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Genetic Regulation of Fatty Acid Biosynthesis in *Brassica napus* Seeds Based on *FAE1* and *FAD2* Genes

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Abstract Fatty acid elongase 1 (FAE1) and delta-12 fatty acid desaturase 2 (FAD2) are two key enzymes in plant fatty acid biosynthesis pathway. Five different types of transgenic lines with identical genetic background were developed by single and double-expression regulation of the genes coding for these two key enzymes in the same *Brassica napus* variety CY2. Seeds of the five types of transgenic lines and the wild-type control, which were planted under the same environmental conditions, were analyzed and compared for fatty acid composition and oil content in the present study. The results showed that the contents of multiple fatty acids, such as oleic, linoleic, linolenic, eicosenoic and erucic acid, could be significantly altered by single and double regulation of the two genes. Particularly in the seeds in which the two endogenous target genes were simultaneously silenced, the oleic acid content was increased to 82.8% from 20.5% of CY2, and in the seeds with grain-specific expression of *Arabidopsis thaliana FAE1* gene, the erucic acid content was raised to 60.2% from 43.9% of CY2. Compared with the wild-type control, the relative ratios of eighteen-carbon unsaturated fatty acids and the oleic desaturation proportion were all markedly changed in the five types of transgenic seeds. In addition, it was found that the seed oil content was improved by enhanced expression of *FAE1* and decreased by silencing of *FAE1* and *FAD2*, suggesting that the synthesis and accumulation of seed oil would be influenced to a certain extent by genetic manipulation of the two key enzyme genes.

Keywords *FAE1* gene; *FAD2* gene; Fatty acid biosynthesis; Genetic regulation; Rapeseed (*Brassica napus*)

In the fatty acid biosynthesis pathway, fatty acid elongase 1 (FAE1), as one of the four constituent enzymes of fatty acid elongase complex, is the rate limiting enzyme of carbon chain extension reaction with oleic acid as precursor in seeds and the key enzyme of the biosynthesis of very long chain fatty acids with more than C20, while delta-12 fatty acid desaturase 2 (FAD2) plays a key role in catalyzing the desaturation of oleic acid to produce linoleic acid and linolenic acid (Wang et al., 2020). Therefore, these two enzymes are two important sites for regulating fatty acid biosynthesis. *Brassica napus* is one of the major oil crops in the world. There have been many reports on the genetic regulation of *FAE1* and *FAD2* genes in *Brassica napus* and its related species. These reports mainly involved the down regulation (Kanrar et al., 2006; Tian et al., 2011a) and up regulation of *FAE1* gene expression (Katavic et al., 2000; Han et al., 2001; Kanrar et al., 2006; Mietkiewska et al., 2007) and down regulation of *FAD2* gene expression (Stoutjesdijk et al., 2000; Sivaraman et al., 2004; Jadhav et al., 2005; Chen et al., 2006; Chen et al., 2009; Jung et al., 2011; Tian et al., 2011b). Most of them studied the regulation of a single gene, while studies on the regulation of two genes at the same time (Peng et al., 2010) were relatively rare. Besides, most of the transformed receptor varieties in the previous studies were different. At present, there is still a lack of results of single-gene and double-gene genetic regulation of two target gene systems under the identical genetic background and the same experimental environment. In this study, in the transgenic improvement of fatty acid composition in *Brassica napus*, the same high-oil variety was used as the receptor parent to systematically study the genetic regulation of *FAE1* and *FAD2* genes, and various types of single-gene and double-gene transformants with the same genetic background were obtained (Shi et al., 2015; Lang et al., 2016; Shi et al., 2016; Shi et al., 2017). In this study, the quality of the seeds of these transgenic lines and their receptor parents planted under the same environmental conditions was analyzed, which could provide a basis for a more comprehensive understanding of the role of these two target genes in the genetic regulation of fatty acid biosynthesis.

