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CRISPR-Based Modification of Fatty Acid Biosynthesis Pathways to Increase Oleic Acid in Rapeseed

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Abstract Recently, high-oleic rapeseed oil has become increasingly popular in the market. After all, this oil is both healthy and easy to use. Last year, our laboratory began to think about how to use CRISPR, the "gene scissors", to improve rapeseed varieties. To be honest, we just wanted to give it a try at the beginning. I remember that the first time we tried to edit the *FAD2* gene, the result was not ideal. Later, we referred to the successful experience of soybeans and adjusted the design of the guide RNA, which gradually made progress. Now, the oleic acid content of several new strains cultivated has indeed increased a lot, and the linoleic acid content has also decreased. However, when it comes to practical application, it still has to pass the regulatory and consumer acceptance. Speaking of CRISPR technology, it does have great potential in rapeseed breeding. This time we mainly improved the fatty acid synthesis pathway, but the same method should be able to be used for other traits. Of course, the technology itself is still being improved, just like an experiment failed last year due to off-target effects. But in any case, this at least provides a new way to improve the quality of rapeseed oil.
Keywords High oleic acid; CRISPR technology; *FAD2* gene; Rapeseed breeding; Fatty acid synthesis

1 Introduction

Rapeseed (*Brassica napus* L.) is mostly grown in China and Canada (Zhang et al., 2019). To be honest, the oil extracted from it is really good. It can be used as cooking oil and biodiesel (Tian et al., 2022). However, the key to the quality of the oil lies in the fatty acid composition. Oleic acid is quite interesting. It is not only good for human health, but also very stable (Okuzaki et al., 2018). Last year, we conducted a comparative experiment and found that high-oleic oil is not easy to deteriorate (Liu et al., 2022a). The demand for this oil is increasing in the market, and food factories and chemical factories are scrambling for it (Lee et al., 2018). However, although high-oleic oil is expensive, the output still cannot keep up with the demand (Wang et al., 2022).

The CRISPR/Cas9 technology has indeed brought revolutionary changes to plant research. I remember that when Okuzaki and his team first tried it in 2018, the results were not very stable. But now it is different. In 2022, Tian's team showed that this technology is much more accurate than traditional breeding methods and can directly operate on genes. Take rapeseed as an example. Our laboratory tried to edit the *FAD2* gene last year, and the oleic acid content really increased a lot (Liu et al., 2022). But to be honest, we encountered a lot of problems when we first started, such as off-target effects. But compared with the old method of hybrid breeding, we can now directly modify key traits such as fatty acid synthesis, which is much more efficient. Of course, this technology is still being improved, and each experiment may have unexpected results.

Our lab is working on an interesting project recently, which is to use CRISPR technology to improve rapeseed oil. We are mainly targeting a gene called *BnFAD2*, which is responsible for fatty acid desaturation. If we manipulate this gene, we may be able to increase the oleic acid content. To be honest, I was not sure when I started. The fatty acid synthesis pathway is quite complicated, and editing a single gene may not be enough. However, after referring to the successful case of soybean, we decided to start with *BnFAD2*. The experimental plan was revised again and again, and it took several rounds of effort to design the gRNA alone. In addition to the technical challenges, we also have to figure out the regulatory network of the entire fatty acid synthesis. Recent data show that the oleic acid content has indeed increased, although it is still far from the expected level. If this approach can be successful, there will be a new way to breed high-oleic rapeseed varieties in the future. Of course, it is still a long way from practical application, but at least it is a good start.

2 Fatty Acid Biosynthesis Pathways in Rapeseed

2.1 Overview of fatty acid synthesis in plants

The production of fatty acids in plants is quite complicated, and it is mainly completed in places like chloroplasts (Karunarathna et al., 2020). At the beginning, acetyl-CoA is used, and it undergoes several steps of changes before it can become fatty acids. Interestingly, a key link in this process is to convert acetyl-CoA into malonyl-CoA (Huang et al., 2020). After that, it has to rely on the "factory" of fatty acid synthase to process and make saturated fatty acids first. However, these saturated fatty acids are not the final products. Liu et al.'s research in 2022 showed (Liu et al., 2022) that they still need to undergo desaturation, elongation and other modifications before they can become unsaturated fatty acids commonly found in our vegetable oils. Of course, the details of this process may be different for different plants, such as rapeseed and soybeans.

