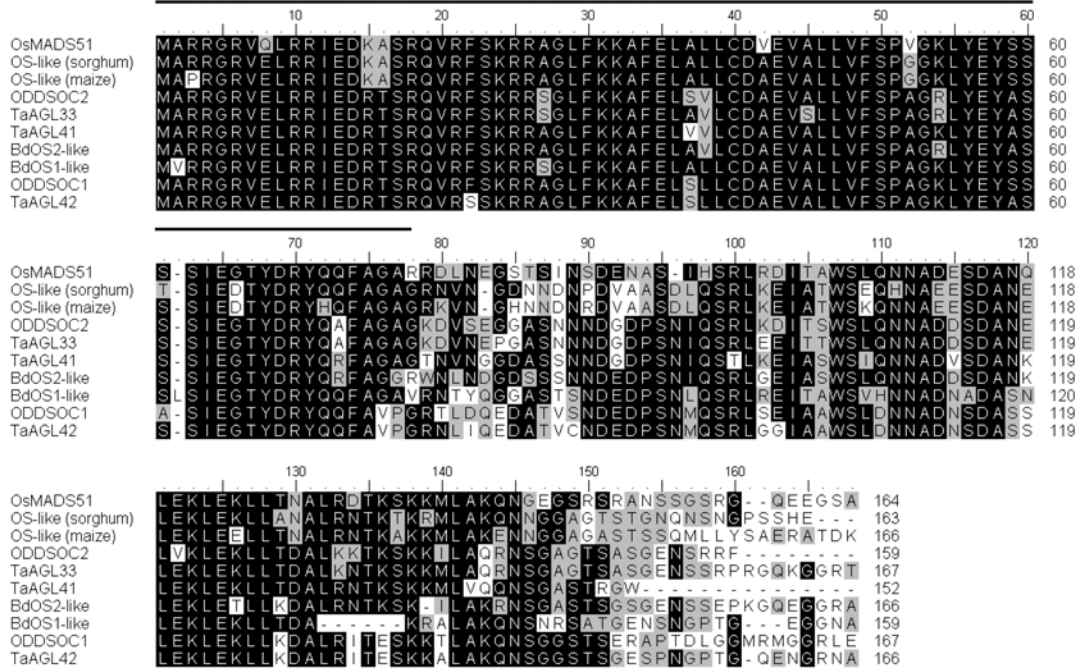


Supplemental Figure S1. Phylogenetic relationships between *ODDSOC-like* genes and other plant MADS box genes. The sequence alignment used to create this tree is provided in Supplemental Fig. S2C.