1 Results and Analysis

1.1 Fatty acid composition of transgenic lines

The results of fatty acid composition analysis showed that the silencing of endogenous *BnFAE1* gene in RNA interference mediated *Brassica napus* effectively inhibited the carbon chain extension reaction with oleic acid as the starting substrate, which directly led to a significant reduction in the synthesis of very long chain fatty acids with more than C20, and a large amount of oleic acid accumulated. According to the data calculation in Table 1, compared with the receptor parent CY2, the contents of eicosenoic acid, erucic acid and total very long chain fatty acids in *BnFAE1-Ri* seeds decreased by 7.4%, 42.6% and 50.7% respectively, while the content of oleic acid increased by 41%. On the one hand, the massive accumulation of oleic acid provided more substrates for its desaturation to produce linoleic acid and linolenic acid, so as to promote the desaturation reaction and significantly improve the content of linoleic acid and linolenic acid. On the other hand, it also produced a certain feedback inhibition on its own synthesis, resulting in a significant increase in the accumulation of palmitic acid and stearic acid in its upstream.

Similar to the interference expression of *BnFAE1* gene, the RNA interference expression of endogenous *BnFAD2* gene also significantly regulated the fatty acid biosynthesis in *Brassica napus*. In these transgenic seeds, the desaturation of oleic acid was greatly inhibited, the synthesis of linoleic acid and linolenic acid decreased significantly, and their contents were 86.6% and 67.4% lower than CY2, respectively, while oleic acid accumulated, and the content was 56.2% higher than CY2. The increase of oleic acid accumulation provided more substrates for its carbon chain extension reaction, promoted the synthesis of very long chain fatty acids to a certain extent, and increased the contents of eicosenoic acid, erucic acid and total very long chain fatty acids.

In seeds with double interference expression of *BnFAE1* and *BnFAD2* genes, the content of oleic acid was as high as 82.8%, which was 62.2% higher than that of receptor parents, while the contents of linoleic acid and linolenic acid decreased by 77.9% and 57.8% respectively, and the contents of eicosenoic acid, erucic acid and total very long chain fatty acids decreased by 6.8%, 42.3% and 50.1% respectively. These data showed that after the silencing of two target genes mediated by RNA interference, the desaturation reaction and carbon chain extension reaction of oleic acid were highly inhibited at the same time, resulting in a significant reduction in the synthesis of various main fatty acids in its downstream, so that it could accumulate more greatly. Therefore, for the improvement of high oleic acid in *Brassica napus*, the double interference expression of *BnFAE1* and *BnFAD2* genes can produce more significant and ideal effect than the single interference expression.

The contents of erucic acid and total very long chain fatty acids in *AtFAE1-SE* seeds increased by 16.3% and 11.9% respectively compared with CY2 (Table 1), while the contents of palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and eicosenoic acid decreased in varying degrees. Except linoleic acid, the contents of other five fatty acids decreased significantly. The results showed that the grain specific positive expression of *AtFAE1* gene in *Arabidopsis thaliana* enhanced the total FAE1 activity in *Brassica napus* and promoted the carbon chain extension reaction. Not only part of oleic acid, but also part of the original eicosenoic acid was further extended to erucic acid, which decreased the contents of oleic acid and eicosenoic acid, and increased the contents of erucic acid and total very long chain fatty acids. After the decrease of oleic acid content, on one hand, the amount of linoleic acid and linolenic acid produced by its desaturation decreased correspondingly, on the other hand, it had a certain pulling effect on its own synthesis, that is, it promoted the transformation from palmitic acid and stearic acid to oleic acid, so as to reduce the accumulation of palmitic acid and stearic acid.

