Chapter 19
Extra Early-Flowering (*exe*) Mutants in Einkorn Wheat Generated by Heavy-Ion Beam Irradiation

Aiko Nishiura, Yusuke Kazama, Tomoko Abe, Nobuyuki Mizuno, Shuhei Nasuda, and Koji Murai

Abstract Four extra early-heading mutants, named *extra early-flowering1* (*exe1*), *exe2*, *exe3*, and *exe4*, were identified in diploid einkorn wheat (*Triticum monococcum* L.) following heavy-ion beam mutagenesis. Based on their phenotypes in the field, the four *exe* mutants were classified into two groups: Type I (moderately extra early-heading type; *exe1* and *exe3*) and Type II (extremely extra early-heading type; *exe2* and *exe4*). Analysis of *VERNALIZATION 1* (*VRN1*), a flowering promoter gene, showed that it was more highly expressed at earlier stages of vegetative growth in Type II mutants than in Type I mutants. Our analyses indicate that the difference in earliness between Type I and Type II mutants is associated with differences in the expression level of *VRN1*.

Introduction

Improving our understanding of the molecular mechanisms of flowering, the phase transition from vegetative to reproductive growth associated with heading time, is one of the most important goals for wheat breeding at the present time. In bread wheat (*Triticum aestivum* L.), heading time is genetically determined by three characteristics: vernalization requirement; photoperiod sensitivity; and narrow-sense earliness (earliness *per se*). Three genes have been identified to determine the requirement of vernalization, namely *VERNALIZATION 1* (*VRN1*), *VRN2* and *VRN3*.

A. Nishiura • K. Murai (✉)
Department of Bioscience, Fukui Prefectural University, Eiheiji-cho, Fukui 910-1195, Japan
e-mail: murai@fpu.ac.jp

Y. Kazama • T. Abe
RIKEN, Nishina Center, Wako, Saitama 351-0198, Japan

N. Mizuno • S. Nasuda
Laboratory of Plant Genetics, Graduate School of Agriculture,
Kyoto University, Kyoto, Japan

Y. Ogihara et al. (eds.), *Advances in Wheat Genetics: From Genome to Field*, DOI 10.1007/978-4-431-55675-6_19

VRN1 encodes an APETALA1/FRUITFULL-like MADS-box transcription factor that is up-regulated by vernalization (Yan et al. 2003; Murai et al. 2003; Trevaskis et al. 2003; Danyluk et al. 2003). Recent studies revealed that expression of *VRN1* is epigenetically suppressed in seedlings at an earlier stage of the vegetative growth phase, and that the repressive histone state is modified by the vernalization signal, leading to the up-regulation of *VRN1* (Oliver et al. 2009; Diallo et al. 2012). The level of *VRN1* transcription gradually increases during the seedling growth stage without the need for further vernalization (Murai et al. 2003; Kitagawa et al. 2012), suggesting that the epigenetic status of *VRN1* is also modified by aging. Furthermore, *VRN1* shows a diurnal expression pattern that is affected by daylight, with a long photoperiod producing up-regulation of its expression level (Shimada et al. 2009). In summary, these observations indicate that the *VRN1* expression is also controlled by autonomous and photoperiodic pathways, as well as the vernalization pathway.

The *VRN2* locus consists of two linked *ZCCT* genes, *ZCCT1* and *ZCCT2*, which encode a protein with a zinc finger motif and a CCT domain (Yan et al. 2004). Natural variations have been identified in the *VRN2* locus. Simultaneous deletion or non-functional mutations of these two *ZCCT* genes result in a plant showing the spring habit (Distelfeld et al. 2009), indicating that *VRN2* is a flowering repressor gene. A high level of *VRN2* expression is observed in seedlings at the 1-leaf stage, while expression is down-regulated by vernalization and aging; by contrast, *VRN1* shows the opposite pattern with low expression in seedlings and up-regulated expression after vernalization (Shimada et al. 2009). It has also been reported that *VRN2* shows a diurnal expression pattern and that a long photoperiod up-regulates its expression level (Dubcovsky et al. 2006; Trevaskis et al. 2006), suggesting that the *VRN2* expression is affected by photoperiod as well as vernalization.