A

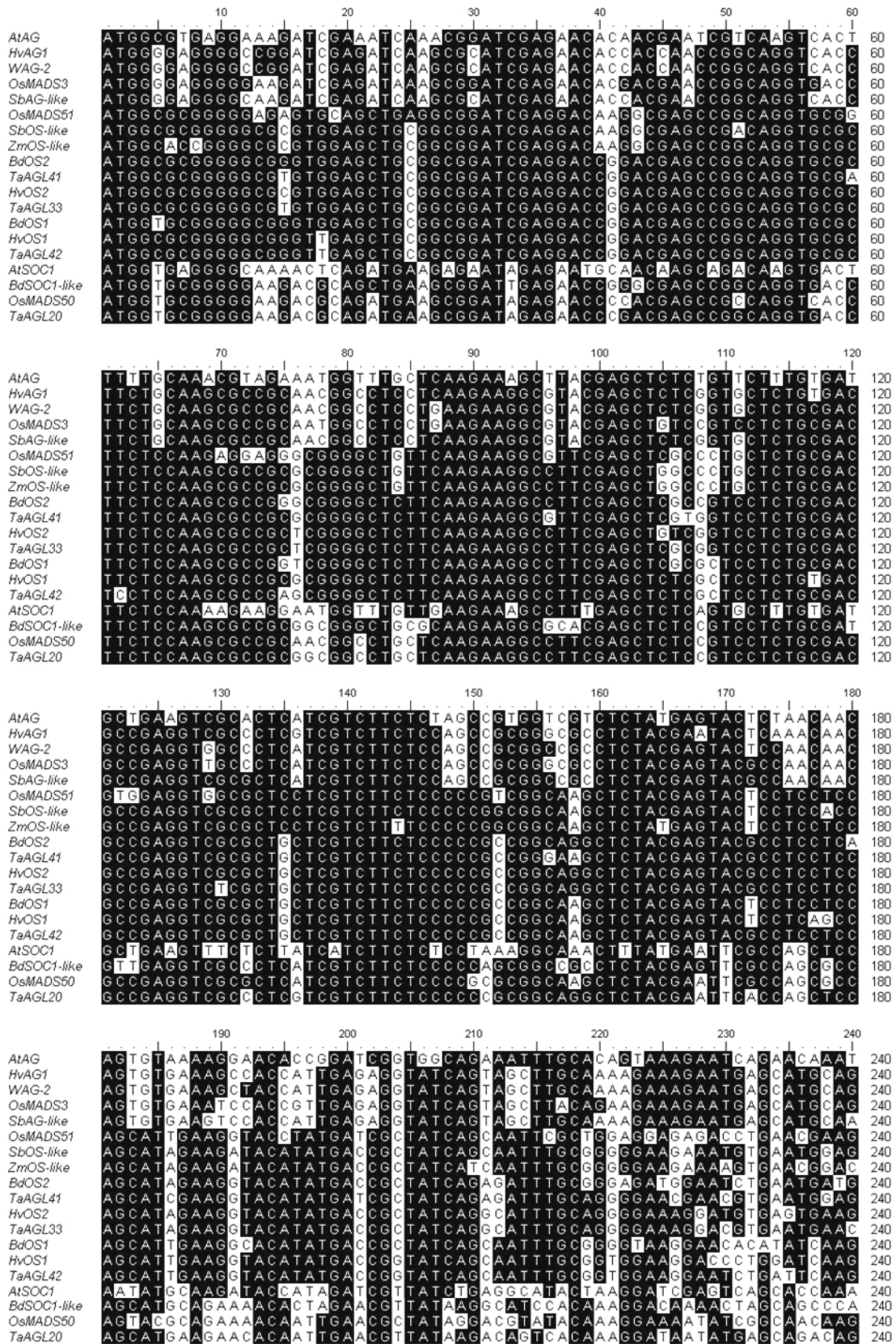
MADS Domain

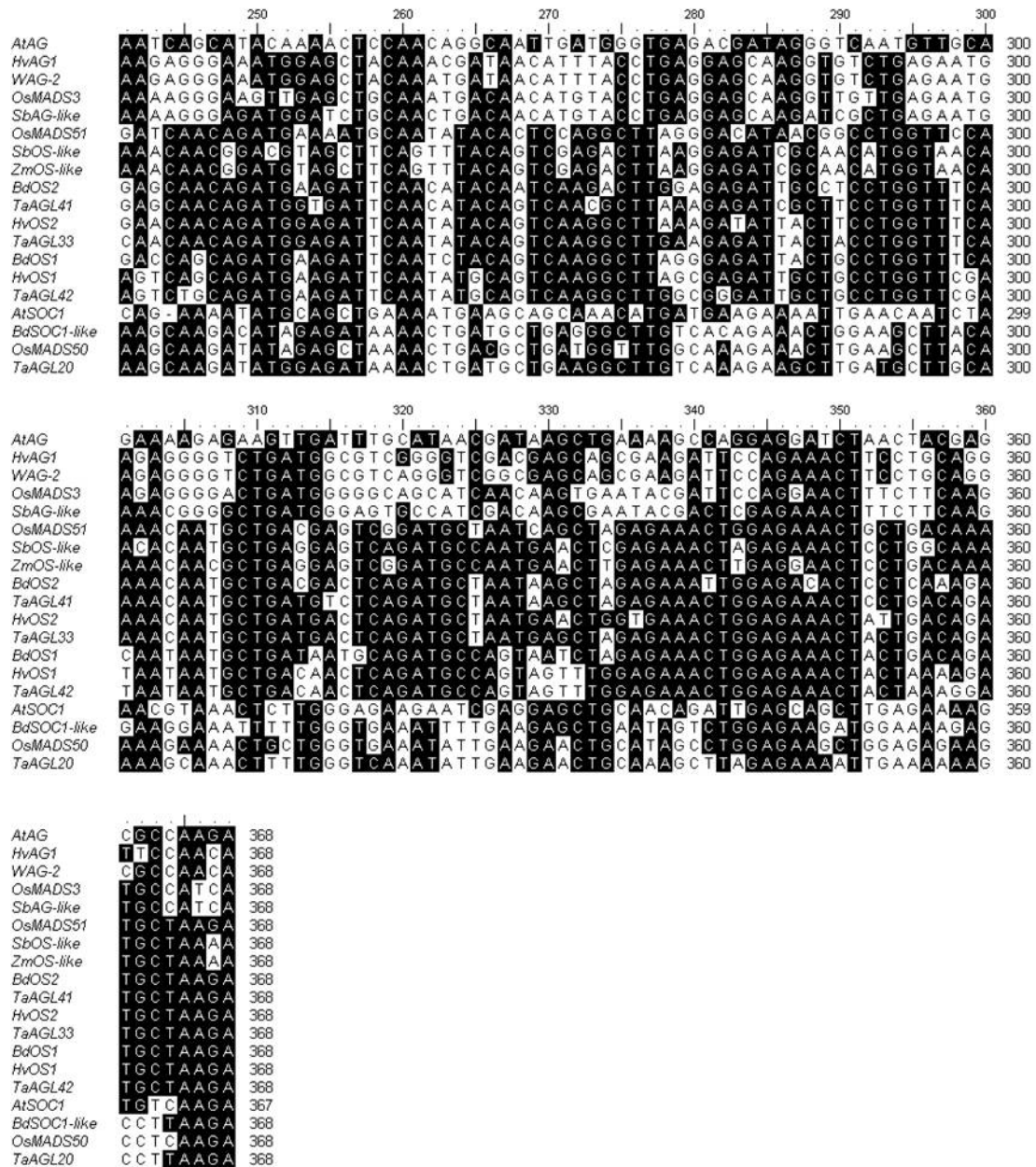


B

	10	20	30	40	50	60	
<i>OsMADS51</i>	ATGGCGCGGAGGGGGAAGTGCAGCTGAGGCGGATCGAGGACAAGGCGAGCCGGCAGGTG						60
<i>SbOS-like</i>	ATGGCGCGGCGCGGGGCGGTGGAGCTCGGGCGGATCGAGGACAAGGCGAGCCGACAGGTG						60
<i>ZmOS-like</i>	ATGGCAACCGCGCGGGGCGGTGGAGCTCGGGCGGATCGAGGACAAGGCGAGCCGGCAGGTG						60
<i>BdOS2-like</i>	ATGGCGCGGCGCGGGGCGGTGGAGCTCGGGCGGATCGAGGACCGGACGAGCCGGCAGGTG						60
<i>TaAGL41</i>	ATGGCGCGGCGCGGGGCGGTGGAGCTCGGGCGGATCGAGGACCGGACGAGCCGGCAGGTG						60
<i>ODDSOC2</i>	ATGGCGCGGCGCGGGGCGGTGGAGCTCGGGCGGATCGAGGACCGGACGAGCCGGCAGGTG						60
<i>TaAGL33</i>	ATGGCGCGGCGCGGGGCGGTGGAGCTCGGGCGGATCGAGGACCGGACGAGCCGGCAGGTG						60
<i>BdOS1-like</i>	ATGGTTCGGGCGCGGGGCGGTGGAGCTCGGGCGGATCGAGGACCGGACGAGCCGGCAGGTG						60
<i>ODDSOC1</i>	ATGGCGCGGCGCGGGGCGGTTGAGCTCGGGCGGATCGAGGACCGGACGAGCCGGCAGGTG						60
<i>TaAGL42</i>	ATGGCGCGGCGCGGGGCGGTTGAGCTCGGGCGGATCGAGGACCGGACGAGCCGGCAGGTG						60
	70	80	90	100	110	120	
<i>OsMADS51</i>	CGGTTCTCCAAGAGGAGCGGGGGCTGTTCAAGAAGGCCTTCGAGCTCGCCCTGCTCTGC						120
<i>SbOS-like</i>	CGCTTCTCCAAGCGCCGCGGGGGCTGTTCAAGAAGGCCTTCGAGCTGGCCCTGCTCTGC						120
<i>ZmOS-like</i>	CGCTTCTCCAAGCGCCGCGGGGGCTGTTCAAGAAGGCCTTCGAGCTGGCCCTGCTCTGC						120
<i>BdOS2-like</i>	CGCTTCTCCAAGCGCCGCGGGGGCTTCAAGAAGGCCTTCGAGCTCGCCCTGCTCTGC						120
<i>TaAGL41</i>	CGATTCTCCAAGCGCCGCGGGGGCTTCAAGAAGGCCTTCGAGCTCGCCCTGCTCTGC						120
<i>ODDSOC2</i>	CGCTTCTCCAAGCGCCGCTCGGGGGCTTCAAGAAGGCCTTCGAGCTGTGGTCTCTGC						120
<i>TaAGL33</i>	CGCTTCTCCAAGCGCCGCTCGGGGGCTTCAAGAAGGCCTTCGAGCTCGCCCTGCTCTGC						120
<i>BdOS1-like</i>	CGCTTCTCCAAGCGCCGCTCGGGGGCTTCAAGAAGGCCTTCGAGCTCGCCCTGCTCTGC						120
<i>ODDSOC1</i>	CGCTTCTCCAAGCGCCGCGGGGGCTTCAAGAAGGCCTTCGAGCTCTCGCTCTCTGC						120
<i>TaAGL42</i>	CGCTTCTCCAAGCGCCGCGGGGGCTTCAAGAAGGCCTTCGAGCTCTCGCTCTCTGC						120
	130	140	150	160	170	180	
<i>OsMADS51</i>	GACGTGGAGGTGGCGCTCTCGTCTTCTCCCGGTCGGCAAGCTCTACGAGTACTCTCC						180
<i>SbOS-like</i>	GACGCCGAGGTGCGGCTCTCGTCTTCTCCCGGTCGGCAAGCTCTACGAGTACTCTCC						180
<i>ZmOS-like</i>	GACGCCGAGGTGCGGCTCTCGTCTTCTCCCGGTCGGCAAGCTCTACGAGTACTCTCC						180
<i>BdOS2-like</i>	GACGCCGAGGTGCGGCTCTCGTCTTCTCCCGGTCGGCAAGCTCTACGAGTACTCTCC						180
<i>TaAGL41</i>	GACGCCGAGGTGCGGCTCTCGTCTTCTCCCGGTCGGCAAGCTCTACGAGTACTCTCC						180
<i>ODDSOC2</i>	GACGCCGAGGTGCGGCTCTCGTCTTCTCCCGGTCGGCAAGCTCTACGAGTACTCTCC						180
<i>TaAGL33</i>	GACGCCGAGGTGCGGCTCTCGTCTTCTCCCGGTCGGCAAGCTCTACGAGTACTCTCC						180
<i>BdOS1-like</i>	GACGCCGAGGTGCGGCTCTCGTCTTCTCCCGGTCGGCAAGCTCTACGAGTACTCTCC						180
<i>ODDSOC1</i>	GACGCCGAGGTGCGGCTCTCGTCTTCTCCCGGTCGGCAAGCTCTACGAGTACTCTCC						180
<i>TaAGL42</i>	GACGCCGAGGTGCGGCTCTCGTCTTCTCCCGGTCGGCAAGCTCTACGAGTACTCTCC						180
	190	200	210	220	230	240	
<i>OsMADS51</i>	TCCAGCATGAAGGTACATATGACCGCTATCAGCAATTTGCGGGAGCAGGAAGAAAGTG						240
<i>SbOS-like</i>	TCCAGCATGAAGGTACATATGACCGCTATCAGCAATTTGCGGGAGCAGGAAGAAAGTG						240