At the same time of positive expression of *AtFAE1* gene, the expression of *BnFAD2* gene was silenced by RNA interference technology, resulting in new changes in fatty acid composition. After calculation and analysis of the data in Table 1, compared with the positive expression of single *AtFAE1* gene, the contents of linoleic acid and linolenic acid in *BnFAD2-Ri*/*AtFAE1-SE* double expression seeds were further reduced by 81.5% and 54.8% due to the great inhibition of oleic acid desaturation reaction, while the content of oleic acid increased by nearly 10%, up to 22.5%, higher than CY2. The contents of eicosenoic acid, erucic acid and total very long chain fatty acids increased slightly, but they did not reach a statistically significant level by t-test. It can be seen from the results that

although the silencing of *BnFAD2* gene in double expression seeds accumulated oleic acid and increased the number of substrates for oleic acid carbon chain extension reaction, the total FAE1 activity enhanced by the positive expression of *AtFAE1* gene was not enough to extend more oleic acid into erucic acid, so the contents of erucic acid and total very long chain fatty acids could not be significantly increased.

Table 1 Seed fatty acid compositions (%) of the transgenic lines

Line	C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	C22:1	VLCFAs
BnFAE1-Ri	3.91±0.25*	1.77±0.26**	61.50±0.76**	17.30±0.79**	9.31±0.33*	4.38±0.23**	1.27±0.07**	6.21±0.28**
BnFAD2-Ri	2.22±0.08**	1.25±0.18	32.07±0.46**	1.44±0.10**	2.67±0.10**	13.53±0.51*	45.43±0.77	60.34±0.36**
BnFAE1-Ri/BnFAD2-Ri	2.94±0.01**	1.70±0.10**	82.76±0.25**	2.37±0.12**	3.46±0.07**	4.98±0.20**	1.61±0.25**	6.83±0.19**
AtFAE1-SE	1.13±0.06**	0.62±0.04**	12.62±0.45**	10.08±0.46	6.70±0.16**	5.43±0.49**	60.16±0.55**	68.85±0.06**
BnFAD2-Ri/AtFAE1-SE	1.45±0.09**	0.97±0.05	22.52±0.47**	1.86±0.18**	3.03±0.10**	6.26±0.33**	60.52±0.61**	70.18±0.76**
CY2	2.57±0.04	1.04±0.08	20.53±0.53	10.73±0.08	8.20±0.29	11.73±0.62	43.90±0.66	56.92±0.45

Note: C16:0: Palmitic acid; C18:0: Stearic acid; C18:1: Oleic acid; C18:2: Linoleic acid; C18:3: Linolenic acid; C20:1: Eicosenoic acid; C22:1: Erucic acid; VLCFAs: Very long chain fatty acids including C20:0 (Arachidic acid), C20:1, C22:0 (Behenic acid), C22:1 and C24:0 (Lignoceric acid); Each value is the mean ± standard deviation of three replications; * and ** indicate statistically significant differences compared with CY2 at 0.05 and 0.01 levels, respectively

1.2 Relative ratios of C18 unsaturated fatty acids of the transgenic lines

In the fatty acid composition of double-low *Brassica napus* seeds, oleic acid, linoleic acid and linolenic acid are the most important components, accounting for more than 90%. These three fatty acids are C18 unsaturated fatty acids, of which oleic acid belongs to monounsaturated fatty acids, while linoleic acid and linolenic acid are polyunsaturated fatty acids. The ratio of the three acids is related to the quality of rapeseed oil. The data showed (Table 2) that the ratios of C18:1/C18:2, C18:1/C18:3 and C18:2/C18:3 among all kinds of transgenic seeds have changed significantly (Except for a few being p <0.05, the rest were all p <0.01). Except *BnFAE1-Ri* seeds, among the other four kinds of transgenic seeds, C18:1/C18:2 was the largest, C18:1/C18:3 was the second, and C18:2/C18:3 was the smallest. The results showed that the ratios of C18:1/C18:2 and C18:1/C18:3 were greatly affected by the regulation of two target genes. Compared with the receptor parent CY2, C18:1/C18:2 and C18:1/C18:3 of four kinds of transgenic seeds increased in different ranges, while these two ratios in *AtFAE1-SE* seeds decreased, and the range was the smallest. The change ranges of three ratios in *BnFAD2-Ri* seeds were greater than those in *BnFAE1-Ri* seeds, indicating that the down-regulation of *BnFAD2* gene expression had a more significant effect on the relative ratios of C18 unsaturated fatty acids than that of *BnFAE1* gene. Among the three single expression and double expression regulation modes involving the down-regulation of *BnFAD2* gene, the change trends of the three ratios were the same, that is, C18:1/C18:2 and C18:1/C18:3 increased, and C18:2/C18:3 decreased, but the change ranges were obviously different, especially the increase ranges of the first two ratios were very different, and the largest increase was in *BnFAE1-Ri/BnFAD2-Ri* seeds, C18:1/C18:2 and C18:1/C18:3 increased by 17.3 times and 8.6 times respectively compared with CY2. The smallest increase was in *BnFAD2-Ri/AtFAE1-SE* seeds, and the two ratios increased by 5.4 times and 2 times respectively.