VRN3 encodes a Raf kinase inhibitor-like protein with a high similarity to the Arabidopsis FLOWERING LOCUS T (FT) protein, which is a florigen (Yan et al. 2006). Transgenic wheat plants overexpressing *VRN3* show an extra early-flowering phenotype without the need for vernalization (Yan et al. 2006; Shimada et al. 2009), indicating that *VRN3* is a strong flowering promoter. Under long day conditions, *VRN3* shows a diurnal expression pattern; however, expression is very low under short day conditions (Shimada et al. 2009; Kitagawa et al. 2012).

Based on data from expression, transgenic and mutant analyses, we developed a gene network model for the interaction of *VRN1*, *VRN2* and *VRN3* in leaves (Shimada et al. 2009). In this model, *VRN1* is upstream of *VRN3* and activates *VRN3* expression under long day conditions. Thus, *VRN1* is proposed to play a role as an integrator of the vernalization and photoperiodic signals. Trevaskis (2010) put forward an alternative gene network model for *VRN1*, *VRN2* and *VRN3*; this model was based on the results of investigations using barley. This alternative model postulates that *VRN1* and *VRN3* mutually up-regulate each other: *VRN1* first activates *VRN3* expression, and then *VRN3* further activates *VRN1*. The model was referred to as "the flowering model for temperate cereals" in a review paper on flowering in plants (Andres and Coupland 2012). More recently, a third model was suggested by Chen

and Dubcovsky (2012). This model proposes that *VRN1* is activated by *VRN3* and then suppresses *VRN2* expression. In this model, *VRN1* is not essential for flowering; this conclusion was drawn from an analysis of a *VRN1* mutant line. However, it is not certain that the mutant line is a true *VRN1* knock-out, because its genotypic alteration is a point mutation and *VRN1* mRNA is transcribed.

To obtain more information about the flowering mechanism in wheat, we are developing a large-scale panel of mutants induced by heavy-ion beam mutagenesis; these mutants are being systematically screened for effects on flowering time (Murai et al. 2013). Heavy-ion beam irradiation is effective at producing gene deletion mutants (null mutations) (Kazama et al. 2011, 2013). Here we describe four newly identified extra early-flowering mutant lines in diploid einkorn wheat, which have been named *extra early-flowering 1* (*exe1*), *exe2*, *exe3*, and *exe4*.

Identification of *exe* Mutants

Seeds of the diploid einkorn wheat (*Triticum monococcum* L., 2n = 2x = 14, genome constitution A^mA^m) strain KU104-1 were given 50 Gy of 50 keV μm^{-1} LET (linear energy transfer) carbon ion beams and then sown in the field. The spikes of M_1 plants were bagged and the harvested selfed seeds of each spike were used to produce the M_2 lines. From approximately 1,200 M_2 lines, we identified plants showing an abnormal extra early-heading phenotype; we termed these mutants *extra early-flowering* (*exe*). The original wild type (WT) strain KU104-1 is a spring habit einkorn wheat having a dominant *VRN1* allele and a null *VRN2* allele. Therefore, the *exe* mutants, *exe1*, *exe2*, *exe3*, *exe4*, identified in this study have no active gene at the *VRN2* locus.

Table 19.1 shows the heading time of the WT and *exe* mutants in the field. Based on the heading time, the *exe* mutants were classified into two groups: Type I showed moderately extra early-heading type (*exe1* and *exe3*); and Type II showed extremely extra early-heading type (*exe2* and *exe4*). In the field, Type I and Type II headed about 30 and 45 days earlier than the WT, respectively.

Table 19.1 Heading time of the *exe* mutants and wild type (WT) plants grown in the field

Lines	Heading time	Difference from WT	Type
WT	6 June	–	–
exe1	7 May	−30	Type I
exe2	22 April	−45	Type II
exe3	7 May	−30	Type I
exe4	25 April	−37	Type II

Data from season 2011/2012

Morphological Characteristics of the *exe* Mutants

WT plants and *exe* mutants were grown in the experimental field at Fukui Prefectural University and their morphological phenotypes were characterized during the maturation stage. Three agronomic characters were assessed: internode length, spike length, and spikelet number per spike.

