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BIOLOGICAL EFFECTS OF 7LITHIUM (^{7}LI) ION BEAM RADIATION ON MUTATION INDUCTION IN *CAPSICUM ANNUUM* L.

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Abstract

Lithium-7 (^{7}Li) ion beam radiation is an effective method for inducing mutations in plants. In this study, the effects of ^{7}Li ion beam radiation on mutation induction in pepper (*Capsicum annuum* L.) were evaluated. The germination rates of irradiated seeds, survival rates of M1 plants and mutation frequencies of M2 plants were statistically analyzed. The results showed that these characteristics were all affected by radiation. A radiation dose of 60 Gy was the balance point between the survival rate of M1 plants and mutation rate of M2 plants, indicating that 60 Gy was a good choice for ^{7}Li ion beam radiation applications in breeding. Sequence-related amplified polymorphism (SRAP) analysis of select M2 plants indicated that radiation caused changes in the DNA. Observations of mutant morphology showed that useful mutants were generated from M2 plants. These results provide both a basis for the application of ion beam radiation in breeding and useful materials for studies of gene function.

Key words: *Capsicum annuum* L.; ^{7}Li ion beam; Mutant; SRAP.

Introduction

Peppers belong to the family Solanaceae and the genus *Capsicum*. Among 35 taxa (species and varieties) 30 are wild and five are domesticated (Carvalho *et al*., 2017). The process of domestication and human interference is always associated with a reduction in the genetic diversity of the selected population (Eyre-Walker *et al*., 1998; Carvalho *et al*., 2014). Although identification and incorporation of wild accessions in a breeding program can cover part of the lost genetic variability, accessions are still deficient. How to generate many more accessions with genetic variability is crucial for breeding.

Today, people find many ways to create plant materials with genetic variability. By altering he DNA structure, artificial mutagenesis with chemical reagents or physical conditions is a very effective way to create materials. Ion beam radiation is a kind of physical mutagenesis with high frequency and broad spectrum applicability, which has been successfully used in mutations induction in different plant species (Hayashi *et al*., 2007; Tanaka *et al*., 1997; Kazama *et al*., 2008a; Wu *et al*., 2001). There are many studies about the effects of ion beams on plants. Ion radiation showed a high mutation rate and wide mutational spectrum, without causing a significant harm to the plant (Yu *et al*., 1991). To optimize the conditions for heavy-ion mutagenesis, Kazama *et al.*, (2008b) irradiated tobacco (*Nicotiana tabacum* L.) tissues at various developmental stages with heavy-ion beams of various doses and examined the effects of radiation by monitoring plant growth and mutation induction. The authors found that the effects differed among irradiated tissues, and the sensitivity to heavy-ion beam radiation increased in the following order: dry seeds, imbibed seeds, and culture tissues. Hayashi *et al*., (2007) indicated that the conditions of C-ion and Ne-ion radiation to maintain high survival rates reached 20 Gy at 23-40 keV/μm and 10 Gy at 60-80 keV/μm, respectively. Seed fertility tended to be unaffected by LET values of 23-70 keV/μm with the same dose of radiation.

In this study, ^{7}Li ion beams within a dose range of 0 to 100 Gy were used to irradiate dry seeds of pepper. The biological effects of ^{7}Li ion beams on *C. annuum* L. were then evaluated by the statistics of the germination rates of irradiated seeds, survival rates of M1 plants and mutation rates of M2 plants, as well as by morphological observations of mutant M2 plants. The results may provide a basis for the application of ion beam radiation in breeding, and the mutants generated in this experiment will be useful for studies of gene function.

Materials and Methods

Plant material: Seeds of *Capsicum annum* L. cultivar "Dragon No. 5" were provided by the Horticultural Sub-Academy of the Heilongjiang Academy of Agricultural Sciences. "Dragon No. 5" displays moderate growth and a low percentage of fertile fruit. There are 25-30 fruits per plant, and the average fruit weight is from 80 to 100 g. The comprehensive disease resistance ability is poor. The fruits are horn shaped and display green or yellow colors. The fruit pulp has a thick flesh that tastes moderately spicy.

