The pea *VEGETATIVE2* gene is an *FD* homolog that is essential for flowering and compound inflorescence development

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Synopsis:

Characterisation of the pea *VEGETATIVE2* (*VEG2*) gene provides new insight into the mechanisms underlying compound inflorescence development in pea.

ABSTRACT

As knowledge of the gene networks controlling inflorescence development in Arabidopsis improves, the current challenge is to characterise this system in different groups of crop species with different inflorescence architecture. Pea (*Pisum sativum* L.) has served as a model for development of the compound raceme characteristic of many legume species, and in this study we characterise the pea *VEGETATIVE2* (*VEG2*) locus, showing that it is critical for control of flowering and inflorescence development, and identifying it as a homolog of the bZIP transcription factor *FD*. Through detailed phenotypic characterizations of *veg2* mutants, expression analyses and the use of protein-protein interaction assays, we find that *VEG2* has important roles during each stage of development of the pea compound inflorescence. Our results suggest that VEG2 acts in conjunction with multiple FT proteins to regulate expression of downstream target genes including *TFL1*, *LFY* and MADS-box homologs, and to facilitate cross-regulation within the *FT* gene family. These findings further extend our understanding of the mechanisms underlying compound inflorescence development in pea, and may have wider implications for future manipulation of inflorescence architecture in related legume crop species.

INTRODUCTION

Inflorescences are the shoot structures that bear flowers, and their form and arrangement has important implications for reproductive success and ease of harvest in agricultural systems (Wyatt, 1982). Angiosperm species exhibit incredible diversity in inflorescence form, which derives from complexity and pattern of branching, the number and position of flowers and the capacity of the inflorescence for continued growth (Weberling, 1992). At the tissue level, this variation can be attributed to differences in the identity and activity of the shoot meristems that produce each component of the inflorescence. Among many genes that have a role in controlling flowering, a subset also have a role in specifying the identity of vegetative, inflorescence or floral meristems, and it is the interaction of these genes that determines how the inflorescence develops. Understanding the genes and regulatory interactions that underlie the development of different inflorescence forms is a crucial step to enable future optimisation of inflorescence architecture for maximal crop productivity.

Like most plant processes, inflorescence development is best understood in the model species Arabidopsis. The Arabidopsis inflorescence is a simple raceme, in which flowers are borne directly on the main stem and the shoot apex remains indeterminate, with organogenesis balanced by self-renewal (**Figure 1A**). Two key genes, *FLOWERING LOCUS T* (*FT*) and *TERMINAL FLOWER1* (*TFL1*), have

a major role in generating this inflorescence form, through antagonistic effects on the expression of meristem identity genes (Kardailsky et al., 1999; Kobayashi et al., 1999; McGarry and Ayre, 2012; Jaeger et al., 2013). FT and TFL1 both belong to the phosphatidylethanolamine binding protein (PEBP) family and individually interact with the basic leucine zipper (bZIP) transcription factors FD and FD PARALOG (FDP) to regulate expression of floral target genes within the apex (Abe et al., 2005; Wigge et al., 2005; Hanano and Goto, 2011). TFL1/FD complexes delay flowering and prevent upregulation of floral genes within the shoot apical meristem (SAM) to maintain shoot indeterminacy (Hanano and Goto, 2011). FT/FD complexes promote expression of floral genes, ultimately resulting in the induction of the MADS-box gene *APETALA1* (*AP1*) in axillary meristems to specify floral identity (Abe et al., 2005; Wigge et al., 2005). *AP1* is also upregulated by the floral integrator and floral identity gene *LEAFY* (*LFY*), which acts independently of the *FT*/*FD* pathway (Ruiz-Garcia et al., 1997; Abe et al., 2005; Wigge et al., 2005). Within floral meristems, *AP1* and *LFY* directly repress *TFL1* expression to maintain determinacy (Wagner et al., 1999; Kaufmann et al., 2010).

In this study, we investigate genes controlling development of the compound raceme of pea. Pea is an important crop plant, and is also representative of other agronomically significant legume species within the Papilionoideae, including lentil, chickpea, common bean and soybean, which share similar inflorescence architecture. Relative to the simple raceme of Arabidopsis, the compound raceme of pea has an additional level of branching, such that flowers are not directly borne on the main inflorescence stem but are instead borne on modified lateral branches, termed secondary inflorescences (I_2s; **Figure 1B**). Pea inflorescence development can thus be considered to consist of three distinct stages. During vegetative growth, the SAM has vegetative (V) identity, and produces the main stem, bearing alternate leaves with vegetative axillary buds, which normally remain dormant. On commitment to flowering, the pea vegetative SAM undergoes a transition to a primary inflorescence (I_1) meristem (Ferguson et al., 1991), which we refer to here as the V/I_1 transition, the first stage of inflorescence development. The I_1 meristem is similar to the vegetative SAM, in that it remains indeterminate and produces the shoot tissues (stem and leaves) of the main stem. However, the I_1 is distinguished by the fact that it bears an axillary I_2 at each stem node instead of a vegetative bud (Singer et al., 1999). Specification of I_2 meristem identity is the second stage of pea inflorescence development. Each I_2 is leafless and determinate, terminating in a hairy stub after bearing several axillary flowers (Hole and Hardwick, 1976). Specification of floral meristem identity is the third and final stage of pea inflorescence development.

A number of key genes that control inflorescence development in pea have been identified based on characterisation of pea mutants with altered inflorescence form (summarised in **Supplemental Figure 1**) . Mutants of *PROLIFERATING INFLORESCENCE MERISTEM* (*PIM*), an *AP1* homolog, fail to correctly specify floral meristems (Taylor et al., 2002). In accordance with a conserved role as a floral meristem identity gene, expression of *PIM* is limited to floral meristems (Berbel et al., 2001). *DETERMINATE* (*DET*), a *TFL1* homolog (*TFL1a*), is expressed within the I_1 meristem, where it promotes SAM indeterminacy (Foucher et al., 2003; Berbel et al., 2012). *det* mutants exhibit conversion of the I_1 to an I_2, which terminates the main stem (Singer et al., 1990). Unlike Arabidopsis *TFL1*, *DET* has no influence on flowering time in pea, and this role is instead played by *LATE FLOWERING* (*LF*), a second pea *TFL1* homolog (*TFL1c*), which acts to delay flowering (Foucher et al., 2003).

Three other pea loci of particular interest for understanding the control of inflorescence development are *GIGAS*, *VEGETATIVE1* (*VEG1*) and *VEGETATIVE2* (*VEG2*). Plants carrying severe mutant alleles for any of these loci exhibit normal vegetative development but fail to develop I_2 or floral structures under long day (LD) photoperiods, suggesting that these loci have critical roles in pea inflorescence development (Reid and Murfet, 1984; Murfet and Reid, 1993; Beveridge and Murfet, 1996). *GIGAS* has been characterized as the *FT* homolog, *FTa1*, which is thought to encode a graft-transmissible floral stimulus that can travel from leaf to apex to promote flowering (Beveridge and Murfet, 1996; Hecht et al., 2011), similar to Arabidopsis *FT* (Corbesier et al., 2007; Mathieu et al., 2007). The most severe *gigas* mutant, *gigas-2*, is non-flowering under LD only, and is late-flowering with normal I_2 and floral morphology under short day (SD) conditions (Murfet, 1992; Taylor and Murfet, 1994; Hecht et al., 2011). *VEG1* has been identified as *FULc*, a pea MADS-box gene from the *AGAMOUS-LIKE79* (*AGL79*) clade, which appears to have a novel role in legume compound inflorescence development as a critical I_2 identity gene (Berbel et al., 2012). The SAM of the *veg1* mutant undergoes the V/I_1 transition and acquires I_1 identity at the same time as wild-type, but fails to subsequently specify I_2 meristems (Berbel et al., 2012). The *VEG2* locus has received the least attention of these three pea loci and has not been described in detail. *VEG2* is represented by two recessive mutant alleles, *veg2-1* and *veg2-2*, both generated by fast neutron mutagenesis (Murfet, 1992; Murfet and Reid, 1993).

Here, we characterize the roles of the *VEG2* locus during each stage of inflorescence development in pea through examination of the two *veg2* mutant alleles. We identify *VEG2* as a pea homolog of *FD* (*FDa*), and further investigate the possible mechanisms of *VEG2* function. Our findings reveal that

VEG2 plays a central role in the regulation of meristem identity, acting in conjunction with multiple FT proteins to regulate expression of *FT*, *TFL1* and *LFY* homologs and key MADS-box genes *VEG1* and *PIM* throughout development of the pea compound inflorescence.

RESULTS

VEG2 acts across all stages of inflorescence development

We first examined *veg2-1* and *veg2-2* mutant phenotypes in order to investigate the role(s) of *VEG2* during compound inflorescence development. Under both LD and SD photoperiods, the *veg2-1* mutant remained non-flowering throughout development and the weaker *veg2-2* mutant flowered later than wild-type (**Figure 2A and B**). A conspicuous feature of both *veg2* mutants was increased aerial branching, with lateral branches occupying aerial nodes in *veg2-1*, and the aerial nodes prior to the first flowering node in *veg2-2* (**Figure 2A and C**; **Supplemental Figure 2**). This may be linked to the absence of flowers and pods in *veg2-1* and their delayed appearance in *veg2-2*, as increased branching is also observed in wild-type plants when flowers/pods are removed (**Figure 2C**; **Supplemental Figure 2**; Lockhart and Gottschall, 1961) and in other non-flowering mutants *veg1* and *gigas* (Gottschalk, 1979; Beveridge and Murfet, 1996).

