In situ visible markers of *Arabidopsis* for radiation biology

Y. Y. Yamamoto, M. Saito,* S. Yoshida, and T. Abe

In this study, two molecular markers were used for *in situ* detection of genetic alterations. The two detection markers were applied to heavy-ion biology for the first time.

The first marker is for the detection of mutation at the mutated generation (M_1). Because of the diploid nature of higher plants, a genetic mutation is not detected as a phenotypic alteration until homozygous mutants are identified at the next generation after mutagenesis ($= M_2$). However, we considered it would be possible to detect mutations at M_1, if we focus on a specific locus.

The *Arabidopsis COP9* gene suppresses anthocyanin biosynthesis, and the loss of *COP9* results in a heavy accumulation of anthocyanins, which are red pigments.[1] A null allele of *COP9*, obtained by the insertion of a kanamycin-resistant gene,[1] enables the preparation of its heterozygous seedlings ($COP9^{+/-}$). Grown on a kanamycin-containing medium, wild-type seedlings have kanamycin-sensitive white cotyledons, and heterozygotes have kanamycin-resistant green cotyledons. The homozygote ($COP9^{-/-}$) plants are also resistant to kanamycin, but have red cotyledons due to the loss of *COP9*. If the intact allele of the heterozygote is damaged by mutagens, the affected cell is expected to show red pigmentation. Because different cells undergo different mutations by mutagenic treatments, a mutagenized M_1 plant is expected to show a chimeric feature.

The seeds of *cop9* heterozygotes obtained by self-pollination, which are a mixture of segregated $COP9^{+/+}$, $COP9^{+/-}$, and $COP9^{-/-}$ seeds, were irradiated with a Ne-ion beam. The treated seeds were grown on a medium containing kanamycin, and heterozygote seedlings were selected and grown in soil. About one month after the transfer, the plants were examined for the presence of red cells. As expected, clear red sectors were observed (Fig. 1). Such sectors were not observed among nonirradiated populations of *cop9* heterozygotes (Table 1) or irradiated wild-type populations (data not shown), strongly suggesting that the sectors are caused by the mutation of *COP9*. Our results demonstrate that mutation rate can be monitored at M_1 generation using this "red sector" assay. In addition, this assay might be useful for the analysis of cell lineage, because one continuous sector is the offspring of a single cell at the time of mutagenic treatments.

We also attempted to examine the *COP1* locus[2] using the same assay, but we could not observe any clear red sectors after Ne-ion beam irradiation (data not shown).

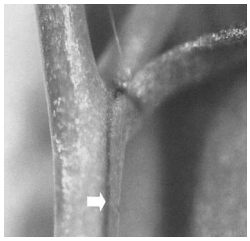

Fig. 1. Red sector on *cop9* heterozygote plant found in population of Ne-ion-beam irradiated plants. The arrow indicates the sector.

Table 1. *cop9* sectors observed on heterozygote plants.

Treatment	Red sectors/examined plants
Control	0/83
Ne-ion beam 100 Gy	6/112

The second marker is for the *in situ* detection of homologous recombination (HR). Swoboda et al. have developed an assay system in which the recombination of neighboring homologous regions results in the recovery of the split *GUS* gene, and the event is detected by GUS staining.[3] Using this assay, their group found that the irradiation with γ ray and UV enhances HR activity.[4,5] Our question is whether HR is also stimulated by irradiation with a heavy-ion beam. Dry seeds carrying the split *GUS* reporter were irradiated with the Ne beam, germinated and grown for two weeks. The seedlings were then subjected to *in situ* GUS staining, and blue areas indicating GUS activity were counted. As shown in Table 2, HR frequency increased threefold following the irradiation. We observed that the blue staining did not form long sectors as shown in Fig. 1, but a spotty staining pattern was observed in tissues that developed after germination, such as the stems and true leaves (data not shown). This observed staining pattern indicates that the stimulation of HR lasts for weeks after the irradiation and the response is systemic.

In summary, our two assays using two visible markers as described above showed reasonable responses to Ne beam irradiations, demonstrating that they are both useful for studies in radiation biology.

Table 2. *In situ* detection of HR by split GUS.

Treatment	Blue spots/examined plants[1]
Control	46/80
Ne-ion beam 150 Gy	130/80

[1]Sum of two independent experiments.

References
1) N. Wei et al.: Cell **78**, 117 (1994).
2) X.-W. Deng et al.: Cell **71**, 791 (1992).
3) P. Swoboda et al.: EMBO J. **13**, 484 (1994).
4) G. Ries et al.: Nature **406**, 98 (2000).
5) O. Kovalchuk et al.: Mutat. Res. **449**, 47 (2000).

* Fukui Agricultural Experiment Station