2.2 Key enzymes and genes involved in rapeseed fatty acid biosynthesis

When it comes to the origin of fatty acids in rapeseed oil, there are two enzymes that are particularly critical. The enzyme FatA is quite interesting. It can break down oleic acid-ACP into free oleic acid, which is the main component of rapeseed oil (Liu et al., 2022a). Last year, when we did an experiment, we found that the oleic acid content would indeed change if the expression of this enzyme was regulated (Figure 1). However, it is interesting that different varieties of rapeseed respond differently to this regulation (Shi et al., 2022). Another important role is the SAD enzyme, which is responsible for converting stearic acid into oleic acid. Jiang et al. (2017) found that activating this enzyme can increase the oleic acid content. In actual operation, we found that although the principle is simple, it is quite troublesome to modify it (Huang et al., 2020). Zhang et al. (2019) showed that after modifying this enzyme, saturated fatty acids were indeed reduced, but sometimes other indicators would be affected.

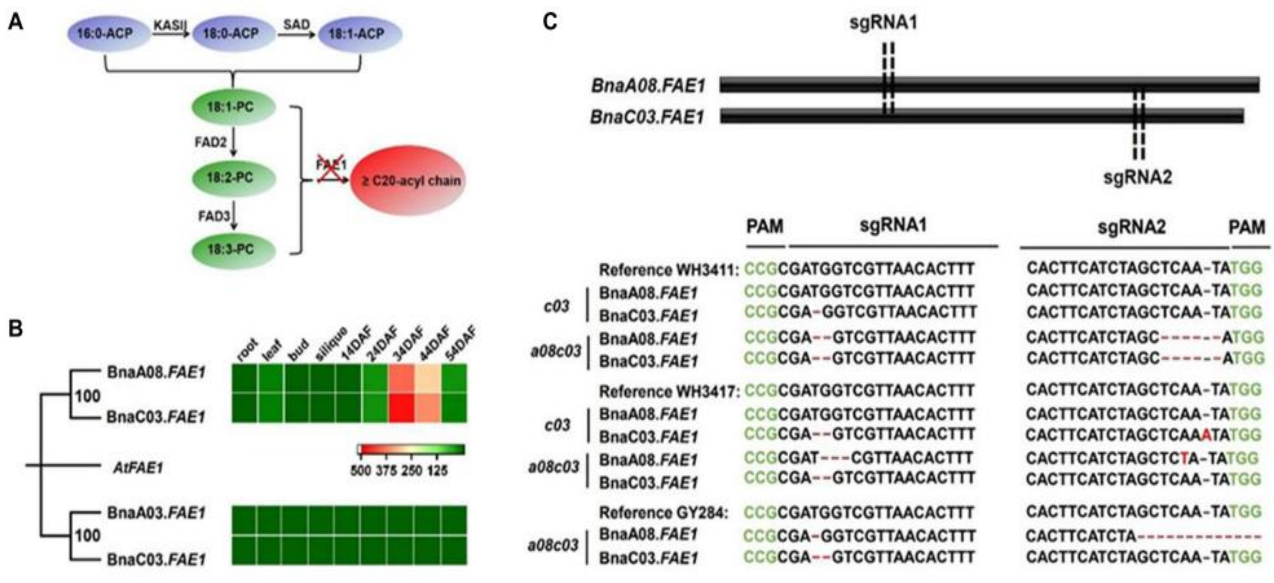

Figure 1 *BnaFAE1* gene analysis and mutant generation (Adopted from Liu et al., 2022b)

Image caption: (A) Illustration of desaturation and elongation of fatty acids. Red cross indicates mutation of *FAE1* genes to block the synthesis of EA. (B) Expression pattern of BnaFAE1s in different tissues. (C) Location of CRISPR/Cas9 sgRNA-1 and sgRNA-2 targeting BnaFAE1 genes and sequencing identification of T2 homozygous mutants. PAM is indicated in green. Red “-” means deletions. Red font indicates nucleotide insertions and substitutions (Adopted from Liu et al., 2022b)

2.3 Role of oleic acid in rapeseed oil quality

Oleic acid in rapeseed oil is a real treasure. It is a monounsaturated fatty acid that has a great impact on the quality of oil. Oil with high oleic acid content is particularly stable and not easy to deteriorate (Karunarathna et al., 2020).

