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Genetic Regulation of Pigmentation in *Brassica napus* Flowers

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Abstract This study focuses on the genetic regulation mechanism of flower pigments in *Brassica napus*, with a particular emphasis on analyzing the roles of anthocyanins and carotenoids in flower color formation. Through metabolomics and RNA sequencing techniques, key genes and metabolites involved in flower color formation were identified, such as *BnaA07.PAP2* and *BnaA03.ANS* genes that regulate red petal coloring, as well as *BnaA09.ZEP* and *BnaC09.ZEP* genes that affect orange petals. The regulatory role of these genes in metabolic pathways through transcription factors was revealed, and the effects of epigenetic and environmental factors on pigment formation were explored. The functions of key genes were validated through CRISPR/Cas9 and other techniques. The research results provide a genetic basis for improving the ornamental value of rapeseed, optimizing pollination efficiency, and enhancing stress resistance. At the same time, it also points out the need for further research on complex genetic networks and environmental gene interaction mechanisms in the future.

Keywords *Brassica napus*; Pigment deposition; Carotenoids; Anthocyanins; Gene regulation

1 Introduction

Brassica napus, also known as rapeseed, is actually a very important crop worldwide. At first, the main reason was that the seeds had a high oil content and were used for oil extraction. The remaining cake meal was also quite rich in protein. However, apart from consumption, it is now also a major source of biodiesel (Shah et al., 2018). Although the impression that most people have of rapeseed may remain at oil extraction, its flowers are actually quite crucial. They can attract pollinating insects. Without this step, seed setting and yield will be affected. Interestingly, this crop has quite strong adaptability. The types grown in different places are also different-there are winter rapeseed, spring rapeseed, and semi-winter ones. The main difference lies in the flowering time, which enables it to grow in various climatic conditions.

In *B. napus*, the color of flowers is not just beautiful, it is actually quite important for both crops and agriculture. Pigments such as anthocyanins and flavonoids are mainly able to attract pollinating insects. Without their help, the fruiting rate and yield may be affected. Moreover, the formation of pigments involves many genes and metabolic pathways, making it a hot topic for botanists to study how colors come about. Studying this is not only interesting, but also has practical applications-for example, it can help us cultivate new varieties that are more attractive to pollinating insects or more resistant to environmental stress (Wang et al., 2023).

Our main purpose in conducting this research is to understand how the flowers of *Brassica napus* display color, especially which genes and metabolic processes are involved. Previously, it was known that pigment deposition was not only related to appearance, but also to pollination and stress resistance, but the specific regulation methods were not fully understood. So we plan to use metabolomics and RNA sequencing to identify key substances and genes related to the coloring of red petals. Not only that, but we will also use methods such as RNA interference and gene overexpression to see how these genes affect color synthesis. I hope that through these works, we can better understand the genetic mechanism of color formation, and lay a foundation for cultivating more beautiful and practical rapeseed varieties in the future.

2 Genetic Basis of Flower Pigmentation

2.1 Key pigments and their roles in flower color

Anthocyanins, as a water-soluble pigment, are quite common in plants, and many colors such as red, purple, or blue are due to them. For example, in the case of *Brassica napus*, whether it is flowers, leaves, or stems, color cannot be formed without these substances. However, you may not know that these colors are not only for beauty, but also help plants cope with environmental pressure. Whether it is extreme weather or when there are pests and diseases, they have a certain defensive effect (Jiang, 2024). In *B. napus*, anthocyanin accumulation is not the the final say of one or two genes. Both structural genes and regulatory genes will participate, especially transcription factors such as MYB, which are particularly critical in the synthesis process.

Carotenoids are also a common type of pigment, such as often making flowers appear yellow, orange or red. Rapeseed like *B. napus* mainly contains lutein and zeaxanthin in its yellow petals. Interestingly, some mutations can cause petals to have different colors-for instance, if the genes *BnaA09.ZEP* and *BnaC09.ZEP* are missing, even orange petals may appear. In addition to coloring, carotenoids are actually quite beneficial to the health of the plants themselves and their environmental resistance. Also, you may have heard of betaine, which comes from tyrosine and can form red and yellow colors in some plants, but it is generally not seen in *B. napus*. In fact, rape mainly relies on anthocyanins and carotenoids to present its color, and these two components play a more significant role in color formation (Grotewold, 2006).

