Molecular characterization of mutations induced in *PLASTOCHRON2* by a heavy-ion beam in dry rice seeds

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Rice is one of the most important crop plants and is also an important model plant, because its entire genome sequence is available. In this work, we studied heavy-ion beam induced mutations in rice plants. Heavy-ion beams have been recognized as a useful mutagen for mutation breeding, because they can induce mutations at high rates with relatively low irradiation doses in plants. For inducing mutations in rice, we used imbibed seeds or dry seeds. When imbibed rice seeds were irradiated with ion beams, mutations could be induced with a relatively low dose of radiation. As a result, the time for ion-beam irradiation was shorter. The disadvantage of using imbibed seeds, however, is that these seeds cannot be used for long as seed germination will occur. Therefore, we cannot irradiate imbibed rice seeds during a period that is not suitable for cultivation of rice plants. However, when dry rice seeds were used for ion-beam irradiation, we could store the irradiated seeds for long time periods (at least several months). Therefore, the dry rice seeds could be irradiated even if the machine time fell during a period unsuitable for cultivation. Previously, we have reported several mutations induced by heavy-ion beam irradiation to imbibed rice seeds [1-3]. However, there is no information in the literature regarding the types of mutations inuduced by heavy-ion beam irradiation on dry rice seeds. To reveal the types of mutations induced by heavy-ion beams on dry rice seeds, we irradiated dry rice seeds (*Oryza sativa* L. cv. Nipponbare) with carbon ions ($^{12}C^{6+}$ ions, 25 Gy, LET: 30 keVμm^{-1}) in the RIKEN RI-beam factory. We obtained one plastochron mutant, named 6-279, from the M_2 population. The mutant line 6-279 showed a phenotype with a short plastochron. For example, when a wild-type (WT) plant prouduced its 4th leaf, 6-279 produced its 7th leaf (Fig. 1). It has been reported that the mutants of *PLASTOCHRON1* (*PLA1*) or *PLASTOCHRON2* (*PLA2*) genes show similar short plastochron phenotypes [4, 5]. A sequence analysis of both *PLA1* and *PLA2* genes revealed that 6-279 showed a 2 bp deletion accompanied by a 1-bp filler DNA in the first exon of *PLA2* gene (Fig. 2). These mutaions caused a frameshft in *PLA2* gene, resulting a premature stop codon. There was no mutation in the *PLA1* gene of this mutant. These results suggest that 6-279 is a mutant of *PLA2* gene.

In the present study, we identified one mutation induced by heavy-ion beam irradiation to rice dry seeds. To characterize the types of mutations induced by heavy-ion beams on dry rice seeds, it is important to analyze a number of rice mutants, which is currently in progress.

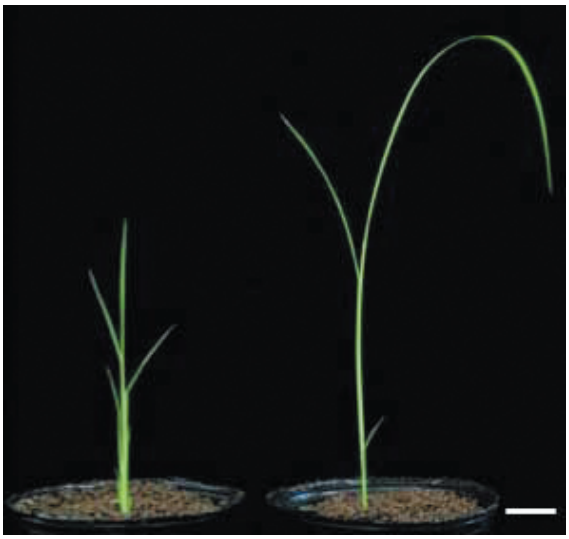

Fig. 1 Phenotipic characterization of 6-279 mutant. 6-279 (left) produced its 7th leaf whereas WT plant (right) produced its 4th leaf. Scale bar = 1 cm.

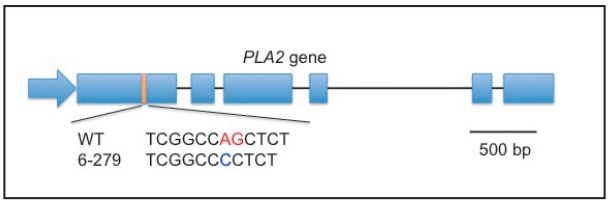

Fig. 2 Schematic representation of *PLA2* gene. *PLA2* gene consists of 6 exons. A 2 bp deletion with a filler DNA was detected in the first exon of the *PLA2* gene. The deleted 2 bp in 6-279 is shown in red, and the filler DNA is indicated in blue. The boxes and lines indicate exons and introns of *PLA2* gene, respectively. The Arrow indicates the direction of the *PLA2* gene.

References

1) R. Morita et al.: RIKEN Accel. Prog. Rep. **45**, 212 (2012).
2) S. Kogure et al.: RIKEN Accel. Prog. Rep. **45**, 213 (2012).
3) S. Kogure et al.: RIKEN Accel. Prog. Rep. **46**, 260 (2013).
4) J. Itoh et al.: Plant Cell **10**, 1511 (1998).
5) T. Kawakatsu et al.: Plant Cell **18**, 612 (2006).

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