The fact that both *veg2* mutations impair the initiation of flowering suggested that they might affect the V/I_1 transition (**Figure 1B**). I_1 meristems are characterized by the expression of the *TFL1* homolog *DET*, and *DET* expression has been used as a developmental marker for I_1 meristem identity (Berbel et al., 2012). In non-flowering *veg1* and *gigas* mutants, the timing of *DET* induction is similar to wild-type, indicating that the V/I_1 transition is not affected under LD in these mutants (Hecht et al., 2011; Berbel et al., 2012; **Supplemental Figure 3**). We first examined *DET* expression in the late-flowering *veg2-2* mutant at weekly time points during development from seedling to flowering adult plant. *DET* induction was delayed by 3-4 weeks in *veg2-2* relative to wild-type, comparable to the approximately 4 week delay in the appearance of floral buds (**Figure 2D**). In a second experiment, we also examined *DET* expression in the more severe allele, *veg2-1*, but focussed on only two time points, in view of limited availability of this sterile genotype. Time points were selected to coincide with the expected peaks in *DET* expression in wild-type and *veg2-2*. At 45 days after sowing, when the presence of floral buds indicated that the V/I_1 transition had occurred in wild-type, *DET* expression was 8- to 9-fold lower in the *veg2* mutants than respective wild-type plants (**Figure 2E**). By day 74, when floral buds were first visible in *veg2-2* apices and wild-type plants had senesced, *DET* expression had increased 4- to 5-fold in the *veg2* mutants. This suggests that *DET* induction is also delayed in *veg2-1*, similar to

veg2-2, and that the V/I_1 transition is therefore delayed in both mutants. In addition, the fact that *DET* is eventually expressed in the non-flowering *veg2-1* mutant, indicates that this mutant does acquire I_1 identity, but as no I_2 structures subsequently develop, we conclude that the next stage of inflorescence development, I_2 meristem specification, must be blocked in this mutant.

Next, we used grafting to investigate whether *VEG2* may contribute to the generation of a long-distance flowering signal, as is the case for the *GIGAS*/*FTa1* gene (Beveridge and Murfet, 1996; Hecht et al., 2011). **Figure 2F** shows that *veg2-2* scions grafted onto wild-type stocks flowered as late as self-grafted *veg2-2* control plants (P = 0.729), while wild-type scions grafted to *veg2-2* stocks flowered at a similar time to self-grafted wild-type plants (P = 1.000). These results indicate that *VEG2* cannot influence flowering across a graft union and suggest that *VEG2* instead acts locally within the shoot apex.

We next examined the weaker *veg2-2* mutant phenotype in more detail for insight into the role(s) of *VEG2* during the later stages of flowering, I_2 and floral development, which do not occur in the non-flowering *veg2-1* mutant. In the *veg2-2* mutant, I_2 morphology was abnormal at all reproductive nodes. Abnormal I_2 structures resembled wild-type in that they bore one or more axillary flowers, but unlike wild-type I_2 structures, which terminate in a stub, these had a bract subtending each flower, and retained an indeterminate apex (**Figure 3A-D, E-J**). After producing one or two flowers, subsequent nodes of the *veg2-2* I_2 bore full compound leaves with vegetative axillary buds or axillary tertiary inflorescence (I_3) structures that reiterate the same abnormal I_2 pattern (**Figure 3I-J**). This phenotype suggests that I_2 identity is initially partly specified in *veg2-2*, but this identity is not maintained, and reversion to I_1 identity occurs. Consistent with this interpretation, *DET* was expressed in the indeterminate apex of the *veg2-2* I_2 at a similar level to that in the *veg2-2* I_1 apex, whereas in wild-type, expression of *DET* was limited to the I_1, and levels in the I_2 stub were negligible (**Figure 3K**). This indicates that *VEG2* has an important role, not only in specifying, but also in maintaining I_2 identity.

We also observed that flowers produced on *veg2-2* I_2 structures were frequently abnormal. Defects were most common in outer (sepal and petal) whorls (**Figure 3L**), and included fusion of floral organs to leaf or other floral tissue, a reduction in organ number, and organ displacement or malformation (**Supplemental Figure 4**). The severity of these floral defects decreased acropetally on the main stem axis, and flowers on I_2 structures at higher reproductive nodes showed normal morphology (**Figure**

193 **3M; Supplemental Figure 4**). These observations indicate a further role for *VEG2* in specification of
194 floral meristems.
195
196 Previous study has shown that *PIM*, a pea *AP1* homolog, has a major role in specification of floral
197 meristems in pea (Taylor et al., 2002). To determine whether *VEG2* could affect floral phenotype in the
198 absence of functional *PIM*, we also examined the phenotype of the *pim-2 veg2-2* double mutant. *pim-2*
199 mutant plants produce I_2 structures but fail to specify floral meristems correctly, and single flowers are
200 typically replaced by groups of abnormal flowers (Taylor et al., 2002; **Supplemental Figure 5**). In
201 contrast, no discrete units recognisable as flowers were observed in the *pim veg2-2* double mutant.
202 Although structures with floral identity did form, these were limited to isolated floral organs that were
203 fused to, or borne in the axils of, leaves or bracts on upper nodes of the main stem or branches
204 (**Supplemental Figure 5**). This more severe phenotype of *pim veg2-2* relative to *pim* indicates that
205 *VEG2* acts at least in part through genes other than *PIM*, to specify floral meristem identity.
206
207 **The *VEG2* locus corresponds to an *FD* gene**
208 Preliminary mapping indicated that *VEG2* was located towards the base of pea LGI (Murfet and
209 McKay, 2012). We exploited the close synteny between pea and Medicago (Duarte et al., 2014), to
210 search for an appropriate candidate for the *VEG2* locus. In the syntenic region of Medicago
211 chromosome 5, we identified a homolog of the Arabidopsis bZIP transcription factor *FD*
212 (**Supplemental Figure 6**). Mutants for *FD* genes in other species typically exhibit delayed flowering
213 time (Abe et al., 2005; Wigge et al., 2005; Muszynski et al., 2006; Park et al., 2014), which is one of
214 the features of the *veg2-2* mutant (**Figure 2B**). Isolation and mapping of the full-length coding
215 sequence for the pea ortholog of this gene, confirmed its location, close to the *VEG2* locus
216 (**Supplemental Figure 6**).
217
218 With this gene as a likely candidate for *VEG2*, we investigated the legume *FD* family further. We
219 identified *FD* genes from Medicago, soybean, common bean and *Lotus japonicus*, excluding homologs
220 of the closely related *AREB3* and other Group A bZIP transcription factors. **Figure 4A** shows that this
221 approach identified three subclades of legume *FD* genes, which we have designated as *FDa*, *FDb* and
222 *FDc*. The *VEG2* candidate was included within the legume *FDa* subclade, which showed the greatest
223 similarity to Arabidopsis *FD* and *FDP*. *FDa* and *FDb* subclades were represented in species from both
224 galegoid and phaseoloid legume clades (**Figure 4A**), but were not apparent in other rosid I orders,
225 including Rosales, Cucurbitales and Malpighiales (**Supplemental Figure 8**). This suggests that *FDa*

and *FDb* subclades resulted from a legume-specific duplication event prior to the divergence of galegoid and phaseoloid lineages, approximately 54 mya (Lavin et al., 2005). Although *FDb* is present in Medicago, BLAST searches of pea transcript databases (Franssen et al., 2011; Kaur et al., 2012) and PCR approaches using degenerate and *MtFDb*-specific primers (**Supplemental Table 3**), provided no evidence for an *FDb* ortholog in pea. *FDc* genes were identified only in soybean and common bean (**Figure 4A**), implying a more recent origin for this clade, specific to the Phaseoleae. The gene present immediately upstream of *FD* in Arabidopsis, *LA RELATED PROTEIN 1C* (*LARP1C*), was found to be conserved close to all legume *FD* genes investigated (**Figure 4B**; **Supplemental Figure 9**; **Supplemental Table 4**). This microsynteny between genomic regions surrounding *FD* in Arabidopsis and legume homologs supports the probable common origin of Arabidopsis *FD* and all three legume *FD* subclades. No microsynteny was apparent between regions containing *FDP* in Arabidopsis and any legume *FD* genes.

Sequencing of *FDa* in the *veg2-2* mutant revealed a SNP (G536A) directing a substitution (R179H) within the DNA-binding, basic region of the bZIP domain (**Figure 4C**). This SNP co-segregated perfectly with *veg2-2* phenotype in a population of 114 F_2 progeny that included 34 *veg2-2* mutants (**Supplemental Figure 6**). An arginine is highly conserved at this position in FD proteins from diverse angiosperm species and in 95% of all Arabidopsis bZIP family proteins (**Supplemental Figure 10**), which comprise 13 divergent groups with minimal sequence similarity outside of the bZIP domain (Correa et al., 2008). This high level of conservation strongly implies that this residue is important for general bZIP transcription factor function. The same arginine to histidine substitution was previously reported for the maize *FD* homolog *DLF1* in the inflorescence mutant *dlf1-N2461A* (Muszynski et al., 2006). Results from 3D modelling indicated that the arginine at this position comes into direct contact with the phosphate groups on target DNA and conversion to a histidine results in distortion of the DNA backbone, which reduces binding strength (Muszynski et al., 2006). The R179H amino acid substitution in the pea *veg2-2* mutant is likely to reduce FDa function in a similar manner.