2.2 Overview of biosynthetic pathways

In B.napus, the synthesis of anthocyanins is not achieved in one step. It requires the conversion of several enzymes step by step-such as Chalketone synthase (CHS), chalketone isomerase (CHI), and anthocyanin synthase (ANS), etc. They basically start with phenylalanine and gradually synthesize anthocyanins. However, these enzymes alone are not enough; in many cases, the expression of regulatory genes also needs to be examined. Genes like *BnaA07.PAP2* and BnaTT8, once activated, can make those enzyme genes expressed more strongly, and anthocyanins accumulate more in the petals. In fact, the entire synthetic pathway is quite delicate and is usually regulated jointly by transcription factors such as MYB, bHLH, and WD40. They form a complex and jointly control the on and off of biosynthetic genes (Yan et al., 2021).

In *B. napus*, the synthesis of carotenoids actually relies heavily on genes like *BnaA09.ZEP* and *BnaC09.ZEP*, which encode zeaxanthin cyclooxygenase. Generally, if these genes are mutated or missing, the composition of carotenoids will be significantly different, and the color will also change accordingly. For example, if there is a problem with the *BnaA09.ZEP* and *BnaC09.ZEP* genes, the content of lutein will increase, while the amount of zeaxanthin in maize will decrease, and eventually the petals may turn from the common yellow to orange. Not only that, but there is also a crucial carotenoid isomerase gene called BnaCRTISO, which is mainly responsible for the conversion of carotenoids. Once mutated, petals may even turn milky white.

2.3 Key genes involved in pigmentation

When it comes to how the flowers of *B. napus* display their colors, it is actually inseparable from two types of pigments-anthocyanins and carotenoids, each of which has a specific structural gene responsible for synthesis. For instance, anthocyanins mainly rely on the enzymes encoded by genes such as CHS, CHI and ANS to catalyze step by step. The synthesis of carotenoids cannot do without the participation of genes such as BnaPSY and BnaPDS3. In simple terms, it is precisely these genes that work diligently that enable the petals to eventually present the colors we see.

In fact, in *B. napus*, flower color is not determined by structural genes themselves-what really plays a regulatory role behind the scenes are some key transcription factors. For instance, BnaA07.PAP2, BnaMYBL2 and BnaTT8 are all "regulars" in the anthocyanin synthesis pathway. Once they become active, the expression of structural genes such as CHS and ANS follows suit, and anthocyanins accumulate more and more. Interestingly, the regulation is not all positive. Inhibitory factors like BnaWRKY41-1, which usually suppress anthocyanin synthesis, can cause the petal color to darken instead once they mutate and become ineffective (Duan et al., 2018).

The situation on the carotenoid side is similar. Although *BnaA09.ZEP* and *BnaC09.ZEP* have names like structural genes, in fact, they are also silently regulating the proportion of different types of carotenoids. Once lacking, the balance of lutein and zeaxanthin is disrupted, and the flower color changes accordingly.

2.4 Epigenetic and environmental influences

In fact, the color formation of *B. napus* is not entirely determined by genes themselves-epigenetic modifications and environmental factors often play a role. For example, although *BnaA07.PAP2* is a crucial regulatory gene, if something is inserted into its promoter region, its expression may be enhanced, ultimately leading to an increase in pigment accumulation. The environment can also be quite "mixed in", as factors such as lighting, temperature, and exposure to drought or pests can all affect the synthesis of pigments. Take drought as an example. Although it may sound like a bad thing, it actually stimulates the accumulation of anthocyanins, helping plants enhance their antioxidant and stress resistance abilities (Chen et al., 2022). So you see, the matter of color is quite complicated. It depends not only on how genes are adjusted themselves, but also on how the external environment changes. The two sides blend together to form the color we see in the end.

