High oleic acid content breeding materials of *Brassica napus* produced by ^{60}Co radiation

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High oleic acid content rapeseed breeding has great significance. Firstly, high oleic acid content oil is a healthy and nutritious oil. It can reduce low density lipoprotein. It reduces cholesterol formation and effectively prevents heart disease. Secondly,high oleic acid content oil con endure a long period of preservation and a long period of shelf life. Thirdly, high oleic acid content oil can become conduct transesterification reaction effectively. This is propitious to producing biodiesel. There are already some reports about high oleic acid content rapeseed breeding at home and abroad. To improve oleic acid content, EMS mutation breeding and transgene breeding have mainly been used. There are few reports about ^{60}Co ionizing irradiation to produce high oleic acid. This research explored the possibilities of selecting high oleic acid content breeding materials by ^{60}Co ionizing radiation used in consecutive selection of high oleic acid content rapeseed.

1. Materials and measures

The varieties XIANGYOU15 (*Brassica napus*) dry seeds were treated by ^{60}Co ionizing radiation dosage 10 Krad. The oleic acid content of XIANGYOU 15 is 59.77%. After treatment the oleic acid content of 13 plants (total 160 plants) was overtaking XIANGYOU15. The frequency of oleic acid content mutation was7.5%.

The highest oleic acid content was 66.37%. Use the 66.37% high oleic acid content mutant we do consecutive directional selection. The plant number selected in per gerenation was 160-600 plants. When the plants blossomed, bags were placed over the plants in the plot to induce self-fertilization. After being harvested and dried, FOSS near infrared analysis instrument was used to analyze the fatty acid formation of every plant seed.

High oleic acid content mutant M604-855(oleic acid content 91.5%) fad2 gene cloning and sequencing: electron PCR was used in promoter design and DNASTAR was used in translation and information analysis, then sequence joint matching and multi-sequence joint matching were used in mutant analysis.

2.Main research results

2.1 The variation of oleic acid content in M1-M5 seeds

The results show that in M3-M4 oleic acid content only increased slightly. Breeding material with oleic acid content higher than 70% did not appear. But in M5 greatly different oleic acid average content and the highest oleic acid content increased dramatically. Oleic acid content of many of the plants was higher than 70%. The highest oleic acid content was 93.5% (Table 1).

Table 1 The variation of oleic acid content(%) in M$_1$-M$_5$ seeds

Generation	Total No. of plants	No. of plants with OAC(%)					Average OAC(%)	The highest OAC(%)
		⩽60	61-70	71-80	81-90	⩾91		
M$_1$	160	25	135	0	0	0	61.12	66.36
M$_2$	160	--	--	--	--	--	--	---
M$_3$	300	8	292	0	0	0	65.25	68.83
M$_4$	300	19	281	0	0	0	66.17	69.85
M$_5$	600	0	352	183	63	3	70.94	93.50

In fatty acid composition of HOAC, 16:0, 20:1, 22:1 were trace or zero and 18:2, 18:3 were very low (Table 2).

Table 2 The fatty acid composition of HOAC in M$_5$ seeds

Plant No.	16:0	18:1	18:2	18:3	20:1	22:1
03-922	0	93.50	5.33	1.05	0	0.05
03-932	0	88.20	8.15	2.34	0.10	0.03
03-923	0	83.30	10.57	3.00	0.54	0.05
03-927	0	81.80	15.48	2.62	0.35	0.01
CK	4.23	61.05	21.12	8.45	2.16	0.55

2.2 High oleic acid content mutant fad2 cloning,sequencing and results analysis

M6 04-855 (oleic acid content 91.5 %) high oleic acid mutant fad2 was compared with fad2 as published at the internet.

Homology of DNA sequencing and protein reached 98%.The base site 270 of high oleic acid content mutant fad2 had changed to A. This induced the codon TGG (Trp) to be converted into TGA (stop codon). The location is at the beta folding area and conservative area of this protein. Base mutations at sites 1044 and 1962 also led to production of stop codon. These changes in structure led to loss of function of fad2.

