Effect of ^{12}C heavy ion beam irradiation on rapeseed (*Brassica napus*)

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Abstract

After treating of 30Gy, 50Gy and 80Gy ^{12}C heavy ion beam irradiation on rapeseed, the effects of botanical characters, quality characters, chromosome behavior and DNA molecular polymorphism were studied. The results are as follows: Treatments of 50Gy and 80Gy ^{12}C heavy ion beam irradiation can make the growth duration earlier and change the botanical characters of rapeseed. These botanical characters include larger leaves of the plant, luxuriant growth, improved agronomy characters. Some plants have variations such as tumor-like root, dwarf stem, light green spoon leave, multi-pistil flower, multi-silique and yellow seed. Treatment of 80Gy ^{12}C heavy ion beam irradiation increases oil content to different degree. The oleic acid contents of some plants are even higher than 70%. Both root top chromosome aberration and pollen mother cell chromosome aberration occur under 30Gy, 50Gy and 80Gy ^{12}C heavy ion beam irradiation on rapeseed. The aberration type includes micronucleus, mininucleus, abnormal tetraspore, bridge, lagging chromosome and fragment, most of which are micronucleus. Treatments of 50Gy and 80Gy ^{12}C heavy ion beam irradiation have effects on molecular biology of rapeseed. By using 40 random primers to amplify the rapeseed after the above treatment, 43 DNA fragments are amplified. It shows polymorphism to some degree in the rapeseed plants after different dosage treatments.

Key words: ^{12}C heavy ion beam, irradiation, rapeseed

Introduction

^{12}C heavy ion beam is a kind of new irradiation source. Compared with the former physical mutation treatments, such as γ ray treatment,×ray treatment, ^{12}C heavy ion beam is much better in physiological, biological and chemical effect. It has higher mutation rate, wider mutation pedigree, easier stability. So it arouses much attention in recent years. There are some reports of ^{12}C heavy ion beam treatments on rice, wheat and corn, but reports on rapeseed are very few. To investigate the effect of ^{12}C heavy ion beam irradiation on rapeseed, some results on rapeseed morphological characteristics, seed quality, chromosome aberration and DNA molecular morphology will be presented and discussed in this work.

1.Experimental Material and Method

1.1 Tested plant species

Original Xiangyou No.15 (*B. napus*) seed.

1.2 ^{12}C heavy ion beam irradiation

α particles were used to bombard ^{9}Be (Beryllium) and make ^{9}Be become ^{13}C. Then ^{13}C becomes ^{12}C, and releases heavy ion beams. The incident energy of the heavy ion beam is 87.5MeV. After the heavy ion beam passes through 50μm Kapton film and 1.5cm air, it remains 69.4MeV, and the LET(linear energy transfer density) is 263V/μm. The treatment dosage is 30Gy, 50Gy and 80Gy. All above work was done at HI-13 tandem accelerator in National Laboratory of Nuclear Physics, China Atomic Energy Science Institute.

1.3 Field planting experiments

Sowed in the experimental field on Oct.20, 2003, plants were designed not to repeat (20 plants per treat, row spacing 20cm, plant spacing 15cm) and self-copulation seed got after maturation. Sowed on Sep. 28 2004, plants were designed not to repeat (120 plants per treatment, row spacing and plant spacing are the same as above) and self-copulation seed got after maturation.

1.4 The observation on the growth period and botanical characters of rapeseed

Courtesy of Prof. Liu H-L, editor in chief (1987), Principle of Oil Seed Rape Production [13], 571-574.

1.5 The character and quality measurement of rapeseed

The oil content in rapeseed, protein content, the fatty acids constitute and glucosinolat content, etc. were measured by a Foss near infrared analyzer.

1.6 Observation of rapeseed chromosomal behavior

Courtesy of Prof. Li Xun, (1991), Introduction of Chromosomal Heredity, 436 ~ 448.

