*mPing*SCAR marker, a powerful tool for genetic analysis of agricultural traits in rice mutants induced using ion-beam irradiation

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Rice is a major cereal crop that is the dietary staple for more than half of the world's population. For sustainable production and increased yield, it is important to perform molecular regulation of various agricultural traits. Mutagenesis study is an effective approach to identify novel genes that impart desired agricultural traits and to investigate their functions. Many of the agricultural traits, however, are quantitative traits and controlled by complex multiple genetic networks. Therefore, it is difficult to identify the mutant gene(s) when the F_2 population derived from the crosses between distantly related varieties is used to develop several available DNA markers. On the other hand, it is difficult to develop available DNA markers for genetic linkage analysis when using the F_2 population derived from the crosses between closely related varieties, to make it easy to identify the mutant gene(s) without multiple genetic segregations.

mPing is reported as the first active miniature inverted-repeat transposable element as well as the first active DNA transposon in rice[1]. Our previous study revealed that the *japonica* rice variety Gimbozu harbored over 1000 copies of *mPing*, whereas most of the closely related *japonica* varieties harbored less than 50 copies[2]. Therefore, polymorphic insertions of *mPing* are available for genetic analysis by using the F_2 population crossed between the closely related *japonica* varieties and Gimbozu[3]. Here, we evaluate the availability *mPing*SCAR (sequence characterized amplified region) marker based on the polymorphic insertions of *mPing* in Gimbozu and mutants derived from closely related varieties.

The imbibition seeds of *japonica* rice variety (cv. Nipponbare) were exposed to C, Ar, and Ne ions accelerated to 135, 95, and 135 MeV/nucleon, respectively. M_1 plants were grown in a paddy field, and M_2 seeds were harvested separately from each M_1 plant. In our paddy field research of the M_2 lines, we isolated a total of 11 mutants in which we observed mutations in the agricultural traits (Table 1). These mutants were crossed with Gimbozu and then the F_2 populations were applied to the genetic analysis for mapping the candidate region of the mutant genes by using the *mPing*SCAR markers.

All the F_2 populations, comprising 24–96 plants, showed bimodal distribution within the parental ranges. The mutant type to wild type ratio fit the 1:3 ratio expected for one-locus segregation. These results indicate that each mutant phenotype is conferred by a single recessive mutant gene. The linkage analyses by using 50 selective *mPing*SCAR markers, which are evenly distributed in all chromosome, identified markers that are closely linked with the mutant phenotypes. Further analyses by using additional *mPing*SCAR markers around the closely linked marker showed that the mutant genes were located in the region at physical distances of 1.13–13.82 Mb on chromosome 1, 3, 4, 5, 8 and 9 (Table 1). The rice annotation project database (RAP-DB: http://rapdb.dna.affrc.go.jp) showed that 195–973 genes were located in each region. Further, we extracted and isolated the DNA segments, including the exon regions of over 30,000 genes from the five mutant lines, and performed exome analysis by using the next-generation sequencing analyzer, Hiseq2000 (Illumina, San Diego, CA, USA). The experimental results showed that a single genomic mutation responsible for the mutant phenotype was identified in the candidate gene (or region) of the four mutant lines.

Although the current progress of next-generation sequencing techniques is remarkable, obtaining the sequence information of the whole genome alone is not enough to identify the candidate gene responsible for the mutant phenotype. Delimiting the candidate genes by using *mPing*SCAR markers in combination with the sequencing techniques and well developed database information would ensure further efficiency in detecting the mutant gene. Thus, we are confident that *mPing*SCAR marker is a powerful tool for the genetic analysis of the agricultural quantitative traits.

Table 1 The mapping summary of mutants in this study.

Number of analyzed mutants	11
Chromosomes for locating the mutant genes	1, 3, 4, 5, 8, 9
The size range of the candidate region	1.13–13.82 Mb
Number of genes located in the candidate region	195–973

References
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