Table 2 Relative ratios of C18 unsaturated fatty acids in seeds of the transgenic lines

Line	C18:1/C18:2	C18:1/C18:3	C18:2/C18:3
BnFAE1-Ri	3.56±0.21**	6.61±0.19**	1.86±0.14**
BnFAD2-Ri	22.29±1.60**	12.02±0.44**	0.54±0.06**
BnFAE1-Ri/BnFAD2-Ri	34.98±1.66**	23.90±0.44**	0.68±0.04**
AtFAE1-SE	1.25±0.10**	1.88±0.08**	1.51±0.09*
BnFAD2-Ri/AtFAE1-SE	12.17±1.00**	7.44±0.10**	0.61±0.04**
CY2	1.91±0.06	2.50±0.11	1.31±0.05

Note: C18:1/C18:2: Oleic acid/Linoleic acid; C18:1/C18:3: Oleic acid/Linolenic acid; C18:2/C18:3: Linoleic acid/Linolenic acid; Each value is the mean ± standard deviation of three replications; * and ** indicate statistically significant differences compared with CY2 at 0.05 and 0.01 levels, respectively

1.3 Oleic desaturation proportions of the transgenic lines

The oleic desaturation proportion (ODP) referred to the proportion of oleic acid converted into linoleic acid and linolenic acid under the catalysis of delta-12 fatty acid desaturase 2 (FAD2) during fatty acid synthesis, that is, the percentage of the two polyunsaturated fatty acids, linoleic acid and linolenic acid, in the total C18 unsaturated fatty acids, which can be used as an indirect evidence to clarify the effect of *FAD2* gene regulation on the activity of endogenous delta-12 fatty acid desaturase. In this study, ODP decreased significantly in seeds with single interference expression and double interference expression of *BnFAD2* gene (Figure 1), among which, the decrease in *BnFAE1-Ri*/*BnFAD2-Ri* seeds was the largest, up to 86.3%. ODP in *BnFAD2-Ri* seeds decreased by 76.3%, which was equivalent to the decrease of BnFAD2 activity. It was worth noting that ODP in *BnFAE1-Ri* and *AtFAE1-SE* seeds also decreased and increased significantly (p<0.01), respectively, but the root cause was obviously not the change of BnFAD2 activity, but the large accumulation and reduction of oleic acid caused by *FAE1* gene regulation.

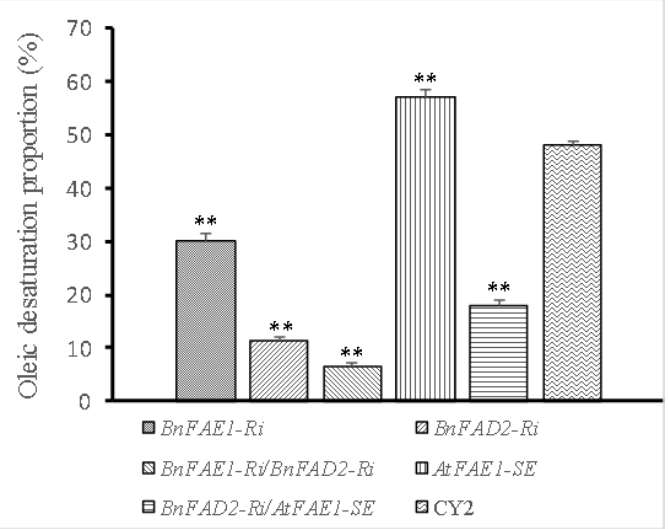

Figure 1 Oleic desaturation proportions (%) in seeds of the transgenic lines

Note: Data presented are mean values of three replications, and standard deviations are indicated by bars; ** indicates statistically significant differences compared with CY2 at 0.01 level