The internode lengths of *exe* mutants were shorter than those of WT plants (Fig. 19.1). In particular, Type II *exe* mutants showed a significantly shorter first internode length than WT plants. Compared to WT plants, *exe* mutant plants produced smaller spikes with fewer spikelets (Fig. 19.2). As a consequence of the smaller numbers of spikelets, spike lengths in *exe* mutants were shorter than in WT plants. Furthermore, the spikes of Type II *exe* mutants were smaller than those of Type I mutants. These observations indicate that shortened culm lengths and fewer spikelets per spike are associated with the extra early-flowering phenotype in the *exe* mutants.

Hypothetical Model for Extra Early-Flowering Phenotype

The expression analysis of *VRN1* indicated that *VRN1* is highly expressed at earlier stages in Type II mutants than in Type I mutants under both short day (SD) and long day (LD) conditions (data not shown). This clearly indicates that the difference in earliness between Type I and Type II mutants is associated with the level of *VRN1* expression. Thus, *VRN1* is essential for flowering in wheat, and the level of expression of *VRN1* determines flowering time (Fig. 19.3).

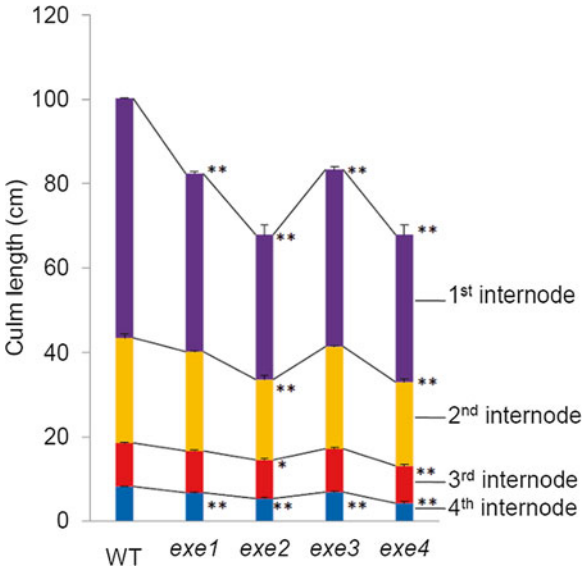

Fig. 19.1 Comparison of internode lengths of *exe* mutants and wild type (WT) plants grown in the field. First to fourth internode lengths are shown. The mean from the main culms of five plants of each line are shown. *Asterisks* indicate significant *P* values (Student's *t*-test): $*p<0.05$ and $**p<0.001$ vs WT

Fig. 19.2 Spikes of *exe* mutants and wild type (WT) plants grown in the field. The *exe* mutants showed a significantly decreased spike length compared to the WT

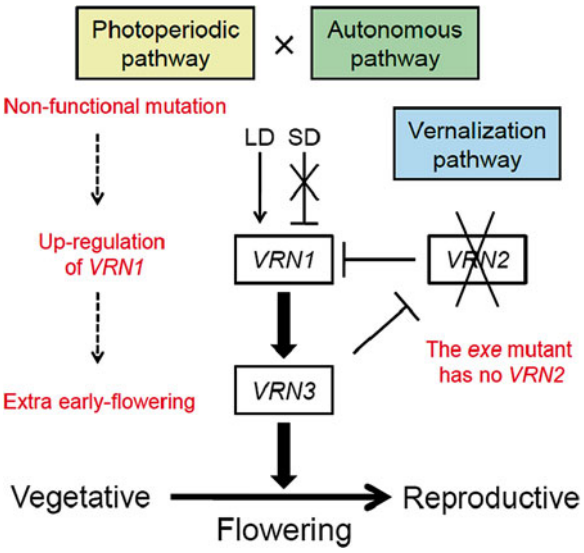

Fig. 19.3 Schematic outline of the proposed mechanism for the extra early-flowering phenotype in *exe* mutants. This is based on the model of Shimada et al. (2009) in which *VRN1* acts as an integrator of the vernalization and photoperiodic pathways that are coordinated with the autonomous pathway. *VRN1* acts by up-regulating the florigen gene *VRN3*. In *exe* mutants, the mechanism for suppressing expression of *VRN1* under SD conditions must be disrupted. Levels of accumulation of *VRN1* transcripts induce *VRN3* expression, resulting in the extra early-flowering phenotype. Note that the *exe* mutant has no *VRN2* gene, because the original strain KU104-1 lacks *VRN2* locus. *Arrows* and *T-bars* indicate promotion and suppression effect, respectively. *Arrows* indicated by *bold lines* show stronger effects

