

## P2-2 Project "Ion Beam Mutagenesis"

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The ultimate goal of our project is to develop applications of quantum beam-based technologies in applied biological fields such as sustainable agriculture or environmental conservation. Ion beams are recognized as useful mutagens for plant and microbe breeding because they are thought to cause mutations via a mechanism distinct from those of chemical mutagens or gamma rays. We have investigated the detailed characteristics of the ion-beam-induced mutations by using specific gene markers or genome-wide sequencing {2-09, 11, and 13 in Part II}. In addition, under collaborations with academic or industrial research organizations, we are aiming to isolate valuable mutants in various organisms such as parasitic plants, plant growth-promoting rhizobacteria, oil-producing algae, sake yeasts, and other bacteria by ion-beam irradiation {2-10, 14, and 17~21}. Uncovering molecular basis of radioresistant organisms is another major business in our project {2-12, 15, and 16}.

### Genome analysis of rice ion-beam mutants successfully tracked down candidate genes responsible for phenotypes of the mutants [1]

Rice is one of the most important crops for human beings and one of the major target plants for ion-beam mutagenesis. To understand the characteristics of the ion-beam-induced mutations in rice at the genomic level, we conducted whole-exome sequencing, which is a method analyzing only gene coding region, and analyzed induced mutations in selected rice mutants produced by carbon-ion beam irradiation. DNA libraries were constructed from five mutant rice lines (two dwarf and three early-heading-date mutants) by capturing DNA fragments covering exon region predicted in the genome. A total of 56 mutations were detected in the five mutant rice lines. The average mutation frequency in the M1 generation was calculated to be  $2.7 \times 10^{-7}$  per base. The breakdown of the 56 mutations was 24 single-base substitutions (SBSs), 23 deletions (DELs), five insertions (INSs), one inversion (INV), and three replacements. Six mutations were frame-shift or loss-of-exon mutations that are supposed to have high impact on gene function (Table 1). A gene functionally related to the phenotype of the mutant was disrupted by the high-impact mutation in four of the five lines examined, suggesting that whole-exome sequencing of ion-beam-irradiated mutants could facilitate the tracking down of candidate genes responsible for phenotypes of the mutants.

### Characterizing genetic effect of different types of radiations, acute/chronic gamma-rays and carbon ions, in plants [2]

Recent availability of genome sequencing enables us to compare the effect of different types of radiations directly at the genomic level. We conducted whole-genome sequencing, characterized the mutations induced by acute and chronic gamma ray irradiation in *Arabidopsis*, and compared with those previously obtained by carbon ion beam irradiation. Dry seeds were used for irradiation by acute gamma rays and carbon ions, while growing plants of vegetative stage were used for chronic gamma ray irradiation, which is performed for five successive generations. The result of dry seed irradiations showed that

acute gamma irradiation induced a greater number of total mutation events (MEs) than carbon ion irradiation, while no difference was detected between the treatments in the number of MEs altering amino acid (AA) sequences. However, the number of genes with altered AAs in the acute gamma irradiation was smaller than that in the carbon ion irradiation (Fig. 1). This could be due to that the gamma rays predominantly induced SBSs, while carbon ion beam frequently induced DELs ( $\geq 2$  bp in size). Chronic gamma irradiation (100–500 mGy/h) resulted in a higher number of MEs per dose as well as a higher frequency of large deletions than the acute gamma irradiation (Fig. 2). In the very low dose rate of chronic gamma irradiation ( $\sim 1$  mGy/h), the transition/transversion ratio decreased as the dose rate increased, although overall mutation frequency was the same as that in non-irradiated control, suggesting that plants actively respond to the very low dose rates of gamma rays and protect DNA by suppressing occurrence of mutations.

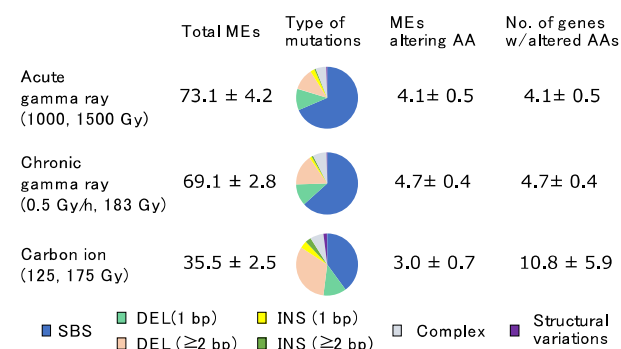
### References

- [1] Y. Oono *et al.*, *Mutat. Res. Fund. Mol. Mech. Mutagen.* **821**, 111691 (2020).
- [2] Y. Hase *et al.*, *Front. Plant Sci.* **11**, 336 (2020).

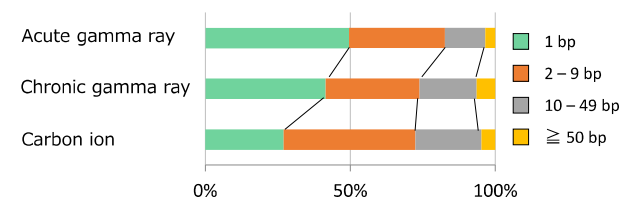
**Table 1**

Phenotype, number and type of high impact mutation, and candidate gene of the five mutant rice lines.

Line	Phenotype	No. of high impact mutation	Type of high impact mutation	Candidate gene for the phenotype
A	Dwarf Small grains,	1	128-bp DEL	<i>GPA1</i>
B	Early heading date Wide leaves Broken flag leaf	3	1-bp DEL × 2 5-bp DEL	<i>PHYB</i>
C	Dwarf Culm bending	0		
D	Early heading date	1	33.6-kb DEL	<i>HD16</i>
E	Early heading date	1	535-kb INV	<i>HD1</i>



**Fig. 1.** Comparison of the MEs. The values (mean ± SD) are number of events or genes per plants.



**Fig. 2.** Comparison of the size of deletions.