1.6.1 Pollen mother cell daub film production method

3 variant plants were taken for every treatment and 2 flower trays for each plant, and then fixed in 3:1 alcohol acetum. For observation, a bud was taken on absorbing paper to remove the alcohol and put on a slice glass. Then anther is taken from

calyx by scalpel to remove medicine and cut. Pollen mother cells (or microspores) were extruded. Hematoxylic dye was added until the image became clear. During the dying process, if dyestuff becomes dry and bubbles appear, dyestuff should be added continually along the edge of the slice glass until the image becomes clear. If Hematoxylic dye is used and then 45% propionic acid dichroism, the way of adding 45% propionic acid 8 is similar to adding the dyestuff. Dyeing time has something to do with temperature. When the temperature is high, dyeing is quick. If the temperature is low, then dyeing is slow. The method of dyeing all anthers can also be adopted. Calyx was stripped from the flower bud by forceps and anther came out. The anther was then put in a small bottle with hematoxylic dye for 1~ 3 days (attention should be paid to keep the dye not dry for film production). 1~2 anther was put on the slice glass and 1~2 drops of 45% propionic acid added. The anther was cut by scalpel and the pollen extruded. The medicine wall was thrown away and slice glass covered for observation. Tetraspores form after the pollen mother cell meiosis process. At this time, the chromosome number has already reduced to half, that is, a napus is 2n=4x=38, but a microspore is n=2x=19. Buds during the period of microspore growth share the great majority on an inflorescence.

1.6.2 Method of pressing root tips

30 seeds were taken to germinate in culture utensil and treated as follows:

(1) Pre-treatment: The purpose was to shorten chromosome relatively. The method was to dip rapeseeds for 1~2 days, then put them into a warm box at 25~28°C for germination, wait until 0.5~l.0cm roots come out, put them into the saturated benzidine solution or 0.1% colchicines solution, handle for 2~4 hours to make chromosome short.

(2) Fixation: After p-dichlorolenzene solution was poured out, the roots were fixed by a new solution and lately transferred into a solution of 70% alcohol. Then they were put into a refrigerator to keep for a long time.

(3) The separation and softening of the cell: The root tips were moved into a 1N hydrochloric acid to handle for 5~ 10 minutes before film production. The processing time has something to do with temperature. If the temperature is high, the processing time is short. If the temperature is low, the processing time is long, which depends upon the degree of root softening.

(4) Dyeing for the film production: A root tip was taken out from the preserving solution, put onto culture utensil with a few preserving solution to prevent drying off. Root tip was taken by forceps to put on absorbing paper to suck surplus solution and then put onto clean slice glass. Calyptrogens were removed by scalpel and a part of root tips was cut and put onto slice glass. The other was placed back to culture utensil. Another slice glass was taken to cover crisscross the former one to have the material spread uniformly into a thin layer. Then these two slice glasses were separated and put on an experimental desk, mixed with drops of aceto-carmine stain respectively, covered by thin glass, dyed for a short moment. Absorbing paper was taken to cover the thin glass, pressed by the thumb to suck away surplus dye. So cells were spread fully and placed under a microscope for observation. If the chromosome is not clear enough, dye can be added along the edge to dye it again and roast film until the chromosome becomes clear.

1.7 The RAPD analysis of rape

1.7.1 The DNA distillation

The CTAB method was adopted and improved slightly for the distillation and detection of DNA genome. During the 5~6 leaf period of rapeseed, five plants were treated with 2 leaves mixed each plant. Then 5~6g lamina tissue were put in the mortar. Liquid nitrogen was added for grinding. Ground powder was poured into the eppendorf tube with 200μl CTABs, mixed fully. Then the mixture was put in 65°C water pot for a 10min warm bath, shaken once or twice during this period. 200μl chloroform was added later. The material was mixed evenly, centrifuged under the condition of 4°C for 2min. Clear liquid was added and then transfer to a clean eppendorf tube with 200μl dimethylcarbinol added, placed at indoor temperature for 15min. It was centrifuged (13000g) under the condition of 4°C again for 5min. The clear liquid was removed and 500μl 70% alcohol was added to flush. It was centrifuged (13000g) under the condition of 4°C again for 5min with precipitate dissolving in 50μl TE solution. Then it was stored in 4°Crefrigerator for standby or at -20°C for long-term storage.

1.7.2 The RAPD analysis

The RAPD total reaction volume was 20μl. The concentration of each reagent was as follows: 1×expand cushion liquid, 1.5mMol/L $MgCl_2$, 0.125mMol/L dNTPs, 2UTaq enzyme,0.45μMol/L 5mer primer (S201-221, coming from Shanghai Biotechnology Company; P15-25, coming from UBC Company); 50μg DNA template. Hot circulation condition: 94°C pre-denaturalization for 3min as a circulation; 94°C 30Sec, 40°C 30sec, and 72°C 45Sec for 35 circulations; 72°C elongation 10min as a circulation; 4°Cstorage.