Seed radiation treatment: Dry seeds (50 seeds each in all exposure dose regimens) of *Capsicum annuum* "Dragon No. 5" were exposed to ^{7}Li ion beams from an H1-3 device at the China Institute of Atomic Energy. The total energy of the ion beams was 42.3 MeV, and the radiation doses were 0, 10, 20, 30, 40, 60 and 100 Gy. The radiation dose rate was approximately 6 Gy/min. Experiments were replicated 3 times.

Seed germination rate statistics and observation of mutant morphology: The exposed and unexposed seeds (control group) were allowed to germinate on a round moistened filter paper in a culture dish at 25°C. Observations were carried out every 24 hours. After germination, the M1 seedlings were transplanted into soil in pots and then grown in a greenhouse under the same conditions to observe the mutants. The seedlings that developed more than two true leaves were scored as surviving plants. M2 seeds were harvested separately from each M1 plant. Ten M1 plants were selected from each radiation dose group. These seeds were then sown and grown in a greenhouse to observe the mutants. The mutation rates were statistically analyzed for each radiation dose treatment group.

DNA extraction and sequence-related amplified polymorphism (SRAP) analysis: Genomic DNA was extracted from true leaves closest to the growing point using a Rapid Plant Genomic DNA Isolation Kit (Sangon, China) according to the manufacturer's instructions. SRAP-PCR was performed in a 15-µL reaction mixture containing 20 ng of template DNA, 1.0 µL of forward primer (10 mM), 1.0 µL of reverse primer (10 mM), and 8.0 µL of 10× Taq Mix (Transgen, China). The primers used in this experiment are listed in Table 1. The thermal conditions were as follows: initial denaturation of 94°C for 5 min; 5 cycles of 94°C for 1 min, 35°C for 1 min, and 72°C for 1 min; 35 cycles of 94°C for 1 min, 35°C for 1 min, and 72°C for 1 min; and a final extension of 72°C for 10 min. The PCR products were separated by electrophoresis on 8 % polyacrylamide gels (acrylamide: bis = 29:1) and visualized with silver staining (Panaud *et al*., 1996).

Results

Statistical results of the characteristics of pepper seeds and plants: Table 2 shows the seed germination rates, germinability, M1 plant survival rates and M2 plant mutant rates under different doses of ^{7}Li ion beams. Both the germination rate and germinability exhibited the same change trend. The values decreased when the dose increased from 0 to 30 Gy and then peaked at 60 Gy. The germination rates and germinability decreased under the 100 Gy dose compared to the 60 Gy, while the value still maintained a relatively high level. The M1 survival rates decreased under the 20-100 Gy dose treatments. Among these treatments, the M1 survival rate was relatively higher in 40 and 60 Gy dose treatments. The M2 mutation frequency increased along with an increase in radiation dose but decreased slightly under the 100 Gy dose treatment. The maximum M2 mutation frequency occurred in the 60 Gy dose treatment.

Statistical results of the mutant morphological characteristics: Radiation treatment in this experiment caused many different morphological types of mutants. Many M1 plants exhibited morphological changes, including leaf deformities, plant etiolation, plant dwarfism and so on. Observations of the M2 plants showed that mutations affected the leaves, flowers, seeds, fruit, plant height, plant width and many other plant characteristics. The mutation frequencies of the different mutant types of M2 plants are shown in Fig. 1. The mutation frequencies of the different mutant types were different under our six kinds of treatment conditions. The average value of the plant growth mutation frequency was relatively higher than the values of other mutation types. The highest value of flower mutation frequency occurred in the treatment with a dose of 100 Gy, the highest value of leaf mutation frequency occurred in the treatment with a dose of 40 Gy, the highest values of both fruit and seed mutation frequencies occurred in the dose of 60 Gy, and the highest values of plant mutation frequency occurred in the treatment with a dose of 100 Gy. Of all the M2 mutants, some (considered to be useful for breeding or were very interesting) were selected for obtaining M3 plants and for checking the genetic stability of the mutant characteristics. The selected M2 mutants are shown in Fig. 2.