1.4 Seed oil contents of the transgenic lines

In this study, the regulation of *FAE1* and *FAD2* genes not only significantly changed the fatty acid composition of seeds, but also affected the oil content of seeds. The oil content in two *Brassica napus* seeds with single interference expression and double interference expression of endogenous target genes was lower than CY2 (Figure 2), in which the oil content in *BnFAE1-Ri* seeds and *BnFAE1-Ri*/*BnFAD2-Ri* seeds decreased by 2.4% and 3.4% respectively, and the difference was very significant (p< 0.01), while the oil content in the seeds with single and double expression of *AtFAE1* gene was 1.7% and 1.5% higher than that of the receptor parents, and the difference also reached a significant level of 0.01. The results clearly showed that high-efficiency inhibition of oleic acid carbon chain extension reaction and desaturation reaction in fatty acid synthesis pathway would affect the synthesis and accumulation of oil and reduce the oil content of seeds, and the greater the degree of inhibition was, the more obvious the decrease of oil content would be; On the contrary, promoting the whole fatty acid synthesis process by enhancing the carbon chain extension reaction of oleic acid would be conducive to the synthesis and accumulation of oil, and the oil content would be improved to a certain extent.

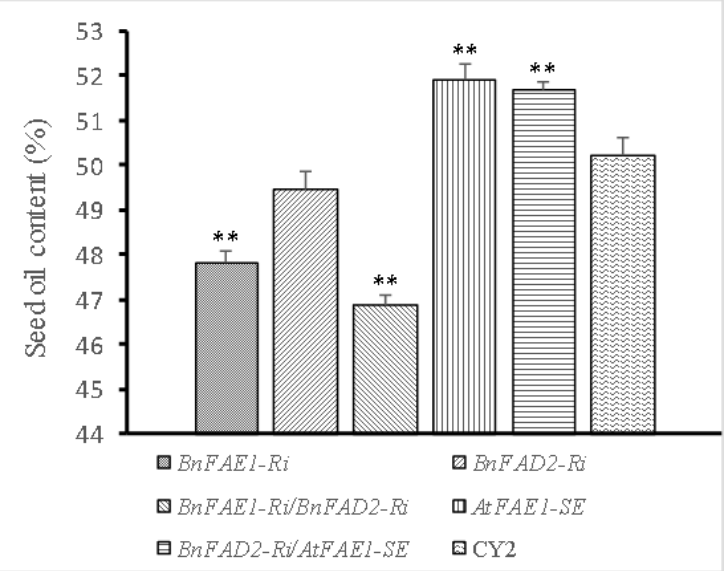

Figure 2 Seed oil contents (%) of the transgenic lines

Note: Results are mean values of three replications, and standard deviations are indicated by bars; ** indicates statistically significant differences compared with CY2 at 0.01 level