PCR amplifer was separated by 1.4% agarose gel. Buffer was 1×TAE. Results were observed and taken pictures under one-off imaging system.

2 Results and Analysis

2.1 The effect of ^{12}C heavy ion beam irradiation on rape growth duration

2.1.1 Effect on the growth duration of M_1

According to field observation, the growth duration was almost the same under the same treatment. Dissimilarity occurs under different treatments. As shown in Table 1, the treatment of 80Gy results in the earliest stage. The growth duration is 196 days. The treatment of 50Gy results in a middle stage, which is 197 days. The treatment of 30Gy and CK results in the latest

stage with 199 days.

Table 1 Effect of ^{12}C heavy ion beam irradiation on growth duration of rapeseed M_1

Treatment(Gy)	Seeding date	Seedling stage	Start flowering stage	Flowering end stage	Ripening	Growth duration(D)
30	10/20	10/30	3/12	4/11	5/8	199
50	10/20	10/30	3/10	4/8	5/6	197
80	10/20	10/30	3/10	4/8	5/6	196
CK	10/20	10/30	3/12	4/11	5/8	199

2.1.2 Effect on the growth duration of M_2

As shown in Table 2, M_2 have the same trend with M_1, that is, the 80Gy treatment results in the earliest stage of 218 days. The 50Gy treatment results in the middle one with 219 days. The 30Gy treatment and CK results in the latest stage of 225 days. The same treatment has almost the same growth duration. Three plants in the 80Gy treatment have the earliest growth duration, whose start flowering stage is on March 15, flowering end stage on April 1, and mature time on May 5. The total growth duration is only 216 days.

Table 2 Effect of ^{12}C heavy ion beam irradiation on growth duration of rapeseed M_2

Treatment (Gy)	Seeding date	Seedling stage	Squaring stage	Start flowering stage	Flowering end stage	Ripening	Growth duration (D)
30	9/28	10/6	2/8	3/21	4/6	5/12	225
50	9/28	10/5	2/5	3/19	4/5	5/8	219
80	9/28	10/4	2/4	3/17	4/4	5/7	218
CK	9/28	10/6	2/8	3/21	4/6	5/12	225

2.2 Effect of ^{12}C heavy ion beam irradiation on the botanical characters of rapeseed under different treatments

Because the M_1 sowed late and plants were few, a systematical observation on the botanical characters was not carried out. Only the botanical characters of M_2 were observed. The observation results for start flowering stage of M_2 were shown in Table 3. It was found that the plants under 50Gy and 80Gy treatments are taller and bigger than those under 30Gy and CK treatments. Their agronomic characters were observed before harvest. Results showed that the plants under 50Gy and 80Gy treatments still have better performance. As shown in Table 4, these plants are taller and bigger.

Table 3 Effect of ^{12}C heavy ion beam irradiation on botanical character of beginning flowering of rapeseed

Treatment (Gy)	Plant height (cm) ($\bar{X}\pm S$)	Green leaf number ($\bar{X}\pm S$)	Maximum leaf length (cm) ($\bar{X}\pm S$)	Maximum leaf width (cm) ($\bar{X}\pm S$)
30	120±1.6 a	13±1.4 a	26±2.1 a	12.2±1.2 b
50	123±1.8 ab	14±2.2 ab	27±2.2 ab	13.0±1.3 bc
80	125±2.0 b	15±2.4 b	27±2.3 ab	13.0±2.0 bc
CK	110±1.3 a	12±1.2 a	25±2.0 a	11.0±1.0 a

Investigation date: Mar 10, 10 plants were observed in every treatment. a, b, c in Tab. 3 represent 0.05 level of difference.