3 Molecular Mechanisms in *Brassica napus* Flower Pigmentation

3.1 Recent advances in genomic studies

When it comes to the research on the color formation mechanism of rape flowers in recent years, it is truly thanks to the progress of genomics and various omics methods. The combination of technologies such as metabolomics, BSA-seq and RNA-seq has helped us identify many key genes and metabolites involved in flower color formation. Take the research of Ye et al. (2022) for example. They found that there are both yellow carotenoids and red anthocyanins in apricot petals, while pink petals mainly accumulate colorless carotenoids and red anthocyanins-you see, color is not determined by a single pigment at all, but by the mixture of several components. Behind the scenes, there is a bunch of genes pulling at each other, and only then do the colors we see become visible.

3.2 Identification of candidate genes for pigmentation

Now we already know quite a few genes related to the flower color of *B. napus*, although the mechanisms by which each of them functions are rather complex. For instance, the gene *BnaA07.PAP2* is now generally regarded as the key to regulating anthocyanin synthesis-it is particularly active in both apricot and pink flowers. In addition, the formation of orange petals is actually closely related to *BnaA09.ZEP* and *BnaC09.ZEP*. If the functions of these two genes are deficient, lutein will increase, while zeaxanthin will decrease, and eventually the color will lean towards orange. Another point that cannot be overlooked is *BnaA03.ANS*, which is almost irreplaceable in red petals. Without it, red pigment could not accumulate at all.

3.3 Functional characterization using CRISPR-Cas9 and RNA interference

Nowadays, technologies like CRISPR-Cas9 and RNA interference (RNAi) have really helped us a lot, allowing us to see more clearly how various genes in *B. napus* affect flower color. For example, someone used CRISPR to knock out the carotenoid isomerase gene BnaCRTTISO, and surprisingly, the petals turned creamy white and even the leaves appeared yellow-which effectively confirmed its role in carotenoid synthesis. Similarly, after silencing the *BnaA03.ANS* gene using RNAi, the original raspberry red petals became noticeably lighter in color, turning into rice red or even zinc yellow. This once again demonstrates how crucial this gene is for anthocyanin synthesis. To be honest, without these technologies, we might still be guessing what role these genes play.

3.4 Gene expression patterns across developmental stages

In fact, to understand how a flower gradually shows its color, one really needs to look at how its genes work at different stages of development. The transcriptome data tells us that many genes related to the synthesis of carotenoids and flavonoids are not expressed from beginning to end-some are active in the early stage, while others come into play later. Jia et al. (2021) found that compared with white flowers, the total amount of carotenoids in yellow flowers was much higher throughout the development process, indicating that the color was not formed all at once but accumulated gradually. Genes like BnaPSY and BnaC4H are also quite interesting.

Their expression patterns in petals and leaves are completely different. It is obvious at a glance that they play a key role in the synthesis of carotenoids and flavonoids. So you see, the matter of flower color is not as simple as just turning a few genes on and off. When and where it is expressed is actually very delicate.

4 Case Study: Role of *BnMYB111* in Anthocyanin Accumulation

4.1 Selection criteria for the case study

The reason why *BnMYB111* was chosen as the research subject this time is mainly because this gene is extremely crucial in the anthocyanin synthesis pathway of rape-after all, anthocyanins directly determine whether the petals can show red, purple or blue. Although flower color formation is influenced by many factors, MYB transcription factors such as BnMYB111 are definitely the "core figures" in the regulatory camp. They can not only activate downstream anthocyanin synthesis genes, but also directly affect the accumulation level of pigments (Figure 1) (Hao et al., 2022).