Fad2 gene DNA sequencing as published on the internet and fad2 gene DNA sequencing of high oleic acid content mutant were compared:

Fad2 gene DNA sequencing as published on the internet:

ATGGGTGCAG	GTGGAAGAAT	GCAAGTCTCT	CCTCCCTCCA	GCTCCCCCGG	AACCAACACC
CTCAAACGCG	TCCCCTGCGA	GACACCGCCC	TTCACTGTCG	GAGAACTCAA	GAAAGCAATC
CCACCGCACT	GTTTCAAACG	CTCGATCCCT	CGCTCTTTCT	CCTACCTCAT	CTGGGACATC
ATCATAGCCT	CCTGCTTCTA	CTACGTCGCC	ACCACTTACT	TCCCTCTCCT	CCCTCACCCT
CTCTCCTACT	TCGCCTGGCC	TCTCTAC TGG	GCCTGCCAAG	GGTGCGTCCT	AACCGGCGTC
TGGGTCATAG	CCCACGAGTG	CGGCCACCAC	GCCTTCAGCG	ACTACCAGTG	GCTTGACGAC
ACCGTCGGTC	TCATCTTCCA	CTCCTTCCCC	CTCGTCCCTT	ACTTCTCCTG	GAAGTACAGT
CATCGACGCC	ACCATTCCAA	CACTGGCTCC	CTCGAGAGAG	ACGAAGTGTT	TGTCCCCAAG
AAGAAGTCAG	ACATCAAGTG	GTACGGCAAG	TACCTCAACA	ACCCTTTGGG	ACGCACCGTG
ATGTTAACGG	TTCAGTTCAC	TCTCGGCTGG	CCGTTGTACT	TAGCCTTCAA	CGTCTCTGGA
AGACCTTACA	GCGACGGTTT	CGCTTGCCAT	TTCCACCCGA	ACGCTCCCAT	CTACAACGAC
CGCGAGCGTC	TCCAGATATA	CATCTCTGAC	GCTGGCGTCC	TCTCCGTATG	TTACGGTCTC
TACCGCTACG	CTGGTTCGCG	AGGAGTGGCC	TCGATGGTCT	GTGTCTACGG	AGTTCCGCTT
ATGATTGTCA	ACTGTTTCCT	CGTCTTGATC	ACTTACTTGC	AGCACACGCA	CCCTTCGCTG
CCTCACTATG	ATTCTTCGGA	GTGGGATTGG	TTGAGAGGAG	CTTTGGCTAC	TGTGGATAGA
GACTATGGAA	TCTTGAGCAA	GGTGTTTCAT	AACATCACGG	ACACGCACGT	GGCGCATCAT
CTGTTCTCGA	CGATGCCGCA	TTATAACGCG	ATGGAAGCGA	CCAAGGCGAT	AAAGCCGATA
CTTGGAGAGT	ATTACCGTTT	GATGGAACGC	CGGTGGTTAA	GGCGATGTGG	AGGGAGGCGA
AGGAGTGTAT	CTATGTTGAA	CCGGATAGGC	AAGGTGAGAA	GAAAGGTGTG	TTCTGTACAA
CAATAAGTTA	TGAGGATGAT	GGTGAAG			

3. Discussion

3.1 Main reasons that ^{6}℃o radiation led to high oleic acid content variation

According to former research in lipin biochemistry (Ohlrogge et al. 1995; Mekhedov et al.2000; Thelen et al.2002), the main gene that causes stearic acid to be converted into oleic acid is fad1.The the main gene that causes oleic acid to be converted to linoleic acid is fad2.The increase in mutant oleic acid content is likely to be because the function of fad1 is strengthened and that induces the oleic acid formation, or because of fad2 gene aberrance or loss of function that means the oleic acid cannot be converted into linoleic acid and thus stays at the oleic acid stages (Fig 1). This also can increase oleic acid content. In this research, fad2 gene DNA sequencing as published on the internet and fad2 gene DNA sequencing of the high oleic acid content mutant were compared. The base site 270 of the high oleic acid content mutant fad2 had changed to A. This induced codon TGG (Trp) to be converted to TGA (stop codon). These changes in structure led to loss of function of fad2. More research needs to be done to confirm whether such variation will occur in every high oleic acid content mutant and whether fad1 gene function will be strengthened.