Attempts to amplify *FDa* by PCR from *veg2-1* genomic DNA were unsuccessful, whether using primers within or spanning the *FDa* coding sequence (**Figure 4D**), and this failure to amplify *FDa* clearly distinguished all *veg2-1* mutant plants (n = 37) in a segregating population (n = 210; data not shown). The simplest interpretation of these findings is that a deletion encompassing *FDa* has occurred in this mutant. As the pea genome sequence is not yet available, we again made use of microsynteny to investigate the extent of this apparent deletion. **Figure 4B** shows that the two genes flanking *FDa* in

Medicago, *LARP1C* and a *RING-H2* gene, also have conserved positions flanking *FDa* in soybean, and in view of the close relationship of pea to Medicago, we considered it likely that this arrangement was also preserved in pea. The pea homologs of these genes were isolated, mapped, and found to be closely linked to each other and to *FDa*, as expected (**Supplemental Figure 6**), and full length coding sequence for both genes was found to be intact in the *veg2-1* mutant (**Figure 4D**). In addition, a fragment 1.4kb downstream of the *FDa* stop codon was found to be present in *veg2-1*, revealing that the 3' boundary for the *veg2-1* deletion is close to *FDa* coding sequence (**Figure 4D**). Further attempts to define the precise boundaries of the deletion by isolating the region between *LARP1C* and this fragment in the wild-type and *veg2-1* mutant were unsuccessful. However, as both the flanking genes predicted by microsynteny were found to be intact in the *veg2-1* mutant, our results suggest that *veg2-1* contains a deletion restricted to *FDa*. Therefore, based on the evidence of distinct functionally significant mutations that specifically affect *FDa* in both of the *veg2* mutants, and the correlation between the molecular nature of the mutations and the severity of the respective mutant phenotypes, we conclude that the *VEG2* locus corresponds to *FDa*, and subsequently refer to *FDa* as the *VEG2* gene.

VEG2 is expressed in the apex throughout development

In view of observations that *VEG2* is important for multiple stages of inflorescence development, it was of interest to investigate the developmental and spatial pattern of *VEG2* expression. We first examined the expression of *VEG2* by quantitative reverse transcription PCR (qRT-PCR) in shoot apex and leaf tissues of wild-type plants throughout development from seedling to flowering adult plant in LD. **Figure 5A** shows that *VEG2* was not significantly expressed in expanded leaves, but was expressed in the shoot apex throughout development, where *VEG2* transcript levels increased during early vegetative growth and showed a further increase during later floral development.

VEG2 expression pattern in the wild-type apex was next investigated in more detail by *in situ* hybridization during the vegetative phase (**Figure 5F**), the V/I_1 transition (**Figure 5G**) and early flowering stages (**Figure 5H-J**). Apical samples from the *veg2-1* mutant, were included as negative controls for the *VEG2 in situ* probe (**Figure 5B**). Expression patterns for *VEG1* (**Figure 5C**) and *PIM* (**Figure 5D-E**) were determined on serial sections of the same apices used for *VEG2*, in order to identify I_2 and floral meristem boundaries, respectively (Taylor et al., 2002; Berbel et al., 2012). Consistent with previous reports, expression of *VEG1* was observed in I_2 meristems, and *PIM* expression was observed in floral meristems and floral primordia, in the petal region of the petal/stamen common primordia and in the sepals (**Figure 5C-E**; Taylor et al., 2002; Berbel et al.,

2012). *VEG2* was expressed in the vegetative SAM, axillary meristems, the I_1 meristem, I_2 meristems, vasculature and tips of leaf primordia (**Figure 5F-J**). Expression was also seen in floral meristems during early development (**Figure 5I**), but was restricted to floral vasculature during later stages of development (**Figure 5J**). This expression pattern is consistent with roles for *VEG2* during the V/I_1 transition, in specification and maintenance of I_2 meristem identity, and in specification of floral meristem identity.

Flowering and meristem identity genes are misregulated in the *veg2* mutants

In order to identify possible regulatory targets of *VEG2*, we next examined the expression of floral integrator and meristem identity genes in the *veg2* mutants, including members of the *FT/TFL1* and MADS-box gene families and the *LFY* ortholog *UNIFOLIATA* (*UNI*), under LD conditions. Gene expression was investigated in the same experiments described above for *DET* expression, firstly in a detailed time-course in *veg2-2*, and then in both *veg2* mutants at specific time points chosen to coincide with the appearance of floral buds in wild-type and *veg2-2* plants (45 and 74 days after sowing, respectively).

FTa1 and *FTc* are significantly expressed in wild-type apical tissue and are upregulated after commitment to flowering (Hecht et al., 2011; **Figure 6A**). A comparison of the two time points in **Figure 6B** suggests that upregulation of *FTa1* was delayed in both *veg2* mutants, and more specifically an approximately 3 week delay was apparent in *veg2-2* in the detailed time-course (**Figure 6A**). *FTc* expression was reduced by 4-fold at both time points in *veg2-1* and showed delayed induction, by approximately 1 week, in *veg2-2* (**Figure 6**). Also, in addition to *DET*, which was shown above to exhibit delayed upregulation in both *veg2* mutants (**Figure 2D and E**), a second pea *TFL1* homolog, *LF* (*TFL1c*), was also misregulated, but in an opposite manner. This gene, which inhibits flowering (Foucher et al., 2003), was expressed more than 7-fold higher in the *veg2* mutants relative to wild-type levels (**Figure 6B**).

In Arabidopsis, the floral integrator and floral identity gene *LFY* defines an FT/FD-independent pathway for *AP1* upregulation (Ruiz-Garcia et al., 1997; Abe et al., 2005; Wigge et al., 2005). In wild-type pea, the *LFY* ortholog *UNI* is expressed at a low level in the apex during early vegetative development and upregulated at the time of flowering, but after induction of *FTa1* and *FTc* (Hecht et al., 2011; **Figure 6A**). This upregulation was delayed by approximately 4 weeks in *veg2-2* and *UNI*

expression remained at a low level in *veg2-1*, more than 3-fold lower than wild-type levels at day 45 (**Figure 6**), indicating that *UNI* is downstream of *VEG2* in pea.

Several MADS-box genes were also mis-expressed in the *veg2* mutants. The I_2 identity gene *VEG1* and the floral identity gene *PIM* have important roles in specifying meristem identity, and consistent with previous reports these genes were upregulated in the wild-type apex immediately prior to floral development, at a similar time to *FTa1* and *FTc* (Hecht et al., 2011; Berbel et al., 2012; **Figure 6**). In the *veg2-1* mutant, *VEG1* and *PIM* were not expressed and in *veg2-2*, these genes showed an approximately 4 week delay in induction, roughly corresponding to the delay in flowering time (**Figure 6**). A similar pattern was also seen for expression of floral organ identity genes *AP3* and *SEPALATA1* (*SEP1*), consistent with the absence of flowers in *veg2-1* and delayed occurrence of floral development in *veg2-2* (**Figure 6**).

FDa/VEG2 interacts with all pea FT proteins

Studies in diverse species have shown that the physical interaction of FD and FT proteins is widely conserved and functionally significant (e.g. Wigge et al., 2005; Taoka et al., 2011; Tsuji et al., 2013). However in maize, where expansion of the *FT* family has resulted in functional divergence between family members, there is evidence that these FT proteins differ in their ability to interact with an FD homolog (Danilevskaya et al., 2008; Meng et al., 2011). Differences in expression pattern, differing effects in transgenic Arabidopsis and inferences from the *gigas* phenotype all indicate a divergence of function within the pea *FT* family (Hecht et al., 2011), which could in part be determined by differences in interaction with VEG2/FDa.

To examine whether this was indeed the case, we tested the interactions of VEG2 with the five pea FT proteins using bimolecular fluorescence complementation (BiFC) analysis in *Nicotiana benthamiana* leaves. For all five combinations, reconstitution of YFP fluorescence was observed in the nuclei of *N. benthamiana* leaf epidermal cells (**Figure 7**), at levels clearly above background (**Supplemental Figure 11**), indicating that VEG2 can interact with all five pea FT proteins *in planta*.

DISCUSSION

The pea inflorescence is typical of many legumes and is distinguished from the simple Arabidopsis inflorescence by an additional level of branching, with a so-called secondary inflorescence (I_2) that displaces flowers from the main inflorescence stem (**Figure 1**). Three pea loci, *GIGAS*, *VEG1* and

VEG2 affect the formation of I_2s, and thus have the potential to provide insight into the genetic mechanisms that direct compound inflorescence development (Benlloch et al., 2007). *GIGAS* and *VEG1* have been recently characterized as homologs of *FT* and *AGL79*, respectively (Hecht et al., 2011; Berbel et al., 2012). Here, we have characterized the third of these loci, *VEG2*, as a pea homolog of the bZIP transcription factor *FD*, and investigated its roles and interactions in the initiation of flowering and throughout inflorescence development.

VEG2 participates in the initiation of flowering

From a developmental perspective, the initiation of flowering in pea is closely associated with the acquisition of I_1 identity by the SAM, which is marked by *DET/TFL1a* expression (Berbel et al., 2012). In *veg2* mutants, the induction of *DET* is delayed relative to wild-type and the other non-flowering mutants, *gigas* and *veg1* (**Figure 2D and E**; **Supplemental Figure 3**). This indicates that *VEG2* has an important role in promoting the V/I_1 transition in wild-type plants under LD conditions, whereas *GIGAS/FTa1* and *VEG1* do not, despite the apparent similarity of their mutant phenotypes. The eventual induction of the I_1 marker gene *DET* in the *veg2-1* deletion mutant (**Figure 2E**) does however suggest that at least one *VEG2*-independent pathway also promotes the V/I_1 transition in pea.

