people have begun to try to combine CRISPR with traditional breeding hybridization technology, which may be able to breed "hexagonal warrior" rice that is both disease-resistant and drought-resistant and has high yield faster. Of course, for these ideas to become a reality, they still have to rely on a lot of experiments to verify.

Farming is becoming more and more difficult nowadays-either drought or flooding, and there are more and more saline-alkali lands. At this time, CRISPR technology can help a lot. It can cultivate particularly "resistant" rice varieties that can produce stable yields no matter how the weather changes. This is very important for ensuring food security. And these new varieties have an additional benefit: with strong disease and insect resistance, farmers don't have to use pesticides desperately; with improved resistance to barrenness, less fertilizer can be used. To put it bluntly, this technology can not only produce more food from the land, but also reduce the burden on the environment, which is a two-pronged approach. Although it is still in the promotion stage, it is indeed a good way to develop sustainable agriculture.

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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.

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