Our laboratory conducted a comparative experiment last year (Liu et al., 2022a) and found that high oleic rapeseed oil is indeed storable and not easy to produce harmful substances when heated. Wang et al. (2022) also confirmed this. However, it is interesting that the oleic acid content of rapeseed from different origins varies greatly. This oil has many uses. It can be used as a high-grade cooking oil and as a biofuel. Huang et al. (2020) found that eating foods rich in oleic acid regularly is good for the heart. So now breeders are trying to find ways to increase the oleic acid content of rapeseed, mainly by regulating the two key enzymes FatA and SAD. Shi et al. (2017) started to study this. Although the progress is good, it will take some time to fully master the regulation method.

3 Application of CRISPR Technology in Fatty Acid Biosynthesis Pathway Modification

3.1 CRISPR/Cas9 mechanism and its suitability for targeted editing

When it comes to the gene editing tool CRISPR/Cas9, the most critical thing is the design of the guide RNA (gRNA). Our laboratory experienced this when we did the rapeseed experiment last year-choosing the right gRNA is very important. Zhang et al. (2019) tried it in 2019, and designed gRNA for key genes in fatty acid synthesis such as *BnFAD2* and *BnLPAT2*, and the effect was really good. But to be honest, we also made mistakes at the beginning, and the efficiency of the designed gRNA was very low (Huang et al., 2020).

The problem of off-target effect is quite annoying. I remember that in an experiment, I wanted to edit *BnFAD2*, but I ended up changing several genes next to it. Later, I referred to the method proposed by Doench et al. (2015), and used computational tools to optimize gRNA design, and the situation was much better. Jiang and Doudna (2017) also suggested that high-fidelity Cas9 variants could be used instead. We tried this and it really reduced accidental injuries. Studies have shown (Liu et al., 2022a) that editing efficiency can be greatly improved as long as the gRNA is properly designed. However, it should be noted that different varieties of rapeseed may respond differently to the same set of gRNA, and we are still exploring this.

3.2 Specific modifications for increasing oleic acid content

When it comes to increasing the oleic acid content of rapeseed, the *FAD2* gene plays a key role. In 2019, Do et al. found (Do et al., 2019) that after knocking out *BnFAD2* with CRISPR, the oleic acid content did increase. The principle is actually quite simple, which is to prevent oleic acid from turning into linoleic acid. However, it is not so easy to operate in practice. Huang encountered some troubles in 2020 (Huang et al., 2020).

The most interesting is the experiment done by Pham et al. (2010), who specifically selected the two sites A5 and C5 on *BnFAD2* for editing. Later, Park followed up in 2021 and found that the oleic acid content of rapeseed modified in this way can exceed 85%. Of course, the effects of different varieties will vary. For example, some strains reported last year were not very stable (Liu et al., 2022a). In fact, modifying *FAD2* alone is not enough. Zhang et al. (2019) tried to edit *BnLPAT2* and *BnLPAT5* at the same time (Figure 2), which had a better effect. These two genes control the Kennedy pathway, and after modification, linoleic acid decreased more significantly. However, this combination editing technology is relatively difficult. Our laboratory has been working on this recently, and the progress has been mixed.

3.3 Experimental approaches for CRISPR-based editing in rapeseed

When it comes to gene editing of rapeseed, transformation technology is actually quite critical. Agrobacterium-mediated methods are widely used, and experiments such as *BnFAD2* have been successful (Do et al., 2019; Huang et al., 2020), but the transformation rate of some varieties cannot be improved. Liu et al. (2022) found that dual gRNA design can indeed improve efficiency, but sometimes multiple gRNAs will increase the risk of off-target effects (Zhang et al., 2019).

The verification process cannot be sloppy, and genotyping must be done first to see the mutation situation. For example, changes in oleic acid content are an important indicator. Huang et al. found in 2020 that some mutant offspring will have trait separation. Of course, sequencing to confirm off-target effects is also necessary-although no obvious problems were found in the study of Do et al. (2019). Now the most direct way to increase oleic acid content is to knock out the *FAD2* gene (Zheng et al., 2019). However, in actual operation, gRNA design is very particular, and Wang et al. (2021) have encountered poor targeting efficiency. When it comes to improving rapeseed quality, the solution proposed by Tian et al. (2022) is more reliable, which is to optimize the transformation process and combine it with strict screening standards.