The majority of work on *FD* genes in other species has focused on their participation in florigen signalling. Several studies have now shown that FD proteins physically associate with FT proteins and are essential for their flower-promoting function, providing a crucial link between FT proteins and their transcriptional targets (Abe et al., 2005; Wigge et al., 2005; Taoka et al., 2011). The FD/FT interaction has been examined in most detail in rice, where it has been shown that the OsFD1 protein does not bind directly to the FT protein Hd3a, but that their interaction is mediated by 14-3-3 proteins (Taoka et al., 2011; Tsuji et al., 2013). These FT/14-3-3/FD complexes have been referred to as florigen activation complexes (FACs), and it is likely that VEG2 also acts as part of one or more FACs in pea. Pea has five *FT* genes and analysis of expression patterns, mutant phenotypes and activity in transgenic Arabidopsis suggest that these genes may have distinct roles in the flowering process (Hecht et al., 2011). Our results indicate that VEG2 can interact with each of the five pea FT homologs in *planta* (**Figure 7**), which suggests that participation in FACs with VEG2 may be important for the function of all pea FT proteins, but appears to rule out differential VEG2 binding as an explanation for differences in their function. Future investigations should instead explore the recent hypothesis that FT functional specificity may in fact derive from interactions with other proteins via a domain distinct from residues required for FAC binding (Ho and Weigel, 2014).

Among the five pea *FT* genes, only three (*FTa1*, *FTb2* and *FTc*) show clear developmental regulation, consistent with roles in initiation of flowering and/or inflorescence development (Hecht et al., 2011). Because the V/I_1 transition is the first stage of inflorescence development, it is likely that the role of VEG2 in this process involves interaction with the FT protein/s that act as florigens and move from leaf to apex following perception of appropriate environmental signals. Grafting experiments with *gigas* mutants suggest that GIGAS/FTa1 may function as one such mobile signal (Beveridge and Murfet, 1996; Hecht et al., 2011). However, the fact that induction of the I_1 marker *DET* is not affected by the *gigas* mutation shows that the FAC involving FTa1 is probably not important for initiating the V/I_1 transition. This is also consistent with the fact that induction of *FTa1* in leaves is delayed relative to floral commitment, and with the fact that the *gigas* phenotype indicates a role later in inflorescence development, promoting I_2 specification rather than the V/I_1 transition. A second *FT* gene, *FTb2*, is also a strong candidate for a florigen signal, as it is induced in leaves within the time-frame of the physiological commitment to flowering in LD and is severely misregulated in photoperiod response mutants (Hecht et al., 2011). Furthermore, *FTb2* is expressed normally in the *gigas* mutant in LD (Hecht et al., 2011), which could account for the fact that the V/I_1 transition is not affected in the *gigas* mutant under these conditions. Functional analysis of *FTb2*, and in particular whether it regulates expression of *DET*, will be important to clarify its involvement in the V/I_1 transition.

***VEG2* is essential for secondary inflorescence development**

We recently showed that expression of the MADS-box gene *VEG1* is crucial for the formation of the I_2, and proposed that I_2 meristem identity is specified by *VEG1* (Berbel et al. 2012). The non-flowering *veg2-1* and *gigas* mutants are unable to form I_2 structures and do not show *VEG1* expression (**Figure 6**; Berbel et al., 2012), which indicates that *VEG2/FDa* and *GIGAS/FTa1* are both required for induction of *VEG1* under LD conditions. In addition, our data indicate that the VEG2 and FTa1 proteins can interact (**Figure 7**), implying that they participate in a FAC that acts to initiate *VEG1* expression and specify I_2 meristem identity. The fact that the abnormal I_2s in the weaker *veg2-2* mutant are indeterminate and revert to I_1 identity (**Figure 3**), shows that *VEG2/FDa* also has a role not only in initial specification of I_2 identity, but also in maintenance of this identity. The incomplete specification of I_2 identity in *veg2-2* is accompanied by a reduction in *VEG1* expression levels relative to wild-type (**Figure 6**), which is consistent with the idea that *VEG1* expression is a critical limiting factor in I_2 development.

We previously proposed a model for inflorescence development in pea, which elaborates on the simple *TFL1/AP1* negative feedback loop described in Arabidopsis (Ratcliffe et al., 1999; Kaufmann et al., 2010). In this model, *DET* prevents upregulation of *VEG1* and *PIM* in the I_1, meristem and *VEG1* prevents upregulation of *DET* in the I_2 meristem, allowing expression of *PIM* in axillary floral meristems (Berbel et al., 2012). The incomplete and transient specification of I_2 identity in the hypomorphic *veg2-2* mutant illustrates how disruption of a regulatory loop can destabilize a sharp developmental transition (Sablowski, 2007). This role for *VEG2/FDa* in the maintenance of I_2 identity is comparable to the role recently described for *FT* in Arabidopsis, in stabilising inflorescence meristem identity after flowering to prevent floral reversion (Liu et al., 2014; Müller-Xing et al., 2014).

One significant point of contrast between *VEG2* and *FD* genes in other species lies in the severity of its null mutant phenotype. Whereas the *veg2-1* mutant is completely unable to flower, *FD* mutants in both Arabidopsis and maize are merely late-flowering (Koornneef et al., 1991; Abe et al., 2005; Wigge et al., 2005; Muszynski et al., 2006). Even when FD/FT function is completely absent in Arabidopsis, in *fd fdp* or *ft tsf* double mutants, flowering will still occur, albeit considerably later than in any single mutant (Jang et al., 2009; Jaeger et al., 2013). However, a non-flowering phenotype is seen in Arabidopsis when *fd* or *ft* mutations are combined with *lfy*, indicating that *LFY* acts in parallel with *FD* and *FT* genes to upregulate *AP1* for specification of floral meristems (Ruiz-Garcia et al., 1997; Abe et al., 2005; Wigge et al., 2005). In pea, the *LFY* ortholog *UNI* is expressed at a low level in vegetative seedlings where it has a role in leaf development (Hofer et al. 1997), but is upregulated at the time of flowering, and this upregulation is dependent on *FTa1* (Hecht et al., 2011). Our results show that the upregulation of *UNI* is also dependent on *VEG2* (**Figure 6**), which suggests that *UNI* is acting downstream of the *VEG2* and *FTa1* in pea, and not in parallel, as is seen for *LFY* in other systems.

A second factor that may contribute to the severity of the *VEG2* null phenotype is the existence of I_2 specification as an intermediate step in pea inflorescence development, and the essential role of *VEG1* in this process. All three non-flowering mutants (*gigas*, *veg1* and *veg2-1*), show a correlation between the absence of *VEG1* expression and failure to express *PIM* (**Figure 6B**; Hecht et al., 2011; Berbel et al., 2012), suggesting that *VEG1* expression is an absolute requirement for *PIM* expression to occur in secondary inflorescences in the presence of functional *DET*. The fact that *PIM* is expressed in the *veg1 det* double mutant (Berbel et al. 2012) suggests that lack of *PIM* expression in *veg1* may reflect a strong suppression of *PIM* by *DET* that is relieved through repression of *DET* by *VEG1*. The delay in

PIM induction observed in the *veg2-2* mutant (**Figure 6**) suggests that *VEG2* promotes *PIM* expression, either directly or indirectly through repression of the floral repressor *LF/TFL1c*.

VEG2 activity in floral meristems implies a role for *FT* genes in flower development

The *veg2-2* mutant phenotype also reveals that *VEG2* has a role in correct specification of floral identity, especially for sepal and petal whorls (**Figure 3L**). The observed acropetal decrease in severity of floral defects (**Figure 3M**) indicates that *VEG2* is especially important for correct floral development at early reproductive nodes, but this importance decreases with plant age. This could be due either to slow accumulation of downstream targets through partial *VEG2* function in the *veg2-2* mutant, or their activation via an alternative age-related pathway. The floral abnormalities seen in *veg2-2* are similar to those seen in mutants for *PIM*, which is misregulated in the *veg2-2* mutant. Like *veg2-2*, *pim* mutants exhibit replacement of sepals with leafy bracts, second and third whorl organs are missing or mosaic, and severity of floral morphology defects decreases acropetally (Singer et al., 1999; Taylor et al., 2002). However, the severity of the *pim veg2-2* double mutant phenotype indicates that *VEG2* probably has other targets in addition to *PIM*; most likely other MADS-box genes such as *AP3* and *SEP1*, which are also misregulated in the *veg2-2* mutant (**Figure 6**). MADS-box transcription factors are known to act in a combinatorial fashion to guide different stages of the flower initiation and development process (Smaczniak et al., 2012), and the persistent effects of *VEG2* might reflect the participation of a limited number of direct *VEG2* targets in MADS tetramers throughout reproductive development. Alternatively, *VEG2* may be required for direct induction of flower-specific MADS genes, which would also imply an extended post-flowering role for *FT* genes and associated FACs. Our results here and in the previous study of Hecht et al. (2011) show that expression of *FTa1* and *FTc* genes is only induced in shoot apical tissue after the initial commitment to flowering, and continues well beyond this transition, consistent with a role in later reproductive development.

VEG2 and the integration of *FT/TFL1* signaling

In a previous study, we presented evidence for potential cross-regulation among *FT* genes; specifically, for the positive regulation of *FTc* at the shoot apex by *FTa1* and *FTb2* (Hecht et al., 2011). Results from this study show that *FTc* is also misregulated in the *veg2* mutants (**Figure 6**), providing support for the conclusion that one or more other *FT* genes may be required for full activation of *FTc*. In addition, *FTa1* expression in the shoot apex is also altered in the *veg2* mutants (**Figure 6**), implying that it too is dependent on one or more *FT* genes acting together with *VEG2*. This is consistent with a scenario in which both *FTa1* and *FTc* are transcriptional targets of FT proteins that arrive to the shoot

apex, a conclusion also supported by the fact that induction of *FTa1* and *FTc* in the apex only occurs after the plant becomes committed to flower.