2 Discussion

Previous studies have shown that the *BnFAE1* gene from high erucic acid varieties was specifically expressed in the seeds of *Brassica napus* acceptor varieties with an erucic acid content of less than 1%, and the erucic acid and very long chain fatty acid contents were increased by nearly 30% and 40% respectively (Han et al., 2001). Under the genetic background of high erucic acid content (more than 35%), the up regulation of *FAE*1 gene can increase the erucic acid content by more than 10% (Katavic et al., 2000; Kanrar et al., 2006; Mietkiewska et al., 2007), while its antisense down regulation can reduce the erucic acid content by 30% (Kanrar et al., 2006). Silencing the expression of *FAE1* gene by RNA interference technology can reduce the erucic acid content by nearly 40%, while the content of oleic acid increased by nearly 38% (Tian et al., 2011a). In *Brassica napus* and *Brassica juncea*, the expression of *FAD2* gene was down regulated by co-inhibition, antisense and RNA interference. The content of oleic acid generally increased by about 20%, and the low increase was more than 10% as well, while the contents of linoleic acid and linolenic acid decreased correspondingly (Stoutjesdijk et al., 2000; Sivaraman et al., 2004; Chen et al., 2006; Chen et al., 2009; Jung et al., 2011). In this study, the *AtFAE1* gene was specifically expressed in the seeds of *Brassica napus* receptor varieties containing 43.9% erucic acid, and the content of erucic acid and very long chain fatty acids increased by 16.3% and 11.9% respectively; the endogenous *BnFAE1* gene in this variety was silenced by RNA interference, the content of erucic acid and very long chain fatty acids decreased by 42.6% and 50.7%, while the content of oleic acid increased by 41%; the endogenous *BnFAD2* gene was silenced in the same way, and the content of oleic acid increased by 11.5%, while the content of linoleic acid and linolenic acid decreased by 14.8%. These data showed that the results of this study were basically consistent with the previous studies. Based on the existing research results, it can be seen that the main fatty acid components affected by the regulation of *FAE1* and *FAD2* genes were different. The former regulation mainly changed the content of oleic acid and very long chain fatty acids with erucic acid as the main body, while the latter regulation mainly changed the content of three C18 unsaturated fatty acids, namely oleic acid, linoleic acid and linolenic acid; Based on the change ranges of main fatty acid content affected by regulation, the effect of *FAE1* gene regulation on fatty acid synthesis was greater than that of *FAD2* gene regulation.

Under the genetic background of high erucic acid in *Brassica napus*, although the down-regulation of *FAE1* and *FAD2* genes would significantly increase the content of oleic acid, the effect of *FAD2* gene down-regulation on

promoting oleic acid accumulation was not as good as that of *FAE1* gene down-regulation, and the effect of *FAD2* gene down-regulation on the relative ratio of C18 unsaturated fatty acids was greater than that of *FAE1* gene down-regulation. When the two genes were down-regulated at the same time, not only the oleic acid was highly accumulated to more than 80%, but also the relative ratio of oleic acid, linoleic acid and linolenic acid was increased to a higher level than that down-regulated by *FAD2* gene alone. As the three main fatty acid components in double-low rapeseed oil, oleic acid, linoleic acid and linolenic acid have their own nutrition and health care functions. Among them, oleic acid can reduce the content of harmful cholesterol and maintain the level of beneficial cholesterol, so as to slow down atherosclerosis, prevent cardiovascular diseases such as coronary heart disease and protect the heart (Chang and Huang, 1998; Nicolosi et al., 2004; Gillingham et al., 2011). Moreover, as a monounsaturated fatty acid, oleic acid has stronger antioxidant capacity and thermal stability than polyunsaturated linoleic acid and linolenic acid. It is relatively not easy to oxidize and deteriorate under long-term storage and high-temperature cooking conditions. Therefore, high oleic acid has become an important goal of *Brassica napus* quality breeding. Two strategies can be adopted for genetic regulation aiming at high oleic acid in *Brassica napus*. One is to silence the endogenous *BnFAD2* gene in double-low *Brassica napus*, and the other is to silence the endogenous *BnFAE1* and *BnFAD2* genes at the same time in double-low or non-double-low receptor varieties. Both strategies can achieve high enrichment of oleic acid and increase the relative ratio of oleic acid to linoleic acid and linolenic acid, so as to improve the nutritional quality of rapeseed oil and extend the shelf life of products. However, compared with the former strategy, the latter strategy has a potential advantage, that is, the content of low linoleic acid, low linolenic acid and low erucic acid in seeds of high oleic acid transgenic *Brassica napus* with two target genes silenced at the same time can remain basically unchanged even if cross pollination occurs with foreign pollen (Peng et al., 2010; Shi et al., 2015; Lang et al., 2016), which can better ensure the high oleic acid quality of *Brassica napus*.

