P2-1 Ion Beam Mutagenesis Research Project

Leader : Yutaka Oono

The long-term objective of our project is to develop applications of quantum beam technology in applied biological fields such as sustainable agriculture and environmental conservation. Ion beams are recognized as useful mutagens for plant and microbe breeding because they are thought to cause mutations by distinct mechanism from chemical mutagens or gamma rays. To develop more efficient ion-beam mutagenesis techniques, we have tried to understand the characteristics of the ion-beam-induced mutations by using specific gene markers or genome wide sequencing {2-10, 11, 16, and 25 in Part II}. In addition, under collaborations with academic or industrial research organizations, many valuable mutants in plants, algae, fungi, and bacteria have been successfully generated by ion-beam irradiation {2-12~14, 18~22, 24, 27~29}. Revealing molecular biology basis of radio resistant organisms is another major business of our project {2-15, 23, and 30}.

Seeds or seedlings? Which should be irradiated for effective mutagenesis? [1]

The first step of ion-beam breeding toward to creating new plant varieties is mutagenesis, irradiating plant tissues with ion beams. Dry seeds, seedlings, or excised tissues are usually used as initial materials for ion-beam irradiation. Although, in many cases, several types of the plant tissues can be irradiated technically, choosing which type of plant tissues to be irradiated is dependent on living style of the plant and ease in handlings before and after irradiation. It has been concerned that choice of tissue type may affect proportion of induced mutations. However, little is known about effect of tissue type used for irradiation on induced mutations.

To evaluate the effect of tissue type on frequency and types of mutations, Hase et al. irradiated Arabidopsis dry seeds and seedlings by carbon ion beams with doses corresponding to 50% and 75% of the shoulder of the survival curve [125 Gy and 175 Gy for dry seeds (shoulder dose; 241 Gy), and 30 Gy and 20 Gy for seedlings (shoulder dose; 41 Gy), respectively]. M2 (next generation of mutagenized population) seeds were harvested from the M1 plants grown from both the irradiated seeds and seedlings. Then, DNA extracted from randomly chosen individual M2 plants were subjected to a whole genome sequencing to investigate characteristics of the induced mutations. As a result, the total mutation frequency in the plants from dry seeds irradiation was 1.4 - 1.9 times higher than that from seedling irradiation (Fig. 1). This difference mainly depended on the frequency of InDels (insertions and the deletions) in DNA, which was 3 times higher in dry seed irradiation than seedling irradiation. The results clearly suggested that the physiological status of the plant tissue used for irradiation affects the frequency and types of mutations. Because InDels disrupt gene function with high possibility if they arise within DNA region encoding a gene, the result also suggested that using dry seeds as irradiating materials is more preferable to obtain loss of function mutations.

Metabolic changes caused by ion-beam irradiation revealed a carbon flow relevant to a toxic compound in plant cells [2]

Our research has mostly focused on genetic effects of ion beams in plants and microbes. However, as well as other ionizing radiation, ion-beam irradiation directly affects plant growth, development, and also biochemistry in a plant cell. Miyagi et al. evaluated the effects of ion- beam irradiation on oxalate and other primary metabolites in leaves of *Rumex obtusifolius*. *R. obtusifolius* is a weed that contains high amounts of vitamin C and amino acids but also contains high amounts of soluble oxalate, a toxic metabolite, in its leaves. The work was conducted as a part of an effort to create a *R. obtusifolius* variety with low-oxalate contents and to establish it as an edible plant. The results showed that oxalate content in *R. obtusifolius* leaves grown from carbon-ion-beam-irradiated seeds was increased compared with that from un-irradiated seeds. Metabolome analysis of ion-beam effects using Capillary Electrophoresis-Mass Spectrometry (CE-MS) revealed that contents of citrate and ascorbate showed strong positive correlations with the contents of oxalate, whereas negative correlations were observed between the contents of oxalate and amino acids. This observation indicates that ion-beam irradiation caused an increase and a decrease in the carbon flows from glycolysis and to amino acids, respectively, and that oxalate content correlates with extent of the carbon flow from the TCA cycle to the isocitrate pathway. Therefore, manipulation of the carbon flow to the isocitrate pathway would be effective to regulate the oxalate level in plants.

References

[1] Y. Hase *et al.*, Sci. Rep. **8**, 1394 (2018).

[2] A. Miyagi *et al.*, Plant Physiol. Biochem. **122**, 40 (2018).

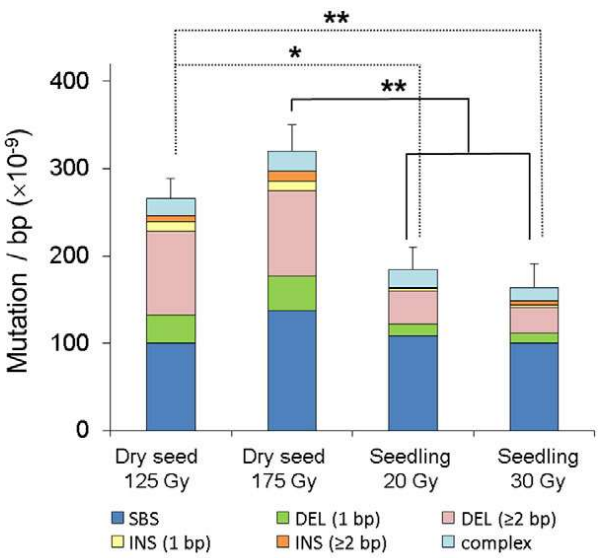

Fig. 1. Frequency and types of mutations induced by carbon-ion irradiation. Error bars represent standard errors for total mutation frequency. Asterisks indicate significant difference in total mutation frequency (t-test, $p < 0.05$ * and $p < 0.01$ **).