

# Determination of the mutation which affect plant height and seed length of rice induced by Ar-ion irradiation

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Rice serves as an important staple food for more than half of the world’s population. The plant height of rice is one of the most important agronomic traits as it affects grain yield. In this paper, we report the genetic analysis of a rice semi-dwarf mutant line called Ar-82. This mutant was isolated from the M<sub>2</sub> population (160 M<sub>2</sub> lines, each line contained ten individuals) created by Ar-ion irradiation (10 Gy, LET: 290 keV/μm) to dry seeds of rice cultivar Nipponbare. The plant height of Ar-82 decreased compared to that of Nipponbare (Fig. 1). The size of both seeds and husked grains also decreased in this mutant line (Fig. 2). The objective of this work was to identify the responsible gene associated with the semi-dwarf phenotype in Ar-82.

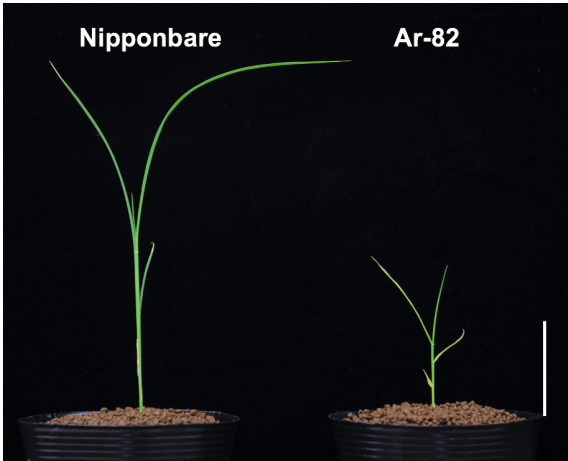


Fig. 1. Plant morphology 17 days after sowing. Scale bar = 5 cm.



Fig. 2. Seeds and husked grains of Nipponbare (a and c) and Ar-82 mutant (b and d). Scale bar = 3 mm.

In order to identify the gene for the semi-dwarf phe-

notype, we performed MutMap analysis.<sup>1)</sup> Briefly, we grew F<sub>2</sub> plants obtained from the cross-pollination between Ar-82 and Nipponbare in a paddy field. We selected 10 F<sub>2</sub> plants that exhibited semi-dwarf phenotype, extracted genomic DNA from each F<sub>2</sub> plant, then mixed equal amounts of each DNA and subjected it for whole genome resequencing analysis. We found several hundred mutations as candidates using the in-house bioinformatic pipeline for mutation detection.<sup>2)</sup> Among these, only four mutations (one single nucleotide variant (SNV), 2 insertions, and 1 large deletion) in chromosome 6 were detected as homozygous mutations. Using the Rice annotation project database (<https://rapdb.dna.affrc.go.jp>) revealed that both 1 SNV and two insertions occurred in the intergenic region, whereas the remaining large deletion (149-kb) disrupted 14 genes (Table 1). These results indicate that the presence of the gene for the semi-dwarf phenotype with 14 genes disrupted by the large deletion. We found that there was a gene named *Short Grain6* (*SG6*), previously reported as a gene responsible for the short grain phenotype,<sup>3)</sup> existed in the deleted region (Table 1). The authors also showed that the *sg6* mutant reduced the plant height.<sup>3)</sup> Based on the observed similarities in the phenotype of the *sg6* mutant and Ar-82, we have concluded that the *SG6* gene was the gene responsible for both the semi-dwarf and short-grain phenotype of Ar-82.

Table 1. Fourteen genes disrupted by the 149-kb deletion.

No.	Locus ID	Description or Gene Name
1	Os06g0665800	Similar to heavy metal ATPase
2	Os06g0665900	Similar to thioredoxin domain-containing protein 2
3	Os06g0666100	Short Grain6
4	Os06g0666183	Non-protein coding transcript
5	Os06g0666400	Valine-glutamine (VQ) motif-containing protein
6	Os06g0666500	Zinc finger, RING/FYVE/PHD-type domain containing protein
7	Os06g0666600	Similar to Glyceraldehyde-3-phosphate dehydrogenase
8	Os06g0666650	Non-protein coding transcript
9	Os06g0666750	Hypothetical protein
10	Os06g0666800	Conserved hypothetical protein
11	Os06g0667000	Serine/threonine protein kinase domain containing protein
12	Os06g0667100	Nuclear factor Y (NF-Y) transcription factor C4
13	Os06g0667200	MADS-box transcription factor, Vegetative development
14	Os06g0667300	Non-protein coding transcript

References  
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2) H. Ichida *et al.*, Plant J. **98**, 301 (2019).  
3) S. R. Zhou and H. W. Xue, J. Integr. Plant Biol. **62**, 847 (2019).

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