In this study, while the *AtFAE1* gene was specifically expressed in seeds, the expression of the *BnFAD2* gene was down-regulated. The purpose was to inhibit the oleic acid desaturation reaction to accumulate oleic acid, provide more substrates for the oleic acid carbon chain extension reaction, and further promote the synthesis of very long chain fatty acids. From the analysis data of fatty acid component, the oleic acid in *BnFAD2-Ri*/*AtFAE1-SE* double expression seeds did accumulate, and its content even exceeded that of the receptor parents, but the contents of eicosenoic acid, erucic acid and total very long chain fatty acids were similar to those in the seeds with single expression of *AtFAE1*, and there was no statistically significant difference. From this result, it was speculated that the FAE1 activity in the seeds of the receptor parent itself was the rate-limiting factor for the carbon chain extension reaction to synthesize more very long chain fatty acids. After the *AtFAE1* gene was specifically expressed in the seeds, the total FAE1 activity in the seeds was enhanced and part (only part) of the oil acids were transformed into erucic acid, at this time, the enhanced FAE1 activity had become the rate-limiting factor for the further synthesis of more very long chain fatty acids. Therefore, when the total FAE1 activity in the seeds with single expression of *AtFAE1* had been in the state of speed limit, it was difficult to extend more oleic acid into very long chain fatty acids such as erucic acid by increasing the number of oleic acid substrates through RNA interference expression of *BnFAD2* gene.

It is generally believed if the delta-12 fatty acid desaturase encoded by the *FAD2* gene in seeds has strong activity, more oleic acid will convert into linoleic acid and linolenic acid, and the oleic desaturation proportion (ODP) will be relatively higher. On the contrary, if the delta-12 fatty acid desaturase has low activity, less oleic acid will convert into linoleic acid and linolenic acid, and the ODP will be correspondingly lower. Based on this cognition, some researchers have evaluated the effect of *FAD2* down-regulation on inhibiting the activity of delta-12 fatty acid desaturase by calculating ODP (Cartea et al., 1998; Sivaraman et al., 2004; Chen et al., 2009; Tian et al., 2011b; Belide et al., 2012). However, it was worth noting that under certain circumstances, the increase of ODP did not necessarily mean that it was the result of increased activity of delta-12 fatty acid desaturase. For example, the content of linoleic acid and linolenic acid in the seeds with grain-specific expression of *AtFAE1* decreased in this study, but ODP increased significantly. This phenomenon was obviously not caused by the increased activity of delta-12 fatty acid desaturase, but because the grain-specific expression of *AtFAE1* gene enhanced the total FAE1 activity, so that

the content of oleic acid decreased significantly after part of oleic acid extending into very long chain fatty acids. Similarly, the decline of ODP did not necessarily mean that it was result of decreased activity of delta-12 fatty acid desaturase. For example, the content of linoleic acid and linolenic acid in the seeds with interference expression of *BnFAE1* gene increased significantly, while ODP decreased significantly. The fundamental reason was that *BnFAE1* gene silencing led to a large accumulation of oleic acid, which greatly increased its content, not because the activity of delta-12 fatty acid desaturase was inhibited.

Oils are formed by the combination of glycerol and fatty acids. Since enhancing or silencing the expression of the key enzyme genes *FAE1* and *FAD2* in fatty acid biosynthesis can significantly regulate the synthesis and accumulation of various fatty acids, theoretically it will also have a certain impact on the assembly and accumulation of oil. Previous studies have found that the up-regulation of *FAE1* gene expression can increase the oil content of *Brassica napus* transgenic seeds by 1.5% to 10.9%, compared with the untransformed control (Katavic et al., 2000; 2001; Kanrar et al., 2006). However, the impact of down-regulation of these two key enzyme genes on oil content of *Brassica napus* seeds is still rarely reported. In this study, the single interference and double interference expression of *BnFAE1* and *BnFAD2* genes reduced the oil content of transgenic seeds, among which the seeds with double interference expression decreased the most, up to 6.7%, while the oil content of transgenic seeds with grain-specific expression of *AtFAE1* increased by 3.4%. Based on these results, it is preliminarily believed that the regulation of these two key enzyme genes of fatty acid synthesis will indeed affect the oil content of seeds. Enhancing their expression can promote the synthesis of fatty acids and their combination with glycerol, so as to improve the oil content of seeds, while silencing their expression will inhibit the synthesis of fatty acids and reduce the assembly with glycerol, resulting in the decrease of oil content of seeds. Therefore, it is suggested that in the practical application research of down-regulating *FAE1* and *FAD2* gene expression, varieties with high-oil content should be selected as receptor parents as far as possible, and transformed to produce more transgenic plants for future generations to identify and screen, and then to obtain transgenic materials that can achieve the goal of fatty acid synthesis regulation and maintain a high level of oil content.