<i>ZmOS-like</i>	TCCAGCATGAAGGTACATATGACCGCTATCAGCAATTTGCGGGAGCAGGAAGAAAGTG						240
<i>BdOS2-like</i>	TCCAGCATGAAGGTACATATGACCGCTATCAGCAATTTGCGGGAGCAGGAAGAAAGTG						240
<i>TaAGL41</i>	TCCAGCATGAAGGTACATATGACCGCTATCAGCAATTTGCGGGAGCAGGAAGAAAGTG						240
<i>ODDSOC2</i>	TCCAGCATGAAGGTACATATGACCGCTATCAGCAATTTGCGGGAGCAGGAAGAAAGTG						240
<i>TaAGL33</i>	TCCAGCATGAAGGTACATATGACCGCTATCAGCAATTTGCGGGAGCAGGAAGAAAGTG						240
<i>BdOS1-like</i>	TCCAGCATGAAGGTACATATGACCGCTATCAGCAATTTGCGGGAGCAGGAAGAAAGTG						240
<i>ODDSOC1</i>	TCCAGCATGAAGGTACATATGACCGCTATCAGCAATTTGCGGGAGCAGGAAGAAAGTG						240
<i>TaAGL42</i>	TCCAGCATGAAGGTACATATGACCGCTATCAGCAATTTGCGGGAGCAGGAAGAAAGTG						240
	250	260	270	280	290	300	
<i>OsMADS51</i>	AACGGAAATAACAAGCATCAACAGTGATGAATGCAAGTATACACTCAGGCTTAGGAG						300
<i>SbOS-like</i>	AATGGAGATAACAACGATAAACCCAGACGATAGCTGCAGATTTACAGTCGAGACTTAGGAG						300
<i>ZmOS-like</i>	AATGGAAATAACAACGATAAACCCAGGATGATAGCTGCAGATTTACAGTCGAGACTTAGGAG						300
<i>BdOS2-like</i>	AATGGAGATTTCAAGTAGCAACAATGATGATGATCCAAACATACAGTCAAGGCTTAGGAG						300
<i>TaAGL41</i>	AATGGAGATTTCAAGTAGCAACAATGATGATGATCCAAACATACAGTCAAGGCTTAGGAG						300
<i>ODDSOC2</i>	AATGGAGATTTCAAGTAGCAACAATGATGATGATCCAAACATACAGTCAAGGCTTAGGAG						300
<i>TaAGL33</i>	AATGGAGATTTCAAGTAGCAACAATGATGATGATCCAAACATACAGTCAAGGCTTAGGAG						300
<i>BdOS1-like</i>	TATGGAGGCGCAAGTACCAACAATGATGATGATCCAAACATACAGTCAAGGCTTAGGAG						300
<i>ODDSOC1</i>	GATGAAGATTTCAAGTAGCAACAATGATGATGATCCAAACATACAGTCAAGGCTTAGGAG						300
<i>TaAGL42</i>	ATTGAAGATTTCAAGTAGCAACAATGATGATGATCCAAACATACAGTCAAGGCTTAGGAG						300
	310	320	330	340	350	360	
<i>OsMADS51</i>	ATAACGGCCTGGTCTCTCAAAACAATGCTGACGAGTGGGATGCTAATCAGCTAGAGAAA						360
<i>SbOS-like</i>	ATCGCAACATGGTCTGAACAACAATGCTGAGGAGTCAGATGCCAATGAACTGAGAGAAA						360
<i>ZmOS-like</i>	ATCGCAACATGGTCTAAACAACAACAGCTGAGGAGTCAGATGCCAATGAACTGAGAGAAA						360
<i>BdOS2-like</i>	ATTCGCTCCTGGTCTTTCAAAACAATGCTGACGACTCAGATGCTAATGAACTAGAGAAA						360
<i>TaAGL41</i>	ATTCGCTCCTGGTCTTTCAAAACAATGCTGATGCTCAGATGCTAATGAACTAGAGAAA						360
<i>ODDSOC2</i>	ATTACTTCTGGTCTTTCAAAACAATGCTGATGACTCAGATGCTAATGAACTGAGAAA						360
<i>TaAGL33</i>	ATTACTTCTGGTCTTTCAAAACAATGCTGATGACTCAGATGCTAATGAACTAGAGAAA						360
<i>BdOS1-like</i>	ATTACTTCTGGTCTTTCAACAATAATGCTGATGACTCAGATGCCAGTAACTAGAGAAA						360
<i>ODDSOC1</i>	ATTGCTGCTGGTCTCTGATAAATGCTGACAATCAGATGCCAGTAACTTAGAGAAA						360
<i>TaAGL42</i>	ATTGCTGCTGGTCTCTGATAAATGCTGACAATCAGATGCCAGTAACTTAGAGAAA						360
	370	380					
<i>OsMADS51</i>	CTGGAGAAACTGCTGACAAAT						381
<i>SbOS-like</i>	CTAGAGAAACTCCTGGCAAT						381
<i>ZmOS-like</i>	CTTGAGAAACTCCTGACAAAT						381
<i>BdOS2-like</i>	TTGGAGCAACTCCTCAAAGAT						381
<i>TaAGL41</i>	CTGGAGAAACTCCTGACAGAT						381
<i>ODDSOC2</i>	CTGGAGAAACTATTGACAGAT						381
<i>TaAGL33</i>	CTGGAGAAACTACTGACAGAT						381
<i>BdOS1-like</i>	CTGGAGAAACTACTGACAGAT						381
<i>ODDSOC1</i>	CTGGAGAAACTACTAAAGAT						381
<i>TaAGL42</i>	CTGGAGAAACTACTAAAGAT						381