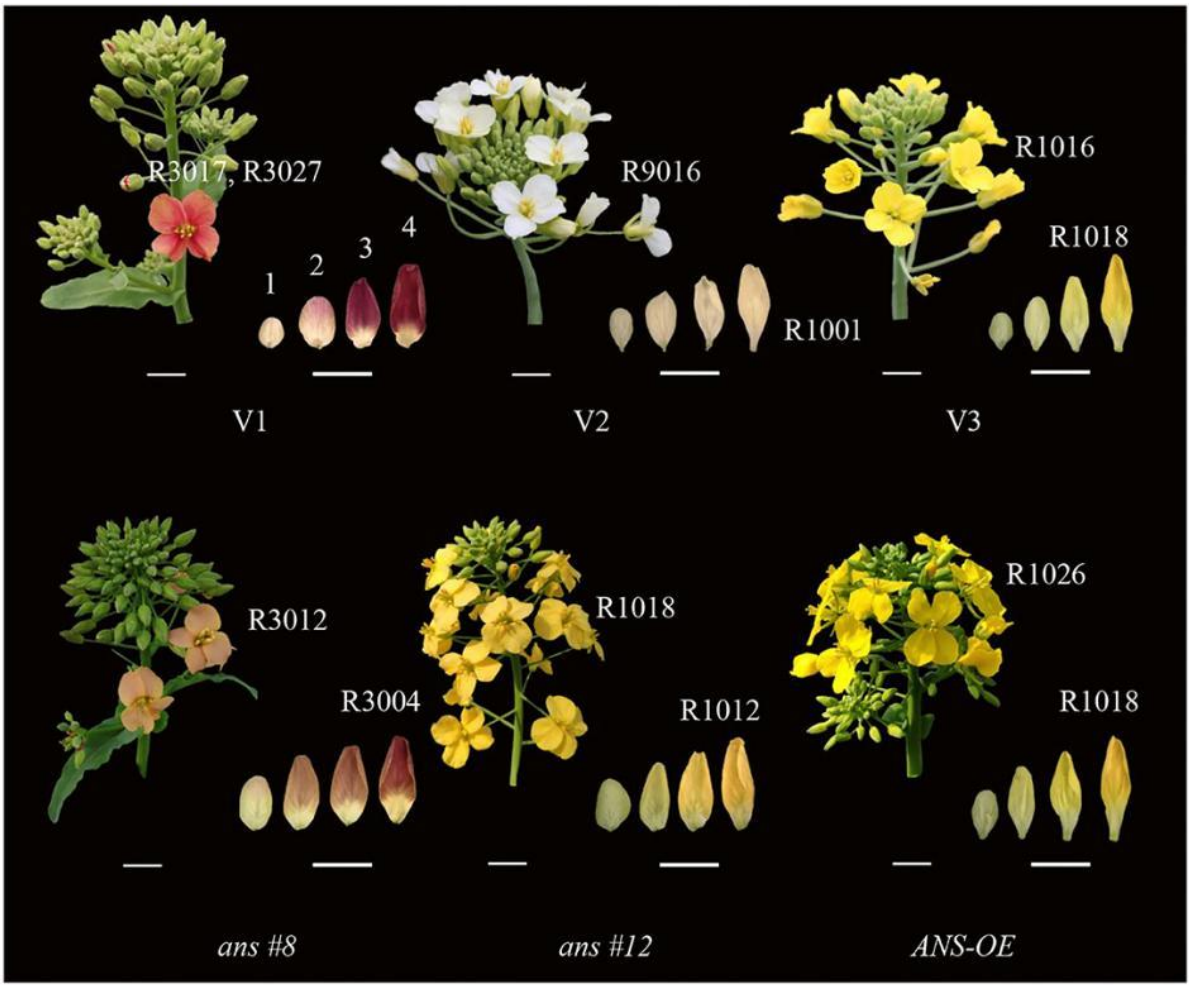

Figure 1 Inflorescence individuals and unopened petals phenotypes of *Brassica napus* with red (Zhehuhong, V1), white (Zhehubai, V2), yellow (Zheyou 50, V3) color, and the transgenic lines (ans#8 and ans#12 indicated RNAi-1 and RNAi-2 plants; ANS-OE indicated overexpression plants). Numbers on the top of unopened petals in V1 represented 4 development stages. R numbers represent colors alignment to RAL methods. Bars at the left bottom represent 1 cm and bars at the right bottom represent 0.5 cm (Adopted from Hao et al., 2022)

For instance, previous genetic analyses and multi-omics data have found that BnaA07.PAP2, which is closely related to it, is actually one of the main switches for the formation of flower color in rape. It is particularly active in flowers with darker colors such as apricot and pink, and encodes a protein located in the cell nucleus. When actually conducting functional verification, it was also found that once some editing was made to the MYB gene, anthocyanin coloring would be significantly deepened, and incidentally, the stress resistance of the plant was also improved-whether it was pests and diseases or environmental stress, it was easier to cope (Klees et al., 2021).

