Arabidopsis thaliana VOZ (Vascular plant One-Zinc finger) transcription factors are required for proper regulation of flowering time

Helena Celesnik*, Gul S. Ali‡, Faith M. Robison and Anireddy S. N. Reddy*

Department of Biology, Program in Molecular Plant Biology, Program in Cell and Molecular Biology, Colorado State University, Fort Collins, CO 80523-1878, USA

*Authors for correspondence (reddy@colostate.edu; celesnik@lamar.colostate.edu)

‡Present address: Mid-Florida Research and Education Center, Apopka, FL 32703, USA and Department of Plant Pathology, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611, USA

Biology Open 2, 424–431
doi: 10.1242/bio.20133764
Received 26th November 2012
Accepted 1st February 2013

Summary

Transition to flowering in plants is tightly controlled by environmental cues, which regulate the photoperiod and vernalization pathways, and endogenous signals, which mediate the autonomous and gibberellin pathways. In this work, we investigated the role of two Zn^{2+}-finger transcription factors, the paralogues AtVOZ1 and AtVOZ2, in *Arabidopsis thaliana* flowering. Single *atvoz1-1* and *atvoz2-1* mutants showed no significant phenotypes as compared to wild type. However, *atvoz1-1 atvoz2-1* double mutant plants exhibited several phenotypes characteristic of flowering-time mutants. The double mutant displayed a severe delay in flowering, together with additional pleiotropic phenotypes. Late flowering correlated with elevated expression of *FLOWERING LOCUS C* (*FLC*), which encodes a potent floral repressor, and decreased expression of its target, the floral promoter *FD*. Vernalization rescued delayed flowering of *atvoz1-1 atvoz2-1* and reversed elevated *FLC* levels. Accumulation of *FLC* transcripts in *atvoz1-1 atvoz2-1* correlated with increased expression of several *FLC* activators, including components of the PAF1 and SWR1 chromatin-modifying complexes. Additionally, AtVOZs were shown to bind the promoter of *MOS3/SAR3* and directly regulate expression of this nuclear pore protein, which is known to participate in the regulation of flowering time, suggesting that AtVOZs exert at least some of their flowering regulation by influencing the nuclear pore function. Complementation of *atvoz1-1 atvoz2-1* with AtVOZ2 reversed all double mutant phenotypes, confirming that the observed morphological and molecular changes arise from the absence of functional AtVOZ proteins, and validating the functional redundancy between AtVOZ1 and AtVOZ2.

Key words: VOZ, Flowering, *Arabidopsis*, FLC, MOS

Introduction

Flowering plants possess an intricate regulatory network to properly time the transition to flowering. Floral initiation has evolved over time to optimize the reproductive success of plants in a variety of environments. Flowering is induced in response to environmental and endogenous cues. Genetic studies have defined four major pathways that sense and respond to flowering cues: the photoperiod, autonomous, vernalization, and gibberellin (GA) pathways (Simpson and Dean, 2002; Srikanth and Schmid, 2011). These pathways are tightly connected and converge on a small number of flowering integrators.

Many genes that specifically regulate flowering time have been identified in *Arabidopsis thaliana*. Mutations in genes that promote flowering result in a late-flowering phenotype. Two major groups of delayed mutants have been categorized in flowering-time studies based on responsiveness to day length (Rédei, 1962; Koornneef et al., 1991). In the first group, late-flowering mutants that are delayed only in long days (LDs) but not in short days (SDs) were proposed to affect the day length sensing pathway, which functions to initiate flowering following exposure to inductive photoperiods. These photoperiod-pathway mutants involve a set of genes that promotes flowering through inductive long-day photoperiods. On the other hand, late-flowering mutants that are delayed in both LDs and SDs involve genes that enable flowering independently of day length. These mutants impact a flowering pathway designated as the autonomous pathway (Rédei, 1962; Koornneef et al., 1991; Simpson and Dean, 2002). In many plant species flowering cannot happen until a progression has been made from juvenile to adult phase through the autonomous pathway, resulting in competence to flower (Poethig, 2003).

Prolonged exposure to cold is known to render plants competent to flower. This process, known as vernalization, is required by winter-annual types of *Arabidopsis* to prevent flowering in the fall season and promote it in the spring. The vernalization response is conferred by dominant alleles of *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*) (Koornneef et al., 1991; Lee and

Amasino, 1995; Michaels and Amasino, 1999; Schmitz and Amasino, 2007). The flowering inhibition is largely a result of *FRI*-mediated upregulation of *FLC*, which encodes a potent suppressor of flowering. Prolonged cold turns off *FLC* expression and renders the plant capable of undergoing floral transition. Plants that contain *FLC/FRI* exhibit delayed flowering that is reversed completely by vernalization (Lee and Amasino, 1995). The late flowering of *FRI*-carrying lines is phenotypically similar to that of mutants in the autonomous pathway, as their late flowering is abolished by loss of *FLC* function or vernalization (Koornneef et al., 1991; Michaels and Amasino, 1999; Sheldon et al., 1999; Michaels and Amasino, 2001). While *FRI* serves to upregulate *FLC* and repress flowering, the genes in the autonomous pathway, such as *FCA*, *FPA*, *FVE*, *FLOWERING LOCUS D* (*FLD*), *FLOWERING LOCUS K HOMOLOGY DOMAIN* (*FLK*), *LUMINIDEPENDENS* (*LD*) and *FY* function to promote flowering by suppressing *FLC* (Lim et al., 2004). The FRI complex is known to supersede the repressive effects of the autonomous pathway (Schmitz and Amasino, 2007).

In this study, we analyzed the involvement of two transcription factors, AtVOZ1 and AtVOZ2, in *Arabidopsis* flowering and development. Our results show that AtVOZs regulate flowering time and other aspects of plant growth and development. Double *atvoz1-1 atvoz2-1* mutant plants exhibit severely delayed flowering time, which correlates with increased mRNA levels of the floral repressor *FLC* and several *FLC* activators, as well as *MOS3/SAR3*, a nuclear pore protein known to play a role in transition to flowering. Both late flowering and upregulation of *FLC* are completely reversed by vernalization. Double *atvoz1-1 atvoz2-1* mutants display additional phenotypes: they are smaller than wild-type (WT) plants, show signs of senescence, exhibit delayed transition from juvenile to adult phase, and show defects in seed production.

Materials and Methods

Plant materials

Experiments were carried out with *Arabidopsis* ecotype Col-0 (WT) and mutant lines in the same ecotype. Seeds of putative T-DNA insertion mutants of *AtVOZ1* (*atvoz1-1*, WiscDsLox489-492010) and *AtVOZ2* (*atvoz2-1*, salk_115813c) were obtained from ABRC, Ohio State University (http://www.arabidopsis.org) and homozygous lines were identified using genomic PCR and RT-PCR with gene-specific and T-DNA-specific primers listed in supplementary material Table S3. The double *atvoz1-1 atvoz2-1* mutant was generated by crossing and identified in the segregating population by PCR with gene-specific and T-DNA-specific primers. To create AtVOZ2; *atvoz1-1 atvoz2-1*, the *atvoz1 atvoz2* double mutant was complemented with a *pC-TAP-VOZ2* construct driven by the 35S promoter using the *Agrobacterium*-mediated transformation (Clough and Bent, 1998; Rubio et al., 2005) and the expression of *pC-TAP-VOZ2* was detected with the TAP-specific primer listed in supplementary material Table S3.