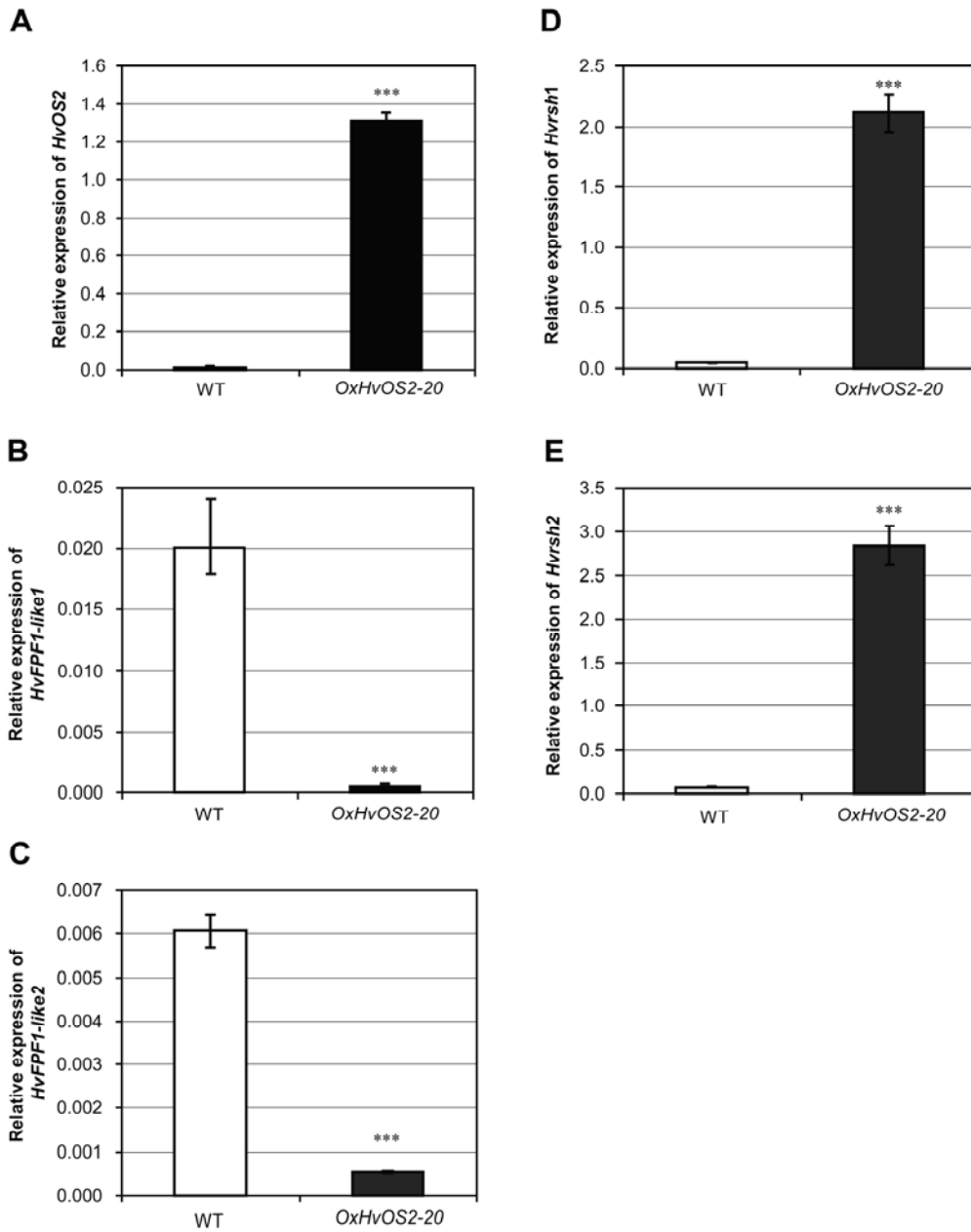
C



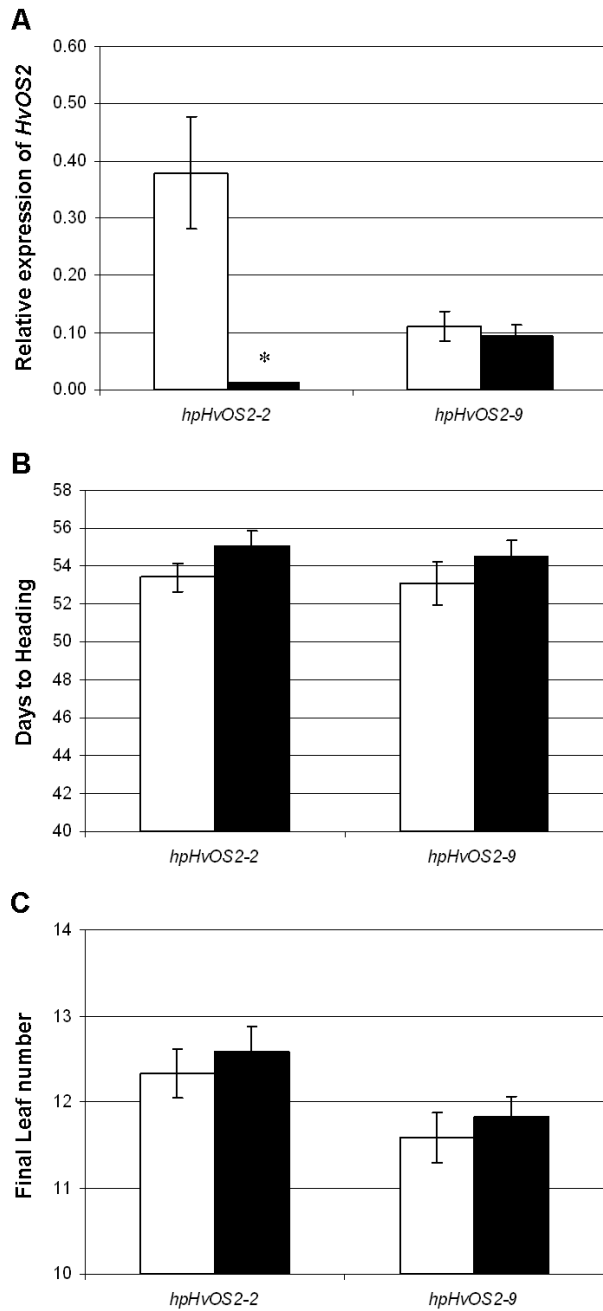


Supplemental Figure S2. Alignments of cDNA and predicted protein sequences. A, Alignment of the predicted protein sequences of *ODDSOC-like* genes B, Alignment of cDNA sequences used in constructing phylogeny of *ODDSOC-like* MADS box genes (Fig 1B). C, Alignment of nucleotide sequences used in constructing phylogeny of *ODDSOC-like* MADS box genes with *AGAMOUS-like* genes and *SOC-1-like* genes as out groups (Supplemental Fig. S1). *OsMADS51* (NM_001051770), *SbOS-like* (XM_002456815), *ZmOS-like* (NP_001140218), *BdOS2-like* (Bradi2g59190), *TaAGL41* (DQ512357), *ODDSOC2* (Unigene 5425), *TaAGL33* (DQ512366), *BdOS1-like* (Bradi2g59120), *ODDSOC1* (Unigene 27247), *TaAGL42* (DQ512358), *AtAG*

(NM_118013), *SbAG-like* (XM_002454940), *WAG-2* (AB465688), *OsMADS3* (L37528.1), *HvAG-1* (AF486648), *AtSOC1* (NM_130128), *OsMADS50* (AY332476.1), *BdSOC1-like* (Bradi1g77020), *TaAGL20* (DQ512338).