One interpretation is that these genes may act as functional integrators of mobile FT signals. However, the induction of *DET*, which marks the V/I_1 transition, occurs with very similar timing to that of *FTa1* and *FTc* in the shoot apex, with no evidence of the delay that might be expected if it was dependent on the expression of these genes. *GIGAS*/*FTa1* is clearly required for *VEG1* induction and I_2 specification, but the role of *FTc* is less clear. *FTc* is still expressed in *gigas*, albeit at a lower level than WT, and the fact that *gigas* plants do not flower under LD implies that *FTc* alone cannot substitute for *FTa1* in the initiation of I_2 formation under these conditions. One explanation is that *FTc* may play a subsidiary role to *FTa1*, reinforcing its expression and/or activity and ensuring a clear induction of *VEG1* and a sharp developmental transition from vegetative branch to I_2.

The observation that *VEG2* regulates *DET* expression provides the first reported evidence for transcriptional regulation of a *TFL1* gene by an *FD* gene. Given the mechanism for *FD* action, this also implies the involvement of *FT* family members, and some evidence for this does exist. In strawberry, the *FT* gene *FvFT1* has been shown to activate *FvTFL1* expression indirectly via *FvSOC1*, to repress flowering and allow production of vegetative shoots under LD conditions in spring and summer (Mouhu et al., 2013). Similarly in Arabidopsis, *TFL1* is strongly upregulated in the SAM at the onset of flowering, and is expressed in proportion to *FT* (Jaeger et al., 2013). Furthermore, a computational model designed to simulate flowering in Arabidopsis required the inclusion of a term for activation of *TFL1* by FT/FD proteins to correctly model the maintenance of SAM indeterminacy during flowering (Jaeger et al., 2013). Collectively, these findings suggest that direct or indirect transcriptional regulation of *TFL1* homologs by *FT* and *FD* homologs is also likely to occur more widely, and the functional significance of this and the mechanisms by which it is achieved will be of interest to determine.

In contrast to *DET*, the expression of the related *LF*/*TFL1c* gene was increased in both *veg2* mutants relative to wild-type (**Figure 6**). The tissue-specificity of *LF* expression is not yet known, but the fact that *lf* mutants are early flowering without any other apparent defects can be interpreted to indicate that *LF* acts to prevent the acquisition of I_2 identity in lateral meristems. In this respect it is similar to *DET*, which also prevents the acquisition of I_2 identity, but in the SAM. The finding that *VEG2* regulates these two *TFL1* homologs in opposite ways (i.e. promoting induction of *DET* and repressing expression

of *LF*) is consistent with the meristem identity changes that occur during the transition to flowering, as *DET* expression is positively associated with inflorescence development, while *LF* expression is not. It also implies that one or more FT proteins may act via FACs to relieve suppression of I_2 identity by *LF* in lateral meristems. In other angiosperms, including eudicot and monocot species, FD proteins have been found to also interact with TFL1 proteins, in complexes that inhibit the transcription of floral target genes (e.g. Pnueli et al., 2001; Danilevskaya et al., 2010; Hanano and Goto, 2011). The possibility that VEG2/FDa may interact with DET/TFL1a and/or LF/TFL1c in a similar manner in pea, remains to be investigated.

The observations and hypotheses resulting from this study are summarized in the model in **Figure 8**, which suggests that FTb2 arriving at the shoot apex in LD may form a FAC with VEG2 and regulate a number of other *FT*/*TFL1* genes in the SAM and lateral meristems. The collective action of these secondary FACs may then allow specific expression of *VEG1* and formation of I_2s in lateral meristems, where *LF* has been downregulated, and promote upregulation of *DET* in the SAM to maintain SAM indeterminacy. Future work to test these ideas should include a detailed analysis of expression dynamics of *FT* and *LF* genes within the SAM, an examination of the interactions between VEG2 and TFL1 proteins, and characterization of *FTb2* and *FTc* mutants.

Overall, the findings from this study have extended our previous work to make a significant contribution to understanding of how differences in inflorescence architecture are generated. They will also assist the investigation of this process in a range of other important legume crop species that share similar inflorescence architecture. Understanding the complex network of genes controlling inflorescence development may ultimately contribute to crop improvement through optimisation of inflorescence architecture for efficient harvest and maximal yield.

METHODS

Plant Material and Growth Conditions

The origins of *veg2-1*, *veg2-2*, *pim-2*, *gigas-2* and *veg1* mutants have been described previously (Gottschalk, 1979; Murfet and Reid, 1993; Taylor et al., 2002; Hecht et al., 2011). Molecular characterisation of the *veg2-1* allele compared the mutant line in the original cv. Kaliski background with cv. Kaliski. All other experiments used mutant lines derived from multiple backcrosses in the dwarf NGB5839 background, as previously described (Hecht et al., 2007). In the case of non-flowering *veg1* and *veg2-1* mutants, wild-type siblings were used as controls. Plants for the qRT-PCR experiment

554 shown in **Figures 5A** and **6A** were grown in growth cabinets at 20°C, and plants for all other
555 experiments were grown in the phytotron. Growth media, light sources, phytotron conditions and
556 grafting protocols have been described previously (Weller et al., 1997; Hecht et al., 2007).
557
558 **Gene Isolation and Phylogenetic Analysis**
559 Partial length *PsFDa* was isolated from a cDNA library screen of 1,000,000 clones from a 5' Stretch
560 Plus λgt11 cDNA library (ClonTech, CA, USA) from pea apical buds (Lester et al., 1997) using partial
561 *MtFDa* as a probe. Partial *PsFDa* sequence was extended using rapid amplification of cDNA ends
562 (SMART RACE cDNA amplification kit; Clontech), genome walking (GenomeWalker Universal kit;
563 Clontech), and standard PCR techniques to obtain full length coding sequence. Putative *FDa* flanking
564 genes and molecular marker genes for mapping of *FDa*/*VEG2*, were isolated using primers designed
565 from either pea sequence, where available, or conserved regions of Medicago orthologs. Primer details
566 are given in **Supplemental Table 3**. PCR fragments were purified and sequenced directly or cloned in
567 pGEM-T Easy (Promega) and then sequenced at the Australian Genome Research Facility (Brisbane,
568 Australia) or Macrogen Inc. (Seoul, Korea).
569
570 For phylogenetic analyses, *FD* genes were identified by performing BLAST searches using
571 Arabidopsis FD protein sequence as a query and identity was confirmed by reciprocal BLAST search
572 against Arabidopsis at TAIR (www.arabidopsis.org) and preliminary phylogenetic analysis (data not
573 shown). For full sequence details, including source, see **Supplemental Table 2**. For each alignment,
574 full length amino acid sequence was aligned using ClustalX (Thompson et al., 1997) and adjusted
575 manually, where necessary, using GeneDoc (Version 2.7.000; Nicholas and Nicholas, 1997;
576 http://www.psc.edu/biomed/genedoc). Using these alignments, distance-based methods were used for
577 phylogenetic analyses in PAUP* 4.0b10 (http://paup.csit.fsu.edu/).
578
579 **Gene Expression Studies**
580 For qRT-PCR, harvested tissue for each sample consisted of both leaflets from the uppermost fully
581 expanded leaf or dissected apical buds (~2mm) from two plants. Samples were frozen in liquid nitrogen
582 and total RNA extracted using the SV Total RNA isolation system (Promega). RNA concentrations
583 were determined using a NanoDrop 8000 (Thermo Scientific). Reverse transcription was conducted in
584 20 µL with 1 µg of total RNA using the Tetro cDNA synthesis kit (Bioline) according to the
585 manufacturer's instructions. RT-negative (no enzyme) controls were performed to monitor for
586 contamination with genomic DNA. First-strand cDNA was diluted five times, and 2 µL was used in

each real-time PCR. Reactions using SYBR green chemistry (Sensimix, Quantace, Bioline) were set up with a CAS-1200N robotic liquid handling system (Corbett Research) and run for 50 cycles in a Rotor-Gene RG3000 (Corbett Research). Two technical replicates and at least two biological replicates were performed for each sample. All primer details are given in **Supplemental Table 3**.

RNA *in situ* hybridization with digoxigenin-labelled probes was performed as previously described (Ferrándiz et al., 2000). Probes used for *VEG1* and *PIM* have been described previously (Berbel et al., 2001; Berbel et al., 2012). Primer details for the *VEG2/FDa* probe are given in **Supplemental Table 3**.

BiFC Assay

Full length coding sequences of *VEG2/FDa*, *FTa1*, *FTa2*, *FTb1*, *FTb2* and *FTc* were amplified from NGB5839 cDNA, cloned in-frame into the pCR8/GW/TOPO entry vector (Invitrogen) and transferred by Gateway LR reaction (Invitrogen) into $pYFP^N43$ and $pYFP^C43$ destination vectors. Primer details are given in **Supplemental Table 3**. Constructs for the positive control interaction between Arabidopsis proteins AKIN10 and AKINβ2 (**Supplemental Figure 11**) have been described previously (Belda-Palazón et al., 2012). Constructs were introduced into *Agrobacterium tumefaciens* C58C1 (pGV2260) and used to infiltrate young fully expanded leaves of 4-week-old tobacco plants as previously described (Scacchi et al., 2009). Leaves were examined after 3 to 4 days with a Leica TCS-SL confocal microscope and a laser scanning confocal imaging system.

Statistical Analysis

Statistical analysis was conducted using IBM® SPSS® Statistics (Version 21), using a significance level of 0.05. Levene's test for homogeneity of variance was applied, and one-way analysis of variance (ANOVA; with Tukey's HSD post-hoc test) or Welch's test for ANOVA (with Games-Howell post-hoc test) were conducted, as appropriate.