Table 4 Effect of ^{12}C heavy ion beam irradiation on agrinomy character of rapeseed

Treatment (Gy)	Plant height (cm) ($\bar{X}\pm S$)	No. of primary branch ($\bar{X}\pm S$)	Site of branch ($\bar{X}\pm S$)	No. of siliques/plant ($\bar{X}\pm S$)	No. seeds/silique ($\bar{X}\pm S$)	1000 seeds weight (g) ($\bar{X}\pm S$)	Average 1000 seeds weight (g) ($\bar{X}\pm S$)
30	175.6±1.2 a	7.2±1.1 a	38.1±0.8 a	311.1±1.0 a	18.8±0.8 a	3.5±0.08 a	9.5±0.1 a
50	176.9±1.5 ab	7.7±1.2 a	39.4±0.9 a	328.5±1.1 ab	20.0±0.9 ab	3.8±0.09 a	10.5±0.2 a
80	179.4±2.1 ab	8.3±1.4 ab	40.0±0.9 ab	343.6±1.2 b	21.9±1.0 b	4.0±0.08 ab	11.9±1.2 ab
CK	175.5±1.1 a	7.0±1.2 a	38.0±0.7 a	304.2±1.1 a	19.2±0.8 a	3.5±0.07 a	9.5±0.1 a

10 plants were observed in every treatment. a, b in Tab. 4 represent 0.05 level of difference.

In addition, the botanical variation of plants with different treatments was observed. Results are shown in Table 5. Plants under 50Gy and 80Gy treatments show botanical variations and plants under 80Gy treatment have more variations (several mutations even occur in the same plant). These variations include tumor-like root, dwarf stem, light green spoon leaves, multi-pistil flower, multi-silique or yellow seed (Figure 1 and 2). Botanical variations can occur under 50Gy treatment of ^{12}C heavy ion beam irradiation, but appear quite few. The frequency is only 0.98% in 120 plants. The variation types are as follows: one plant has light green spoon leaves, one plant has multi-pistil flower and another plant has multi-silique. More variation types appear under 80Gy treatment. The frequency is also higher. 3 plants have tumor-like root (2.5%), 3 plants have stem variations (2.5%), 3 plants have light green spoon leaves (2.5%), 5 plants have multi-pistil flower (4.2%), 3 plants have disilique (2.5%), 4 plants have yellow seed (3.3%).

2.3 Effect of ^{12}C heavy ion beam irradiation on the rape quality with different treatments

The results of self-incompatibility posterity seed composition of 10 different individual plants of M_2 were listed in Table 6. Few variations (such as oil content, fatty acid composition, protein content and glucosinolate content) occur under 30Gy treatment and 50Gy treatment. But variations occur more often under 80Gy treatment. Seed oil contents increase to different degree and 3 plants have oil content higher than 70%. The highest content reaches 80.2%.

Table 5.1 Observation on botanical morphology variant plants by different treatments of ^{12}C heavy ion beam irradiation on rapeseed

Treatment (Gy)	Root variation			Stem variation			Leave variation		
	Plant No.	Morphology	Frequency %	Plant No.	Morphology	Frequency %	Plant No.	Morphology	Frequency %
30	0	-		0	-		0	-	
50	0	-		0	-		1	Light green spoon leave	0.98
80	3	Tumor-like root	2.5	3	Plant height 155cm	2.5	3	Light green spoon leave	2.5
CK	0	-		0	-		0	-	

Table 5.2 Observation on botanical morphology variant plants by different treatments of ^{12}C heavy ion beam irradiation on rapeseed

Treatment (Gy)	Flower variation			Silique variation			Seed variation		
	Plant number	Morphology	Frequency %	Plant number	Morphology	Frequency %	Plant number	Morphology	Frequency %
30	0	-		0	-		0	-	
50	1	Multipistil	0.98	1	Disiliques	0.98	0	-	
80	5	Multipistil	4.2	3	Disiliques	2.5	4	Yellow seed	3.3
CK	0	-		0	-		0	-	

Table 6 Effect of ^{12}C heavy ion beam irradiation on quality character of rapeseed

