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Mutation induction by ion beams in plants

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Introduction

Ever since Stadler showed mutation induction in maize by X-rays in 1928, a great deal of studies on the mutagenesis in plants by ionizing radiation have been carried out for the breeding or basic researches. The effect of ion beams was also investigated and found that ion beams show high relative biological effectiveness (RBE) of lethality, mutation, and so on (Blakely 1992, Lett 1992), but the characteristics of ion beams on the mutation induction were not clearly elucidated yet. As ion beams deposit high energy on a target densely and locally as opposed to low linear energy transfer (LET) radiation such as gamma-rays, it is well suggested that ion beams predominantly induce single- or double-strand DNA breaks with damaged end groups whose reparability would be low (Goodhead, 1995). Therefore, it seems possible that ion beams can frequently produce large DNA alteration such as inversion, translocation and large deletion rather than point mutation, resulting in producing characteristic mutants induced by ion beams. However, mutation frequency and its spectrum by ion beams are hardly obtained in plants, although only Mei *et al.* (1994) reported that a rearrangement was induced in the semi-dwarf mutant of rice by argon ions.

Takasaki Ion Accelerators for Advanced Radiation Application (TIARA) has been established as the first facility of ion beams for biological use in 1993. We have investigated the characteristics of ion beams for the biological effects and the induction of mutation (Tanaka *et al.* 1997a, Shikazono *et al.*, 1998, Hase *et al.*, 1999). Here, I will introduce the characteristics of ion beams as well as new mutants induced by ion beams.

Irradiation

Several kinds of ion beams such as C, He and Ne ions were used in this study. All ions were generated by the AVF-cyclotron (JAERI, Takasaki, Japan). The physical properties of the 220 MeV carbon ions that are preferentially used in this study are as follows: Incident energy at the target surface was 17.4 MeV/u, mean linear energy transfer (LET) in a target (0.25 mm thickness) was estimated to be 110 keV/ μm as water equivalent, and the range of ions was ca. 1.0 mm. These physical properties were calculated by ELOSS code program, a type of modified OSCAR code program (Hata and Baba 1988). Particle fluences of the ions were determined using a diethyleneglycol-bis-allylcarbonate (CR-39) film track detector. 2 MeV electron beams (JAERI, Takasaki, Japan) were also used as a low LET ionizing radiation control.

In general, ion beams were scanned at more than 50 X 50 mm, and exited the vacuum chamber through the beam window made of a 30 μm titanium foil. The irradiation sample was placed in the air at the distance of 10 cm from the beam window. In the case of Arabidopsis or tobacco seeds, 100-3,000 seeds were sandwiched between kapton films (7.5

µm thickness) to make a monolayer of the seeds for homogeneous irradiation. In the case of rice or barley seeds, embryo side was kept facing toward ion beams. Whereas, in the case of calli or explants in a petridish, the lid of petridish was changed to a kapton-film cover in order to decrease the loss of the energy of ion beams. Samples were irradiated for less than 3 min for all doses.

Mutation frequency

In order to estimate mutation frequency of a locus induced by ion beams, we chose *Arabidopsis* visible phenotype loci, i.e., transparent testa (*tt*) which seed coat is transparent because of pigmentless, glabrous (*gl*) which has no hair on their leaves and stems, and long hypocotyl (*hy*) which hypocotyl is longer than wild type in the light condition. In this study, irradiation doses for the induction of mutation were determined from the RBE of carbon ions compared with that of electrons on the survival of plants, which was approximately 5, and further determined from the fact that both doses are at the three quarters of the shoulder dose of each survival curve (Tanaka *et al.* 1997a).

First of all, complementation analysis has been performed to determine the locus of the *tt*, *gl* or *hy* phenotype. Unexpectedly, complementation analysis revealed that new *tt* loci (tentatively named as *ttA* and *ttB*) were found. Mutation frequencies per locus per diploid cell per dose for carbon-ion induced *tt*, *gl*, and *hy* were 2.6×10^{-6} , 1.9×10^{-6} , 2.3×10^{-6} , respectively and for electron-induced *tt*, *gl*, and *hy* mutation frequencies as controls were 0.08×10^{-6} , 0.25×10^{-6} , and 0.14×10^{-6} , respectively (unpublished data). Thus, mutation frequencies by carbon ions were 8 fold to 33 fold higher than those by electrons.