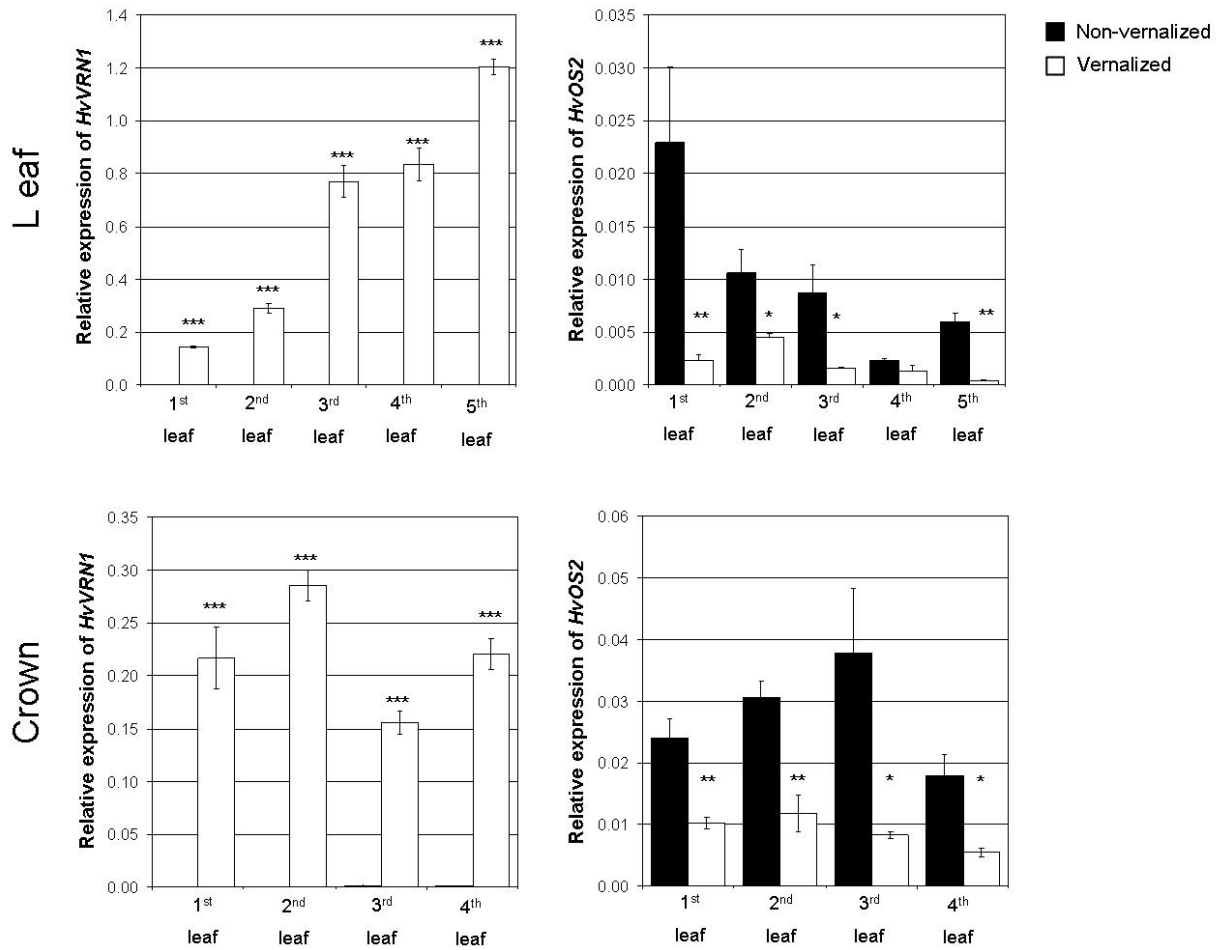


Supplemental Figure S3. Quantitative RT-PCR analysis of gene expression in *HvOS2* over-expression lines. Expression levels of candidate genes were assayed by qRT-PCR, in transgenic barley seedlings over expressing *HvOS2* (black) and compared to wildtype null (WT) siblings (white) at the 2nd leaf stage. Error bars show SE. Asterisks indicate P values of ANOVA: ***, P, < 0.001. (min. of 3 biological repeats). A, *HvOS2* (Contig12031). B, *HvFPF1-like1* (HU14G14r) C, *HvFPF1-like2* (Contig18182) D, *Hvrsh1* (Contig5185). E, *Hvrsh2* (Contig5058/9).

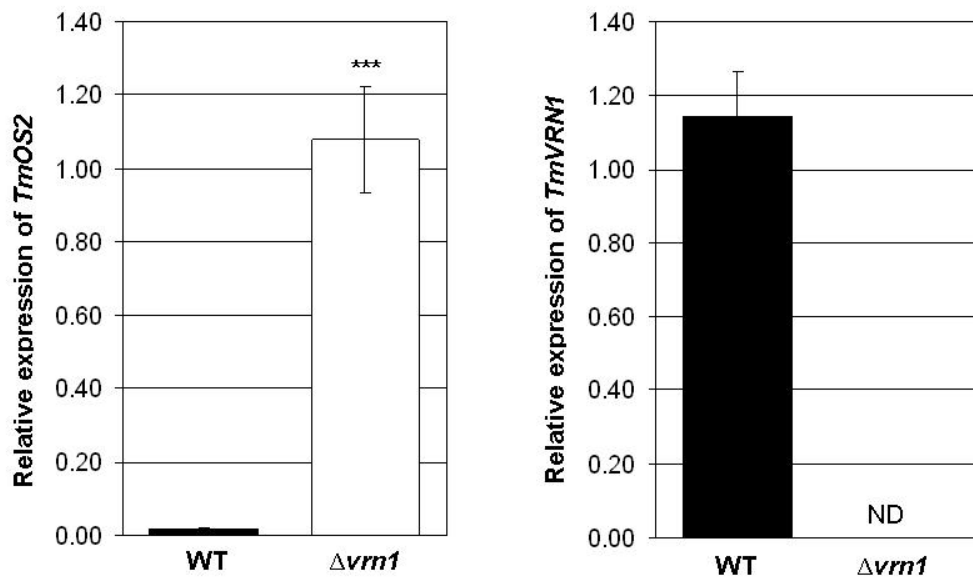


Supplemental Figure S4. Phenotypes and expression levels of *HvOS2* in RNAi transgenic plants. A, Expression of *HvOS2* in RNAi transgenic plants. Data is shown for two transgenic barley two independent lines transformed with gene-specific RNA interference (RNAi) constructs (black) versus wild-type null siblings (white). Expression was assayed by qRT-PCR and is shown relative to *ACTIN*. Error bars show SE. Asterisks indicate P values of ANOVA test: *, P , < 0.05 (min. of 3 biological repeats). B, The average number of days until heading and C, final leaf number for transgenic plants (black) and wild-type siblings (white) Error bars show