Treatment (Gy)	Plant name	Seed oil content (%)	Fatty acid composition (%)					Protein content (%)	Glucosinolate content (μmol/g)
			16:0	18:1	18:2	18:3	22:1		
30	1	41.3	3.8	60.0	20.2	4.3	0.5	23.3	33.0
	2	38.7	5.1	61.2	20.1	4.3	0.0	25.5	21.8
	3	37.5	4.4	61.2	18.8	6.0	3.2	26.6	25.5
	4	40.7	6.2	58.9	20.7	6.6	1.2	19.9	40.0
	5	38.1	7.1	60.7	21.2	5.5	2.4	24.4	39.9
	6	38.9	6.6	61.2	20.0	4.9	3.3	20.2	12.4
	7	39.6	7.0	58.2	22.2	5.6	1.2	23.3	33.6
	8	42.2	5.5	61.1	18.1	4.4	0.8	20.4	32.1
	9	39.9	4.2	63.5	18.4	5.0	4.1	20.5	25.1
	10	38.4	4.3	63.3	18.8	5.8	0.5	24.6	35.5
($\underline{X}$±S)		39.53±1.5	5.42±1.50	60.93±1.66	19.85±1.32	5.24±0.79	1.72±1.42	22.87±2.45	29.89±8.69
50	1	38.8	5.1	58.6	17.3	4.4	1.5	22.4	35.2
	2	41.5	3.2	69.1	17.5	5.2	0.2	22.3	12.6
	3	39.9	5.5	58.2	20.2	4.1	0.1	27.8	22.6
	4	42.6	6.0	59.9	21.1	3.5	0.2	23.5	23.1
	5	43.1	4.2	60.1	18.4	5.1	0.7	24.0	33.6
	6	38.2	3.3	64.1	18.8	5.5	0.6	28.3	33.3
	7	38.1	2.2	58.2	20.1	6.2	2.1	22.6	35.5
	8	42.2	4.1	55.3	19.7	7.7	3.1	21.8	41.2
	9	39.7	4.2	59.3	18.1	6.0	0.0	23.7	40.0
	10	38.3	5.1	56.9	17.4	8.1	0.2	24.5	35.7
(X±S)		40.24±1.95	4.31±1.17	59.97±4.02	18.86±1.34	5.58±1.48	0.87±1.04	24.09±2.25	28.02±9.63
80	1	39.5	6.8	78.1	18.0	5.3	0.6	28.4	33.1
	2	41.1	7.4	66.5	19.2	3.4	0.6	23.5	32.2
	3	38.2	1.6	80.2	10.9	3.3	0.9	31.2	26.1
	4	37.9	5.3	75.2	15.5	3.9	0.5	22.4	28.4
	5	42.2	5.4	64.2	10.6	4.4	0.1	23.6	25.5
	6	43.3	4.6	63.1	19.2	3.5	3.1	22.6	32.2
	7	40.0	8.3	65.9	21.4	3.7	2.5	23.3	18.5
	8	42.5	7.2	66.4	21.5	5.2	2.6	27.7	13.9
	9	45.0	9.1	60.7	18.7	6.1	0.8	20.8	28.6
	10	43.3	7.2	66.1	16.4	8.7	0.4	25.5	40.1
(X±S)		41.30±2.36	6.29±2.15	68.64±6.69	17.14±3.86	4.75±2.22	1.21±1.08	24.9±3.24	27.86±7.51
CK	1	40.0	5.3	60.6	18.8	7.3	1.2	21.7	40.0
	2	39.9	4.8	59.1	20.1	7.7	1.7	25.3	38.1
	3	41.2	5.5	58.2	19.4	8.1	3.2	20.1	30.2
	4	41.0	5.2	59.9	20.5	8.2	1.6	22.7	30.5
	5	38.5	6.3	58.7	21.5	6.5	0.6	24.4	29.1
	6	44.1	6.0	61.2	18.3	6.1	2.1	22.9	28.4

Treatment (Gy)	Plant name	Seed oil content (%)	Fatty acid composition (%)					Protein content (%)	Glucosinolate content (μmol/g)
			16:0	18:1	18:2	18:3	22:1		
	7	41.5	7.1	62.1	18.4	5.4	2.3	22.8	15.2
	8	39.2	6.6	58.4	20.6	4.4	2.2	24.6	23.1
	9	40.7	5.4	57.2	21.2	5.8	1.0	22.8	25.5
	10	41.1	4.9	61.5	22.1	7.1	0.2	23.1	21.1
(X±S)		40.72±1.66	5.71±0.76	59.69±1.62	20.09±1.33	6.46±1.33	1.61±0.89	23.04±1.49	28.12±7.45

2.4 Effect of ^{12}C heavy ion beam irradiation on the chromosomal behavior of rape with different treatments

2.4.1 Chromosome aberration at root tip

10000 cells at root tip were observed under different treatments. The results showed that chromosome aberration rate at root tip increases with the increasing amount of irradiation. The aberration rate are 0.08%, 0.15% and 0.18% respectively. As shown in Table 7 and Figure 3, the aberration type includes micronucleus, mini-nucleus, abnormal tetraspore, bridge, lagging chromosome and fragment, most of which are micronucleus.