Interestingly, the regulatory methods of such genes are rather complex. Take the homologous gene *BnaPAP2.A7* of BnMYB111 for example. Different splicing variants can have completely opposite effects on anthocyanin synthesis, which also indicates that we cannot make a blanket statement. Later, some people examined the metabolomics and transcriptomic data together and found that the accumulation patterns of anthocyanins and related flavonoids in petals of different colors were indeed different, and it was precisely the differential expression of genes such as *BnMYB111* behind this. So, although color is just an appearance, the regulatory network hidden inside is much more intricate than we think.

4.2 Functional validation of *BnMYB111* in regulating anthocyanin biosynthesis

Recently, people have been paying particular attention to the *BnMYB111* gene, mainly to figure out exactly how it regulates anthocyanin synthesis in rapeseed. As a member of the MYB transcription factor family, BnMYB111 is not alone-it often works together with "relatives" such as BnMYB90 and BnMYB114 to affect the expression of key anthocyanin synthesis genes like *DFR* and *F3H* (Chen et al., 2021). It was also found during the actual experiment that once the plants were overexpressed with BnMYB111, the anthocyanins in the petals increased significantly. No problem, it is a positive regulatory factor. Interestingly, not only rapeseed itself has this mechanism, but also in *Arabidopsis thaliana*, knocking out certain negative regulatory factors-such as AtMYB60, AtCPC or AtMYBL2-can deepen the coloring of anthocyanins. This can't help but make people wonder if there is a similar "brake gene" hidden in rapeseed, but we just haven't fully discovered it yet.

4.3 Experimental evidence: trANScriptomics and metabolomics analysis

Through transcriptome and metabolome analysis, we now have a more specific understanding of the regulatory mechanism of rapeseed anthocyanin synthesis. For instance, the results of RNA-seq and metabolic profiling showed that genes such as *ANS*, *DFR* and *UF3GT* had significantly higher expression levels in red petals than in white and yellow petals, indicating that they were indeed directly related to red pigment formation. In fact, not only structural genes but also alleles play a prominent role. For instance, the type BnaA07.PAP2$^{\text{In-184-317}}$, once introduced, can widely activate multiple genes along the anthocyanin synthesis pathway, turning the originally yellow flower into an apricot color and significantly increasing its accumulation. However, color formation does not rely on just one pathway. Transcriptional data also show significant differences in the expression of carotenoid and flavonoid related genes, indicating that the overall regulatory network is more complex than imagined (Zhang et al., 2020). These omics pieces of evidence, when put together, gradually pieced together the regulatory puzzle behind the patterns.

4.4 Implications for breeding and genetic engineering

Understanding how anthocyanin synthesis in rapeseed is regulated is actually quite useful for both breeding and genetic engineering. The discovery of key genes such as *BnMYB111*, *BnaA07.PAP2* and *BnaA03.ANS* has provided many clues for the future breeding of new varieties with brighter colors. These genes themselves can also be used as targets for genome editing, and perhaps some rapeseed with special flower colors and better resistance to environmental pressure can be produced. In practice, whether it is overexpression or gene knockout, it has been verified that they can indeed change the accumulation level of anthocyanins-in this way, not only is it good-looking, but perhaps it can also enhance some practicality in agriculture. In addition, the transcriptome and metabolome data are becoming increasingly abundant nowadays. There is more basis for selecting candidate genes, and it should be much smoother to cultivate what ideal traits in the future.

5 Evolutionary and Comparative Genomics Perspective

5.1 Evolutionary conservation of pigmentation pathways in Brassica species

In fact, if you take a closer look at several cruciferous plants, you will find that the pathways through which they synthesize pigments are quite similar-whether it's carotenoids or anthocyanins, the functions of many key genes remain quite consistent. Take *BnaA09.ZEP* and *BnaC09.ZEP* in rape for example. Both of these genes encode zeaxanthin cyclooxygenase and are basically expressed only in flowers. Functionally, they can almost replace each other. It is obvious that they are quite important in flower color formation. This pattern seems to be quite common in other cruciferous plants as well. The situation is similar for anthocyanins. Regulatory genes like

BnaA07.PAP2 are not only involved in rapeseed but also in similar synthetic pathways in cabbage (*B. oleracea*) and Chinese cabbage (*B. rapa*) (Figure 2) (Chen et al., 2020). Looking at it this way, although the species are different, the "toolbox" for regulating colors seems to be passed down from generation to generation.