Growth conditions, vernalization treatment, phenotypic analyses

Arabidopsis plants were grown in Pro-mix BX Mycorise soil (Premier) in growth chambers with cold fluorescent light under controlled conditions (temperature 22℃, 80% relative humidity and 90–110 µmol photons m^{-2} s^{-1} light intensity). Long-day photoperiods consisted of 16 hours of light and 8 hours of dark and short-day conditions were 8 hours of light and 16 hours of dark. To study the effect of vernalization on flowering, seeds were vernalized for 12 weeks and then transferred to normal growth conditions in the growth chamber. Flowering time was measured by recording the number of days from sowing to opening of the first flower and by counting the total number of rosette leaves at bolting. Three experimental replications were performed and statistically analyzed (20 to 60 plants per replication). Trichome analysis was performed on plants as they opened the first flower, with the help of a dissecting microscope. Three replications were performed (17 to 30 plants each) and statistically analyzed. DAPI (4′,6-diamino-phenylindole) staining of pollen and microscopic analysis was performed as described previously (Golovkin and Reddy, 2003). Flowers, anthers, siliques and seeds were visualized and abortive seed count was performed under a dissecting microscope.

Expression analysis of flowering genes

Transcript levels of flowering-time genes were measured in 28-day-old WT and mutant plants by RT-PCR. Aerial parts were collected from plants grown in LD conditions 10 hours after dawn. Total RNA was extracted by using the Omega RNA extraction kit (Omegabiotek). After treatment with RNase-free DNase I (Fermentas) to remove any contaminating genomic DNA, cDNA was synthesized with Superscript II RNase H^- reverse transcriptase (Invitrogen) and subjected to PCR. Specific primers for *FLC*, *VIP3*, *ELF7*, *PIE1*, *SEF*, *FD*, *FT*, *CO* and *MOS3* are described in supplementary material Table S3. Bands were visualized by agarose-gel electrophoresis.

Protein purification

AtVOZ2 in pET-32a (N-terminally His-tagged) and AtVOZ2m in pET-28a (N-terminally His-tagged) were expressed in *Escherichia coli* BL21 at 30℃ by adding IPTG (1 mM final concentration) when the culture density reached OD_{600}~0.6. Following 4 hours of incubation, bacteria were harvested by centrifugation for 10 minutes at 4℃ (5000 g). Pellets were resuspended in Bind/Wash buffer (20 mM Tris-HCl [pH 7.5], 50 mM NaCl, 0.1% Triton X-100) containing 1× Complete protease inhibitor cocktail without EDTA (Roche) and sonicated. The lysate was centrifuged (4℃, 10 minutes, 15000 g) and the supernatant was filtered through a 0.45 µm filter (Life Science Products, Inc.). AtVOZ2 and AtVOZ2-m proteins were isolated from the filtrate by binding to His•Bind resin (Novagen) following manufacturer's instructions. Purified proteins were desalted by centrifugation with Amicon Ultra PL-10 Centrifugal Filter Devices (Millipore) and stored in 50 mM Tris-HCl, pH 8. Protein concentration was measured by using the Bio-Rad Protein Assay (Bio-Rad).

Electrophoretic mobility shift assay (EMSA)

Fluorescently labeled *pMOS3* and *pmos3-m* probes that were used to test the interaction of AtVOZ2 with the *MOS3/SAR3* promoter were prepared by mixing equal volumes of the sense oligonucleotide (0.1 nmol/µl in 10 mM Tris-HCl [pH 8.0]) and the Cy5-labeled antisense oligonucleotide (0.1 nmol/µl in 10 mM Tris-HCl [pH 8.0]) (Integrated DNA technologies), then heating the mixture at 65℃ for 5 minutes and slow-cooling it to 30℃. The oligonucleotides used to prepare the *pMOS3* probe were sense (5′-GACGTCCGGCGCAGCGTTTATCAGACGC-TGGGATTAAAACA-3′) and the *Cy5-labeled* antisense oligonucleotide (5′-/Cy5/TGTTTTAATCCCAGCGTCTGATAAACGCTGCGCCGGACGTC-3′). To prepare the *pmos3-m* probe the mutated sense (5′-GACGTCCGGCGCATTTTT-TATTTTTTTTTGGGATTAAAACA-3′) and mutated antisense (5′-/Cy5/TGT-TTTAATCCCAAAAAAAAATAAAAAATGCGCCGGACGTC-3′) probes were used.

EMSA reaction mixtures (10 µl final volume) with purified AtVOZ2 and AtVOZ2m proteins and *pMOS3* and *pmos3-m* promoter probes were combined in microfuge tubes on ice in the following order: 6 µl water, 1 µl 5× EMSA buffer (5× EMSA buffer: 20 mM HEPES [pH 7.9], 500 mM KCl, 40% glycerol [v/v], 25 mM EGTA [pH 8.0], 0.5 mM DTT, 1.25 mM $ZnCl_2$. DTT and $ZnCl_2$ were added to 5× buffer immediately before use), 2 µl purified protein (up to 2.4 µg), 1 µl fluorescently labeled probe (at 2 or 20 pmole/µl). Reactions were incubated at room temperature for 30 minutes. Loading dye (25 mM Tris-HCl [pH 7.5], 4% [v/v] glycerol, 0.02% [w/v] Bromophenol blue) was added (1 µl) and the samples were run immediately on 6% non-denaturing acrylamide gels for 1 hour at 100 V in TAE buffer. Fluorescent bands on gels were visualized using the Typhoon Trio imaging system.

Chromatin immunoprecipitation (ChIP)

ChIP analyses were performed as described previously with minor changes (Du et al., 2009). In short, protoplasts from ~4-week-old WT plants were transfected with 40 µg of *YFP* control, *AtVOZ1-YFP* or AtVOZ2-YFP plasmids and cross-linked. Cross-linking and DNA isolation were done as before (Du et al., 2009). The sheared lysates were pre-cleared with agarose beads and incubated with 60 µl anti-GFP beads (D153-8, MBL) for 6 hours at 4℃. Beads were washed five times, resuspended in elution buffer and cross-linking was reversed by heating at 65℃ for 12 hours. The immunoprecipitated DNA was purified using a DNA purification kit (Qiagen) and used as a template in PCR reactions (28 cycles) with MOS3 UE-F (5′-AGGAGGGAAAACGAATTGAGTC-3′) and MOS3 UE-R (5′-CCGAATT-CCTTTCCAATTAAAGTCAAC-3′) that flanked the $GCGT_{TTATCAG}ACGC$ sequence in the *MOS3/SAR3* promoter. PCR amplification using primers in the *actin-2* promoter served as a control for ChIP specificity; ACT2 UE-F (5′-GCCATCAAAGCAAAAGAACTAATC-3′) and ACT2 UE-R (5′-ATGAATT-TATATAGGCGGGTTTATCTC-3′).

