# Identification of genetic and phenotypic variations induced by ion-beam irradiation in soybean (*Glycine max*)

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## 1. Introduction

Mutagenesis using soybean (*Glycine max*) as a plant material has obstacles due to the characteristics of its genome; the size is relatively large (approximately 1.1 Gb; Schmutz et al., 2010) and it has duplicative nature that may mask the effects of mutations by the presence of an intact copy of the gene in the genome (Otto, 2007; Mikuriya et al., 2017).

Nevertheless, in the previous study, soybean mutants were successfully obtained by irradiating soybean seeds with ion beam (Arase et al., 2011) which has been reported to be effective in inducing mutants with high frequency and a broad spectrum of mutation phenotype (Tanaka et al., 2010). The current study conducted analyses aiming to identify genetic variations and the extent of phenotypic variation in the ion-beam irradiated soybean plants.

### 2. Methods

For analyzing the extent of phenotypic variation of the mutant plants, mutant plants of different generations were grown in the field for multiple years and were analyzed in terms of chlorophyll content and flowering time. Plants of different generations were also grown concurrently to exclude the effect of differences in environmental condition. For identification of genetic variations, the genomes of eight mutant lines derived from two cultivars were compared with those of their respective wild types using whole genome sequencing (next generation sequencing platform) analysis.

#### 3. Results and Discussion

Field experiments revealed that there were both mutant lines that constantly had altered phenotypes over multiple years and those that did not. Nevertheless, the extent of phenotypic variation between generations was low when plants of different generations were grown concurrently under the same condition. These results indicate the existence of non-conditional and conditional mutant lines in the mutant population.

Whole genome sequencing analysis revealed that there were several mutation hotspots shared between the mutant lines. It also revealed a difference in mutation frequency between mutant lines derived from different cultivars. Small alterations were detected concomitant with large ones and were predominant, which may reflect characteristics of mutations induced by ion-beam irradiation.

### 4. Conclusion

In the current study, several characteristics of mutations induced by ion-beam irradiation in soybean were revealed. The information could be useful for conducting mutagenesis using ion-beam irradiation in soybean and may hopefully contribute to the advancement of mutation breeding technology.