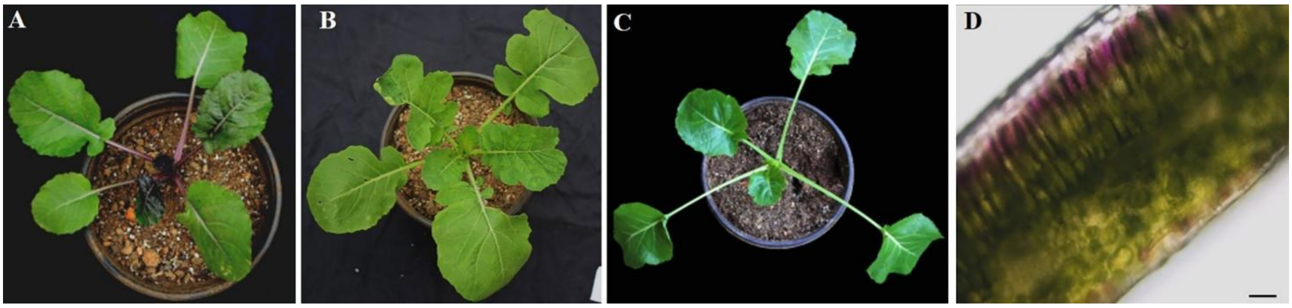

Figure 2 Characterization of anthocyanin accumulation in leaves of purple rapeseed (Adopted from Chen et al., 2020)
Image caption: (A-C) Different phenotype of young plant of PR (A) and ZS11 (B) planted outdoor and PR in the glass greenhouse (C), (D) Hand section of a young leaf of PR. Scale bar: 10μm (Adopted from Chen et al., 2020)

5.2 Insights from comparative genomics across related species

Comparative genomics has indeed helped us understand many things in recent years, especially on the genetic basis of pigment regulation in rapeseed and its related species. Take the carotenoid isomerase gene *BnaCRTISO* for example. Once it mutates, the color of petals and leaves will change quite significantly-this actually indicates that behind pigment regulation, many genes are cooperating with each other, and the movement of one alone may trigger a series of reactions. In addition, genes like *CONSTANS-LIKE* (*COL*) are also quite interesting. They are originally closely related to photoperiod and flowering time. However, in the cruciferous family, both the gene structure and the promoter region are quite conserved, which makes people can't help but speculate Coloring and flowering may be evolutionarily related. There are also homologous genes like *SOC1*. Although they all regulate flower color and flowering time, their expression patterns are not exactly the same in different species, indicating that transcription factor binding sites are gradually differentiating during evolution while still retaining some core functions (Sri et al., 2020). From this perspective, the evolutionary process not only retains old debts but also adds new tricks, making the regulatory mechanism increasingly complex.

5.3 Adaptive significance of pigmentation variations

The appearance of different flower colors in cruciferous plants is actually due to adaptive considerations-after all, color directly affects whether it can attract pollinating insects, which is closely related to the success rate of reproduction. Take rapeseed as an example, petals like apricot and pink, which contain anthocyanins (which are closely related to the regulation of BnaA07.PAP2), are usually more attractive to pollinators than pure yellow or white. In addition, pigments are not only used to attract insects, such as the *BnaA03.ANS* gene, which is almost irreplaceable in the formation of red petals. Its differential expression is likely to enable plants to live better in specific environments. Interestingly, it has recently been found that the distribution of epigenetic markers between different subgenomes of rapeseed is actually asymmetric, indicating that color regulation during evolution may also respond to environmental selection pressure. From this perspective, the color scheme is just a "face saving project", which is not quite fair-it hides a whole set of survival strategies underneath.