Results

AtVOZ transcription factors regulate transition to flowering

Arabidopsis Zn^{2+}-finger transcription factors AtVOZ1 and AtVOZ2 share high sequence similarity and contain an

N-terminal region with transactivating activity, and a C-terminal region that functions as the DNA-binding domain comprising a Zn^{2+}-finger motif and a conserved basic region structurally similar to the NAC domain (Mitsuda et al., 2004; Jensen et al., 2010). To investigate the role of AtVOZs in plant growth and development, three *Arabidopsis* T-DNA insertion lines were examined: the single mutants *atvoz1-1* (WiscDsLox489-492010) and *atvoz2-1* (salk_115813c) obtained from ABRC and the double mutant *atvoz1-1 atvoz2-1* generated by crossing single mutant lines (Fig. 1A). When plants were grown in long-day conditions (16 hours light, 8 hours dark), a severe delay in flowering time was observed for the double *atvoz1-1 atvoz2-1* mutants compared to wild type (WT; ecotype Col-0) (Fig. 1B,C; supplementary material Table S1). In contrast, initiation of flowering differed only slightly, although significantly ($P<0.05$) for single mutants compared to WT, suggesting functional redundancy between the AtVOZ proteins (Fig. 1B,C, top panels; supplementary material Table S1). The number of rosette leaves at bolting was 2.5-fold higher (an average of 23.8 more leaves) for *atvoz1-1 atvoz2-1* than for WT (Fig. 1B; supplementary material Table S1). Moreover, the flowering time measured as days to opening of the first flower was greatly delayed for the double mutant, which flowered at 65.5±1.5 days or 23.4 days later than WT (Fig. 1C; supplementary material Table S1). At that age, WT plants were fully mature with the mean height of 7.8±1.2 inches ($n=70$) and producing seeds (Fig. 1C, bottom panel). In short days (8 hours light, 16 hours dark), the flowering time of *atvoz1-1 atvoz2-1* double mutant (153.9±7.4 days) was also strongly delayed in comparison to WT (112.0±3.4 days) (supplementary material Table S1). Interestingly, in spite of the greatly delayed flowering time, the *atvoz1-1 atvoz2-1* leaf count in SDs was similar to that of WT plants (supplementary material Table S1), suggesting that overall slower growth of the double mutant may have contributed to delayed flowering time in SD. Complementation of the double mutant line with AtVOZ2 (AtVOZ2; *atvoz1-1 atvoz2-1*) reversed all double mutant phenotypes in LDs and SDs (Fig. 1; supplementary material Table S1). In fact, in long days AtVOZ2 overexpression in

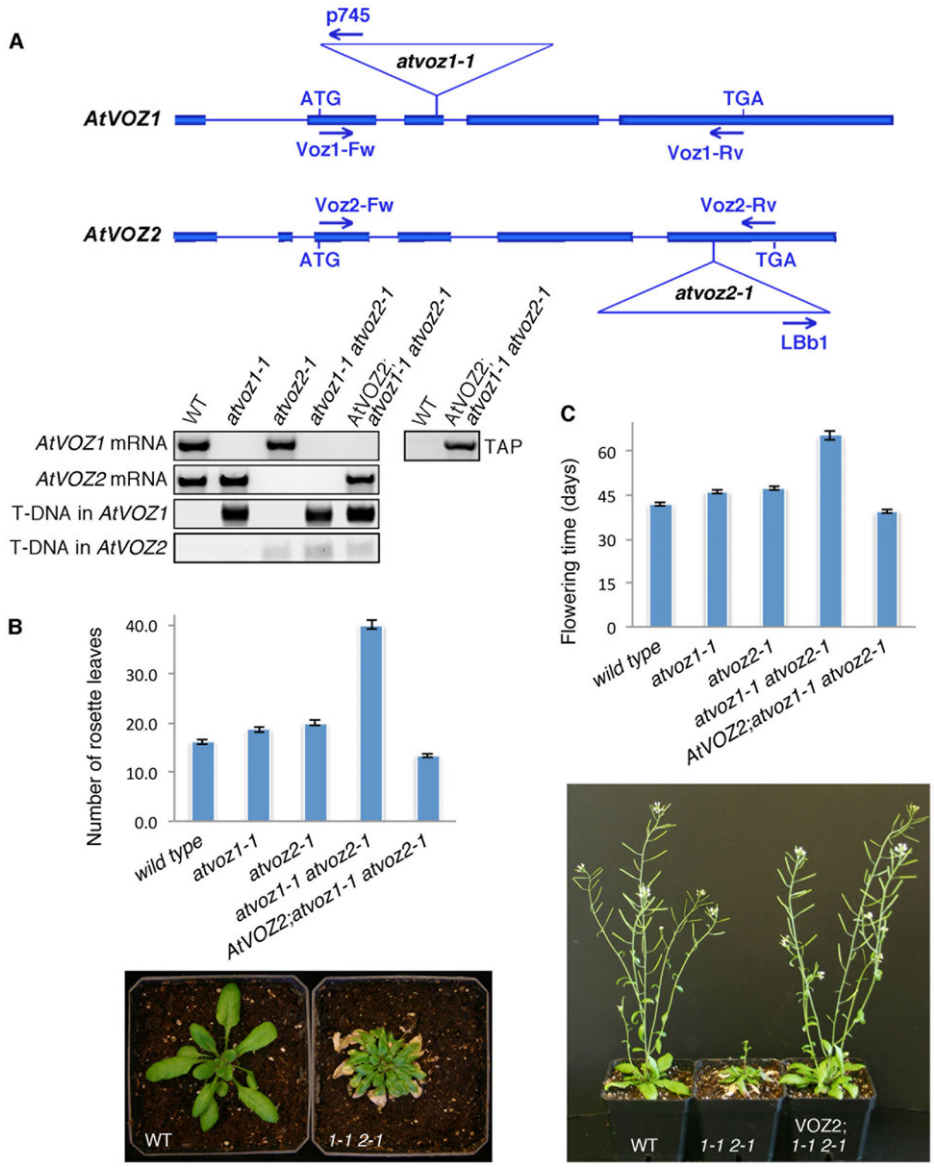

Fig. 1. AtVOZ proteins are activators of floral transition in *Arabidopsis*. (**A**) Top: diagrammatic representation of *AtVOZ1* and *AtVOZ2* genes. Exons, blue rectangles; introns, blue lines. T-DNA insertion sites in *atvoz* mutants are indicated by triangles. Bottom: genotyping of *atvoz1-1* and *atvoz2-1* single mutants, *atvoz1-1 atvoz2-1* double mutant, and AtVOZ2; *atvoz1-1 atvoz2-1* complemented line with gene-specific or T-DNA-specific primers indicated in the above diagram. TAP-specific primer was used to detect expression of *CTAP-AtVOZ2* in the complemented line. (**B**) Top: number of rosette leaves at bolting for WT, *atvoz* single and double mutants and complemented line under LD conditions. Mean values±s.e.m. are shown. Bottom: phenotypic comparison of WT and *atvoz1-1 atvoz2-1* (*1-1 2-1*) plants at the beginning of bolting. Increased leaf number and extensive senescence is seen for *atvoz1-1 atvoz2-1*. (**C**) Top: flowering time, measured as number of days to opening of the first flower, for WT, *atvoz* single and double mutants and complemented line under LD conditions. Mean values±s.e.m. are shown. Bottom: phenotypic comparison of 9-week-old WT, *atvoz1-1 atvoz2-1* (*1-1 2-1*) and complemented (*VOZ2;1-1 2-1*) plants at the time of opening of the first *atvoz1-1 atvoz2-1* flower.

atvoz1-1 atvoz2-1 resulted in slightly precocious flowering at fewer leaves than WT. These complementation results confirmed that the observed changes for *atvoz1-1 atvoz2-1* were due to lack of AtVOZs and corroborated that these proteins overlap in function. Together, our results show that AtVOZs are important regulators of transition to flowering.

