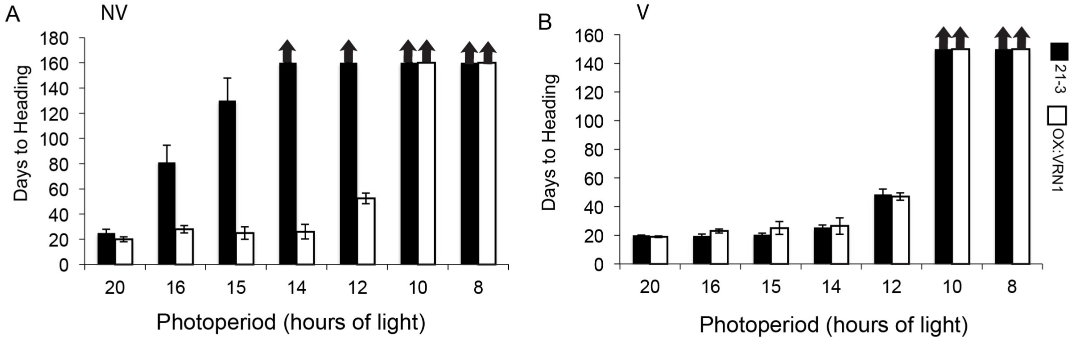
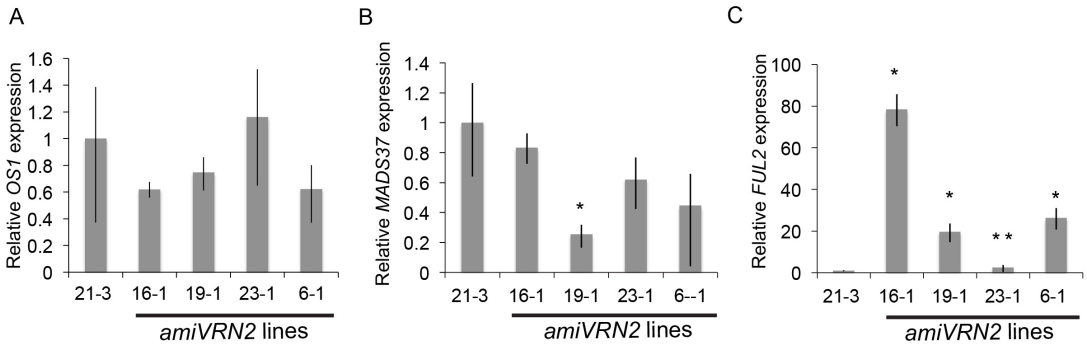
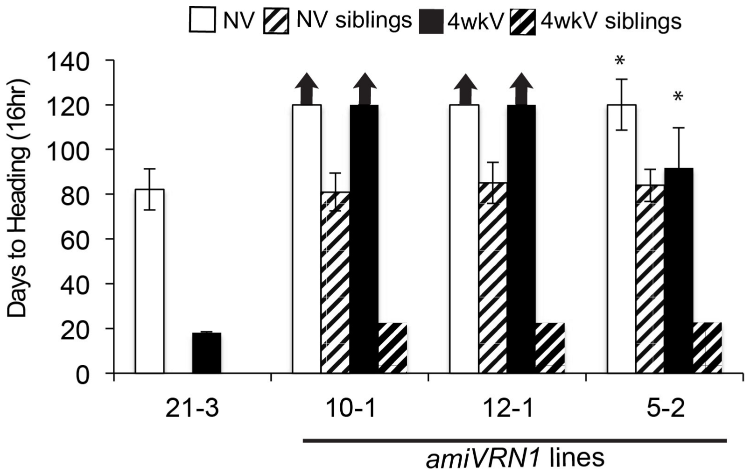


