RIKEN Accel. Prog. Rep. **38** (2005)

Establishment of *Arabidopsis thaliana* lines mutagenized by heavy ion beam irradiation

Y. Y. Yamamoto, H. Saito, H. Ryuto, N. Fukunishi, T. Abe, and S. Yoshida

For plant mutagenesis by heavy ion beam irradiation, irradiated samples (M_1) are usually grown for the next generation (M_2), and phenotypic screening is performed at M_2. The growth step requires a long time (months to years), large space, and considerable labor. Sharing M_2 resources can save such research resources of plant researchers and breeders, thus, it is desirable to establish common M_2 populations, as long as many users are expected. As the first trial, we have decided to prepare an M_2 population of *Arabidopsis thaliana.* There are several reasons for choosing *Arabidopsis.* Firstly, it is one of the most commonly used plant species for plant genetics. Secondly, the growth of *Arabidopsis* does not require a large outdoor space in a field; an *in house* growth facility is sufficient. Thirdly, considering its small genome size, a relatively small population is sufficient for saturated mutagenesis. Fourthly, the most efficient beam conditions for *Arabidopsis* mutagenesis have already been determined.1)

Consulting our previous report,1) we irradiated dry seeds of *Arabidopsis thaliana* ecotype Columbia with a Ne^{10+} beam at a dose of 150 Gy. The irradiated seeds were then grown for self-pollinated seeds. After harvesting, we grew seeds from 9 M_1 plants as one batch. We prepared around 700 M_2 batches, representing 6,000 M_1 plants. Because one M_1 plant contains two germ line cells2), one batch of the prepared M_2 seeds displays 18 independent germ lines. Assuming a heterozygote mutation at M_1, representation of 5 homozygous siblings at M_2 on average requires 360 seedlings per batch.

There are several long hypocotyl mutant loci that have been identified by extensive genetic studies, and among them, *hy1*, *hy2*, and *hy3*3) show significantly long hypocotyls when grown under white light. Examination of the appearance rate of these *hy*-type mutants in the Ne-beam-irradiated M_2 population, we found 4 independent mutant lines out of 200 M_2 batches (Table 1). This rough estimation suggests that 150 batches corresponding to 3,000 germ lines provide one mutant allele for each locus. According to this estimation, the prepared population of 700 batches would provide 4 to 5 alleles per locus. Further analysis would provide more precise information on the quality of our *Arabidopsis* M_2 population.

References

1) H. Saito, T. Matsuyama, Y. Y. Yamamoto, T. Abe, and S. Yoshida: RIKEN Accel. Prog. Rep. **37**, 147 (2004).
2) G. P. Rédei and C. Koncz: in *Methods in Arabidopsis Research*, edited by C. Koncz, N.-H. Chua, and J. Schell (World Scientific, Singapore, 1992), p.16.
3) M. Koornneef, E. Rolff, and C. J. P. Spruitt: Z. Pflanzenphysiol. **100**, 147 (1980).

Table 1. Characteristics of the *Arabidopsis* M_2 population.

Phenotype	No. of screened batches	No. of mutants	Corresponding loci in the *Arabidopsis* genome
hy§1	200	4§2	3§3
albino	200	41	unknown

§1Significantly long hypocotyl phenotype under white light. §2Identity of the genetic loci is not determined. §3*hy1*, *hy2*, and *hy3*.