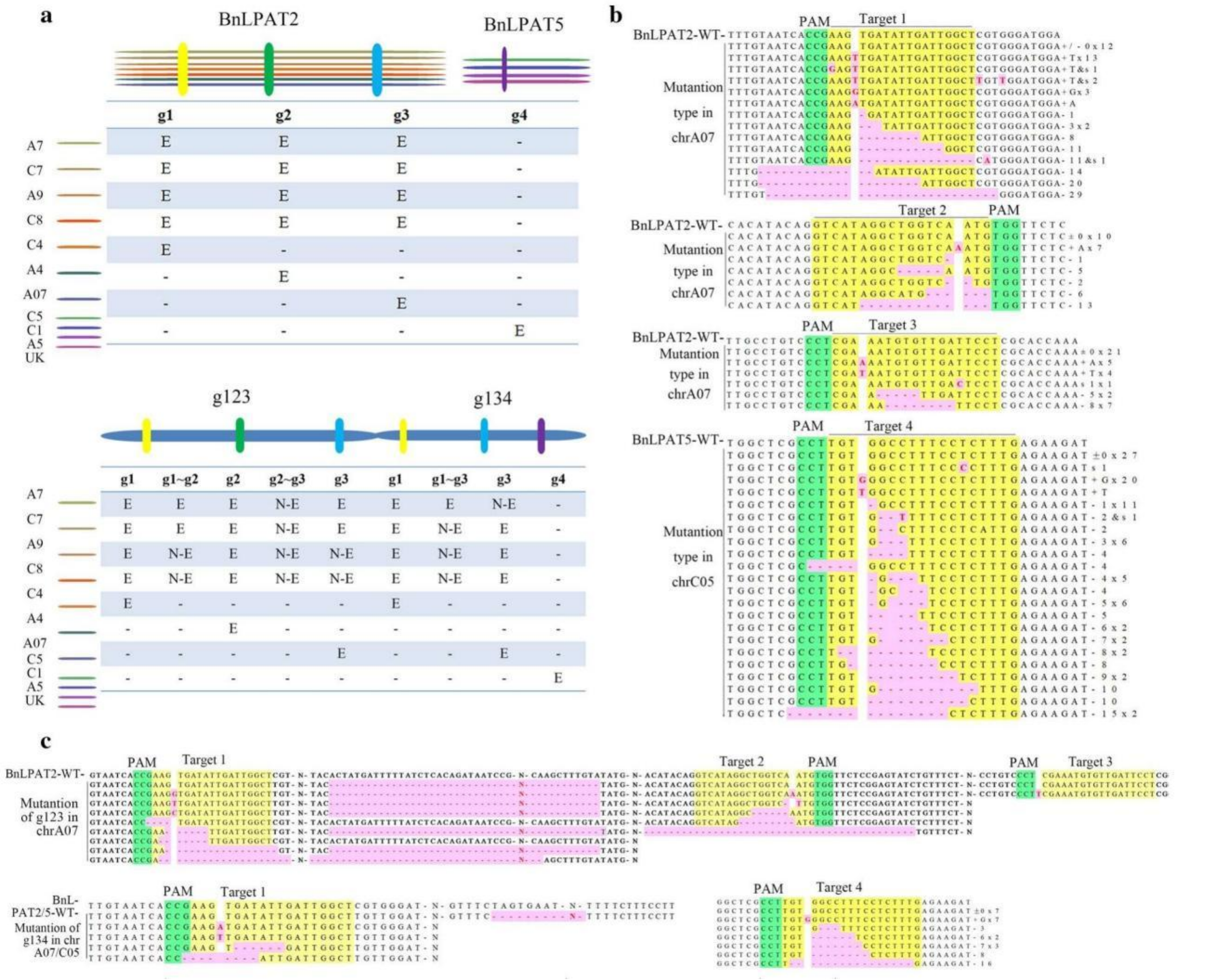

Figure 2 Schematic of gene editing using CRISPR-Cas9 in *B. napus* (Adopted from Zhang et al., 2019)

Image caption: a Single-gRNA (upper) and multi-gRNA (lower) mediated gene editing in *B. napus*. E, editing; N-E, non-editing. b Single-gRNA mediated target for knockout at CRISPR-Cas9-mediated cleavage. Sequencing results in chrA07 are listed in g1 lines, g2 lines and g3 lines, respectively, and in the common site of g4 lines. c Multi-gRNA-mediated target for knockout at CRISPR-Cas9-mediated cleavage. "-" and "+" indicate nucleotide insertions and deletions in the target sequence, while "s" indicates substituted mutations, and "c" indicates combined mutations. The pentagram in red indicates insertions in the target site. gRNAs are coloured yellow, whereas PAMs are coloured green (Adopted from Zhang et al., 2019)