AtVOZ proteins function in early *Arabidopsis* development

Aside from changes in flowering time, *atvoz1-1 atvoz2-1* mutant plants displayed other pleiotropic phenotypes, indicating that AtVOZs perform additional functions in *Arabidopsis* growth. In early vegetative development, double *atvoz1-1 atvoz2-1* mutants appeared smaller than WT, particularly under short-day conditions, with some of their leaves showing senescence (Fig. 2A). Senescence became more extensive and apparent as plants aged (Fig. 1B). Some of the leaves also curled into a distinctive funnel shape (Fig. 2B, right panel). *atvoz1-1 atvoz2-1* leaves mostly had a round lamina and were smaller, which is characteristic of juvenile leaves (Fig. 2B, left panel). Indeed, analysis of leaf trichomes revealed a greater number of juvenile leaves (i.e. those without abaxial trichomes) in the double mutant compared to WT, as well as a large overall increase in the number of juvenile, adult (i.e. with abaxial trichomes) and cauline leaves at the time of flowering in LD conditions (Fig. 2C). The delay in transition from juvenile to adult leaves as compared to WT suggests that AtVOZs influence the vegetative phase change in *Arabidopsis*. Expectedly, single *atvoz1-1* and *atvoz2-1* mutants looked similar to WT and displayed only slight trichome and leaf count differences (Fig. 2A,C; supplementary material Fig. S1). AtVOZ2-complementation of the double mutant reversed all phenotypes (Fig. 2A–C).

AtVOZ proteins affect silique and seed development

Double atvoz1-1 atvoz2-1 mutants also exhibited changes in the reproductive phase following the flowering. *atvoz1-1 atvoz2-1* siliques were smaller than WT siliques (9.7±1.7 mm compared to 14.5±1.2 mm, $n>100$) and consequently contained fewer seeds (Fig. 2D; supplementary material Table S2). A large fraction of double mutant seeds were non-viable (Fig. 2E). Individual siliques on a single plant contained variable numbers of seed abortants, ranging from siliques holding mostly or exclusively viable seeds to siliques containing only abortants or unfertilized ovules. The percentage of abortive seeds varied considerably from one plant to another and between experiments, but an increase in seed abortants was always observed in *atvoz1-1 atvoz2-1* compared to WT plants. Measurements from one experiment are presented in supplementary material Table S2. In contrast to silique differences, inspection of flower architecture (i.e. number and arrangement of floral organs and flower size) under the dissecting microscope showed no dissimilarities between *atvoz1-1 atvoz2-1* and WT.

AtVOZs control expression of *FLC* and *FLC* regulators

To investigate the molecular mechanisms behind the observed phenotypic changes of *atvoz1-1 atvoz2-1* plants, we examined mRNA levels of several flowering-time genes in the *atvoz1-1 atvoz2-1* mutant. Expression of the flowering repressor *FLC* was strongly increased in the double mutant, in agreement with the late flowering phenotype of *atvoz1-1 atvoz2-1* (Fig. 3; supplementary material Fig. S2). Upregulation of *FLC* was reversed in double knockout plants complemented with AtVOZ2 (Fig. 3; supplementary material Fig. S2).

A number of genes have been shown to control *FLC* expression. We examined transcript levels of several *FLC* regulators in *atvoz1-1 atvoz2-1* (Fig. 3). Expression of *VERNALIZATION INDEPENDENCE3* (*VIP3*) and *EARLY FLOWERING7* (*ELF7*) was increased in the *atvoz* double mutant. *VIP3* and *ELF7* encode proteins that are related to components of the yeast PAF1-like complex, known to associate with RNA polymerase II and direct histone modifications by

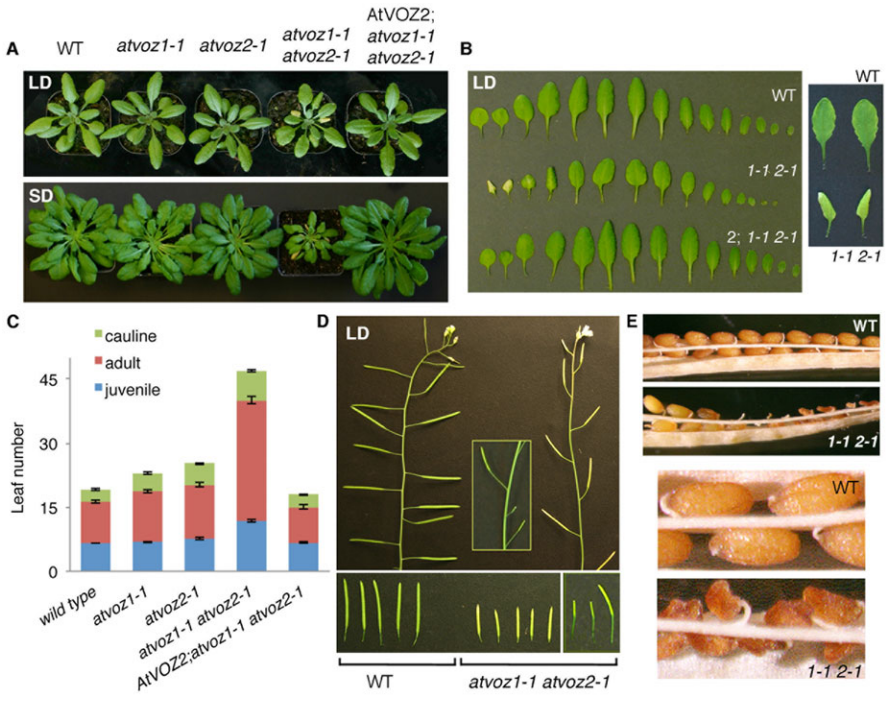

Fig. 2. Pleiotropic phenotypes of *atvoz1-1 atvoz2-1*. (**A**) Phenotypic comparison of WT, *atvoz1-1* and *atvoz2-1* single mutants, *atvoz1-1 atvoz2-1* double mutant, and AtVOZ2; *atvoz1-1 atvoz2-1* complemented line in LDs (4-week-old plants) and SDs (12-week-old plants). (**B**) Left: leaf spread of 4-week-old WT, *atvoz1-1 atvoz2-1* (*1-1 2-1*) and AtVOZ2-complemented *atvoz1-1 atvoz2-1* mutant (2; *1-1 2-1*). Right: characteristic funnel shape of *atvoz1-1 atvoz2-1* leaves. (**C**) *atvoz1-1 atvoz2-1* displays increased number of juvenile, adult and cauline leaves in LDs. Mean values±s.e.m. are shown. (**D**) Top: shorter siliques of *atvoz1-1 atvoz2-1* compared to WT. Inset: some *atvoz1-1 atvoz2-1* plants display an alternating medium-size and very short siliques. Bottom: spread of WT (left) and *atvoz1-1 atvoz2-1* siliques (middle, right). (**E**) Top: *atvoz1-1 atvoz2-1* siliques contain both viable and abortive seeds. Bottom: close-up of WT seeds and *atvoz1-1 atvoz2-1* abortive seeds.

