# Preliminary study on mutagenic effects of $^{60}$Co-γand spaceflight treatment to *Brassica napus*[*]

**LI Haojie, PU Xiaobin, ZHANG Jinfang, JIANG Liangcai**

*Crop Institute of Sichuan Academy of Agricultural Sciences, Chengdu, 610066, china*
*Email: jlcrape@hotmail.com*

**Abstract**

40 double-low seeds of *Brassica napus* were irradiated by γ-ray at dose of 800Gy, 1000Gy and 1200Gy respectively. 12 lines were experienced the spaceflight by "Shenzhou 4" and "Round-trip artificial satellite No.18". Molecular markers detection were performed and the agronomic characters were investigated in $M_1$ (radiation contemporary), SP2, $SP_3$ (progeny of plant carried by spaceflight). The main results are as follow (1) the seedling survival rate, Plant height, height of primary branch, No. of silique per plant and seed weight per plant in $M_1$ significantly decreased comparing with the control, but the difference on 1000-seed weight was not significant. There are Polymorphism of characteristic band on SSR primer Na-$OE_{02}$ between $M_1$ and the control. Early-flowering plant and dwarf plant appeared in $M_1$. (2) $SP_1$ had no obvious effect in germination percentage, seedling survival rate, seed setting and other agronomic characters. Some mutated plants appeared in $SP_2$ however. Useful mutants included the plants with long silique, more pods, dwarf plant, later maturity and bigger seeds et al. And the results of identifying agronomic characters of the mutants in $SP_2$, $SP_3$ indicated that the mutated characters of more branches, more pods, and bigger seeds could be heritable from $SP_2$ to $SP_3$. The later maturity and more pods mutated plant had characteristic band comparing with the control on SSR and RAPD primer.The same to its $SP_3$ plants. It indicated that the spaceflight mutagenesis produced hereditable mutation.

**Key words**: Radiation,$^{60}$Co-γ,Spaceflight, variation, SSR polymorphism, *Brassica napusL*

## Introduction

Abundant materials are the foundation of new crop variety. So, it is a valid way to induce variation by biotechnology for creation breeding resources. Radiation was a simple and effective method of introducing variation, for the novel characters of mutants was stable early. And it was extensively utilized for crop breeding, especially in rice (Fu HW et al., 2002; Huang SHW et al., 2000; Wu KX et al., 1999; Quan CH Y et al., 2006). Mutagenesis by spaceflight was another way to introducing mutants, which was applied in rice, wheat, soybean, green lentil, edible germ, vegetable et al. And many mutants were obtained successfully (Mei M T et al., 1994; Mei M T et al., 1998; Qiu F et al., 1998; Xu JL et al., 2002; Pang BL et al., 2004; Liu JY et al., 2005). Rape is one of the four main oils in world. But having sinapine, which have the role of protection to the radiation of several rays, rape was the crop of bear-radiation or anti-radiation. So, the reports were relatively less in china (Liu Z et al., 2000). The double-low seeds of *Brassica napus* were irradiated by $^{60}$Co-γand spaceflight on "shengzhou4"and "Round-trip artificial satellite No.18"in this study. Some mutants were obtained. The agronomic characters and molecular test showed the genetic variation was real and heredity was stable.

The objective of this study is to create mutation for breeding resources in *Brassica napus* by two ways, observe the mutant traits and purify them for possible uses.

## Materials and Methods

Plant materials Conventional varieties applied in production in Sichuan of china, double-low *Brassica napus L* of self-cross lines from crop institute Sichuan Academy of Agricultural sciences were used.

Radiation by $^{60}$Co-γ Windy-dry seeds of 10 convention varieties were treated by $^{60}$Co-γwith the dose of 800Gy, 1000Gy,1200Gy respectively in the radiation field of institute of Biotechnology and nuclear Technology Sichuan Academy of Agricultural sciences.

Spaceflight Windy-dry seeds of 6 *Brassica napus* lines were carried by "shengzhou 4" in 12, 2002, and another 6 lines were carried by "Round-trip artificial Satellite No.18" in 9, 2003.