4 Expected Impact on Oleic Acid Content and Oil Quality

4.1 Changes in fatty acid composition following CRISPR editing

CRISPR/Cas9 is indeed a good way to increase the oleic acid content of rapeseed, but the effect depends on how it is edited. For example, Liu et al. (2022) found that if only one site, *BnFAD2*, was edited, the oleic acid ratio was less than 80%, but if both A5 and C5 sites were changed at the same time, the T1 and T4 generations could exceed 85%-this gap is quite obvious. The data of Huang et al. (2020) are similar. The wild-type oleic acid is only 66.43%. After all copies of *BnaFAD2* are mutated, it directly rises to more than 80%.

But the role of CRISPR/Cas9 is not only to increase oleic acid, but also to reduce those not-so-good fatty acids. Shi et al. (2022) found that after knocking out *BnFAD2* and *BnFAE1*, the erucic acid content decreased significantly, and polyunsaturated fatty acids (PUFA) also decreased a little. Jiang et al. (2017) also tried this on domesticated rapeseed (Camelina sativa). Once the *FAD2* gene was edited, linoleic acid and linolenic acid were greatly reduced. These two fatty acids are not very stable and are prone to problems during processing. So overall, the edited oil is indeed more stable and of better quality.

4.2 Comparison of CRISPR-modified rapeseed with conventional high-oleic varieties

The high-oleic rapeseed on the market now has better performance after being modified by CRISPR technology. After the oleic acid content is increased, the antioxidant property of the oil is significantly enhanced, which is particularly suitable for use in the industrial field (Jiang et al., 2017). However, traditional varieties also have their own advantages, such as being more stable in certain processing techniques. From a health perspective, the cardiovascular benefits of this type of modified oil have been confirmed (Shi et al., 2022), and after the PUFA and erucic acid content is reduced, it is more convenient for both consumption and industrial application (Liu et al., 2022; Sandgrind et al., 2023).

In terms of economic benefits, this type of rapeseed variety is indeed very promising. Due to the improved quality, the market price may be higher (Shi et al., 2022). The processing link can also save a lot of costs. After all, the unnecessary fatty acids are reduced, and the refining efficiency is naturally improved (Jiang et al., 2017). The environmental advantages are also obvious. Compared with traditional breeding methods, CRISPR technology is cleaner and safer (Tian et al., 2022). Although some people were concerned about the accuracy of gene editing in the early days, subsequent studies have shown that the risk of off-target effects can be completely controlled (Zhang et al., 2019; Karunarathna et al., 2020). Overall, this technology has indeed brought new opportunities to rapeseed breeding. Not only has the quality of oil products been significantly improved, but the entire industry chain from planting to processing can benefit. As market demand changes, this precisely improved rapeseed variety should become more and more popular.