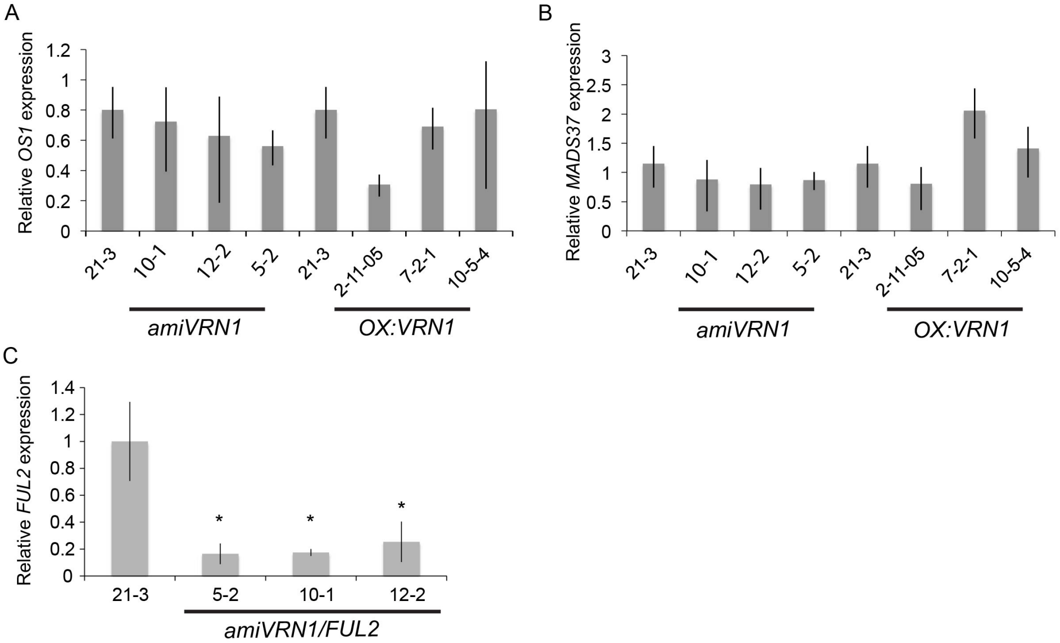
Supplemental Figure 1. Days to heading of Bd21-3 and *OX:VRN1* lines grown in 20,16, 15,14, 12, 10, and 8 hours of lights. Plants were were vernalized (V) as an imbibed seed for 4 weeks before outgrowth in the indicated photoperiods. Non-vernalized (NV) plants and V plants were both moved into the indicated photoperiod at the same time. Arrows indicates that no plants flowered during the course of the experiment.



Supplemental Figure 2. *BdOS1*, *BdMADS37* and *BdFUL2* gene expression in leaves of Bd21-3 and *amiVRN2* plants grown in 16h long days without vernalization. qRT-PCR expression data sampled from the youngest leaf at the third leaf stage of Bd21-3 wild-type (WT) and *amiVRN2* plants for *BdOS1* (A), *BdOS2* (B), and *BdFUL2*. Bars represent the average of three biological replicates +/- SD. Expression analysis was repeated with similar results. Single asterisks indicate P-values < 0.01 and two asterisks indicate P valuse < 0.05.



Supplemental Figure 3. Days to heading of Bd21-3, and *amiVRN1* plants non-vernalized (NV) and vernalized for 4 weeks (4wkV) at 5°C at the 3rd leaf stage in a 16-h photoperiod before outgrowth for 120 days in a 16-h photoperiod. Arrows indicates that no plants flowered during the course of the experiment. Asterisks (*) indicates that some plants in this treatment did not flower during the course of the 120 day experiment. Siblings are plants segregating from the same primary transformant mother plant that does not carry the transgene.



Supplemental Figure 4. *BdOS1* (A) and *BdMADS37* (B) gene expression in the youngest leaves of Bd21-3 wild type (WT), *amiVRN1*, and *OX:VRN1* plants at the three leaf stage grown without vernalization under a 20h photoperiod. Bars represent the average of three biological replicates +/- SD. Expression analysis was repeated with similar results. (C) *BdFUL2* gene expression in the third leaf of Bd21-3 and *amiVRN1/FUL2* lines which knockdown mRNA expression of *VRN1* and *FUL2* simultaneously. See Figure 6 for *VRN1* expression in the *amiVRN1/FUL2* lines. Asterisk indicates P value <.05.