3.2 Generations of high oleic acid content produced by ^{6}℃o radiation treatments

M5 oleic acid content was increased rapidly in this research. And it was produced at the base of successive directional selection. Thus it may be imagined that mutation occurred at the stage of the later generations. However we believe mutation actually occurred under ^{60}Co r ray treatment. There were 14 individual plants with oleic acid content exceeding CK during the first treatment. The highest oleic acid content was 6.59 percent point higher than CK. Because the colony seed was analyzed, it was impossible to separate the mutant plants in the earlier generations. After several successive selections and self-fertilization separations, the plants with highest mutation could be separated. In this research, The base G was converted to base A in fad2 gene DNA sequencing. According to molecular mechanism of gene mutation, no matter what transversion or transition happens, several replications are needed. That is to say several generations are needed. That was also the reason high oleic acid content mutation occurred in later generations.

Fad2 gene DNA sequencing of high oleic acid content mutant:

atgggtgcag	gtggaagaat	gcaagtgtct	cctccctcca	agaagtctga	aaccgacacc
atcaagcgcg	taccctgcga	gacaccgccc	ttcactgtcg	gagaactcaa	gaaagcaatc
ccaccgcact	gtttcaaacg	ctcgatccct	cgctctttct	cctacctcat	ctgggacatc
atcatagcct	cctgcttcta	ctacgtcgcc	accacttact	tccctctcct	cccccaccct
ctctcctact	tcgcctggcc	tctctac**tga**	gcctgacaag	ggtgcgtcct	aaccggcgtc
tgggtcatag	cccacgagtg	cggccaccac	gccttcagcg	actaccagtg	gctggacgac
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catcgacgcc	accattccaa	cactggctcc	ctcgagagag	acgaagtgtt	tgtccccaag
aagaagtcag	acatcaagtg	gtacggcaag	tacctcaaca	accctttggg	acgcaccgtg

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atgttaacgg  ttcagttcac  tctcggctgg  ccgttgtact  tagccttcaa  cgtctctgga
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aaggagtgt   atctatgttg  aaccggatag  gcaaggtgag  aagaaaggtg  tgttctggta
caacaataaa  atcgtcgacc  tgcaggcatg  caagcttggc  actggccgtc  gttttacaac
gtcgtgactg  gaa
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4. Conclusions

4.1 The high oleic acid content breeding materials of rapeseed (B. napus) were obtained by 10 krad $^{6\circ}$Co r ionizing irradiation treatment of dry seeds. The seed oleic acid content exceeded CK during each generation. The highest oleic acid content was 6.59 percent higher than CK.

4.2 Under consecutive directional selection, the oleic acid content of M2, M3 and M4 progeny increased to different degrees. The oleic acid content was higher than 70% for most plants and the highest reached 93.5%.

4.3 Fad2 gene DNA sequencing as published on the internet and fad2 gene DNA sequencing of high oleic acid content mutant were compared. The base G was converted to base A in fad2 gene 270 site of high oleic acid content mutation (M^04-855). This induced codon TGG (Trp) to be converted to TGA (stop codon). These changes in structure led to loss of function of fad2. Oleic acid content was increased rapidly.

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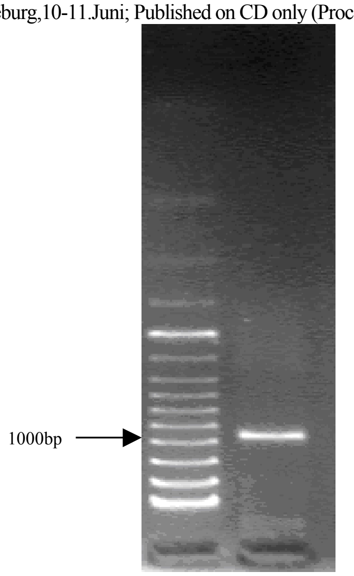

Fig 1 The main genes that determine different desaturation of fatty acid formation

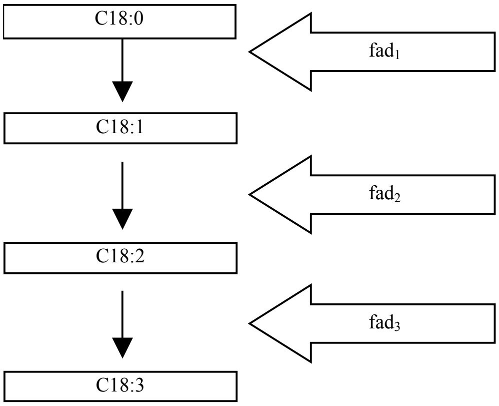

Fig.2 The main genes that determine different desaturation of fatty acid formation