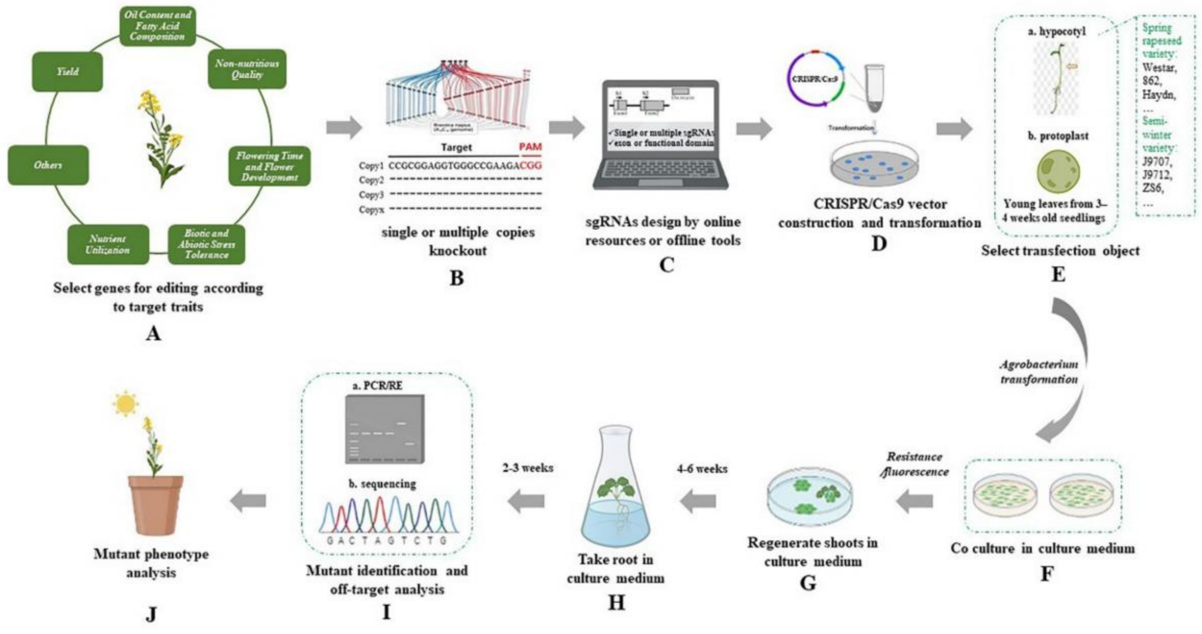

Figure 3 The general workflow of CRISPR/Cas9-based gene editing in rapeseed (Adopted from Tian et al., 2022)

Image caption: (A) Select genes for editing according to target traits. The traits that *B. napus* genes edited by CRISPR/Cas9 are involved in are shown here; (B) perform single or multiple copy knockout according to function differentiation-related information among homologous copies; (C) design sgRNAs for selected target genes using online and offline tools (sgRNAs are usually designed in exon or functional domain, which can lead to a greater probability of functional mutation); (D) CRISPR/Cas9 vector with the sgRNA coding sequence construction and transformation; (E) select genetic transformation object (the representative receptor materials successfully used for transformation for CRISPR/Cas9 vector are shown on the right); (F) deliver CRISPR/Cas9 vector by co-culturing *Agrobacterium* with transfection object in the culture medium; (G,H) differentiate callus and regenerate plants by inducing reagents in the culture medium, and identify positive plants by resistance or fluorescence; (I) identify target and/or off-target editing by (a) polymerase chain reaction restricted enzyme (PCR-RE) and/or (b) sequencing; (J) build target gene-edited rapeseed lines for phenotyping (Adopted from Tian et al., 2022)

5 Case Study: Successful CRISPR-Based Modification in High-Oleic Soybean and Lessons for Rapeseed

5.1 Background of CRISPR success in soybean

The oleic acid content in soybean oil has always been an important indicator, after all, it is related to the quality of the oil. Recently, CRISPR/Cas9 technology has been very useful in this regard. Speaking of which, the *FAD2* gene is a key role-the enzyme it encodes converts oleic acid into linoleic acid. If the function of this gene is inhibited, the oleic acid content will naturally increase (Wu et al., 2020). However, in actual operation, things are not that simple.

Studies have found that editing the *FAD2* gene can indeed significantly increase the oleic acid content while reducing the proportion of polyunsaturated fatty acids (Huang et al., 2020; Liu et al., 2022a). But interestingly, there are some differences in the results obtained by different laboratories (Shi et al., 2022). The latest study by Zhou et al. (2023) pointed out that more influencing factors may need to be considered. For example, the genetic background of some soybean varieties may affect the editing effect, which brings some uncertainty to the actual operation. From an application perspective, this technology-modified soybean oil has great potential in the market. Not only is the nutritional value higher, but the industrial processing performance is also better. However, to achieve large-scale application, some technical details may need to be resolved. After all, the promotion of gene-edited crops is always accompanied by various discussions and considerations.

5.2 Key strategies for targeting similar pathways in rapeseed

The success story of soybeans has inspired us, and perhaps a similar approach can be used on rapeseed. However, the situation of rapeseed is much more complicated. After all, it is polyploid, and there are several copies of the *FAD2* gene (called *BnaFAD2*). This means that multiple gene sites must be edited at the same time to see a significant effect. In practice, researchers have found that editing two specific sites, BnaFAD2.A5 and C5, has a good effect (Huang et al., 2020). Oleic acid content does increase, but the extent of the increase varies from variety to variety (Liu et al., 2022a). Interestingly, if mutations of different alleles are combined, the effect may be better. Of course, the technical difficulty of this multi-target editing has also increased accordingly. From an application perspective, this gene-edited rapeseed seed is indeed very promising. However, compared with soybeans, gene editing of rapeseed faces more challenges, after all, its genome is more complex. This also explains why related research progress is relatively slow.