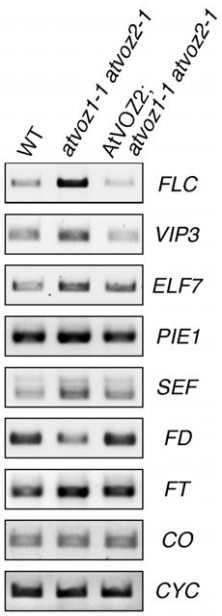

Fig. 3. AtVOZs control the *FLC* flowering pathway. Expression of flowering genes in WT, *atvoz1-1 atvoz2-1* and AtVOZ2; *atvoz1-1 atvoz2-1* plants, measured by RT-PCR. Cyclophilin (*CYC*): template control.

histone methyltransferases (He et al., 2004; Oh et al., 2004). Modifications of *FLC* by the *Arabidopsis* PAF1-like complex were postulated by Oh et al. to possibly involve recruitment of the *FLC*-chromatin remodeling factors such as *PHOTOPERIOD-INDEPENDENT EARLY FLOWERING 1* (*PIE1*) (Oh et al., 2004), a homologue of a member of the yeast SWR1 complex that is involved in histone H2A variant replacement (Deal et al., 2007). *PIE1* exhibited only mild transcript level changes in *atvoz1-1 atvoz2-1* (Fig. 3). However, a significant upregulation in the *atvoz1-1 atvoz2-1* mutant was observed for another component of SWR1, the *FLC* activator *AtSWC6/SERRATED LEAVES AND EARLY FLOWERING* (*SEF*) (Lázaro et al., 2008) (Fig. 3). Together, our data show that AtVOZs regulate *FLC* levels, and that the observed *FLC* upregulation correlates with increased expression of genes involved in *FLC* chromatin modification. In contrast, *CONSTANS* (*CO*), the main photoperiod pathway gene, showed similar expression in WT and the double mutant (Fig. 3).

FLC encodes a MADS box protein that directly represses certain flowering-time genes. An important target of FLC is the *FD* gene, which encodes a bZIP transcription factor preferentially expressed in the shoot apex. FD associates with the flowering pathway integrator FLOWERING LOCUS T (FT) to activate downstream flowering activators (Abe et al., 2005). FT encodes a RAF kinase inhibitor-like protein and functions as a long distance signal between the leaves and the shoot meristem to promote flowering. In *atvoz1-1 atvoz2-1*, the increased *FLC* expression was correlated with decreased expression of *FD*, consistent with the late flowering of the mutant (Fig. 3). The transcript level of the flowering promoter FT showed a slight increase (Fig. 3). Since it is known that FD is required for FT to promote flowering, as an *fd-1* mutation has been shown to suppress early flowering of 35S::FT overexpressing plants (Abe et al., 2005), the decrease in *FD* expression appears to be enough for delayed flowering in the *atvoz1-1 atvoz2-1* mutant.

The *atvoz1-1 atvoz2-1* mutant is responsive to vernalization

The delayed flowering of *atvoz1-1 atvoz2-1* correlates with increased expression of the flowering gene *FLC*. FRI-containing lines and mutants in the autonomous pathway also have elevated *FLC* expression and delayed flowering, and show sensitivity to vernalization, which can abolish their late flowering through an epigenetic shut off of *FLC* expression. In contrast, photoperiod pathway mutants remain relatively uninfluenced by this treatment (Martinez-Zapater and Somerville, 1990; Koornneef et al., 1991; Mouradov et al., 2002; Moon et al., 2003; Lim et al., 2004). Similar to FRI-carrying lines and autonomous pathway mutants, *atvoz1-1 atvoz2-1* plants responded to vernalization. Vernalized *atvoz1-1 atvoz2-1* plants flowered much earlier than their non-vernalized counterparts. In fact, the flowering time was comparable to that of WT plants (Fig. 4A). Moreover, *FLC* transcript level was reduced to WT levels in the vernalized double mutant, in agreement with the complete recovery of flowering time (Fig. 4B).

However, in addition to reversing flowering time to WT levels, vernalization treatment caused several other changes in *atvoz1-1 atvoz2-1* plants (Fig. 4C–I). The flowers of *atvoz1-1 atvoz2-1* plants were mostly smaller and had non-dehiscent or shriveled anthers (Fig. 4D–F). Many had an aberrant number of stamens (~33%). DAPI-staining of *atvoz1-1 atvoz2-1* pollen revealed defects in pollen development in a small fraction (11%) of pollen grains; during normal development, pollen mitosis II of the generative cell creates two sperm cells that associate with the vegetative nucleus, but in abnormal *atvoz1-1 atvoz2-1* pollen grains only one sperm cell was observed (Fig. 4I). Vernalized *atvoz1-1 atvoz2-1* plants generated little or no seed, since they were mostly producing very small siliques that contained only aborted seeds or unfertilized ovules (Fig. 4C,G,H). It is unclear why vernalization treatment caused extensive pleiotropic effects in *atvoz1-1 atvoz2-1* plants. Perhaps a vernalization-responsive gene or genes involved in reproductive organ and seed development is/are specifically deregulated in *atvoz1-1 atvoz2-1*.

AtVOZs directly regulate expression of *MOS3*, which encodes a nuclear pore constituent involved in flowering

To investigate whether AtVOZ transcription factors directly regulate the genes examined in Fig. 3, we searched for the consensus VOZ binding site ($GCGTN_{\times 7}ACGC$) (Mitsuda et al., 2004) in their promoter sequences. Neither the *FLC* gene, nor the abovementioned *FLC* regulators and targets contained $GCGTN_{\times 7}ACGC$ in their promoter regions. In addition, none had either of the 2 suboptimal elements that have been reported to allow reduced AtVOZ binding ($GCGTN_{\times 7}ACG\mathbf{T}$ and $GCGT\mathbf{N}_{\times 8}ACGC$). Considering this, AtVOZs might be regulating these flowering genes indirectly rather than by direct binding. Another possibility is that AtVOZs could recognize other, yet unidentified binding elements in promoter sequences of these genes.

To identify flowering genes that contain the VOZ binding element, we performed a genome-wide search for the presence of $GCGTN_{\times 7}ACGC$ in promoter sequences up to 1000 bp upstream from the translation initiation site. The search identified the gene *MODIFIER OF SNC1,3* (*MOS3*)/*SUPPRESSOR OF AUXIN RESISTANCE 3* (*SAR3*) whose promoter region contains the palindrome $GCGT_{TTATCAG}ACGC$. *MOS3/SAR3* encodes a nuclear membrane protein with homology to human nucleoporin 96, a subunit of a nuclear pore complex involved

