

Genomic distribution of essential genes influences deletion mutation inheritance in *Arabidopsis thaliana*[†]

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The heavy-ion beam, a type of radiation, has been applied to plant breeding as a powerful mutagen.¹⁾ Linear energy transfer (LET; $\text{keV } \mu\text{m}^{-1}$) explains the effectiveness of heavy-ion irradiation. The LET value should be appropriately selected to efficiently induce deletions with on-demand size.²⁾ Many essential genes in *Arabidopsis* have been reported.^{3,4)} A deletion is not homozygously inherited if a mutant possesses a deletion covering an essential gene that is involved in morphogenesis or gametogenesis. Although the disruption of essential genes should affect the hereditary nature and/or size limitation of the induced deletion, the relationship between essential genes and deletion mutations has not been investigated at the genome level.

Dry *Arabidopsis thaliana* Col-0 seeds were irradiated using heavy-ion beams under three conditions: C-ion beams with LETs of 100 and 200 $\text{keV } \mu\text{m}^{-1}$ as well as an Ar-ion beam with an LET of 290 $\text{keV } \mu\text{m}^{-1}$, to investigate the effect of high-LET beams on inducing deletions. We grew 96 M₂ lines from each irradiation treatment, and we used 96 M₂-lines-derived 192 M₃ lines (two M₃ lines per one M₂ line) for array CGH analysis. Detected deletions were classified into two types by PCR and qPCR: homozygously inherited deletions showing Mendelian inheritance (homozygous deletions) and deletions only heterozygously inherited because of homozygous lethality (heterozygous deletions). A total of 103 deletions were detected in 79 out of 288 M₂ lines. The deletion size did not significantly differ between the irradiation treatments with different LETs ($p > 0.05$; Kruskal-Wallis rank-sum test). This result suggested the presence of an upper limit of the deletion scale; however, heavy-ion beams with higher LET induced larger mutations in prior studies.^{2,5)}

We compared the distances between the closest pair of 811 essential genes as the theoretical maximum sizes of the homozygous deletions and the sizes of the observed homozygous or heterozygous deletions regardless of the LET value (Fig. 1). The mean size of the homozygous deletions was significantly smaller than that of the distances between the closest pairs of essential genes ($p < 0.01$; Wilcoxon rank-sum test). The mean size of the homozygous deletions was influenced

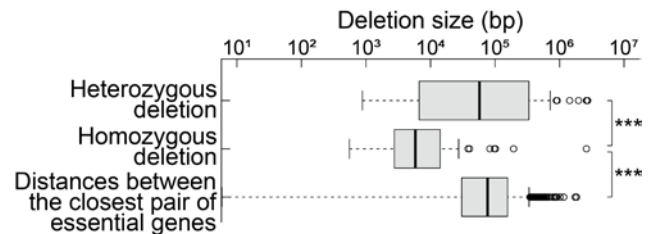


Fig. 1. Box plots of distributions of heterozygous and homozygous deletions and distances between the closest pair of essential genes. ***: $p < 0.01$ in Wilcoxon rank-sum test.

by the essential gene distribution.

We then investigated the influence of the essential genes on the distributions of the homozygous and heterozygous deletions in the mutants. We then collected 94 homozygous and 55 heterozygous deletions (>100 bp) from 94 M₃ lines possessing deletions that we isolated and 22 previously reported mutant lines.^{2,5-7)} Next, we investigated the distribution of 1,740 mutable genes.⁸⁾ A total of 593 and 4,061 genes overlapped in the 94 homozygous and 55 heterozygous deletions, respectively. The mutable genes occupied 10% of the genes in the homozygous deletions (62 out of 593) and 6.6% of the genes in the heterozygous deletions (270 out of 4,061) ($p < 0.01$, chi-square test). We investigated the overlaps of the essential genes with the homozygous or heterozygous deletions. No essential gene (0 out of 593) overlapped homozygous deletions, whereas 2.2% (90 out of 4,061) of the genes in the heterozygous deletions were essential ($p < 0.01$, chi-square test). This finding can be attributed to the nature of the essential genes: deletions including essential genes cannot be homozygously inherited. These results suggest that the distribution of essential genes affects the upper limit of the deletion size.

References

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