5.3 Potential adaptations in protocols to suit rapeseed's genetic profile

Editing the rapeseed genome is indeed a technical job. After all, its complex allotetraploid structure is no joke. In order to deal with multiple gene copies, the CRISPR experimental protocol must be specially designed. For example, designing multiple gRNAs for the conserved region of the *BnaFAD2* gene is a good way to ensure more comprehensive editing. However, interestingly, studies have found that using a single gRNA can sometimes achieve good results (Zhang et al., 2019). Of course, the multi-gRNA strategy is usually safer. But having a good editing protocol is not enough, and the transformation and regeneration links are also critical (Liu et al., 2022a). After all, in order to make the edited traits stably inherited, every step of the entire process must be controlled well. In actual operation, it will be found that the protocols adopted by different laboratories may be very different. Some prefer a simple and direct single gRNA, while others insist on a multi-gRNA strategy. This is not to say who is right or wrong, the key depends on the specific research goals and variety characteristics.

5.4 Outcome and insights gained from soybean CRISPR modifications

CRISPR technology has been used successfully in soybeans, which is indeed of great reference value for rapeseed research. You see, by increasing the oleic acid content and reducing those not-so-good polyunsaturated fatty acids, the quality of the oil will be different immediately (Huang et al., 2020). And the best thing is that this kind of gene editing does not require the introduction of exogenous genes, which not only meets regulatory requirements but is also more acceptable to consumers (Shi et al., 2022). But then again, rapeseed is much more complicated than soybeans. The experience of soybeans tells us that it is important to identify the key genes for fatty acid synthesis (Liu et al., 2022a). Now everyone is focusing on the *BnaFAD2* gene, but how to adjust the experimental plan to deal with the complex genetic background of rapeseed still needs to be carefully considered. Recent studies have pointed out that as long as the method is appropriate, CRISPR technology can completely cultivate higher-quality oil crops (Ali and Zhang, 2023). In fact, it is quite interesting to think about it. The same technology can be applied to different crops in very different ways. The successful case of soybeans has given us a direction, but when it comes to rapeseed, we may have to take some detours. But in any case, this does provide new ideas for improving the quality of vegetable oil.

6 Challenges and Future Directions

6.1 Technical challenges in CRISPR editing of rapeseed

When doing CRISPR editing in rapeseed, the most troublesome thing is the efficiency of gRNA. Sometimes the designed gRNA looks good, but the actual effect is not satisfactory. For example, a study found that the overall editing efficiency can reach about 64%, but when it comes to single-site and double-site editing, the success rate is quite different (Liu et al., 2022a). What's more troublesome is that rapeseed is a polyploid plant with too many homologous genes, making it difficult to deliver CRISPR components (Zhang et al., 2019). In the final analysis, if the delivery system is not powerful, no matter how good the editing tool is, it will be useless.

Speaking of stability is another hurdle. Whether the edited traits can be stably inherited is really uncertain. Although Huang et al. (2020) observed that the oleic acid content of mutant seeds was indeed increased, it is hard to say how many generations it can be maintained. After all, factors such as mutation type and genomic environment will affect stability. For breeding, if the trait disappears after being passed on, wouldn't the previous efforts be in vain? Therefore, if this problem is not solved, it will be difficult to truly use CRISPR technology in breeding.

6.2 Regulatory and public acceptance concerns

The regulatory policies of various countries on gene-edited crops vary widely, which has added a lot of obstacles to the commercial promotion of CRISPR rapeseed. Some places require a lot of safety assessments before it can be put on the market, and it takes several years just to go through the procedures (Ali and Zhang, 2023). The most troublesome thing is that the standards of each country are not unified. If it is approved in one country today, it may need to be re-approved in the neighboring country tomorrow.

When it comes to consumer acceptance, the situation is also quite complicated. Although CRISPR technology can bring real benefits, when it comes to "gene editing", many people's first reaction is still to think of genetic modification, and they are very nervous (Jiang et al., 2017). In fact, there is a big difference between the two, but ordinary consumers can't tell the difference. So the key is to make the words clear, explain the scientific principles clearly, and slowly build trust. Otherwise, no matter how good the technology is, it will be useless if the people don't buy it.