Accession Numbers

Please refer to **Supplemental Table 2** for details of sequences used in phylogenetic analyses, and **Supplemental Table 4** for details of *FD* and flanking genes in Arabidopsis and legume species. GenBank accession numbers for other pea genes are as follows: *AP3* (JN412098), *DET* (AY340579), *FTa1* (HQ538822), *FTa2* (HQ538823), *FTb1* (HQ538824), *FTb2* (HQ538825), *FTc* (HQ538826), *LARP1C* (JI919144, JI924790, JR963915), *LF* (AY343326), *PIM* (AJ291298), *RING-H2*

(XXXXXXXX), *SEP1* (AY884290), *UNI* (AF010190), *VEG1* (JN974184), *VEG2/FDa* (XXXXXXXX).

Supplemental Data

Supplemental Figure 1: Phenotypes for key inflorescence mutants in pea.

Supplemental Figure 2: Branching in the *veg2* mutants.

Supplemental Figure 3: *DET* expression in non-flowering *gigas* and *veg1* mutants.

Supplemental Figure 4: *veg2-2* floral morphology.

Supplemental Figure 5: The *pim-2 veg2-2* double mutant phenotype.

Supplemental Figure 6: Comparative map for pea and Medicago showing relative locations of *VEG2*/*FDa* and surrounding genes.

Supplemental Figure 7: Alignment of legume FD amino acid sequences.

Supplemental Figure 8: Phylogram of the angiosperm FD family.

Supplemental Figure 9: Microsynteny between genomic regions containing *FD* and flanking genes in Arabidopsis and legume species.

Supplemental Figure 10: Conserved nature of the amino acid altered by the *veg2-2* SNP.

Supplemental Figure 11: Positive and negative BiFC controls.

Supplemental Table 1: Mapping loci details.

Supplemental Table 2: Details of sequences for FD proteins and related bZIP transcription factors used for phylogenetic analyses and alignments.

Supplemental Table 3: Primer details.

Supplemental Table 4: Details for *FD* and flanking genes in Arabidopsis and legume species.

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AUTHOR CONTRIBUTIONS

F.C.S., J.L.W., V.H., F.M. and C.F. designed the research, F.S., A.B. and J.K.V.S. performed the research, and F.C.S. and J.L.W. wrote the paper.

FIGURE LEGENDS

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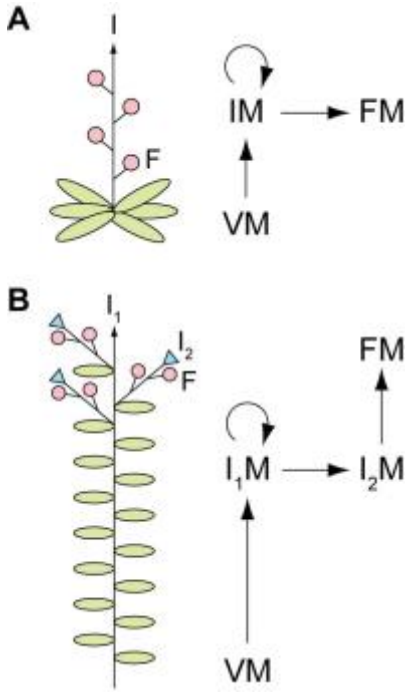

Figure 1. Inflorescence development in Arabidopsis and pea.
(A) The simple raceme of Arabidopsis.
(B) The compound raceme of pea.
For each species, a diagram of inflorescence architecture (left), and schematic of the meristem transitions involved in inflorescence development (right) are shown. In diagrams, arrows indicate indeterminate growth of the inflorescence stem (I; I_1), green ovals are leaves, pink circles are flowers (F) and blue triangles are stubs terminating each secondary inflorescence (I_2) axis. In schematics, straight arrows indicate meristem transitions and products, and circular arrows indicate meristem indeterminacy. Meristem abbreviations are as follows: vegetative meristem (VM), inflorescence meristem (IM), primary inflorescence meristem (I_1M), secondary inflorescence meristem (I_2M), floral meristem (FM).

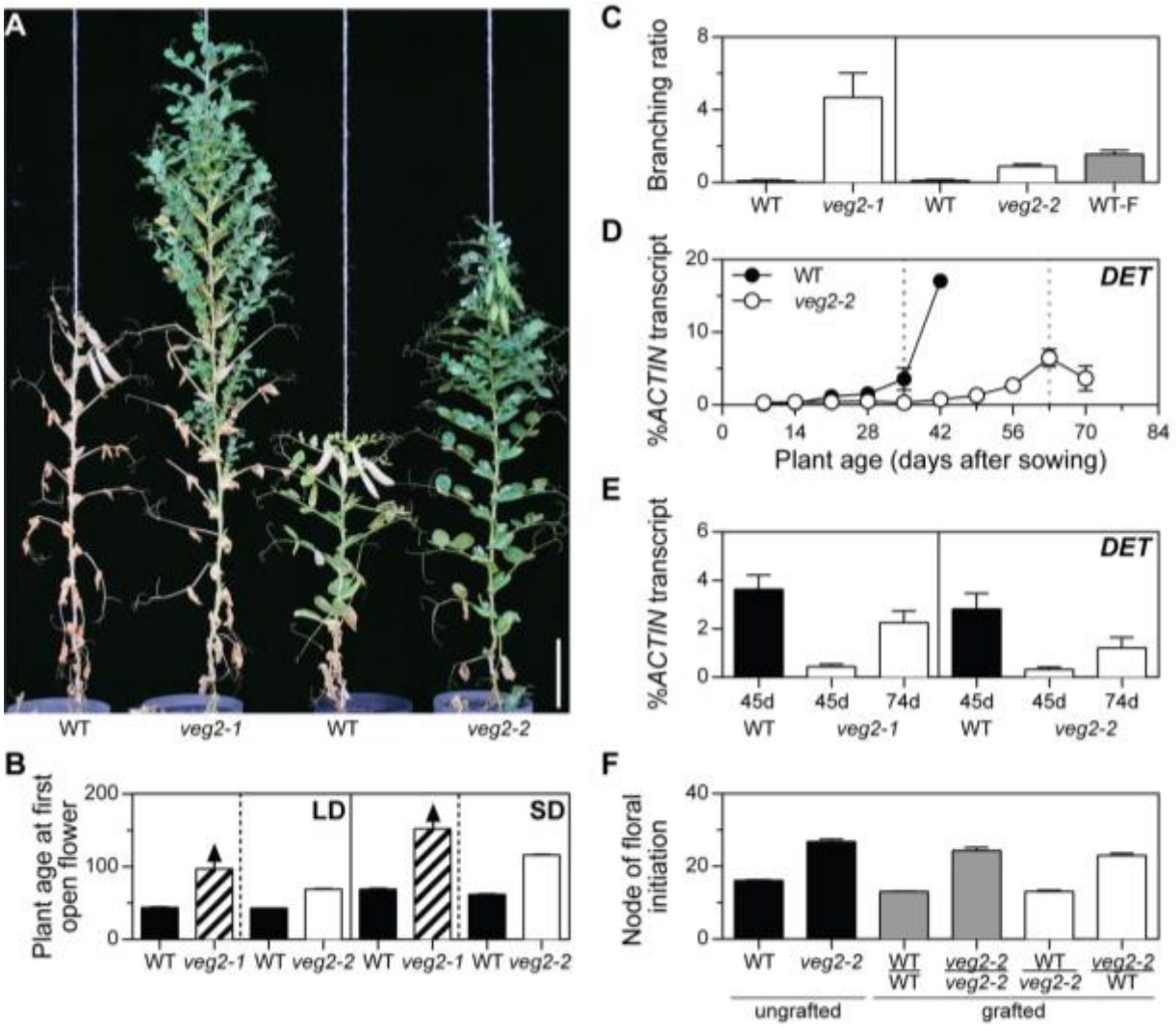

Figure 2. *VEG2* acts locally in the apex to promote flower initiation and inflorescence development.

(A) Representative *veg2-1* and *veg2-2* plants, and their associated wild-type lines (WT; wild-type siblings of *veg2-1***,** and NGB5839, respectively). Plants are shown 97 days after sowing in LD (18h). The scale bar represents 10cm.

(B) Plant age at first open flower (days after sowing) in LD (24h) and SD (8h). Values represent mean ± standard error for n = 3 to 6 plants. For non-flowering *veg2-1* mutants, bars with diagonal hatching and arrow show plant age at the end of the experiment.

(C) Ratio of total branch length to main stem length in intact *veg2* mutants, associated wild-type lines, and deflowered wild-type plants (WT-F; line NGB5839; each flower removed after anthesis). Mean values ± standard error are shown for n = 5 to 6 plants grown in LD (24h) and measured 97 days after sowing. Measurements include all vegetative laterals 5mm or longer in length.

(D) and **(E)** Relative expression of *DET* transcript as a marker of primary inflorescence (I_1) identity in dissected shoot apices at **(D)** weekly time points throughout development in *veg2-2*, and **(E)** specific time points in both *veg2* mutants, in LD (24h and 18h, respectively). In **(D)** developing floral buds were first macroscopically visible in wild-type 35 days after sowing and in *veg2-2* 63 days after sowing (broken lines). In **(E)** time points correspond to early flowering stages in wild-type (45 days after sowing; 45d) and *veg2-2* plants (74 days after sowing; 74d). Mean values ± standard error are shown for n = 2 to 3 biological replicates.