Trial Seeds treated by $^{60}$Co-γand spaceflight were sown in the farm of PI county by direct seeding for three replications and 50 grains per repeation. The land productivity was even. Field management on this experiment was conducted based on standard management for rape in Sichuan. $M_1$and $SP_1$ seeds were bulk-harvested at maturity, and were sown in the next year.

Statistic analysis germination percentage were investigated after sowing a week, and seedling survival rate were investigated at the four-leaf period (the number of plants of four-leaf/number of seed grains being sown×100). The data were analyzed by the statistical program Regional Crop Trial Analysis system.

DNA extraction and SSR, RAPD analysis DNA extraction: total genomic DNA from both mutants and the control

[*] **Fund:** National 863 Project (2001AA241104), "Tenth Five Years Program" on Breeding Tackling Key Problem (200107001-1-7-4) and Applied Basal Research Item of the Science and Technology Hall (05JY029-002-1) in Sichuan

was extracted from fresh leaves according to the CTAB method (Murray and Tompson 1980) with minor modifications. SSR primers from http.www.ukcrop.com and RAPD primers were synthesized by the gene company of Beijing shaibaisheng. SSR reaction: reaction total volume 15 μL: 10×Buffer 1.5 μL,25mM $Mg^{2+}$ 1.5μL, 10mM dNTP 0.6μL, 25ng/μL primers 2 μL, 10ng/μL DNA 7.3μL, 5U/μL Tag enzyme 0.125Ul; PCR reaction procedure:94°C 5min, 94°C 30sec,55°C 45sec,72°C 1.5min, 46cycles,72°C 10min.RAPD reaction: total volume 13.5 μL: 10×Buffer 1.5 μL,25mM $Mg^{2+}$ 1.5μL, 10mM dNTP 0.6μL, 25ng/μL primers 2 μL, 140ng/μL DNA 0.6μL, $ddH_2O$ 7.0 μL, 5U/μL Tag enzyme 0.125μL; PCR reaction procedure:94°C 5min, 94°C 1min, 30°C or 36°C 1min, 72°C 2min,45cycles, 72°C 10min.

Investigation of agronomic characters 10 plants were tested in agronomic characters per replication.

## Results

Germination percentage of $M_1$ treated by $^{6°}$Co-γdecreased to the control, but was not significant.

**Table1. LSD analysis of percentage of the plant and agronomic characteristicistics of the plants in $M_1$ at dose of 800Gy, 1000Gy, 1200Gy**

| Genotype | Dose | Seedling survival rate(%) | Plant height(cm) | Height of primary branch(cm) | No. of silique per plant | Seed No. per silique | 1000-seed weight(g) | seed weight per plant(g) |
|---|---|---|---|---|---|---|---|---|
| Zhong shuang 9 | CK | 90. 00a | 190.00a | 76.50A | 969.20A | 10.27a | 3.95a | 35.50A |
| | 800Gy | 87. 00a | 186.00b | 61.20B | 568.00B | 11.06a | 3.85a | 22.00B |
| | 1000Gy | 70. 00b | 160.00c | 52.00C | 233.50C | 11.57a | 3.70a | 10.00C |
| | 1200Gy | 54. 00c | 130.50d | 22.40D | 180.50D | 8.20b | 3.02a | 4.00D |
| DEYIN-10 | CK | 90. 00A | 173.00a | 56.50a | 639.00A | 13.93A | 3.15a | 22.00a |
| | 800Gy | 40. 00B | 169.50b | 39.50b | 541.00B | 3.98C | 2.85a | 4.60b |
| | 1000Gy | 17. 50C | 160.00c | 33.50c | 211.50C | 8.95B | 2.05b | 3.10bc |
| | 1200Gy | 10. 00D | 130.00d | 12.00d | 158.00D | 8.51B | 1.65c | 1.95c |

Note: Capital and small letter indicate significance at $p \leqslant 0.05$and 0.01 respectively.