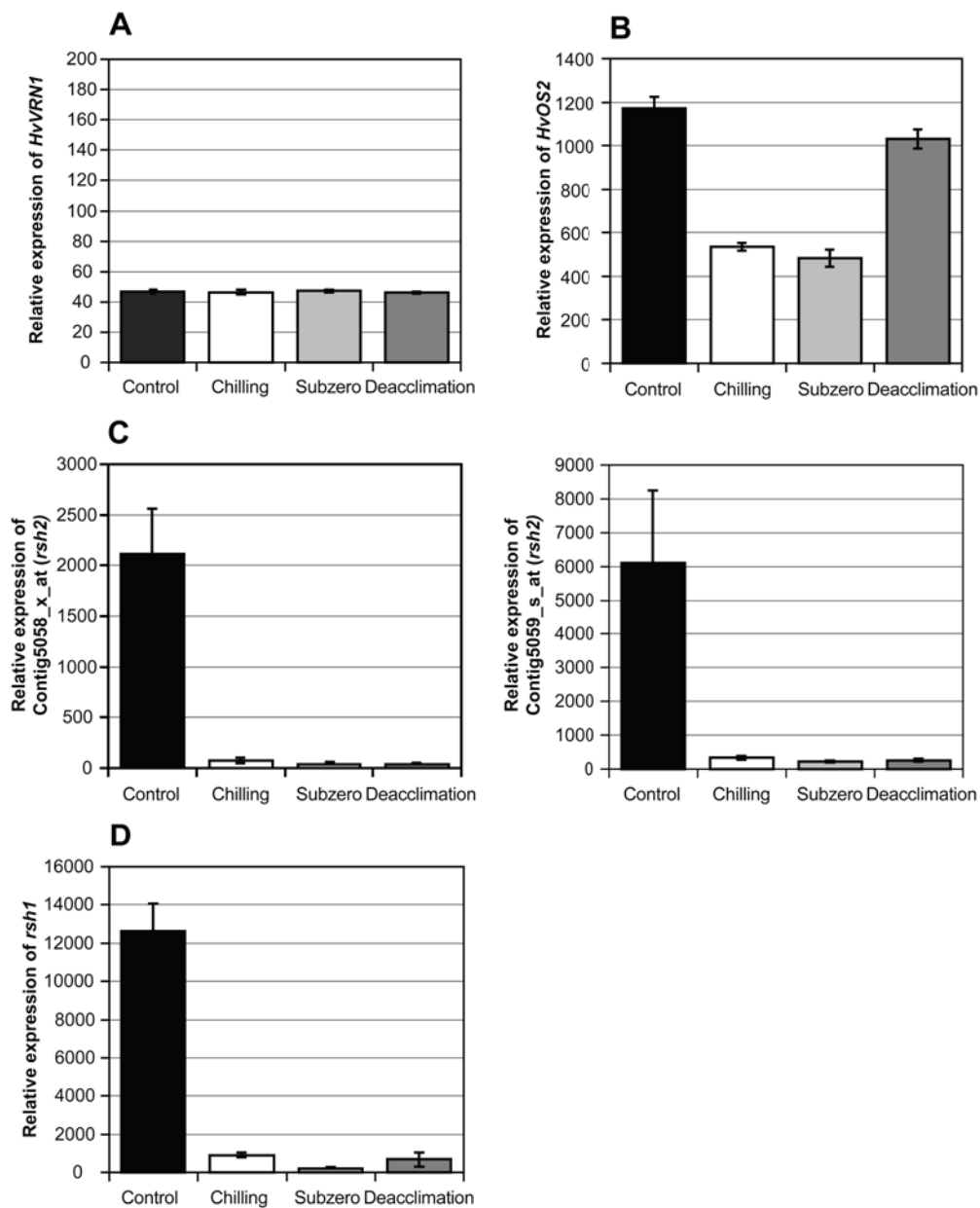
SE. Heading date and final leaf number was calculated from 15 individual plants per genotype.



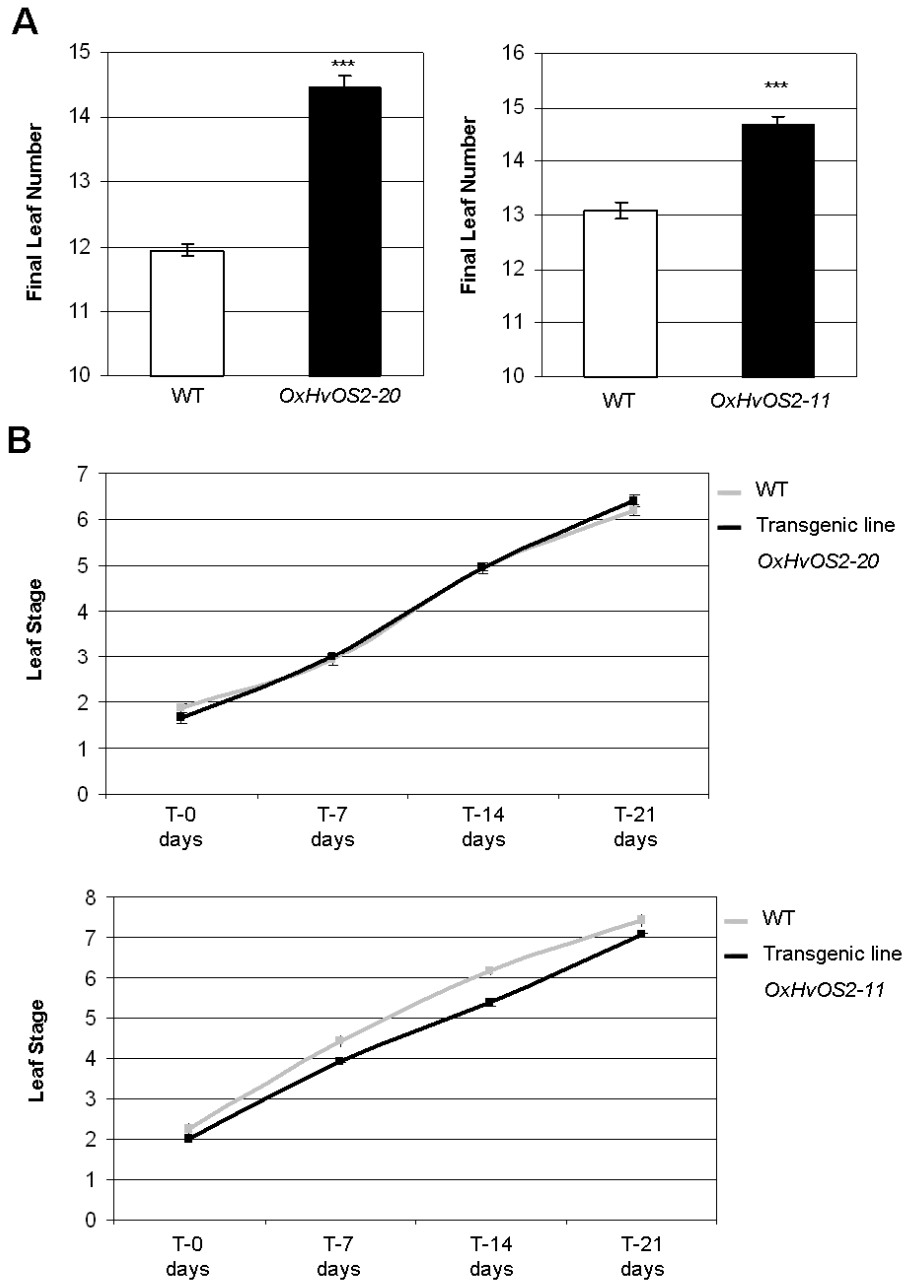
Supplemental Figure S5. Quantitative RT-PCR analysis of *HvOS2* gene expression during development in leaf and crown tissue. Relative expression levels of *HvVRN1* and *HvOS2* (white bars) in leaves and crown tissue from barley plants (cv. Sonja). The fully expanded leaf and crown tissue was taken from non-vernalized plants (black bars) and plants vernalized for 49 days (white bars) at the 1st, 2nd, 3rd, 4th and 5th leaf stage. Expression was assayed in non-vernalized (black bars) and plants vernalized for 49 days (white bars), and is shown relative to *ACTIN*. Error bars show SE from a minimum of 4 biological repeats. Asterisks indicate P values of ANOVA: *, P, <0.05; **, P, <0.01; ***, P, <0.001.



