Detection of structural variations in three responsible genes induced by relatively high-LET ion beams in rice

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The molecular characteristics of mutations induced by a heavy-ion beam with a relatively high LET in rice, a model plant of monocotyledonous, has not yet been revealed. In this study, we irradiated dry seeds of rice (*Oryza sativa* L. 'Nipponbare') with Fe- or Ar-ion beams, isolated 3 mutants for which the responsible gene has been clarified, and characterized the structure of the mutated gene using polymerase chain reaction (PCR) analysis.

A *pla1* mutant (Ar7-165, Fig. 1) was isolated from 239 M_1 lines irradiated with Ar ions (7.5 Gy, LET: 289 keV/μm). In this mutant, no PCR amplification occurred when primers designed for amplification of the central area of the *PLA1* gene (primer pairs F4 and R20, F6 and R21, and uF5 and R21, as shown in Fig. 2) were used, suggesting that structural variations (SVs) such as inversion or translocation occurred in the gene (Fig. 2).

A dwarf mutant (Fe15-235, Fig. 1) that showed a defect in gibberellin biosynthesis was isolated from 236 M_1 lines irradiated with Fe ions (15 Gy, LET: 650 keV/μm). We conducted PCR analysis with the primers for gibberellin biosynthesis-related genes and detected a deletion in the region including the 1st to 6th exon of the *OsKS1* gene (Fig. 2). Additional PCR and sequencing analyses revealed that Fe15-235 harbors a 9,843 bp deletion (Chr04: 31018475 - 31028317) in the *OsKS1* gene.

We isolated the *brd1* mutant (WAr30-204), which showed a dwarf phenotype with a defect in brassinosteroid biosynthesis from 242 M_1 lines irradiated with Ar ions generated by the WACAME line[1)] (30 Gy, LET:184 keV/μm). PCR analysis revealed that this mutant harbors a 27 kb deletion involving the entire *OsBRD1* gene (Fig. 2). Another deletion of approximately 5 kb was also detected 18 kb downstream from the 27-kb deletion. It suggested that a complex mutation with large deletions occurred in the *OsBRD1* gene region. Our findings suggest that a heavy-ion beam with a relatively high LET may predominantly induce large deletions or SVs in the responsible gene region in rice. This tendency was also observed in the PCR and sequence analysis for Arabidopsis mutants induced by Ar-ion irradiation on dry seeds[2)] and rice mutants induced by Ar-ion irradiation on imbibed seeds.[3)]

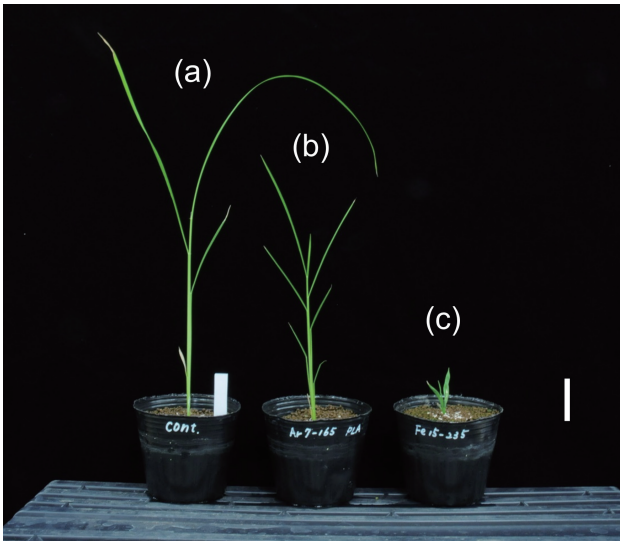

Fig. 1. Morphological comparison of (a) Nipponbare, (b) Ar7-165, and (c) Fe15-235 plants 24 days after sowing. Scale bar = 5 cm.

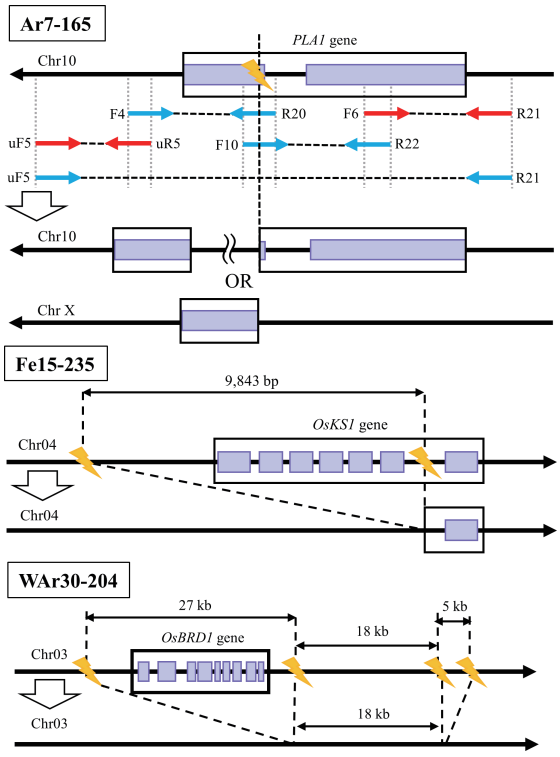

Fig. 2. Schematic representation of mutated genes. Purple boxes indicate protein coding regions (exons), and yellow marks indicate the position of the DNA double-strand breaks induced by heavy-ion beams. In the case of the Ar7-165 mutant, the position of the primers used in PCR are shown. Red arrows indicate primers for which DNA amplification occurs in PCR, whereas blue arrows indicate primers for which DNA amplification did not occur in PCR.

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References

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