Table 7 Effect of ^{12}C heavy ion beam irradiation on chromosomal aberration in root top cell of rapeseed

Treatment (Gy)	Observed cell number	Micronucleus	Mininucleus	Bridge	Lagging chromosome	Fragment	Aberration cell number	Micro nucleus rate (%)	Total aberration rate
30	10000	8	0	0	0	0	11	0.08	0.08
50	10000	11	1	1	1	1	15	0.11	0.15
80	10000	13	2	1	1	1	18	0.13	0.18
CK	10000	0	0	0	0	0	0	0	0

2.4.2 Pollen mother cell chromosome aberration

10000 pollen mother cells were observed under each treatment. The results showed that aberration occur under different treatments. But the aberration rate is low. They are 0.02%, 0.08% and 0.15% respectively. The aberration type includes micronucleus, abnormal tetraspore, bridge, lagging chromosome and fragment (Table 8, Figure 4, 5, 6, 7). The rest are normal cells like the image of pollen mother cells (Figure 8).

Table 8 Effect of ^{12}C heavy ion beam irradiation on chromosomal aberration in pollen mother cell of rapeseed

Treatment (Gy)	Observed cell number	Micronucleus	Bridge	Lagging chromosome	Fragment	Abnormal tetraspore	Aberration cell number	Micro nucleus rate (%)	Total aberration rate (%)
30	10000	2	0	0	0	0	2	0.02	0.02
50	10000	5	1	1	1	0	8	0.05	0.08
80	10000	10	1	2	1	1	15	0.10	0.15
CK	10000	0	0	0	0	0	0	0	0

2.5 RAPD analyses of the effect of ^{12}C heavy ion beam irradiation on rape M_2 with different treatments

Selected from 40 random primers, 6 primers with clear amplified bands were used to amplify rapeseed after different treatments. 43 DNA fragments were amplified. It showed that different treatments on rapeseed have polymorphism to some degree, that is, variations occur after ^{12}C heavy ion beam irradiation. It is the results that primers of S201, S208, S213, P18, P20, P23, etc. amplify the rape respectively under ^{12}C heavy ion beam irradiation with different treatments and CK. Primer amplification is different in number and position. It shows that any particular primer has its own binding site on DNA sequence. Once DNA fragment insertion, absence or base mutation occurs in this area of genome, it would result in the change of the given binding site distribution and thus the amplification number and size, which shows polymorphism. The results of 6 different primers are adopted in this experiment. There are obviously different positions and numbers of band under ^{12}C heavy ion beam irradiation with different treatments compared with CK (xiangyou No.15). DNA molecular structure variations occur under ^{12}C heavy ion beam irradiation with 30Gy, 50Gy, 80Gy treatments and untreated xiangyou No.15. So polymorphism appears here. The RAPD fingerprint diagrams after primer amplification are as follows (Figure 10-Figure15).

3.Discussion

3.1 Effect of ^{12}C heavy ion beam irradiation on rapeseed

This above results show that ^{12}C heavy ion beam irradiation has some effects on rapeseed characters. These include luxuriant growth, earlier growth duration, improved agronomy characters such as increasing silique number each plant, seed number each silique and weight of 1000 seeds. Treatment of 80Gy ^{12}C heavy ion beam irradiation can make botanical character variation such as tumor-like root, dwarf stem, light green spoon leave, multipistil flower, multi-silique or yellow seed and so on. In addition, fatty acid composition has variations such as oil content increase to different degree and the oleic acid content of some plants is higher than 70%. The results in this work are almost identical with the results of predecessors by using γ ray. But tumor-like root, light green spoon leave, multi-silique or yellow seed have not been reported yet. These variations will

provide the foundation material for rapeseed breeding. The observation of root-tip cell and pollen mother cell chromosome behavior show that aberration occurs under ^{12}C heavy ion beams irradiation with different treatments. Aberration rate is higher under 80Gy treatment and perhaps that is an important reason for variation. According to RAPD results there are some differences on DNA fingerprint under different treatments. It shows polymorphism to some degree and variation occurs in irradiated offspring.