Seedling survival rate decreased significantly (table1).And were also different among the dose of 800Gy, 1000Gy and 1200 Gy. There are significant difference between $M_1$ and the control on agronomic characters such as plant height, height of primary branch, No. of silique per plant, seed No.per silique and seed weight per plant. Some high 1000-seed weight (5.29g) mutants were obtained, but the difference was not significant as a population between $M_1$ and the control. Botany characteristic such as plant height, leaf shape, vegetative growth, flowering character and seed-setting were also affected, and induce to reduce on yields characteristic. So the seed weight per plant was decreased.

Polymorphism analysis of SSR in $M_1$ indicated that there were characteristic band between mutants of $M_1$ and the control (Fig 1),which two bands on the control, three bands on the mutated plant.

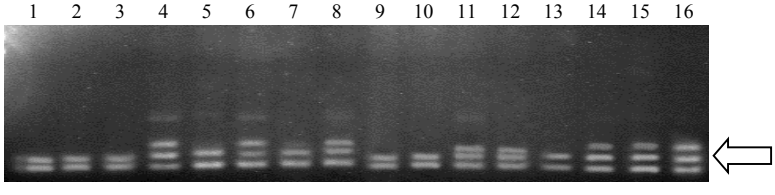


Fig.1. Polymorphism of NaOE02 in $M_1$

(1, 5, 9, 13 indicated CK of Zhongshuang9, Chuanyou18, Chuanyou20 and Shuza6;2-4, 6-8, 10-12, 14-16 indicated $M_1$ radiated by 800 Gy, 1000 Gy, 1200Gy of Zhongshuang9, Chuanyou18, Chuanyou20, Shuza6)

The results of spaceflight showed that botany characters such as seed leaf euphylla, leaf shape, leaf color et al, agronomic characters such as height of plant, Height of primary branch et al of $SP_1$ were similar to the control. However, some characters of the leaf shape, plant body, vegetative growth, flowering character and seed-setting et al of $SP_2$ were different with the control. Useful mutants included more-branch per plants(PR-1), more-pod plants (PR-1) and high-seed-weight plants (PR-1, 9804) et al (table 2). And the characters were heritable from $SP_2$ to $SP_3$ (table3) (Plant height: 9804, No. of branches per plant: PR-1, No. of pods per plant: PR-1, 1000-seed weight: 9804).

**Table 2.Variation characters found in $SP_2$ plants of the three populations**

| Lines | | Plant height(cm) | No. of branches per plant | No. of pods per plant | Seeds per pod | 1000-seed weight(g) | Growth period(d) |
|---|---|---|---|---|---|---|---|
| 9713 | CK | 190.00-210.00 | 7.20-16.00 | 299.00-654.00 | 7.70-16.40 | 4.55-4.70 | 210 |
| | $SP_2$ | 184.00-201.00 | 9.50-15.00 | 111.00-374.00 | 10.30-21.30 | 2.40-5.30 | 212 |
| 9804 | CK | 170.00-202.00 | 11.00-17.00 | 230.00-624.00 | 9.70-19.00 | 2.76-4.78 | 215 |
| | $SP_2$ | 110.00-135.00 | 5.00-12.00 | 178.00-334.00 | 9.00-17.00 | 3.67-8.60 | 213 |
| (PR-1) | CK | 187.00-205.00 | 7.00-15.00 | 359.00-654.00 | 15.70-25.30 | 3.29-4.21 | 210 |
| | $SP_2$ | 171.00-198.00 | 10.00-38.00 | 143.00-2366.00 | 16.20-28.50 | 2.99-6.64 | 215 |

**Table 3. The mutant characters of $SP_3$ plants from populations**

| Lines | | Plant height(cm) | No. of branches per plant | No. of pods per plant | Seeds per pod | 1000-seed weight(g) | Growth period(d) |
|---|---|---|---|---|---|---|---|
| 9713 | CK | 198.00 | 13.00 | 482.00 | 15.00 | 4.66 | 210 |
| | $SP_3$ | 191.00 | 14.33 | 223.00** | 17.50 | 5.00 | 209 |
| 9804 | CK | 189.00 | 12.50 | 361.00 | 21.00 | 4.00 | 211 |
| | $SP_3$ | 124.00** | 9.50 | 207.00** | 19.30 | 6.64* | 212 |
| (PR-1) | CK | 198.20 | 12.20 | 379.90 | 28.80 | 3.71 | 213 |
| | $SP_3$ | 203.30 | 26.60** | 891.20** | 29.30 | 3.82 | 220* |

*and **indicate significance at $p \leqslant 0.05$ and 0.01 respectively.