Table 1. The primer sequences used in the SRAP analysis.

forward primer	3′-5′	reverse primer	3′-5′
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me2	TGAGTCCAAACCGGAGC	Em2	GACTGCGTACGAATTTGC
Me3	TGAGTCCAAACCGGAAT	Em3	GACTGCGTACGAATTGAC
Me4	TGAGTCCAAACCGGACC	Em4	GACTGCGTACGAATTTGA
Me5	TGAGTCCAAACCGGAAG	Em5	GACTGCGTACGAATTAAC
Me6	TGAGTCCAAACCGGTAA	Em6	GACTGCGTACGAATTGCA
Me7	TGAGTCCAAACCGGACA	Em7	GACTGCGTACGAATTCAA
Me8	TGAGTCCAAACCGGTGT	Em8	GACTGCGTACGAATTAGC

Table 2. Characteristics of pepper seeds under different radiation dose conditions.

Dose (Gy)	Seeds (No.)	Germination rate (%)	Germinability (%)	M1 survival rate (%)	M2 mutation frequency (‰)
0	100	96	92	95	0
10	100	96	92	96	8
20	100	90	80	88	13
30	100	94	85	80	10
40	100	96	92	82	21
60	100	99	92	81	25
100	100	96	92	70	20

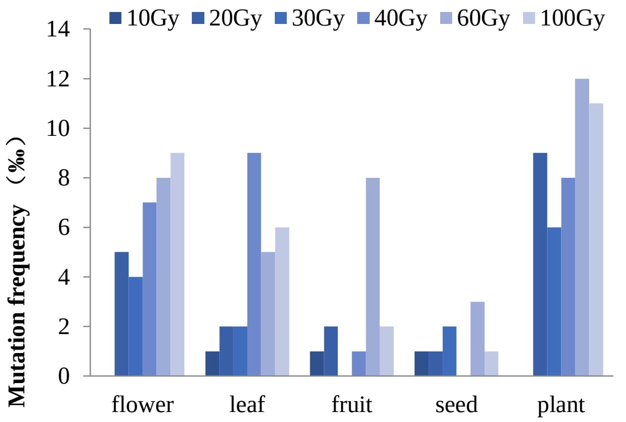

Fig. 1. Mutation frequency of different mutant types in M2 plants.