Interestingly, the acceptance level varies greatly in different regions. Some countries are relatively open, while others are particularly cautious. This difference sometimes has little to do with science itself, but is more related to cultural traditions and eating habits. Therefore, to promote CRISPR rapeseed oil, it is not enough to rely on technological advantages alone, but we must also learn to "adapt to local customs."

6.3 Future prospects in CRISPR-based fatty acid biosynthesis enhancement

When it comes to improving the fatty acid composition of rapeseed, CRISPR technology does show a lot of possibilities. Recently, a study successfully increased the oleic acid content by knocking out the *BnFAD2* and *BnFAE1* genes (Shi et al., 2022). However, in actual operation, the design of gRNA is critical, which must be both efficient and accurate to ensure that only the target gene is modified.

Now the more cutting-edge approach is to combine CRISPR with other omics technologies. For example, through metabolomics analysis, the specific pathway of fatty acid synthesis can be more clearly understood (Karunarathna et al., 2020). This can not only improve the accuracy of editing, but also discover new regulatory targets. Of course, this multi-omics method is not cheap and requires high laboratory conditions. Although the technology has a promising prospect, it still has to go through several levels to promote it. In addition to optimizing editing efficiency, it is also necessary to consider the different regulatory policies of various countries and whether consumers can accept it. However, as the research deepens, I believe that these problems can be gradually solved. After all, compared with traditional breeding, CRISPR's advantages are still very obvious, especially in the precise regulation of fatty acid composition.

7 Concluding Remarks

CRISPR technology is changing the way we improve the fatty acid composition of rapeseed. Recent studies have shown that by precisely editing specific genes, such as *BnFAD2* and *BnFAE1*, oleic acid content can indeed be significantly improved (Shi et al., 2022). However, there are always some unexpected situations in actual operation. Sometimes the editing efficiency cannot be improved, which may be related to the genetic background of different rapeseed varieties. Now more and more laboratories are beginning to try to combine CRISPR and metabolomics (Karunarathna et al., 2020). This will not only provide a more comprehensive understanding of the fatty acid synthesis pathway, but also discover some unexpected regulatory nodes. Of course, this method requires more resources, which not every research team can afford. When it comes to practical applications, things are much more complicated. In addition to the optimization of the technology itself, we must also consider the differences in regulatory policies in different countries and the acceptance of consumers for gene-edited foods. But it is undeniable that compared with traditional breeding methods, CRISPR does have obvious advantages in precisely regulating fatty acid composition. If these practical problems can be solved in the future, the application prospects of this technology in rapeseed improvement should be more and more broad.

There has been a new breakthrough in rapeseed breeding recently. By using CRISPR/Cas9 technology to tinker with genes that control fatty acid synthesis, we have indeed produced new varieties with better oil quality. However, there are many problems in actual operation, especially for polyploid crops such as rapeseed, which have too many gene copies and are particularly difficult to edit. The effect of increasing oleic acid content is quite obvious. Not only has the nutritional value increased, but the stability of the oil has also improved a lot. This is good news for the biofuel industry, but how much change it can bring depends on subsequent applications (Liu et al., 2022a). Interestingly, this editing method may also work on other polyploid crops, such as wheat and cotton. When it comes to improving oil quality, it is not just rapeseed. Oil crops such as soybeans and sunflowers should be able to learn from this method (Huang et al., 2020). Of course, each crop has its own characteristics and cannot be copied completely. The problem now is that although the effect is good in the laboratory, it still has to go through several hurdles to promote it on a large scale, including practical issues such as regulatory approval and farmer acceptance.

CRISPR/Cas9 is a very useful tool for plant gene editing. Its most powerful feature is that it can accurately locate the gene to be modified, and its efficiency is much higher than the old method. In our experiment, we found that it worked well to adjust the oleic acid content of rapeseed, but there were still some minor problems in actual operation. Polyploid crops like rapeseed have a large number of gene copies, and it was almost impossible to edit multiple copies at the same time. But using CRISPR/Cas9 is much more convenient, although the success rate is not 100% (Zhang et al., 2019). When it comes to improving oil quality, increasing oleic acid content is indeed a breakthrough, but the specific improvement depends on subsequent stability tests (Liu et al., 2022a). This technology is developing very fast, and it is estimated that it will be able to show its prowess in rapeseed breeding in a short time. However, to truly promote it, it may be necessary to optimize the editing efficiency. After all, agricultural applications have to consider the cost issue, and farmers will not pay for flashy technology.

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Conflict of Interest Disclosure

The author affirms 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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