Polymorphism analysis of RAPD (Fig 2) and SSR (Fig 3) in $SP_2$ and $SP_3$ indicated that 4 polymorphic RAPD primers BRK07, BRK11, BRK15 and BRK17 were selected from 119 primers. BRK07 could stably amplify the characteristic band BRK07-410 in the randomly selected plants of $SP_2$ and $SP_3$ population, but there was no the band in CK. The polymorphic SSR primer pair 9L/R was selected from 132 primer pairs, which were in connection with flower organ development of Arabidopsis thaliana. 9L/R could amplify the characteristic band in the randomly selected plants from $SP_2$ and $SP_3$ population stably, but there was no the band in CK, which M.W was 175 bp.

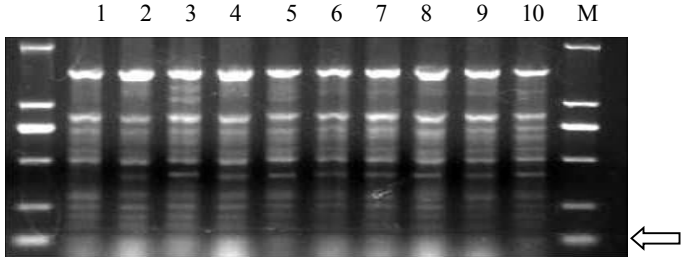


Fig.2. RAPD analysis between the mutated plant of $SP_2$, $SP_3$ and their CK by primer BRK07

(M indicate DNA marker DL2000, 1 indicate CK plants, 2 indicate the mutated plant of $SP_2$, 3 to 10 indicate the $SP_3$ plants from the mutant of $SP_2$)

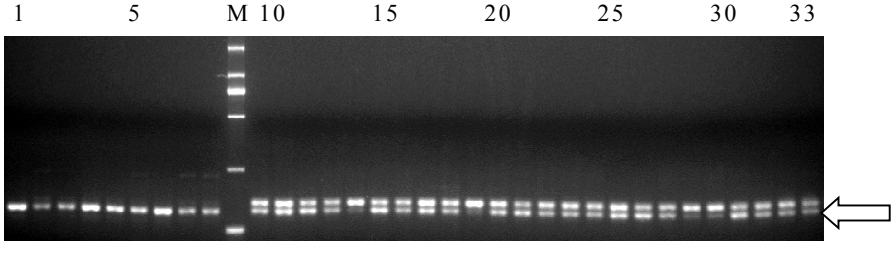


Fig.3. SSR analysis between the mutated plant of $SP_2$, $SP_3$ and their CK by primer pair 9L/R

(M indicate DNA marker DL2000, 1-9 indicate CK plants, 10-19 indicate the mutated plant of $SP_2$, 20-33 indicate the $SP_3$ plants from the mutant of $SP_2$)

## Discussion and Conclusions

Radiation of $^{60}$Co-γand spaceflight as way of mutagenesis were applied in crop breeding in several studies before (Fu HW et al.,2002;Huang SHW et al.,2000;Wu KX et al.,1999;Quan CH Y et al.,2006; Mei MT et al., 1998;Qiu F et al.,1998;Zhou YM et al.,1998;Chen L et al.,1997; Xu JL et al., 2002;Liu JY et al.,2005). Effects of the former were more direct than the later. Mutants often appeared on contemporary by $^{60}$Co-γ,but in $SP_1$,$SP_2$ even $SP_{10}$ by spaceflight. Having the traits of simple-manipulate, cost-low and treated-more, radiation as a valid way of inducing variation was applied extensively to now though the mutagenesis direction were uncertainly. In this study, some useful mutants such as low-Height of primary branch and high-1000-grain-weight were obtained. Effect of mutagenesis by spaceflight was decided to the role of shielding space cabin to the ray of cosmos, difference among materials and how long being treated. Mutants appeared in $SP_1$ in some reports(Zhang J et al.,2000;Liu Z et al.,2000), but in $SP_{10}$ in other reports(Yang CY et al.,2003; Liu JY et al.,2005)In our experiment, some useful mutants such as low-Height of primary branch, high-1000-grain-weight, more-branch and more-mod were obtained in $SP_2$. And the characters were stably heritable from the identification of agronomic and molecular. Now, lines ($SP_4$) of the late-maturity, more-branch and more-pod mutated plant were obtained, which could be possibly used for gene cloning and location in future.