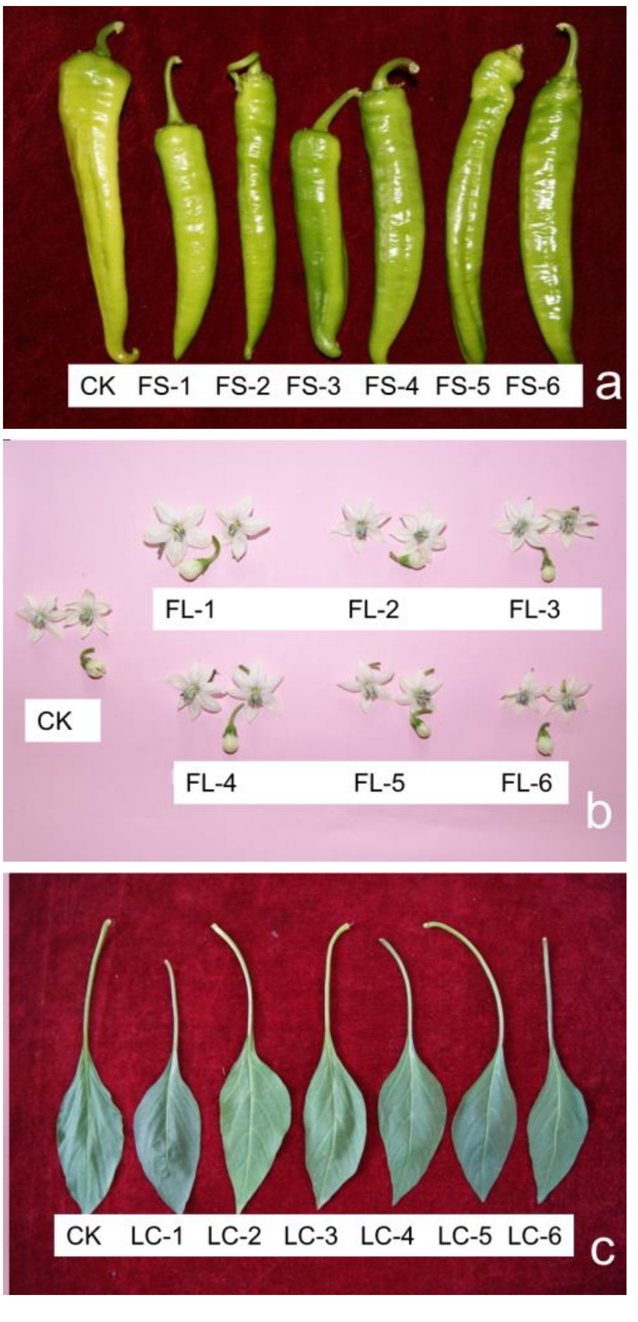

Fig. 2. Select mutants with different mutation types. a. Fruit size and shape mutations; b. Flower mutations; c. Leaf color mutations. CK: Wild type. FS-1–FS-6, FL-1–FS-6, LC-1–LC-6: Mutants codes.

SRAP analysis: Seven M2 plants that were morphological mutants were analyzed using SRAP primers. Eleven pairs of primers showed polymorphism among the seven samples. The fragments that were amplified in this experiment ranged from 70-500 bp in size. Partial results of the SRAP analysis are shown in Fig. 3. The arrows show differentially amplified bands among the different samples. These results mean that the radiation of the ^{7}Li ion beams caused changes in the DNA of the seeds.

Discussion

We examined the biological effects of ^{7}Li ion beam radiation using several different dose conditions. The results show that radiation doses between 10 and 40 Gy inhibited seed germination, while radiation doses greater than 40 Gy promoted seed germination. Although the seed germination sensitivity to the ion beam at different doses was different, the changes were not very marked. In the study of *Nicotiana tabacum*, heavy-ion beam radiation did not affect seed germination (Kazama *et al*., 2008b). This is somewhat different from our results; the reason may be due to differences in plant species and ion beams. Survival rates were significantly affected under the 20-100 Gy dose treatments and were relatively high under the 40 and 60 Gy dose treatments. Observations of mutant M2 plant morphology indicate that ion beam radiation induced a high mutation frequency at a relatively low dose without severely damaging the plants. Our results indicate that the radiation dose of 60 Gy represents the balance point between the survival rate of M1 plants and the mutation frequency of M2 plants and is the optimal dose condition for ^{7}Li ion beam radiation of pepper seeds.

Many M1 plants showed morphological changes, including leaf deformity, plant etiolation, plant dwarfism and so on. Of all the morphological change types, dwarfism is the most common. This phenomenon has also been reported in other studies (Nishizawa *et al*., 1994; Tanaka *et al*., 1997; Zhang *et al*., 1998; Kazama *et al*., 2008a). There are many morphological change types in M2 plants. According to the mutation frequency of the M2 plants, which is related to different radiation doses, we can see that the radiation dose can affect the distribution of mutation types. While we are limited by the scale of our experiment, it is hard to determine a relationship between radiation dose and mutation type in this study.