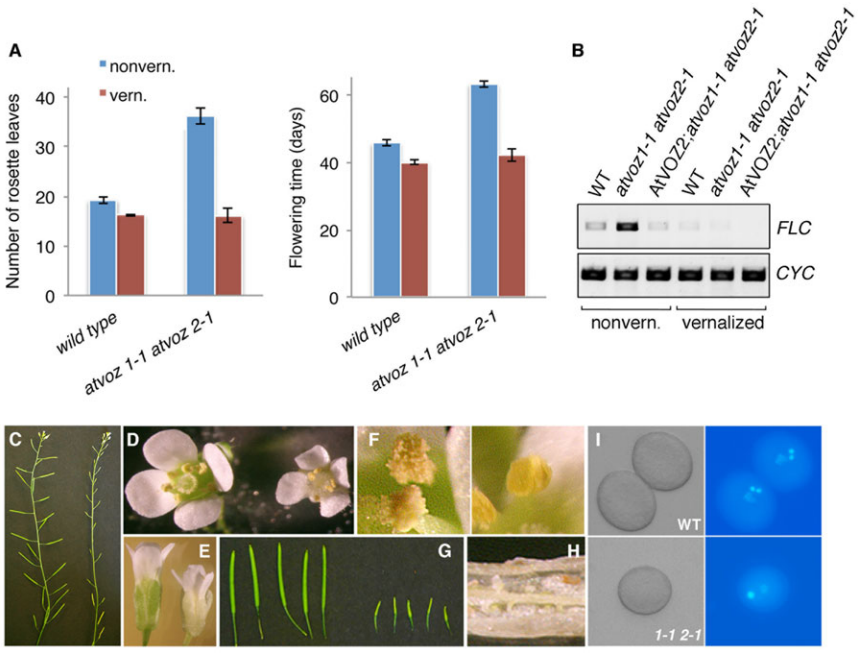

Fig. 4. Vernalization reverses *FLC*-mediated delayed flowering of *atvoz1-1 atvoz2-1* mutants. (**A**) Rosette leaf number at bolting (left) and time to opening of the first flower (right) of non-vernalized (blue) and vernalized (red) WT and *atvoz1-1 atvoz2-1* plants. Mean values±s.e.m. are shown. (**B**) *FLC* expression in non-vernalized and vernalized plants, measured by RT-PCR. Cyclophilin (*CYC*): template control. (**C–I**) *Pleiotropic effects of vernalization on atvoz1-1 atvoz2-1*. (C) Inflorescences of vernalized WT (left) and *atvoz1-1 atvoz2-1* (right) plants. (D,E) Flower size of vernalized WT (left) and *atvoz1-1 atvoz2-1* (right) plants. (F) WT anthers (left) and non-dehiscent *atvoz1-1 atvoz2-1* anthers (right) from vernalized plants. (G) Silique sizes of vernalized WT (left) and *atvoz1-1 atvoz2-1* (right) plants. (H) Silique from vernalized *atvoz1-1 atvoz2-1* containing unfertilized ovules and abortive seeds. (I) Pollen from vernalized WT and *atvoz1-1 atvoz2-1* flowers seen under bright field (left) and stained with DAPI (right).

in nucleocytoplasmic trafficking of macromolecules (Zhang and Li, 2005). MOS3/SAR3 regulates export of mRNAs, together with two components of the same nuclear pore subcomplex, Nup160/SAR1 and Seh1 (Parry et al., 2006; Wiermer et al., 2012). Mutants of *MOS3/SAR3* are smaller and less robust than WT plants and flower significantly earlier and with fewer leaves (Zhang and Li, 2005; Parry et al., 2006). *Nup160/sar1* are also early flowering (Dong et al., 2006) and double mutants *mos3/sar3 nup160/sar1* exhibit severe developmental defects (Parry et al., 2006). The *mos3/sar3* mutation strongly promotes flowering in the FRI-containing background, connecting MOS3/SAR3 with the *FLC* flowering pathway (Jacob et al., 2007). Another nuclear pore protein, AtTPR, has been shown to regulate flowering time and upregulate expression of *FLC*, further linking nuclear pore function with the flowering time (Jacob et al., 2007). Gene expression analysis in *atvoz1-1 atvoz2-1* showed a substantial increase in *MOS3/SAR3* expression, indicating that AtVOZs control this gene (Fig. 5A; supplementary material Fig. S2). The observed upregulation was restored to WT levels in the complemented line AtVOZ2; *atvoz1-1 atvoz2-1*.

To test whether AtVOZs regulate *MOS3/SAR3* directly by binding to its promoter, we performed electrophoretic mobility shift assay (EMSA) using a fluorescently labeled 41-bp-long promoter probe encompassing the $GCGT_{TTATCAG}ACGC$ element (Fig. 5B). EMSA revealed that purified AtVOZ2 bound to the probe in a dose-dependent manner. The binding was dependent on the zinc finger domain of AtVOZ2, as point mutations in two conserved amino acids (C249A/H253A) in that region completely abolished this interaction (AtVOZ2m, Fig. 5B). Furthermore, introducing mutations in the $GCGT_{TTATCAG}ACGC$ sequence (*pmos3-m*, Fig. 5B) diminished AtVOZ2 binding, indicating that AtVOZ2 interacts with the consensus VOZ-binding element. Due to difficulties expressing AtVOZ1, this protein was not tested by EMSA. The binding of both proteins was confirmed in plant cells by chromatin immunoprecipitation (ChIP) analysis. PCR of immunoprecipitated DNA using primers flanking the AtVOZ binding site in the *MOS3/SAR3* promoter revealed that AtVOZ1 and AtVOZ2 associate with the *MOS3* promoter *in vivo* (Fig. 5C). Together, our results show that AtVOZs directly regulate *MOS3/SAR3*, which is required for proper timing of flowering, by binding to its promoter and repressing its expression, and suggest that at least part of AtVOZ activities are exerted through influencing the nuclear pore complex.

Discussion

Our data demonstrate an important role of AtVOZ1 and AtVOZ2 transcription factors in regulating the transition from vegetative growth to flowering. *atvoz1-1 atvoz2-1* plants exhibit delayed flowering, which correlates with increased *FLC* expression and shows a robust response to vernalization. Vernalization causes a decrease in *FLC* transcript abundance and restores flowering time of *atvoz1-1 atvoz2-1* to WT levels. Recently, Yasui et al. showed that phytochrome B interacts with AtVOZs (Yasui et al., 2012). They have also reported that *atvoz1 atvoz2* double mutant is late flowering. In addition to flowering-time control, we observed other phenotypes, such as delayed juvenile phase and defects in seed production, in the *atvoz1-1 atvoz2-1* double mutant, suggesting a broader role for AtVOZs in plant development. Functions additional to flowering-time control are often observed for genes in flowering pathways. For instance, *MSI1*, a flowering-time gene in the autonomous pathway, is also involved in gametophyte and seed development (Bouveret et al., 2006). Furthermore, due to additional functions, double mutants in some autonomous pathway genes display severe pleiotropic phenotypes or even lethality (Koornneef et al., 1998; Veley and Michaels, 2008). Homologues of AtVOZ1 and AtVOZ2 are found in various vascular plants as well as in the moss *Physcomitrella patens* (Mitsuda et al., 2004), indicating that AtVOZs are not specific to flowering plants. This is consistent with their additional role in growth and development.

Our experimental data indicate that AtVOZs directly regulate expression of the nuclear pore protein MOS3/SAR3, required for export of mRNA from the nucleus. Like AtVOZs, MOS3/SAR3 is required for proper plant growth and flowering time, further

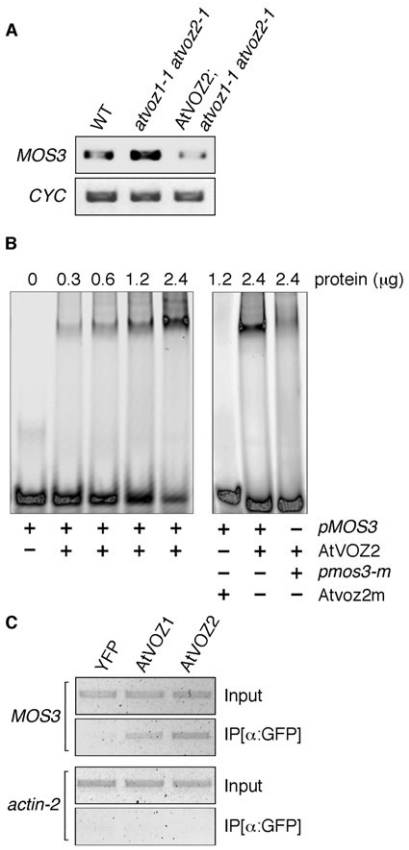

Fig. 5. AtVOZs directly repress expression of the *MOS3/SAR3* gene, which encodes a nuclear pore protein involved in regulation of flowering time. (**A**) *MOS3/SAR3* expression measured by RT-PCR. Cyclophilin (*CYC*): template control. (**B**) Left: electrophoretic mobility shift assay (EMSA) showing dose-dependent binding of purified AtVOZ2 to *MOS3* promoter probe (*pMOS3*; 2 pmoles). Right: mutated AtVOZ2 (Atvoz2m) does not bind to *pMOS3* (20 pmoles). AtVOZ2 binding is reduced when the consensus VOZ binding element in *MOS3* probe is mutated (*pmos3-m*; 20 pmoles). (**C**) Chromatin immunoprecipitation (ChIP) analysis showing *in vivo* binding of AtVOZs to *MOS3* promoter. Complexes of AtVOZ1-YFP and AtVOZ2-YFP bound to DNA were precipitated with GFP-antibody beads (IP [α:GFP]). Precipitated sequences were PCR-amplified using *MOS3*-specific primers spanning the consensus VOZ binding element. YFP, negative control; *actin-2*, control for assay specificity. Input: PCR amplification of *MOS3* and *actin-2* promoter regions in extracts prior to immunoprecipitation.