References

Andres F, Coupland G (2012) The genetic basis of flowering responses to seasonal cues. Nat Rev Genet 13:627–639

Chen A, Dubcovsky J (2012) Wheat TILLING mutants show that the vernalization gene *VRN1* down-regulates the flowering repressor *VRN2* in leaves but is not essential for flowering. PLoS Genet 8:e1003134

Danyluk J, Kane NA, Breton G et al (2003) TaVRT-1, a putative transcription factor associated with vegetative to reproductive transition in cereals. Plant Physiol 132:1849–1860

Diallo AO, Ali-Benali MA, Badawi M et al (2012) Expression of vernalization responsive genes in wheat is associated with histone H3 trimethylation. Mol Genet Genomics 287:575–590

Distelfeld A, Tranquilli G, Li C et al (2009) Genetic and molecular characterization of the *VRN2* loci in tetraploid wheat. Plant Physiol 149:245–257

Dubcovsky J, Loukoianov A, Fu D et al (2006) Effect of photoperiod on the regulation of wheat vernalization genes *VRN1* and *VRN2*. Plant Mol Biol 60:469–480

Kazama Y, Hirano T, Saito H et al (2011) Characterization of highly efficient heavy-ion mutagenesis in *Arabidopsis thaliana*. BMC Plant Biol 11:161

Kazama Y, Fujiwara MT, Hirano T et al (2013) Characterization of a heavy-ion induced white flower mutant of allotetraploid *Nicotiana tabacum*. Plant Cell Rep 32:11–19

Kitagawa S, Shimada S, Murai K (2012) Effect of *Ppd-1* on the expression of flowering-time genes in vegetative and reproductive growth stages of wheat. Genes Genet Syst 87:161–168

Murai K, Miyamae M, Kato H et al (2003) *WAP1*, a wheat *APETALA1* homolog, plays a central role in the phase transition from vegetative to reproductive growth. Plant Cell Physiol 44:1255–1265

Murai K, Nishiura A, Kazama Y, Abe T (2013) A large-scale mutant panel in wheat developed using heavy-ion beam mutagenesis and its application to genetic research. Nucl Inst Methods Phys Res B 314:59–62

Oliver SN, Finnegan EJ, Dennis ES et al (2009) Vernalization-induced flowering in cereals is associated with changes in histone methylation at the *VERNALIZATION1* gene. Proc Natl Acad Sci U S A 106:88386–88391

Shimada S, Ogawa T, Kitagawa S et al (2009) A genetic network of flowering time genes in wheat leaves, in which an *APETALA1*/*FRUITFULL*-like gene, *VRN1*, is upstream of *FLOWERING LOCUS T*. Plant J 58:668–681

Trevaskis B (2010) The central role of the *VERNALIZATION1* gene in the vernalization response of cereals. Funct Plant Biol 37:479–487

Trevaskis B, Bagnall DJ, Ellis MH et al (2003) MADS box genes control vernalization-induced flowering in cereals. Proc Natl Acad Sci U S A 100:13099–13104

Trevaskis B, Hemming MN, Peacock WJ, Dennis ES (2006) *HvVRN2* responds to daylength, whereas *HvVRN1* is regulated by vernalization and developmental status. Plant Physiol 140:1397–1405

Yan L, Loukoianov A, Tranquilli G et al (2003) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci U S A 100:6263–6268

Yan L, Loukoianov A, Blechl A, Tranquilli G et al (2004) The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. Science 303:1640–1644

Yan L, Fu D, Li C, Blechl A et al (2006) The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. Proc Natl Acad Sci U S A 103:19581–19586