Germplasm resources represent a very important factor in breeding (Lalarukh *et al*., 2020). Artificial mutagenesis is a fast and efficient method for creating new germplasm with useful mutant characteristics. Many useful mutants for breeding and gene functional analyses were obtained in this study. The relatively high germination rate and M1 survival rate in our study indicated that ^{7}Li ion beam radiation caused little physiological injury to pepper seeds, and this result is similar to those of previous studies in rice (Hayashi *et al*., 2007). Our results also showed high mutation frequencies and wide mutation ranges in M2 plants, which indicated that ^{7}Li ion beam radiation was appropriate for pepper germplasm generation.

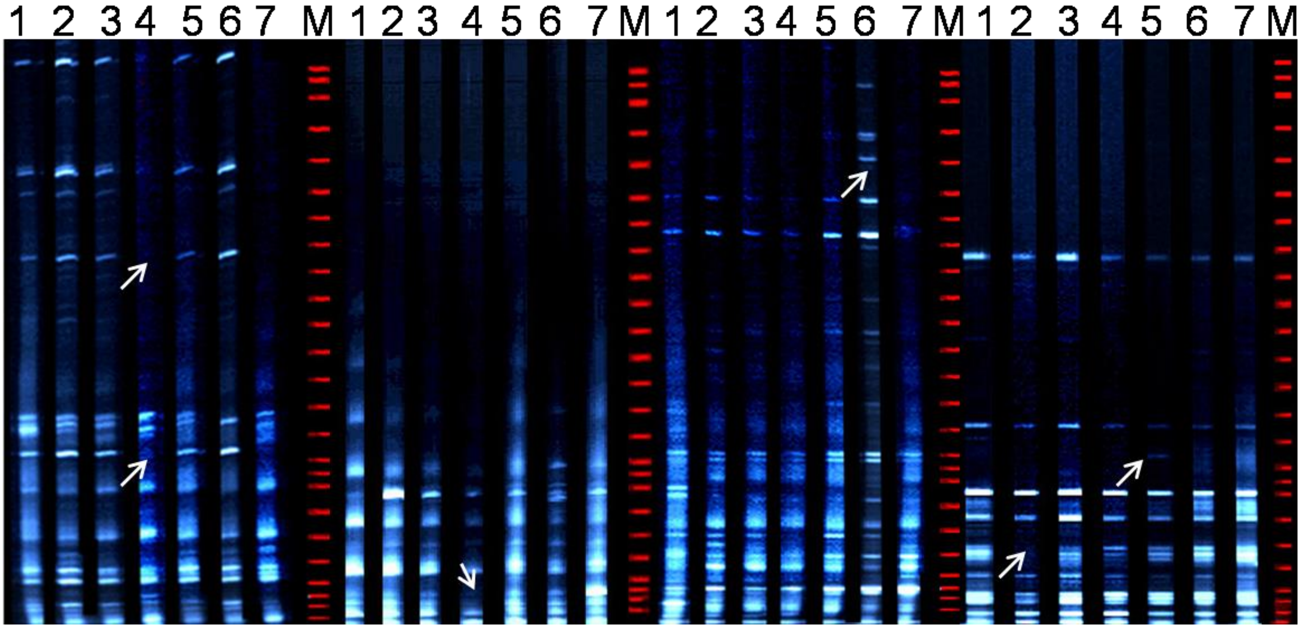

Fig. 3. Partial results of a SRAP analysis of 7 M2 plant samples. The arrows show the unique bands. 1-7: seven M2 plants; M: marker.

Conclusion

In this study, the effects of different doses of ^{7}Li ion beam radiation on pepper seeds were evaluated. The germination rates of irradiated seeds, survival rates of M1 plants and mutation frequencies of M2 plants were all affected by radiation. Both phenotype and DNA were altered by ^{7}Li ion beam radiation. The radiation dose of 60 Gy was found to be the balance point between the survival rate of M1 plants and the mutation rate of M2 plants, which means that 60 Gy is a good choice for ^{7}Li ion beam radiation applications in breeding. Morphological observations of mutants revealed that useful mutants were generated from M2 plants. These results provide both a basis for the application of ion beam radiation in breeding and also useful materials for developing new plant varieties as well as for functional studies of genes.

Acknowledgments

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