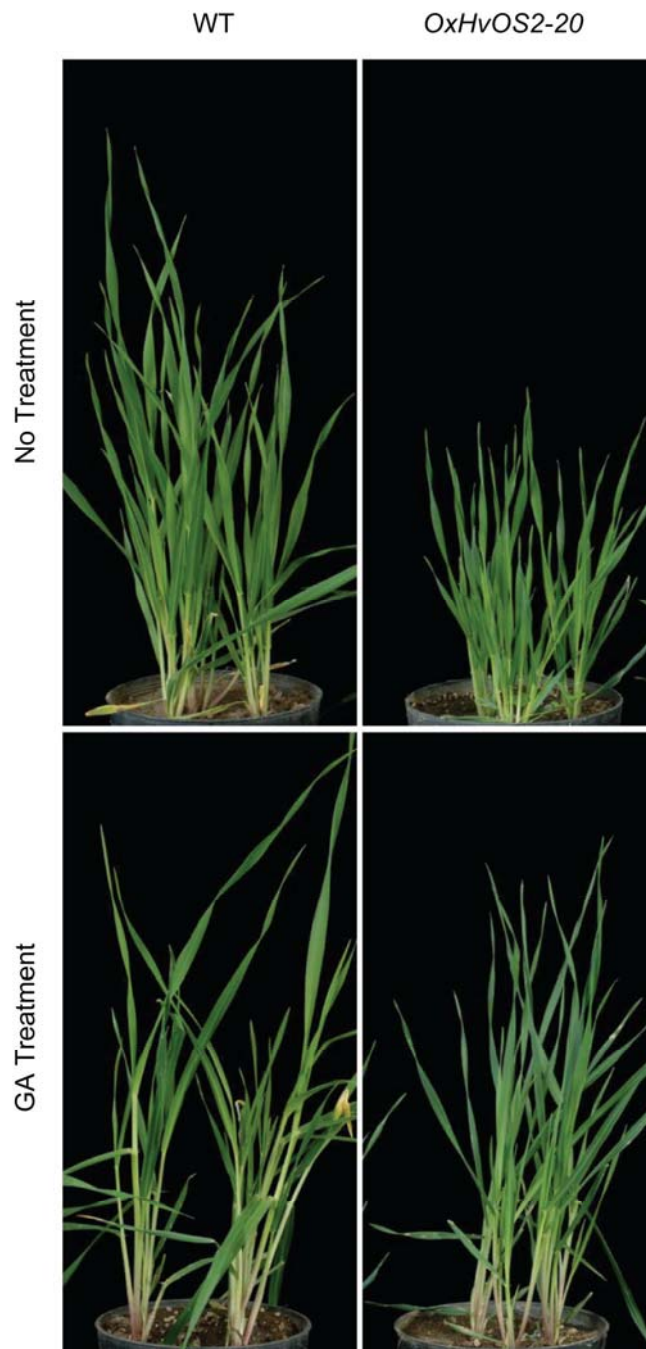
Supplemental Figure S6. Quantitative RT-PCR analysis of *TmOS2* gene expression in the $\Delta VRN1$ mutant grown in long days. Relative expression levels of *TmOS2*-like (*TmAGL33*) in the *TmVRN1* deletion mutant ($\Delta VRN1$) (white bars, $n = 4$) versus the wild type parent strain (black bars, $n = 5$). Expression was assayed in leaves from plants grown in long days, and shown relative to *ACTIN*. Error bars show SE. Asterisks indicate P values of ANOVA: ***, $P < 0.001$, ND, not detected.



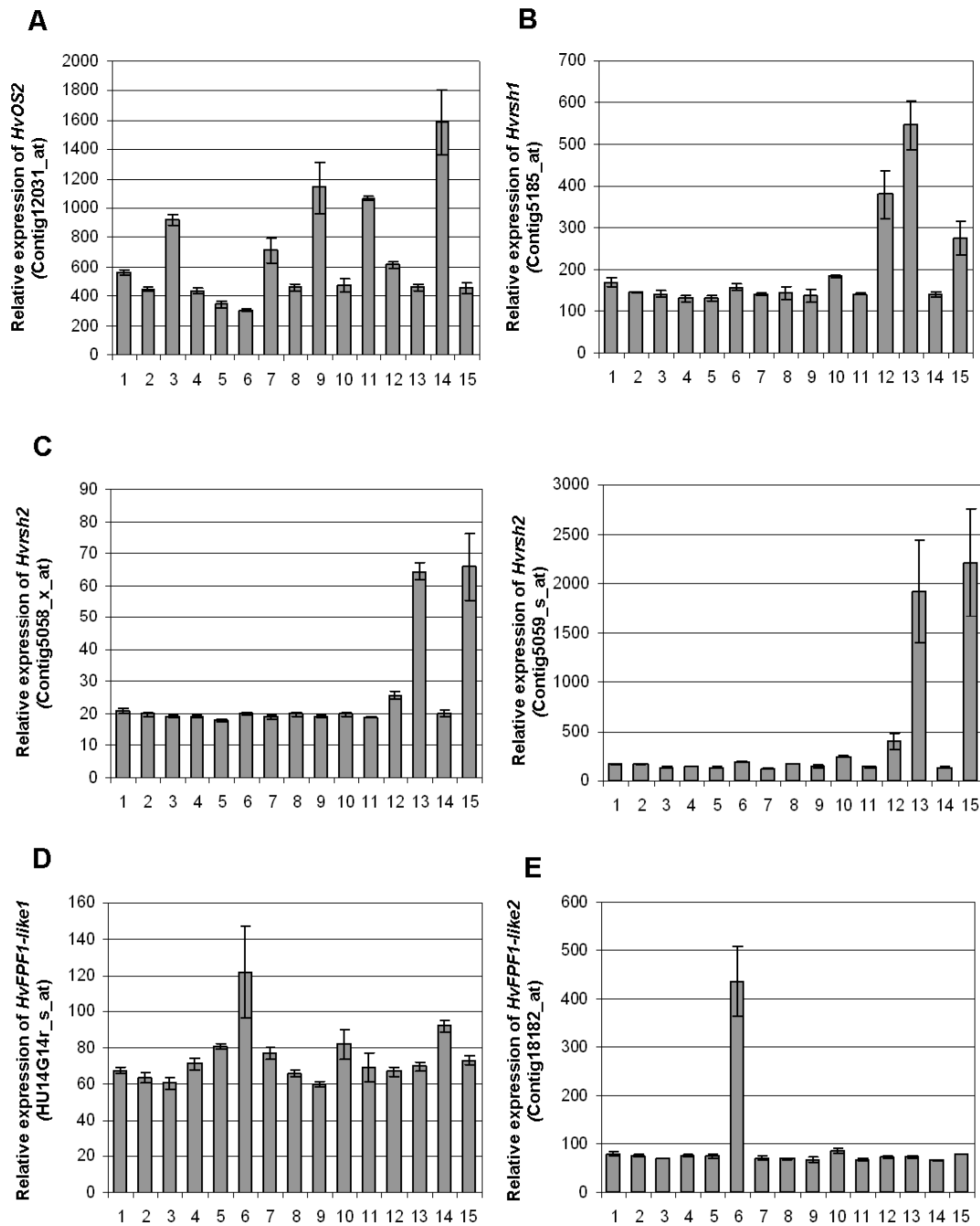
Supplemental Figure S7. Selected data from low temperature stress microarray experiment (cv. Dicktoo). Mean expression levels for A, *HvVRN1* B, *HvOS2* C, *Hvrsh2* and D, *Hvrsh1*. Error bars show standard error of 3 biological repeats. Data was sourced from the Plant Expression Database (www.plexdb.org) Experiment Accession No. BB81.



