Trials of mutation detection programs to detect structural variations induced by heavy-ion beams in rice

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We screened rice mutants generated by heavy-ion-beam irradiation and identified mutations using whole-genome sequencing (WGS). A heavy-ion beam induces both small mutations (single-nucleotide variants and small insertions/deletions) and structural variations (SVs) such as large deletions, inversions, and translocations.[1] The detection of SVs using short-read WGS data is a challenging task compared with the detection of small mutations.[2] We used the Pindel[3] software to detect SVs. Pindel can detect break points of large deletions (< 10 kb) and small-size insertions (1–20 bp).[3] However, the heavy-ion beam can generate deletions with lengths of over 10 kb.[1] Therefore, besides Pindel, another program is needed to detect the SVs induced by the heavy-ion-beam irradiation.

In the present study, we tested two programs, Delly[4] and Manta,[5] for detecting the SVs induced by heavy-ion irradiation. We used a high-performance bioinformatics pipeline[6] incorporated with Delly and Manta. The candidate mutations were visually confirmed by using Integrated Genomics Viewer (IGV). We analyzed WGS data of 11 rice mutants induced by carbon ion irradiations ($^{12}C^{6+}$, 50–175 Gy, LET: 30 keVμm^{-1}). In the present study, we ignored the candidates of heterozygous mutations detected by Delly and Manta because all heterozygous mutations were false-positives in our initial experiment (Data not shown).

In the 11 mutants, there were 10 and 19 homozygous candidates detected by Delly and Manta, respectively. Of the 10 and 19 candidates detected by Delly and Manta, 8 and 9 were positive mutations, respectively. These data suggest that both programs detected positive mutations induced by carbon ions. Of the 8 and 9 positive mutations detected by Delly and Manta, 4 and 2 were also detected by Pindel. In other words, both 4 mutations detected by Delly and 7 mutations detected by Manta were not detected by Pindel. In total, 8 independent mutations that were not detected by Pindel were identified (Table 1), *i.e.* one mutation (No. 1) was detected by Delly only, 4 mutations (No. 2, 5, 7, and 8) were detected by Manta only, and 3 mutations (No. 3, 4, and 6) were detected by both programs. Using IGV, we determined the types of each mutation. One mutation (No. 1) was determined to be a deletion (Table 1). The remaining mutations (No. 2, 3, 4, 5, 6, 7, and 8) were estimated to be translocations (Table 1). We tried to design primer pairs around the breakpoints of each mutation to confirm the existence of mutation by PCR analysis. Around the mutation

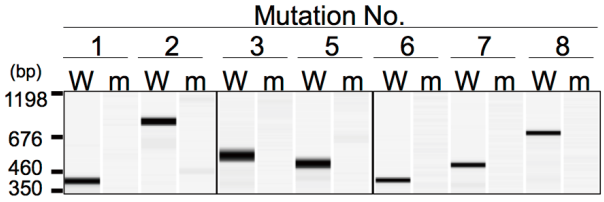

Fig. 1. PCR confirmation of 7 mutations. W indicates the result of using wild-type DNA as the template, and m indicates the result of using mutant DNA as the template. The PCR pruducts are analyzed using MultiNA MCE-202 (Shimadzu). DNA size markers are shown on the left side.

Table 1. Structural variations detected by Delly and Manta.

No.	Program	Types of mutation	Position of structural variation breakpoints
1	Delly	deletion	chr06: 19711627 ~ chr06: 20041214
2	Manta	translocation	chr10: 4549172 ~ ND
3	Both	translocation	chr01: 4662084 ~ chr08: 25179978
4	Both	translocation	chr08: 25179965 ~ chr01: 5523529
5	Manta	translocation	chr01: 5523529 ~ chr08: 25179965
6	Both	translocation	chr03: 964315 ~ chr02: 627397
7	Manta	translocation	chr02: 627397 ~ chr03: 964315
8	Manta	translocation	chr03: 5844008 ~ ND

ND, not determined by

No. 4, a primer pair specific to the wild-type allele could not be designed. When we performed PCR with the 7 primer pairs using wild-type genomic DNA as a template, each band was detected (Fig. 1, lane W). However, no band was

detected when we used mutant genomic DNA as a template (Fig. 1, lane m), indicating that the wild-type alleles do not exist in these mutants. Our present study indicated that both Delly and Manta are useful programs to detect SVs in rice mutants induced by heavy-ion irradiation.

References

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