(F) Node of floral initiation for graft combinations of wild-type (NGB5839) and *veg2-2* created by grafting seven-day-old scions on to three-week-old stocks, controls comprising grafted plants with stock and scion of identical genotype, and ungrafted control plants. For each graft combination, the genotypes of scion (top) and stock (bottom) are shown, separated by a horizontal line. Values represent mean ± standard error for n = 5 to 11 plants grown under LD (18h).

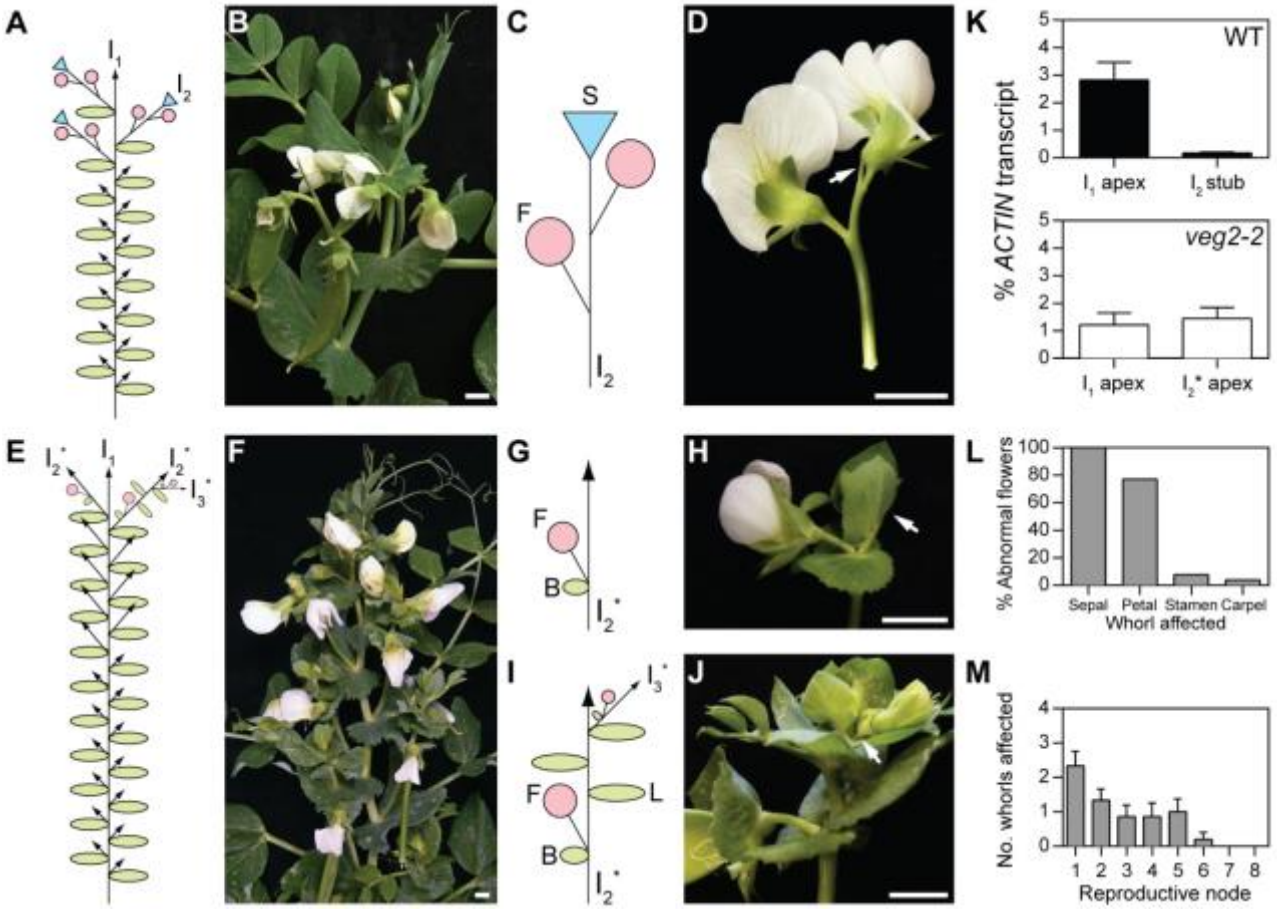

Figure 3. Inflorescence and floral morphology is abnormal in the *veg2-2* mutant.

(A-D) Wild-type inflorescence structure in pea line NGB5839. **(A)** Diagram of plant architecture. **(B)** Photograph of reproductive nodes on the main stem. **(C)** Diagram and **(D)** photograph of the secondary inflorescence (I_2) which bears axillary flowers (F) and terminates in a stub (S; arrow).

(E-J) Inflorescence structure in *veg2-2*. **(E)** Diagram of plant architecture. **(F)** Photograph of reproductive nodes on the main stem. **(G)** Diagram and **(H)** photograph of a typical *veg2-2* I_2, which bears an axillary flower with subtending bract (B) and retains an indeterminate apex (arrow). **(I)** Diagram and **(J)** photograph of an older *veg2-2* I_2, which has a pod and subtending bract at the first node, three nodes with full compound leaves (L) and a flower on an axillary tertiary inflorescence (I_3; arrow in photograph). Note all *veg2-2* I_2 structures are indeterminate, similar to **(H)** and may develop additional nodes after bearing axillary flowers, similar to **(J)**.

(K) Relative expression of *DET* transcript as an indicator of primary inflorescence (I_1) identity in the dissected shoot apex during early flowering stages (I_1 apex; 45 and 74 days after sowing in wild-type NGB5839 and *veg2-2*, respectively) and in the I_2 (wild-type I_2 stub 59 days after sowing, *veg2-2* I_2 apex 74 days after sowing) under LD (18h) conditions. Mean values ± standard error are shown for n = 2 to 3 biological replicates.

(L) Proportion of abnormal flowers in *veg2-2* defective in each of the four floral whorls for n = 26 flowers grown under LD (24h) conditions.

(M) Number of whorls affected by floral defects at each reproductive node on *veg2-2* plants. Values represent mean ± standard error for n = 7 plants grown under LD (24h) conditions.

In diagrams, arrows indicate the potential for indeterminate growth, circles are flowers, triangles are terminal stubs, ovals are leaves or bracts, and asterisks indicate abnormal nature of structures. In photographs, scale bars indicate 1cm.

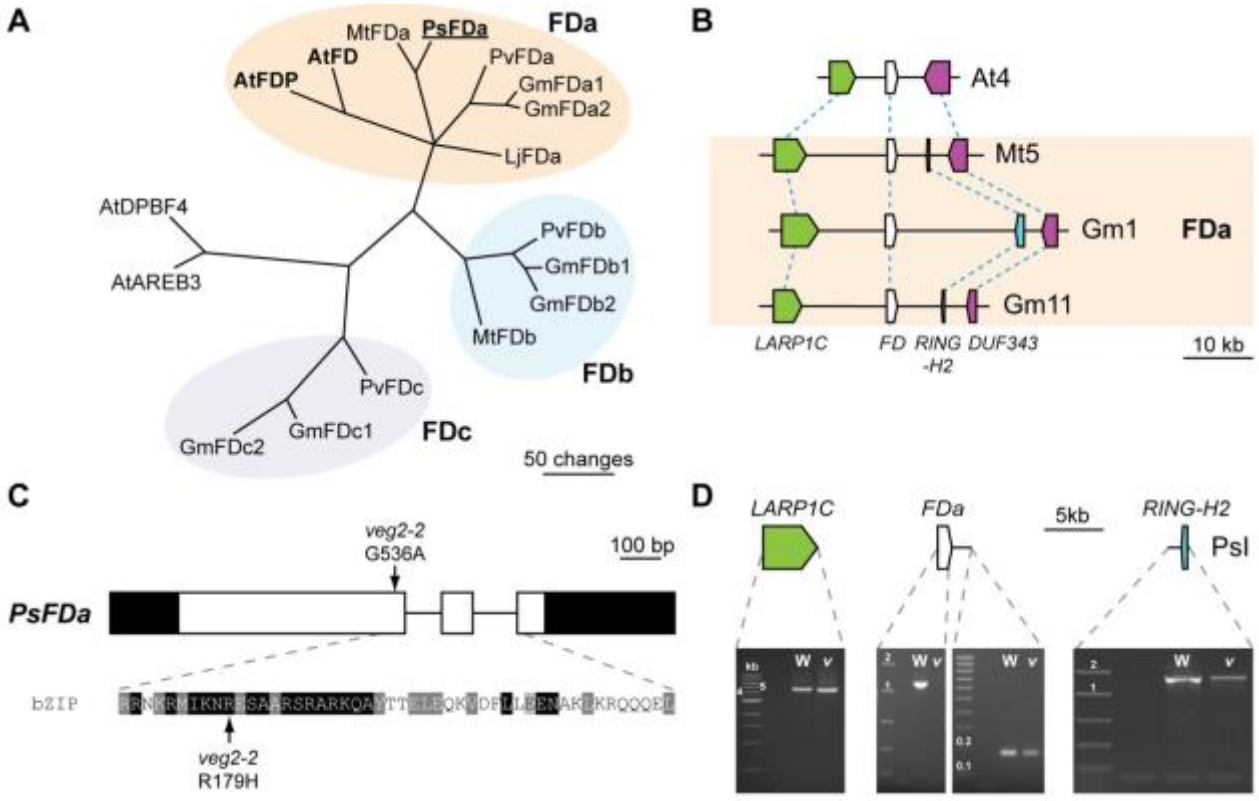

Figure 4. The *VEG2* locus corresponds to *FDa.*

(A) Phylogram of the legume FD family. Branches with bootstrap values <55% obtained from 10,000 trees have been collapsed. Two related group A bZIP transcription factors from Arabidopsis, DPBF4 and AREB3, are included as an outgroup. Alternative names for previously identified soybean proteins GmFDL02 (GmFDb1), GmFDL04 (GmFDc1) and GmFDL0602 (GmFDc2) are adopted to better reflect wider phylogenetic relationships. The analysis is based on the sequence alignment shown in **Supplemental Figure 7**. Sequence details are available in **Supplemental Table 2**. Ps, *Pisum sativum*; At, *Arabidopsis thaliana*; Mt, *Medicago truncatula*; Lj, *Lotus japonicus*; Gm, *Glycine max*; Pv, *Phaseolus vulgaris*.

