

Identification of mutated sites induced by Ar-ion-beam irradiation in rice

S. Kogure,^{*1} R. Morita,^{*1} Y. Hayashi,^{*1} K. Ichinose,^{*1} M. Yamada,^{*1} T. Wakana,^{*1}
H. Tokairin,^{*1} K. Ishii,^{*1} T. Sato,^{*2,*3} and T. Abe^{*1,*2}

Our team has studied the mutation induction in rice as an effect of heavy-ion-beam irradiation. Rice is a model plant of monocots, and it is useful for identifying mutation sites because its entire genome sequences are available. In previous studies, we showed that C-ion beams (15 Gy, LET 50 keV/μm) and Ne-ion beams (15 Gy, LET 63 keV/μm) cause small size deletion (6 mutant lines include 2 to 12 bp, 1 mutant line include 72348 bp) in rice genome.⁽¹⁻⁴⁾ In this study, we report the screening and identification of mutated genes and sites induced by Ar-ion beams.

Last year, we grew 1370 lines of M₂ generation obtained by irradiation of imbibed rice seeds with Ar-ion beams (2.5 or 5 Gy, LET 290 keV/μm) in both a greenhouse and a field. Over 100 mutant lines were isolated by screening, and some were suitable for PCR and sequence analysis.

Two mutant lines were selected in a greenhouse 2–3 weeks after germination and identified as mutated sites. Ar5-587 showed the phenotype of plastochron (PLA) mutants, which cause the rapid initiation of vegetative leaves without affecting phyllotaxy⁽⁵⁾ (Fig. 1a). A sequence analysis revealed that it contained 176-bp deletion and 7-bp insertion in the 1st exon of *PLAI*. Ar5-672 showed the phenotype of rice gibberellin (GA)-related mutants, which cause severe dwarfness with wide leaf blades and dark

green leaves⁽⁶⁾ (Fig. 1b). It contained 2,627-bp deletion in the GA positive regulator, *GIBBERELLIN-INSENSITIVE DWARF 2 (GID2)*.⁽⁶⁾

Three mutant lines were selected in a field, and identified as mutated sites. Ar5-62 exhibited heading 20 days earlier than wild type (Nipponbare). It contained 65,534-bp deletion and 2-bp (TG) insertion in chromosome 7, and lacked whole *GRAIN NUMBER, PLANT HEIGHT AND HEADING DATE 7 (Ghd7)*,⁽⁷⁾ which is an important regulator of heading date and yield potential in rice. Ar5-154 exhibited tall phenotype at the heading stage. It contained 47,930-bp deletion in chromosome 5, and lacked whole *ELONGATED UPPERMOST INTERNODE 1 (EUI1)*.⁽⁸⁾ Ar5-90 showed short grains (Fig. 1c) and semi-dwarfness. TAIL-PCR and several sequence analyses revealed that it contained 22,148-bp deletion in chromosome 1, and lacked whole *DAIKOKU DWARF1 (DI)*.⁽⁹⁾

The data from these five mutant lines show that Ar-ion beams (5 Gy, LET 290 keV/μm) cause large deletions (>100 bp) in the rice genome.

It is necessary to identify more mutated regions of rice mutants for characterizing the mutations induced by heavy-ion-beam irradiation. We have isolated various rice mutants, and the research is in progress.

Table 1. Isolated mutants by Ar-ion-beam irradiation

Line	Phenotype	Gene	Mutation size
Ar5-62	Early heading	<i>Ghd7</i> (Os07g0261200)	65534-bp + 2-bp in
Ar5-90	Short grain	<i>DAIKOKUDWARF1</i> (Os05g0333200)	22148-bp del
Ar5-154	Elongation at heading stage	<i>EUI1</i> (Os05g0482400)	47930-bp del
Ar5-587	Plastochron	<i>PLAI</i> (Os10g0403000)	176-bp del + 7-bp in
Ar5-672	Severe dwarf	<i>GID2</i> (Os02g0580300)	2627-bp del

del: deletion, in: insertion

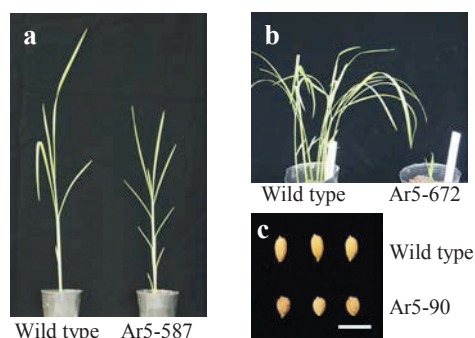


Fig. 1. Photograph of 4-weeks-old seedlings of Ar5-587 (a), 2-weeks-old seedlings of Ar5-672 (b), and seeds of Ar5-90 (c). Bar = 1 cm.

References

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*¹ RIKEN Nishina Center

*² RIKEN Innovation Center

*³ Graduate School of Life Sciences, Tohoku University