Genetic analysis of an early flowering rice mutant induced by argon ions

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Heading date, also known as flowering time, is one of the important traits for a crop such as rice to achieve a high yield by making effective use of sunlight and temperature.[1)] Although numerous genes influencing the heading date have been identified and characterized in rice, it is necessary to identify novel genes that determine the heading date to understand the mechanism to control this trait. To identify a novel gene influencing the heading date, we isolated a rice mutant (Ar-G150) that exhibited an early flowering phenotype from a rice (*Oryza sativa* L. cv. Nipponbare) M_2 population derived from imbibed seeds irradiated with argon ions (2.5 Gy, 95 MeV/n, LET: 286 keVμm^{-1}). To determine the mode of inheritance of the early flowering trait, we produced F_2 progenies derived from a cross between Ar-G150 and their original variety, Nipponbare. We grew Nipponbare, Ar-G150, and the F_2 progenies in a greenhouse under natural photoperiod conditions from June 17, 2016, and recorded the heading date of each plant. The heading date of Nipponbare were from September (Sept.) 2nd to Sept. 6th. The heading date of Ar-G150 were from August (Aug.) 23rd to Aug. 26th, approximately 10 days earlier than Nipponbare. Segregation for the early-flowering phenotype was observed in F_2 progenies, *i.e.*, seven F_2 plants showed normal flowering (the heading dates were from Sept. 2nd to Sept. 5th), and 23 F_2 plants showed early flowering (the heading dates were from Aug. 23rd to Aug. 27th). The segregation of normal- and early-flowering phenotypes in the F_2 progenies (7:23) showed a good fit to a 1:3 ratio (the two-tailed p-value from Fisher's exact test was 1.00), indicating that Ar-G150 possessed a single dominant mutated gene with an early-flowering trait. To characterize the mutated phenotype in detail or determine the causative gene of early flowering, the homozygous line for the mutated gene is needed. Thus, we cultivated 16 F_3 lines (approximately 20 plants per line) derived from the self-pollination of early-flowering F_2 plants to select the homozygous line using a paddy field in 2019. In 5 out of the 16 F_3 lines, all F_3 plants exhibited early flowering, indicating that the mutated gene in their parental plant was homozygous. In the remaining 11 lines, segregation for the early- and normal-flowering phenotype was observed in F3 plants, indicating that the mutated gene in the parental plant was heterozygous. The number of homozygous F_2 and heterozygous F_2 (5:11) showed a good fit to a 1:2 ratio (the two-tailed p-value from Fisher's exact test was 1.00).

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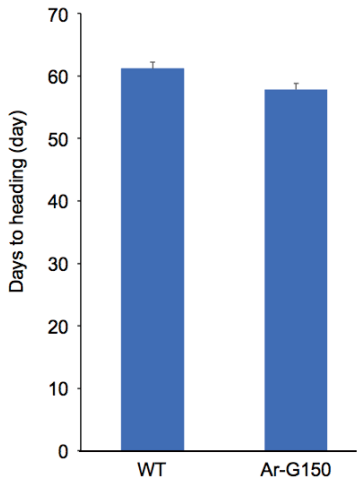

Fig. 1. Days to heading of WT and Ar-G150 grown under a short-day condition. Values are means ± standard error ($n = 5$). Student's t-test was used to compare the two groups.

These results indicated that the early-flowering character was determined by a single gene.

Using the homozygous F3 plants, we started to characterize the early-flowering phenotype of Ar-G150. First, we determined the days to heading under a controlled photoperiod condition using a growth chamber to reveal whether the mutated gene affects the photoperiod response. We grew Nipponbare and Ar-G150 plants under a short-day condition (10 h light/14 h dark), and measured the days to heading. Since rice is a short-day plant and the critical photoperiod of Nipponbare is estimated to be 15.6 h,[2)] this condition prompts rice plants to develop flowers. Under this condition, the days to heading of Ar-G150 (57.8 ± 1.0 d, average ± standard error) was slightly less than the days to heading of Nipponbare (61.2 ± 1.2 d), although no significant differences were observed between the days to heading of both lines ($p > 0.05$), suggesting that the mutated gene have little or no effect of promoting flowering under the short-day conditions (Fig. 1). We also started to perform the whole genome re-sequencing analysis of homozygous F_3 mutants to reveal the causative gene. The information of the causative gene will help us understand the mechanism to control the heading date of rice in more detail.

References

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