3 Materials and Methods

3.1 Experimental materials

The test materials included the interference expression of endogenous *FAE1* gene *BnFAE1* in *Brassica napus* (*BnFAE1-Ri*), the interference expression of endogenous *FAD2* gene *BnFAD2* in *Brassica napus* (*BnFAD2-Ri*), the interference expression of *BnFAE1*/*BnFAD2* double genes (*BnFAE1-Ri/BnFAD2-Ri*), the positive expression of *FAE1* gene *AtFAE1* in *Arabidopsis thaliana* (*AtFAE1-SE*) and the interference expression of *BnFAD2* gene/ positive expression of *AtFAE1* gene (*BnFAD2-Ri*/*AtFAE1-SE*). There were 5 types of transgenic lines of *Brassica napus* and their common receptor parent CY2. These five types of transgenic lines had been independently transformed by the author's laboratory. At present, they had reached more than 8 generations. The transgenic lines were pure and had stable inheritance and expression.

3.2 Material planting and harvesting

The experimental materials were planted completely randomly in the same field, and we repeated for 3 times with 40 plants for each repetition. The cultivation management during the whole growth period was the same as that in the general field. During flowering, normal plants were selected for bagging the main inflorescence to avoid the influence of foreign pollen. After maturity, the bagged main inflorescence seeds were harvested for quality analysis.

3.3 Analysis of fatty acid composition

The capillary gas chromatography of fatty acid methyl ester (Shi et al., 2016) was used to analyze the fatty acid composition in the seeds of each test material on the TRACE 1300 gas chromatograph produced by THERMO FISHER in the United States. Each material was repeated 3 times (that is, 3 cells), and the measurement was performed 3 times for each repetition (cell). The relative content of each fatty acid component was calculated according to the peak area normalization method, which was expressed as the percentage of single fatty acid to total fatty acid.

3.4 Determination of oil content

The Soxhlet extraction method (Wang et al., 2011) was used to determine the oil content in the seeds of each test material, and the 3 cells of each material were independently sampled and determined 3 times.

3.5 Calculation of oleic desaturation proportion

The oleic desaturation proportion (ODP) was calculated according to the relative contents of three kinds of C18 unsaturated fatty acids, namely oleic acid, linoleic acid and linolenic acid. The formula was as follows: ODP = (linoleic acid content + linolenic acid content) / (oleic acid content + linoleic acid content + linolenic acid content) × 100% (Chen et al., 2009; Belide et al., 2012).

3.6 Data statistical analysis

In Microsoft Office Excel 2007, F-test and t-test methods were used to statistically compare and analyze the data of fatty acid composition, relative ratio of C18 unsaturated fatty acid, oleic desaturation proportion and oil content in the seeds of various transgenic lines and common receptor parents.

Authors' contributions

LCX was responsible for genetic transformation, molecular identification and writing the first draft of this manuscript; WFL participated in molecular identification and quality analysis; LRH participated in quality analysis; ZT participated in molecular identification; HZH participated in some genetic transformation work; WXL was responsible for the construction of expression vectors; WGT participated in various research work throughout the whole process, mainly responsible for experimental design, transgenic material planting and screening, data statistical analysis and manuscript finalization. All authors read and approved the final manuscript.

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