3.2 Rapeseed character mutation analyses under ^{12}C heavy ion beam irradiation

A heavy ion beam is particles without electricity. It can be produced by an accelerator or a nuclear reactor. Its energy is very high and the frequency that causes the mutation is also higher. In the past it was normally thought that most of variation caused by mutation is recessive gene and does not appear in M_1. But in this work it is not fully like that. The mutation has both dominance and recessivity. The most typically one is earlier growth duration that belongs to dominant character and exhibits in M_1. M_2 shows the same as M_1. The others such as luxuriant growth, tumor-like root, multipistil flower, di-silique are all dominant characters. The recessive mutation in this work includes shorter plants and yellow seeds. As for a dominant mutation in mutant, it relates possibly to the stronger gene function that controls gibberellin formation, which needs further study.

3.3 Micronucleus formation after ^{12}C heavy ion beam irradiation

In this work aberration cells that include root-tip cell and pollen mother cell under 12C heavy ion beam irradiation are the same as those under γ ray treatment. So it can be used as an index for irradiation effect. Micronucleus variation can also be used to determine frequency of chromosome aberration. There are different views about micronucleus formation. It is possible that when chromatin forms chromosome, multiform heterochromatin particles appear, and their fibrils are very sensitive to the irradiation effect and easy to split. As a result the heterochromatin particles gather to form micronucleus. Because the interphase of cell division needs long time and includes three stages of G1, S, G2, the frequency is high and easily observed. This viewpoint needs further confirmation by experiments.

3.4 Bridge, lagging chromosome and fragment formation after ^{12}C heavy ion beam irradiation.

Chromosome bridge and fragment: Chromosome bridge and fragments normally form by inversions during pachytene. After nonsister chromatids cross and exchange segments, the inversion chromosome, dicentric chromosome, acentric chromosome and normal chromosome form. Dicentric chromosome forms during anaphase I. At anaphase the dicentric chromatid forms a bridge between the poles and breaks up, and the acentric fragment disappears.

Lagging chromosome formation: the chromosome number of *Brassica napus* is 2n=38. During the zygotene stage, 19 bivalents form. Their centromeres are attached by their spindle fibers to the two poles. The chromosomes locate at the metaphase plate by equivalent forces from the two poles. The balance may be broken by ^{12}C heavy ion beam irradiation to rapeseed. Chromosome behavior abnormities are induced and individual chromosomes lag behind the others. Perhaps that is the reason that the lagging chromosome was seen during metaphase.

As for the reason that the lagging chromosomes were also seen during anaphase I or anaphase II, probably it is because the normal function of spindle fibers moving chromosomes toward the two poles are disrupted, the movement of chromosomes during anaphase cannot be of synchronization.

3.5 Analysis of RAPD polymorphism after ^{12}C heavy ion beam irradiation on rapeseed

RAPD shows the molecular difference of rapeseed after ^{12}C heavy ion beam irradiation. ^{12}C heavy ion beam irradiation has the function to change genetic structures. Rapeseed chrosome contains DNA, histone, nonhistone and RNA, in which DNA is the main genetic substance. ^{12}C heavy ion beam irradiation may induce mutations such as G-C→A-T transition or C-G→A-T transversion. Thus frameshift mutation and other sub-effects of irradiation can occur.

Ionizing radiation in the form of ^{12}C heavy ion beam irradiation cause chromosome aberration and structural variation of DNA molecules more easily than X-rays and γ-rays and its energy can be absorbed more easily by organism. So its relative biological effect(RBE) is higher and the mutation is more difficult to be repaired by the plant. The mutation percentage is high and stable. Directed or oriented mutation can be achieved because the energy can be adjusted. Therefore mutation induced by ^{12}C heavy ion beam irradiation will be very useful in rapeseed breeding.

Though the biggest weakness of RAPD analysis is regarded as its bad reappearance, the emersion of RAPD marker can improve as long as experimental conditions are strict and standard.

3.6 Suitable ^{12}C heavy ion beam irradiation dosage to rapeseed

After treating of 30Gy, 50Gy and 80Gy 12C heavy ion beam irradiation on rapeseed, the effects shown in this work are not enough extensive. The mutation percentage is high and the variation range is extensive when 12C heavy ion beam irradiate corn (Courtesy of LUO H-B, 2003). Perhaps rapeseed has a strong capacity of anti-irradiation. Former studies also showed that crucifer is not sensitive to irradiation because their seed has propylene mustard oil as a barrier to irradiation (Courtesy of HU Y-J, 2003). It seems 12C heavy ion beam irradiation dosage to rapeseed should increase if we want to get more mutation and enlarge mutation range.

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