6 Applications in Breeding and Biotechnology

6.1 Enhancing ornamental value of *Brassica napus* flowers

In fact, there is a rather complex genetic regulatory mechanism behind the color of rape flowers, which also leaves much room for improvement in terms of its ornamental value. For instance, if we can adjust certain genes in the synthesis pathways of carotenoids or anthocyanins, there will be a chance to make the color of flowers more diverse-not just the common yellow, but also possibly orange, pink and even red. Previous experiments have found that if the genes *BnaA09.ZEP* and *BnaC09.ZEP* are silved, the composition of carotenoids will change, with an increase in lutein accumulation and a decrease in neoxanthin, and finally the petals will show orange. Anthocyanins also have similar regulatory roles. For instance, *BnaA07.PAP2* is regarded as one of the key genes.

Once activated, it can drive the expression of a batch of downstream genes, enabling rape to produce apricot or pink flowers. These discoveries actually offer quite a few ideas for breeding. In the future, it might be possible to cultivate more aesthetically pleasing and popular rapeseed varieties that not only have high yields but also take into account ornamental value.

6.2 Implications for pollination biology and crop yield

The role of flower color in the pollination process of rapeseed is actually more important than we imagine-after all, color is the first thing that pollinating insects notice, which directly affects whether they are willing to come, whether they can effectively pollinate after coming, and ultimately also affects seed setting and yield. Take carotenoids for example. If the *PHYTOENE DESATURASE 3* gene mutates, petals will turn yellowish-white, pigment components and the structure of pigment bodies in cells will change accordingly, and the behavior of pollinators may also change as a result (Zhao et al., 2021). In addition, not only by color to attract external pollination, but also some people have attempted to change the pollination method through genetic means-for example, by causing chromosomal inversion through MITE transposions, closed-flower pollination, that is, mainly self-pollination lines can be cultivated. This not only helps maintain seed purity, but also accelerates the breeding process (Wan et al., 2022). So, if the genetic mechanism behind flower color can be clarified, breeders can design pollination strategies more precisely. Whether it is to attract insects or control self-pollination, the ultimate goal is to further increase yield and quality.

6.3 Future directions for genetic improvement

If you want to have a deeper understanding of how rapeseed flower color is regulated in the future, there are actually several directions that are particularly worth pondering. Firstly, it is necessary to identify the genes involved in the synthesis pathways of flavonoids and carotenoids that have not been fully studied one by one, and then verify their roles-although some are now known, there is still a long way to go before fully understanding the molecular mechanisms of flower color formation. Secondly, gene editing technologies such as CRISPR/Cas9 will definitely be key tools that can more accurately adjust target genes, and perhaps create flower color types that we cannot even imagine now. In addition, with the increasing maturity of multi omics methods, integrating metabolomic, transcriptomic, and genomic data into one analysis can not only discover new genetic resources, but also have the opportunity to develop practical molecular markers, making breeding screening more efficient (Wan et al., 2023). Applying these tools and techniques should not only enhance the ornamental value of rapeseed, but also optimize pollination efficiency, ultimately taking yield and quality to the next level.

7 Challenges and Future Perspectives

Although we have gained a good understanding of the genetic regulation of rapeseed color formation, there are still many details that have not been clarified. For example, the molecular mechanisms behind why carotenoids and anthocyanins accumulate differently between flowers of different colors are still not fully understood. Although genes like *BnaA07.PAP2* have been shown to be closely related to anthocyanin coloring, we still know very little about how they are regulated and how they interact with the carotenoid pathway. In addition, genes like BnaZEPs, which have functional redundancy in carotenoid synthesis and are only expressed in specific tissues, require further exploration of how they operate (Liu et al., 2020). More complicated is the lack of systematic research on how regulatory SNPs, various transcription factors, and the target genes they control work together to ultimately affect flower color. Fill in these blanks bit by bit, and it's probably the most important area for further research to focus on.

7.1 Integration of multi-omics approaches

To fill these knowledge gaps, it is probably still necessary to rely on the integrated approach of multi-omics-piling up genomic, transcriptomic, proteomic and metabolomic data for review, in order to piece together a more complete picture of flower pattern regulation. For instance, previously, BSA-seq, RNA-seq and metabolome analysis have been combined and indeed helped us identify many key genes and metabolites related to flower color. The advantage of this type of method is that it can simultaneously grasp clues from multiple levels, which is much more effective than going it alone. In addition, nowadays gene editing is becoming increasingly convenient.