functionally linking these proteins (Zhang and Li, 2005; Dong et al., 2006; Parry et al., 2006). As AtVOZs directly regulate *MOS3/SAR3*, it is possible they exert at least part of their activities by controlling the nuclear pore function. It is conceivable that the effect of AtVOZs on flowering could result from altered nucleocytoplasmic localization and consequently activity of some flowering-time transcripts, a possibility that requires further functional analyses. A similar mechanism was proposed by Faria et al. for vertebrate Nup96 (Faria et al., 2006), which is required in mice immunity for nuclear mRNA export of specific interferon-regulated genes in response to viral infection. They observed that reduced levels of Nup96 resulted in nuclear retention of these transcripts and postulated that such retention likely contributes to the lower levels of the immune proteins encoded by these mRNAs at the plasma membrane. Nuclear pore proteins have also been directly linked to transcript abundance (Dong et al., 2006; Wiermer et al., 2012). It is conceivable that the observed changes in transcript accumulation in *atvoz1-1 atvoz2-1* could partly be ascribed to that aspect of nuclear function. Additionally, the yeast nuclear pore Nup84 complex (equivalent to vertebrate Nup107-160 complex) is capable of activating transcription *in vivo* by tethering target genes to the nuclear pore and coupling transcription with mRNA export (Menon et al., 2005). Since none of the AtVOZ-controlled flowering genes mentioned in Fig. 3 carry the consensus VOZ binding site, it is intriguing to think that AtVOZs may control their expression indirectly via regulation of the nuclear pore complex.

Recently *MdVOZ1*, one of 5 predicted apple *VOZ1* and *VOZ2* genes, was implicated in reproduction in apple (Mimida et al., 2011). Like *Arabidopsis VOZ* transcripts, *MdVOZ1* mRNA was detected in multiple tissues, with a strong accumulation in fruit, sepals and petals, and weak expression in stamens and carpels. In <10% of transgenic *Arabidopsis* plants constitutively expressing apple MdVOZ1, sterile flowers with enhanced elongation of flower stalks and abnormal inflorescences were observed. This suggested involvement of MdVOZ1 in the development of reproductive organs (Mimida et al., 2011). In contrast to morphological changes in *Arabidopsis* plants constitutively expressing MdVOZ1, overexpression of AtVOZ2 in *atvoz1-1 atvoz2-1* double mutant resulted in plants that were visually indistinguishable from WT, suggesting that apple and *Arabidopsis* VOZ homologues diverged in their functions. However, lack of functional AtVOZs in *Arabidopsis* resulted in plants that produced smaller siliques and displayed substantial seed abortion, providing a correlation between the role of apple and *Arabidopsis* VOZ proteins in seed production.

Interestingly, MdVOZ1 was shown to interact with MdFT1 and MdFT2, apple orthologues of *Arabidopsis* FT. Based on this association and the expression patterns of apple VOZ1 and FTs, it was suggested that apple FTs might be involved in reproductive organ and fruit development in addition to flowering-time control. It is not known whether AtVOZ1 or AtVOZ2 also interact with FT in *Arabidopsis*. If so, such association could represent another level of flowering regulation, perhaps by: (i) targeting a specific subset of flowering genes, (ii) promoting modulation of AtVOZ and/or FT activities by their protein–protein interactions, or (iii) an intriguing idea is that AtVOZs could assist the FT florigen in leaf-to-meristem movement. In line with the third possibility is a recent report suggesting that *Nicotiana benthamiana* homologues of AtVOZ1, sc4i21 and Ni67, are utilized by plant-adapted rhabdoviruses for cell-to-cell movement (Min et al., 2010). Also, *AtVOZ1* is primarily expressed in the phloem (Mitsuda et al., 2004), in accord with FT movement.

Acknowledgements

This work was supported by a grant from the National Science Foundation (MCB-5333470 to A.S.N.R). We are grateful to Amy Lowery and Sarah-Marie Lyons for help with maintaining plant lines and assistance in *atvoz1-1 atvoz2-1* studies. We thank Drs Denghui Xing and Irene Day for helpful comments on this manuscript and the ABRC for providing T-DNA insertion lines of the *AtVOZ* single mutants. Plasmid constructs and mutants generated in this research are available upon request. We thank the Colorado State University Libraries Open Access Research and Scholarship Fund for paying the publication charges for this article.

Competing Interests

The authors have no competing interests to declare.

References

Abe, M., Kobayashi, Y., Yamamoto, S., Daimon, Y., Yamaguchi, A., Ikeda, Y., Ichinoki, H., Notaguchi, M., Goto, K. and Araki, T. (2005). FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* **309**, 1052-1056.

Bouveret, R., Schönrock, N., Gruissem, W. and Hennig, L. (2006). Regulation of flowering time by *Arabidopsis MSI1*. *Development* **133**, 1693-1702.

Clough, S. J. and Bent, A. F. (1998). Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* **16**, 735-743.

Deal, R. B., Topp, C. N., McKinney, E. C. and Meagher, R. B. (2007). Repression of flowering in *Arabidopsis* requires activation of *FLOWERING LOCUS C* expression by the histone variant H2A.Z. *Plant Cell* **19**, 74-83.

Dong, C. H., Hu, X., Tang, W., Zheng, X., Kim, Y. S., Lee, B. H. and Zhu, J. K. (2006). A putative *Arabidopsis* nucleoporin, AtNUP160, is critical for RNA export and required for plant tolerance to cold stress. *Mol. Cell. Biol.* **26**, 9533-9543.

Du, L., Ali, G. S., Simons, K. A., Hou, J., Yang, T., Reddy, A. S. and Poovaiah, B. W. (2009). Ca^{2+}/calmodulin regulates salicylic-acid-mediated plant immunity. *Nature* **457**, 1154-1158.

Faria, A. M., Levay, A., Wang, Y., Kamphorst, A. O., Rosa, M. L., Nussenzveig, D. R., Balkan, W., Chook, Y. M., Levy, D. E. and Fontoura, B. M. (2006). The nucleoporin Nup96 is required for proper expression of interferon-regulated proteins and functions. *Immunity* **24**, 295-304.

Golovkin, M. and Reddy, A. S. N. (2003). A calmodulin-binding protein from *Arabidopsis* has an essential role in pollen germination. *Proc. Natl. Acad. Sci. USA* **100**, 10558-10563.

He, Y., Doyle, M. R. and Amasino, R. M. (2004). PAF1-complex-mediated histone methylation of *FLOWERING LOCUS C* chromatin is required for the vernalization-responsive, winter-annual habit in *Arabidopsis*. *Genes Dev.* **18**, 2774-2784.