## References

Huang SH W, YU LQ. Inhibiting efficacy of metabolites of Streptomyces lavendulohygtroscopicus and its ultraviolet induced strain on two rice diseases. Chinese Rice Research Newsletter.2000, 8(2):5~6.

Guan CHY, Liu CHL, Chen SHY, Peng Q, Li×and Guan M(2006). High Oleic Acid Content Materials of Rapeseed (*Brassica napus*) Produced by Radiation Breeding. Acta Agronomic Sinica32(11):1625-1629.

Mei MT, Deng H,Lu Y,Zhuang C,Liu Z,Qiu Q,Qiu Q and Yang T C(1994). Mutagenic effects of heavy ion radiation in plants[J]. Advances in Space Research14 (10):363-372.

Mei M T,Qiu Y L,Sun Y,Huang R,Yao J,Zhang Q,Hong M and Ye J (1998).Morphological and molecular changes of maize plants after seeds been flown on recoverable satellite[J]. Advances in Space Research22 (12):1691-1697.

Pang BL, Peng XM, Zhu XQ, Deng GQ, Pang AJ, Liu QY (2004).The breeding of new rice variety by space mutation and irradiation. Acta Agriculturae Nucleatae Sinica18(4):284-285.

Qiu F, Li JG, Weng ML, Jin DM, Gao HY, Wang PS, Jiang XC, Wang B (1998). Molecular analysis of long pod mutant line of Mung bean generated by space mutagenesis. Scientia Agricultura Scinica31(6):38-43.

Yang CY, Chen FY, Wang YX, Fan XW, Yuan QH (2003). Polymorphic analysis of microsatellite markers in mutants of japonica cultivar "Akibikari"induced by the space flight. Acta Botanica Boreali-occidentalia Sinica 23(9):1550-1555.

Xu JL, Wang JM, Luo RT, Zhang MX, Jiang XC, Li JG (2002). Studies of inheritance and application in rice breeding of the large grain mutant induced in space environment. Heredita 24(4):431-433.

Zhang J, Li JG, Wang PS, Wang XQ, Jiang XC (2000). Molecular analysis of space mutant line of kidney bean. Space Medicine & Medical Engineering 13(6):410-413.

Lu JY, Liu M, Xue H, Pan Y, Zhang CH, Galina S.Nechitailo(2005).Random amplified polymorphic DNA analysis of tomato from seeds carried in Russian Mir space station. Space Medicine & Medical Engineering 18(1):72-74.

Liu Z, Zhao RQ (2000). The effects of space treatment on *Brassica napus* L.. Chinese Journal of Oil Crop Sciences 22(2):6-8.

LI×Y, Wan ZHL, XU D-Yet al(1994).THE MECHANISM FOR THE DIFFERENCE IN SENSITIVITY TO 60Co-γ-RATS BETWEEN GLYCINE.MAX MERRILL AND *BRASSICA* NAPUS L. Journal of Southwest Agricultural University 16(4):365-368.

ZHOU Y M., TAN Y L,LIU MENG et al(1998).Studies on radiation induced mutation in *Brassica* napus. Chinese journal of oil crop Science20(4):1-5.

CHEN LI, Li J N, Wang R et al(1997). EFFECT OF 60Cογ-RAT ON GERMINATING ABILITY OF YELLOW-SEEDED.RAPE(*BRASSICA* NAPUS L.)IN DIFFERENT GENETIC SOURCES. Chinese journal of oil crop Science19(4):7-10.

Murray, M. G., and W. F. Thompson, 1980: Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research 8, 4321- 4325.