Using CRISPR/Cas9 to knockout or adjust candidate genes, combined with high-throughput sequencing for verification, not only can the gene functions be clarified, but also there is an opportunity to discover those regulatory elements hidden in non-coding regions (Li et al., 2022). Only by integrating and applying all these technologies can we truly clarify the intricate genetic and biochemical networks behind the formation of flower patterns.

7.2 Ethical and regulatory considerations

As more and more research focuses on improving the ornamental and agronomic traits of rapeseed, we also need to start seriously considering the ethical and regulatory issues behind it-especially after gene editing technologies like CRISPR/Cas9 are used too much, people inevitably worry about the potential ecological impact and safety of genetically modified crops. The existing regulatory framework must keep up to ensure that these genetically modified rapeseed varieties do not have negative impacts on the environment and human health. In fact, it is not only about regulations, but also crucial to communicate with the public: the benefits and potential risks of genetic modification need to be clearly explained in a transparent manner, so that more people can participate in the discussion. Ultimately, only by properly handling these ethical and regulatory aspects can genetically engineered rapeseed varieties be truly and responsibly promoted.

8 Conclusion

In the process of studying how the flower color of *Brassica napus* is formed, we have noticed some rather interesting phenomena. For instance, if the functions of the *BnaA09.ZEP* and *BnaC09.ZEP* genes are lost, the composition of carotenoids in the petals will change-lutein accumulates more, while vitamin E decreases instead, eventually making the petals appear orange. On the other hand, the BnaA07.PAP2 gene has also been confirmed to be a key factor in regulating anthocyanin coloring. Once activated, it can promote the massive accumulation of red anthocyanins in petals. The formation of red pigment also cannot do without BNAa0.ans. Basically, it can be said that it is indispensable. It was also found in the experiment that if BnaCRTISO mutates, its petals will turn milky white and the color of its leaves will also become lighter. It is obvious at a glance that it simultaneously affects the synthesis of carotenoids and flavonoids. Also, when there is a problem with the *PHYTOENE DESATURASE 3* gene, the synthesis of carotenoids will be blocked, and the petals will turn yellowish-white. When these results are put together, they gradually piece together the somewhat complex yet rather exquisite network behind the regulation of rape flower color.

These findings are not just laboratory results, they are actually quite meaningful for plant genetics and crop breeding. For example, by understanding how the color is regulated, it is possible to design ornamental rapeseed with colors that better meet market demand in the future-whether it is a darker red or a brighter orange, they may be "customized", which can enhance both ornamental and commercial value. Moreover, once the synthesis pathways of carotenoids and flavonoids are clarified, metabolic engineering will have room for operation, not only for color adjustment, but also for improving crop stress resistance or nutritional composition. Moreover, the functions of key genes such as *BnaA07.PAP2* and BnaZEPs are gradually becoming clear, which is equivalent to providing a large number of ready-made genetic tools for breeding work-in the future, it may be much easier to cultivate varieties with novel colors and agronomic traits. So, studying flower colors may seem like pursuing "beauty", but the genetic mechanisms and potential applications hidden behind them are far more important than we imagine.

If we are to study the flower color of rape next, we may have to go in a more complex direction-for instance, how exactly do the carotenoid and flavonoid pathways interact with each other, and whether there are any regulatory genes that we haven't discovered yet involved. In addition, environmental factors are also quite worth considering. When external conditions such as light and temperature change, will gene expression also change, eventually leading to different flower colors? Technically, it is still necessary to rely on gene editing tools like CRISPR/Cas9, combined with multi-omics analysis, to verify the functions of the genes that have been identified while continuing to explore new regulatory targets. In fact, the ultimate goal is still to serve breeding: only by gradually clarifying these genetic mechanisms can there be a chance to cultivate rapeseed varieties that are both novel in color and stable in inheritance. At that time, whether for ornamental purposes or for sale, the value can be further increased.

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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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