Jacob, Y., Mongkolsiriwatana, C., Veley, K. M., Kim, S. Y. and Michaels, S. D. (2007). The nuclear pore protein AtTPR is required for RNA homeostasis, flowering time, and auxin signaling. *Plant Physiol.* **144**, 1383-1390.

Jensen, M. K., Kjaersgaard, T., Nielsen, M. M., Galberg, P., Petersen, K., O'Shea, C. and Skriver, K. (2010). The *Arabidopsis thaliana* NAC transcription factor family: structure–function relationships and determinants of ANAC019 stress signalling. *Biochem. J.* **426**, 183-196.

Koornneef, M., Hanhart, C. J. and van der Veen, J. H. (1991). A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol. Gen. Genet.* **229**, 57-66.

Koornneef, M., Alonso-Blanco, C., Blankestijn-de Vries, H., Hanhart, C. J. and Peeters, A. J. (1998). Genetic interactions among late-flowering mutants of Arabidopsis. *Genetics* **148**, 885-892.

Lázaro, A., Gómez-Zambrano, A., López-González, L., Piñeiro, M. and Jarillo, J. A. (2008). Mutations in the *Arabidopsis SWC6* gene, encoding a component of the SWR1 chromatin remodelling complex, accelerate flowering time and alter leaf and flower development. *J. Exp. Bot.* **59**, 653-666.

Lee, I. and Amasino, R. M. (1995). Effect of vernalization, photoperiod, and light quality on the flowering phenotype of Arabidopsis plants containing the *FRIGIDA* gene. *Plant Physiol.* **108**, 157-162.

Lim, M. H., Kim, J., Kim, Y. S., Chung, K. S., Seo, Y. H., Lee, I., Kim, J., Hong, C. B., Kim, H. J. and Park, C. M. (2004). A new Arabidopsis gene, *FLK*, encodes an RNA binding protein with K homology motifs and regulates flowering time via *FLOWERING LOCUS C*. *Plant Cell* **16**, 731-740.

Martinez-Zapater, J. M. and Somerville, C. R. (1990). Effect of light quality and vernalization on late-flowering mutants of *Arabidopsis thaliana*. *Plant Physiol.* **92**, 770-776.

Menon, B. B., Sarma, N. J., Pasula, S., Deminoff, S. J., Willis, K. A., Barbara, K. E., Andrews, B. and Santangelo, G. M. (2005). Reverse recruitment: the Nup84 nuclear pore subcomplex mediates Rap1/Gcr1/Gcr2 transcriptional activation. *Proc. Natl. Acad. Sci. USA* **102**, 5749-5754.

Michaels, S. D. and Amasino, R. M. (1999). *FLOWERING LOCUSô C* encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* **11**, 949-956.

Michaels, S. D. and Amasino, R. M. (2001). Loss of *FLOWERING LOCUS C* activity eliminates the late-flowering phenotype of *FRIGIDA* and autonomous pathway mutations but not responsiveness to vernalization. *Plant Cell* **13**, 935-941.

Mimida, N., Kidou, S., Iwanami, H., Moriya, S., Abe, K., Voogd, C., Varkonyi-Gasic, E. and Kotoda, N. (2011). Apple FLOWERING LOCUS T proteins interact with transcription factors implicated in cell growth and organ development. *Tree Physiol.* **31**, 555-566.

Min, B. E., Martin, K., Wang, R., Tafelmeyer, P., Bridges, M. and Goodin, M. (2010). A host-factor interaction and localization map for a plant-adapted rhabdovirus implicates cytoplasm-tethered transcription activators in cell-to-cell movement. *Mol. Plant Microbe Interact.* **23**, 1420-1432.

Mitsuda, N., Hisabori, T., Takeyasu, K. and Sato, M. H. (2004). VOZ; isolation and characterization of novel vascular plant transcription factors with a one-zinc finger from *Arabidopsis thaliana*. *Plant Cell Physiol.* **45**, 845-854.

Moon, J., Suh, S. S., Lee, H., Choi, K. R., Hong, C. B., Paek, N. C., Kim, S. G. and Lee, I. (2003). The *SOC1* MADS-box gene integrates vernalization and gibberellin signals for flowering in *Arabidopsis*. *Plant J.* **35**, 613-623.

Mouradov, A., Cremer, F. and Coupland, G. (2002). Control of flowering time: interacting pathways as a basis for diversity. *Plant Cell* **14 Suppl. 1**, S111-S130.

Oh, S., Zhang, H., Ludwig, P. and van Nocker, S. (2004). A mechanism related to the yeast transcriptional regulator Paf1c is required for expression of the Arabidopsis *FLC/MAF* MADS box gene family. *Plant Cell* **16**, 2940-2953.

Parry, G., Ward, S., Cernac, A., Dharmasiri, S. and Estelle, M. (2006). The *Arabidopsis* SUPPRESSOR OF AUXIN RESISTANCE proteins are nucleoporins with an important role in hormone signaling and development. *Plant Cell* **18**, 1590-1603.

Poethig, R. S. (2003). Phase change and the regulation of developmental timing in plants. *Science* **301**, 334-336.

Rédei, G. P. (1962). Supervital mutants of Arabidopsis. *Genetics* **47**, 443-460.

Rubio, V., Shen, Y., Saijo, Y., Liu, Y., Gusmaroli, G., Dinesh-Kumar, S. P. and Deng, X. W. (2005). An alternative tandem affinity purification strategy applied to Arabidopsis protein complex isolation. *Plant J.* **41**, 767-778.

Schmitz, R. J. and Amasino, R. M. (2007). Vernalization: a model for investigating epigenetics and eukaryotic gene regulation in plants. *Biochim. Biophys. Acta* **1769**, 269-275.

Sheldon, C. C., Burn, J. E., Perez, P. P., Metzger, J., Edwards, J. A., Peacock, W. J. and Dennis, E. S. (1999). The *FLF* MADS box gene: a repressor of flowering in Arabidopsis regulated by vernalization and methylation. *Plant Cell* **11**, 445-458.

Simpson, G. G. and Dean, C. (2002). *Arabidopsis*, the Rosetta stone of flowering time? *Science* **296**, 285-289.

Srikanth, A. and Schmid, M. (2011). Regulation of flowering time: all roads lead to Rome. *Cell. Mol. Life Sci.* **68**, 2013-2037.

Veley, K. M. and Michaels, S. D. (2008). Functional redundancy and new roles for genes of the autonomous floral-promotion pathway. *Plant Physiol.* **147**, 682-695.

Wiermer, M., Cheng, Y. T., Imkampe, J., Li, M., Wang, D., Lipka, V. and Li, X. (2012). Putative members of the Arabidopsis Nup107-160 nuclear pore sub-complex contribute to pathogen defense. *Plant J.* **70**, 796-808.

Yasui, Y., Mukougawa, K., Uemoto, M., Yokofuji, A., Suzuri, R., Nishitani, A. and Kohchi, T. (2012). The phytochrome-interacting VASCULAR PLANT ONE-ZINC FINGER1 and VOZ2 redundantly regulate flowering in *Arabidopsis*. *Plant Cell* **24**, 3248-3263.

Zhang, Y. and Li, X. (2005). A putative nucleoporin 96 is required for both basal defense and constitutive resistance responses mediated by *suppressor of npr1-1,constitutive 1*. *Plant Cell* **17**, 1306-1316.