Supplemental Figure S8. Leaf appearance rate and final leaf number in *HvOS2* over-expression lines versus null sibling control lines. A, Final leaf number and B, leaf emergence rate for lines *OxHvOS2-20* and *OxHvOS2-11*. Leaf emergence rate was recorded 7, 14 and 21 days after the majority of plants reached the 2 leaf stage (T-0 days). Error bars show SE from a minimum of 12 biological repeats. Asterisks indicate P values of ANOVA: ***, P , <0.001.



Supplemental Figure S9. Images of transgenic plants over-expressing *HvOS2* and wild-type siblings with or without GA treatment. A single application (10 μ L) of GA₃ (2.5 μ g/ μ L) in ethanol was applied to leaves.



Supplemental Figure S10. Selected data from microarray analysis of gene expression during barley development (cv. Morex). Expression of *HvOS2* (A), *Hvrsh1* (B), *Hvrsh2* (C) *HvFPF1like-1* (D) and *HvFPF1like-2* (E). Treatments 1-15; 1) Germinating seed, coleoptyle. 2) Germinating seed, radicle. 3) Germinating seed, embryo. 4) Seedling, root. 5) Seedling, crown. 6) Seedling, leaf. 7) Immature, inflorescence. 8) Floral bracts, before anthesis. 9) Pistil, before anthesis. 10) Anthers,

before anthesis. 11) 5 DAP caryopsis. 12) 10 DAP caryopsis. 13) 16 DAP caryopsis. 14) 22 DAP embryo. 15) 22 DAP endosperm. Error bars show standard error of 3 biological repeats. Data was sourced from the Plant Expression Database (www.plexdb.org) Experiment Accession No. BB3.

Supplemental Table S1. Best Arabidopsis matches for HvOS1 and HvOS2

Protein Description	Accession	Identity	BLAST Score	E-value
HvOS1				
AGAMOUS-LIKE 19 (AGL19)	NP_194026	57/124 (45%)	108	3e-24
AGAMOUS-LIKE 14 (AGL14)	NP_192925	58/125 (46%)	107	8e-24
AGAMOUS-LIKE 20 (AGL20 / SOC1)	NP_182090	56/142 (39%)	105	2e-23
HvOS2				
AGAMOUS-LIKE 79 (AGL79)	NP_189645	70/143 (48%)	114	5e-26
AGAMOUS-LIKE 20 (AGL20 / SOC1)	NP_182090	46/67 (68%)	107	5e-24
AGAMOUS-LIKE 14 (AGL14)	NP_192925	63/140 (45%)	107	8e-24

Supplemental Table S2. Non -redundant blastn results for *HvOS1* and *HvOS2*

Description	Accession	Organism	Identity	BLAST Score	E-value
<i>HvOS1</i>					
<i>TaAGL42</i>	DQ512358	<i>T.aestivum</i>	95% (479/503)	793	0.0
<i>TaAGL33</i>	DQ512366	<i>T.aestivum</i>	86% (382/444)	464	2e-127
<i>TaAGL41</i>	DQ512357	<i>T.aestivum</i>	83% (407/487)	438	1e-119
<i>OsMADS51</i>	NM_001051770	<i>O. sativa</i>	79% (395/497)	324	3e-85
Hypothetical protein, mRNA	XM_002456815	<i>S. bicolor</i>	91% (206/225)	307	3e-80
<i>HvOS2</i>					
<i>TaAGL33</i>	DQ512366	<i>T.aestivum</i>	95% (449/471)	747	0.0
<i>TaAGL41</i>	DQ512357	<i>T.aestivum</i>	89% (424/472)	599	5e-168
<i>TaAGL42</i>	DQ512358	<i>T.aestivum</i>	84% (389/458)	448	2e-122
Hypothetical protein, mRNA	XM_002456815	<i>S. bicolor</i>	82% (356/429)	372	1e-99
Hypothetical protein, mRNA	NP_001140218	<i>Z.mays</i>	82% (353/430)	350	5e-93

Supplemental Table S3. Differentially expressed genes between plants over-expressing HvOS2 and wild-type siblings - See Excel File

Supplemental Table S4. Primers		
	Forward	Reverse
qRT-PCR primers		
<i>VRN1</i>	GGAAACTGAAGGCGAAGGTTGA	TGGTTCTTCCTGGCTCTGATATGTT
<i>HvOS1</i>	TTGCGGTTCCCTGGAAGGACC	AACTACTGGCATCTGAGTTGTC
<i>HvOS2</i>	CAATGCTGATGACTCAGATGCT	CGCTATTTGTTGCGCCAAT
<i>TmOS2</i>	CAATGCTGATGACTCAGATGCT	TCGTTGCGCCAACATCTTCT
<i>HvFPF1-like1</i>	GTCAAGAACCGCGACCACTT	CACATGCATAATCGCACACG
<i>HvFPF1-like2</i>	CGTCGACCTCATCTCCCTTC	AGTTGATTTGGGGCAGCTTG
<i>rsh1</i>	TGGGAATCAGCGGTCTAAGG	CTTTGGAAACGAGGCGAGAG
<i>rsh2</i>	GGCATCAACCCGGACTACAG	AAGGTCTTGCCGTCCTTGTC
ChIP primers		
<i>HvOS2</i>	CCCATCTCTGCACTCCCTG	GGAAAGCTAGCCTCCTCCTCCTCCTCCGG
<i>TmVRN1</i> genotyping		
	CTGAAACATATCAGATCCAGG	AACTTTATTCTCCTCCTGCAG
		AATGGATTTCGTGCATAAGTTGG