Mutation spectrum

Mutation spectrum has been investigated on the flower color of chrysanthemum cv. Taihei (pink color) by Nagatomi *et al.* (1995, 1997). The explants of leaf and floral petals incubated in agar medium were irradiated with carbon ions of 220 MeV. After irradiation, the culture was transferred to a new medium to induce callus. The mutation induction of the regenerated plants from the callus was investigated, comparing the results with those with gamma-ray irradiation. The mutation rates of flower color induced by ion beams were approximately half of those induced by gamma rays in both floral petal and leaf. The most of flower color mutants induced by gamma rays were light pink, and a few were dark pink color. On the other hand, flower mutants induced by ion beams showed complex and stripe types other than single color. The complex-type flower color mutants increased as the dose of ion beams rose. The color spectrum of the ion beam-induced mutants shifted from pink to yellow. Those specific mutants such as complex and striped color types have never obtained by gamma-ray irradiation in the cultivar.

Molecular analysis of mutants

In order to investigate the DNA alteration of mutations induced by ion beams in plants, polymerase chain reaction (PCR) analysis was performed to compare DNA fragments amplified from carbon ion- and electron-induced *Arabidopsis* mutants (Shikazono *et al.* 1998a). Until now, twenty-two mutants of *TT3-TT6*, *TTG*, *TTA* and *GL1* (Shikazono *et al.* 1998b) loci have been isolated and investigated for the PCR analysis using with several pairs of primers for each locus.

13 loci out of 26 loci possessed point-like mutation because all primer pairs could amplify the corresponding fragments. 6 loci showed the missing fragments for several primer pairs, indicating that those mutants might have rearrangement such as inversion or translocation. Whereas, 7 mutant loci out of 13 loci seemed to possess large deletion covering their own loci, because no amplified PCR fragment with every primer pair was obtained. These results imply that, in the case of mutation induced by ion beams, half of mutants have point-like mutation and the other half of mutants have large DNA alteration such as inversion, translocation and large deletion.

Three mutants that have rearrangements were analyzed in their sequences of the genes. Common feature is that all the DNA strand breaks induced by carbon ions were found to be rejoined using short homologies. These results suggest that the non-homologous end joining pathway operates after plant cells are exposed to ion beams.

New mutants induced by ion beams

Arabidopsis thaliana

(1) UVB resistant mutants (*ultraviolet-light insensitive (uvi)*)

Four UVB resistant mutants (*uvi1~uvi4*) have been isolated in 5,100 M₂ families derived from carbon ion-irradiated 1,280 M₁ seeds. Especially, *uvi2* showed very high abilities of both photoreactivation and dark repair. The reduction of CPD and (6-4) photoproducts that are two major DNA damages caused by UVB, are both faster in *uvi2* than in wild type, indicating that *uvi2* mutation would gain the DNA reparability (Tanaka *et al.*, 1999).

(2) anthocyanin spotted testa (*ast*)

A new phenotype that has the spotted pigmentation on the seed coat has been isolated from 11,960 M₂ plants derived from 1,488 self-pollinated M₁. Accumulation of pigment is observed only in the seed coat. Anthocyanin content was about 6 times higher at 6 days after flowering than that of the wild type seeds (Tanaka *et al.* 1997b).

(3) New *tt* loci

As described above, two new loci of *tt* phenotype (*ttA*, *ttB*) have been found along with the research of mutation frequency experiment (unpublished).

(4) *frill (frl)*

A novel flower mutant, *frl1*, was isolated like *ast*. The *frl1* mutant has serrated petals and sepals but the other floral and vegetative organs appear to be normal. *FRL1* gene should act in petal and sepal development in an organ identity-specific manner (Hase *et al.* in press).

Other plants

(5) Flower color mutants in chrysanthemum

As already described in *mutation spectrum*, complex and strip types of flower color have been obtained in chrysanthemum. A higher mutation frequency of complex flower color mutants were derived from floral petal irradiation than that from leaf irradiation (Nagatomi *et al.*, 1995).

(6) Potato virus Y resistant mutants in tobacco

In order to develop an efficient procedure for obtaining a desired mutation, ion beams were exposed to tobacco anthers, and potato virus Y (PVY) resistant mutants have been selected. High frequency (2.9~3.9%) of resistant mutants was obtained by the irradiation of C and He ions with dose of 5~10 Gy (Hamada *et al.* 1999).