Supplemental Table 1

Genotyping overexpression	
pANIC 6 series AcV5 R	aga cca gcc gct cgc atc ttt cca ag
VRN2 cDNA F no cacc	ATGTCGACATGTGGCATGTGCG
VRN2 cDNA R no stop	TCGGAGCCATCCGAGGTCGAG
amiRNA	
G-11491-GW-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTCATCGGATCCCAGCAGCAGCCACAGCAAA
G-11494-GW-attB2 R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCGGTACCGCTGCTGATGCTGATGCCAT
BdVRN2miR-sI-C	agTACTTGGATCATAGGTACCTGcaggagattcagtttga
BdVRN2miR-aII-C	tgCAGGTACCTATGATCCAAGTActgctgctgctacagcc
BdVRN2miR*sIII-C	ctCAGGTTCCTTTGATCCAAGTAttcctgctgctaggctg
BdVRN2miR*aIV-C	aaTACTTGGATCAAAGGAACCTGagagaggcaaaagtgaa
BdVRN1miR-s1-C	agTTCAGAAATCGACTCGCGCATcaggagattcagtttga
BdVRN1miR-aII-C	tgATGCGCGAGTCGATTTCTGAActgctgctgctacagcc
BdVRN1miR-s*III-C	ctATGCGGGAGACGATTTCTGAAttcctgctgctaggctg
BdVRN1miR-a*IV-C	aaTTCAGAAATCGTCTCCCGCATagagaggcaaaagtgaa
BdVRN1L1/2miR-sI-271	agTTTCCTATATTCGTGACGCCAcaggagattcagtttga
BdVRN1L1/2miR-aII-271	tgTGGCGTCACGAATATAGGAAActgctgctgctacagcc
BdVRN1L1/2miR-*sIII-271	ctTGGCGACACCAATATAGGAAAttcctgctgctaggctg
BdVRN1L1/2miR-*aIV-271	aaTTTCCTATATTGGTGTCGCCAagagaggcaaaagtgaa
Genotyping amiRNAs	
Universal F amiRNA	ctgatgcatatacatgatggc
Phylogenetics	
CO-like_994f	GAGAAGCARATCCGSTAYGMGTC
CO-like_1175r	CGGAACCAYCCGAGGTSRAG
for qPCR	
NassPul_qVRN2_f	AGGAAAGCTTACGCCGAGAT
NassPul_qVRN2_r	GGTGGAGTCTGCTTGGATGT
NassPub_qVRN2_f	GGTACGAGTCCAGGAAAGCA
NassPub_qVRN2_alt.r	GAGGTCGAGTCTGCTTGGATGT
Mnutans_5'_46F	CTGCAGTGGTCTCATTTCCTC
Mnutans_5'_166R	GTTTGTTTGCTCTGGTGGTGT
A_sativa_VRN2_58F	CAAATCCGGTATGAGTCAAGAAAA
A_sativa_VRN2_132R	ATGTACCTTGGCGAAGCGGCCT
Grass_UBQ5_F	CGCCGACTACAACATCCAG
Grass_UBQ5_R	TCACCTTCTTGTGCTTGTGC
Lolium_EF1alphaF	CCTTGCTTGAGGCTCTTGAC
Lolium_EF1alphaR	GTTCCAATGCCACCAATCTT
BdFUL2 qPCR F1	CAAGGGCAAGCTCTACGAGT
BdFUL2 qPCR R1	AAGAGCCTTTTCAGCATACGA

Supplemental Figure 5. Alignment of *VRN2/GhD7/CO9* genes and outgroups used for phylogenetic analysis. Apparent frameshifts in the alignment are due to the removal of unalignable regions, which resulted in higher support values at several nodes, presumably due to reduction of random noise in the data versus true phylogenetic signal. See nexus file.