Extra-early-flowering wheat mutants produced by heavy-ion-beam mutagenesis†

A. Nishiura,[*1] Y. Kazama,[*2] T. Abe,[*2] N. Mizuno,[*3] S. Nasuda,[*3] and K. Murai[*1]

To avoid harvesting in the rainy season, early flowering or early heading is one of the most important characteristics for bread wheat (*Triticum aestivum*) in East Asia, including Japan. A detailed understanding of the flowering mechanism in wheat plants is of value not only for wheat breeding but also for basic scientific research. From the large scale mutant panel of diploid einkorn wheat (*Triticum monococcum*) strain KU104-1 developed by heavy-ion-beam irradiation[1], we identified four extra early-flowering mutants, named *extra-early-flowering1* (*exe1*), *exe2*, *exe3*, and *exe4*. The four *exe* mutants fell into two groups, namely Type I (moderately extra-early-flowering type; *exe1* and *exe3*) and Type II (extremely extra-early-flowering type; *exe2* and *exe4*), based on a field experiment.

In a growth chamber, leaf-emergence timing in wild-type (WT) and *exe* mutant plants was examined under long-day (LD) or short-day (SD) conditions. Under LD conditions, WT plants averaged about 180 days from sowing to flag-leaf unfolding. In contrast, Type I *exe* mutants took about 65 days and Type II about 50 days. WT plants transited from the vegetative to reproductive growth phase at the 9-leaf stage, while *exe* mutants showed earlier phase transition: at the 5-leaf stage for *exe1* and *exe3* (Type I) and the 4-leaf stage for *exe2* and *exe4* (Type II). In WT plants, the flag leaf occurred at the 18-leaf stage, while in *exe1*, *exe2*, *exe3*, and *exe4*, the flag leaf appeared at the 9-leaf, 8-leaf, 9-leaf, and 7-leaf stages, respectively. Under SD conditions, WT plants transited from the vegetative to reproductive growth phase at the 13-leaf stage. In contrast, *exe1* and *exe3* mutants (Type I) transited at the 8-leaf stage, while *exe2* and *exe4* mutants (Type II) transited at the 4-leaf stage. Interestingly, the phase transition was early (at the 4-leaf stage) in Type II *exe* mutants under both SD and LD conditions. Comparing Type I *exe* mutants between SD and LD conditions indicates that these mutants retained photoperiodic sensitivity. However, Type II *exe* mutants had lost almost all photoperiodic sensitivity.

exe

Fig. 1. *Extra-early-flowering* (*exe*) mutant plants grown in the field.

In wheat, it was found that three genes mainly control the flowering, namely *VERNALIZATION 1* (*VRN1*), *VRN2* and *VRN3*. The analysis of *VRN1*, a flowering-promoter gene, showed that it was more highly expressed in seedlings at the early developmental stages in Type II mutants than in Type I mutants. The up-regulation of *VRN1* expression in *exe* mutants was associated with earliness in flowering under LD conditions. The extremely extra-early flowering in Type II mutants was associated with a more rapid up-regulation of *VRN1* expression than in Type I mutants. It is notable that a similar level of *VRN1* expression was observed at the phase transition in each *exe* mutant: at the 5-leaf stage for Type I mutants and at the 4-leaf stage for Type II mutants. This finding suggests that the level of *VRN1* expression is correlated with the phase transition from vegetative to reproductive growth and may act as a threshold for flowering competency in wheat plants.

The original KU104-1 is an einkorn wheat strain that carries a null allele of the *VRN2* gene, a repressor of flowering. Thus, our results indicate that the level of *VRN1* expression controls earliness in flowering in *exe* mutants independently of *VRN2*. In bread wheat, it has been known that winter cultivar quantitatively requires prolonged cold temperature to yield vernalization saturation. Some winter cultivars require 2-4 weeks of low temperature to reach the maximum vernalization effect on heading, and others require more than 4 weeks. Positional cloning of the gene was performed for the duration required for vernalization, and the results demonstrated that this trait is controlled by the recessive *VRN1* gene on the A genome at the protein level[2]. A previous study also revealed that the binding ability of the VRN1 protein to TaHOX1 was associated with the requirement of low-temperature duration to reach vernalization saturation. These findings, together with the present *exe* study, support our model of gene network for flowering: *VRN1* plays a central role in the flowering pathway[3].

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*1 Department of Bioscience, Fukui Prefectural University
*2 RIKEN Nishina Center
*3 Graduate School of Agriculture, Kyoto University

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