(7) Yellow mosaic virus resistant mutants in barley

Two mutant lines of yellow mosaic virus resistant barley were found in ca. 50,000 M2 families. The resistance in field was not changed over three generations (Kishinami *et al.* 1996)

(8) Blast resistant mutants in rice

High efficiency of getting blast resistant mutants of rice was obtained using ion beams, although resistant mutants were already obtained by gamma rays and thermal neutrons (Nakai *et al.* 1995).

Conclusion

The *uvi1~uvi4* mutants were induced from 1,280 M₁, and the *ast* and *fril* mutants were obtained from the offspring of 1,488 M₁ seeds. As ion beams showed high induction of mutation of the known loci such as *tt*, *gl*, *hy*, it would be true that ion beams can highly induce not only known mutants but also novel mutants. In chrysanthemum, complex and striped flower-color mutants that has never induced by gamma-rays irradiation have been produced, indicating that ion beams could induce a lot of kinds of mutants on the similar phenotypes. In conclusion, the characteristics of ion beams for the mutation induction are 1) to induce mutants with high frequency, 2) to show broad mutation spectrum, and 3) to produce novel mutants. For these reasons, chemical mutagen such as EMS and low LET ionizing radiation such as gamma rays will predominantly induce many but small modifications or DNA damages on the DNA strands, resulting in producing several point-like mutations on the genome. On the contrary, ion beams as a high LET ionizing radiation will cause not so many but large and irreparable DNA damages locally, resulting in producing limited number of null mutation (Fig. 1).

Ion beams that produce novel and null mutation with high frequency will be useful as a mutagen for mutation breeding and developing gene resources in plants.

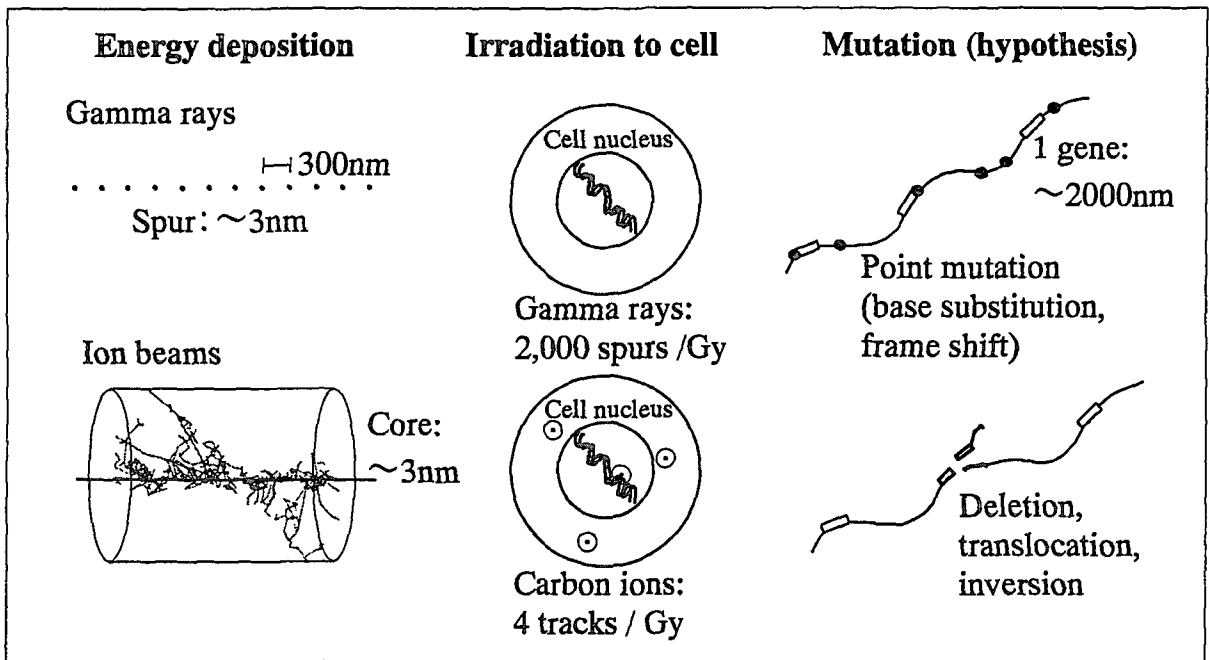


Fig. 1 Characteristic of energy transfer of ion beams for mutation induction

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