(B) Microsynteny between genomic regions containing Arabidopsis *FD*, legume *FDa* genes, and flanking genes. Genes are represented as boxes with point showing putative direction of transcription on black lines representing regions of the genome with chromosome number indicated. Between species, corresponding genes are connected by dashed lines. Microsynteny for legume *FDb* and *FDc* genes is shown in **Supplemental Figure 9**. Gene details are given in **Supplemental Table 4**.

(C) Diagram of the pea *FDa* gene showing nature and location of the SNP in *veg2-2*, which affects a conserved amino acid within the functional bZIP domain. Exons are shown as boxes, with coding sequence in white and untranslated regions in black. Shading levels in bZIP domain sequence indicate degree of conservation (black = 100%, dark grey = 80%, light grey = 60%) from alignment with other FD proteins shown in **Supplemental Figure 10**. Nucleotide numbering begins at the start of the coding sequence.

(D) Representative PCR results for full-length coding sequence for *FDa*, its putative 5' and 3' flanking genes, and a region immediately downstream of *FDa* from wild-type (Kaliski) and *veg2-1* mutant gDNA template. For each gel, lanes containing a DNA ladder and PCR product for a no template negative control, wild-type (W) positive control and *veg2-1* (*v*) mutant gDNA are shown (from left to right). Band size (kb) is indicated for relevant ladder bands. Above each gel, the genomic regions isolated are shown diagrammatically, as for **(B)**. Regions between gene diagrams are not drawn to scale.

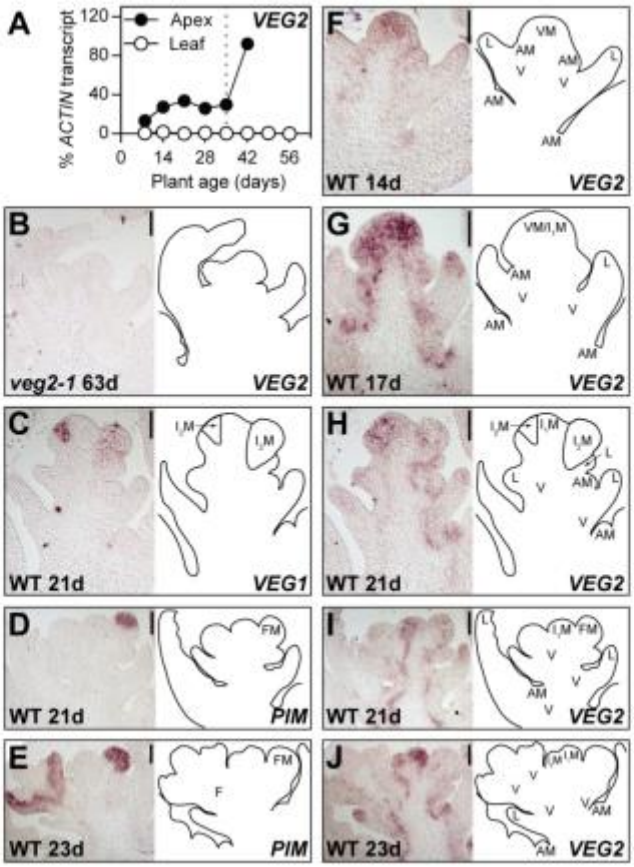

Figure 5. *VEG2* is expressed in the apex throughout development.
(A) Expression of *VEG2* in dissected shoot apices and the uppermost fully expanded leaf of wild-type (NGB5839) plants throughout development. Relative transcript levels were determined by qRT-PCR, normalised to the transcript level of *ACTIN*, and represent mean ± standard error for $n = 2$ biological replicates, each consisting of pooled material from two plants grown in LD (24h). Developing floral buds were first macroscopically visible in the wild-type apex 35 days after sowing (grey line).
(B-J) *In situ* hybridization results for *VEG2* and meristem marker genes. **(B)** *VEG2* expression in the shoot apex of the *veg2-1* deletion mutant, as a negative control for the *VEG2* probe. **(C)** *VEG1* expression domain, as a marker for secondary inflorescence (I_2) meristems. **(D, E)** *PIM* expression domain, as a marker for floral meristems. **(F-J)** *FDa* expression in **(F)** the vegetative apex, **(G)** the apex at the approximate time of the transition from vegetative to primary inflorescence (I_1) meristem identity, **(H-I)** the I_1 apex during early I_2 and floral development, and **(J)** the I_1 apex during development of floral primordia. Each pair **(C, H)**, **(D, I)** and **(E, J)** represents serial sections from the same apex. Shoot apices shown in photographs in **(C-J)** are from wild-type (WT; NGB5839) plants grown in LD (16h). For each sample, plant age in days after sowing (d) is indicated. Regions of expression are indicated by abbreviations on diagrams as follows: axillary meristem (AM), floral meristem (FM), developing flower (F), leaf primordia (L), primary inflorescence meristem (I_1M), secondary inflorescence meristem (I_2M), vasculature (V), vegetative shoot apical meristem (VM).

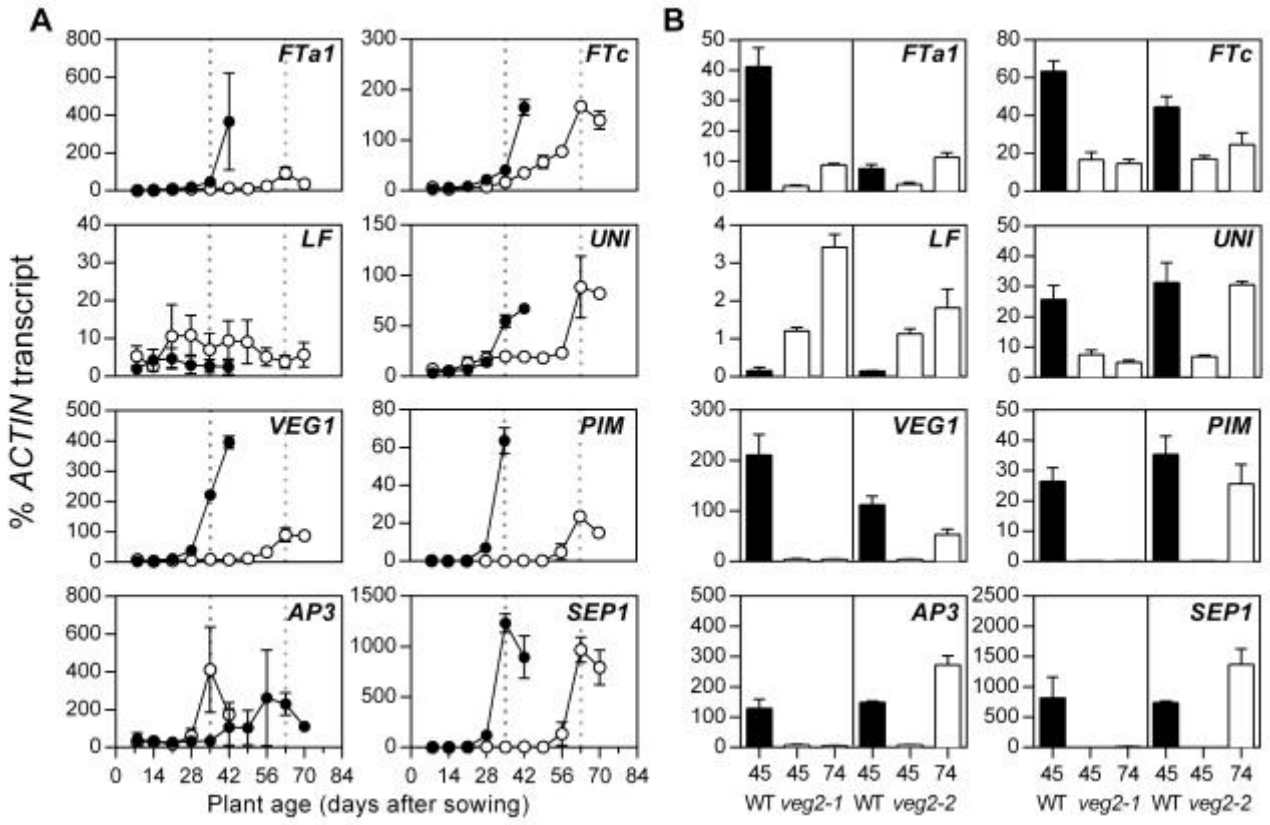

Figure 6. Flowering and meristem identity genes are misregulated in the *veg2* mutants.
Gene expression in dissected shoot apices at **(A)** weekly time points throughout development in wild-type (NGB5839) and *veg2-2*, and **(B)** specific time points in both *veg2* mutants and associated wild-type lines (WT; wild-type siblings of *veg2-1***,** and NGB5839, respectively), in LD (24h and 18h, respectively). In **(A)** developing floral buds were first macroscopically visible in wild-type 35 days after sowing and in *veg2-2* 63 days after sowing (brokem lines). In **(B)** time points correspond to early flowering stages in wild-type (45 days after sowing) and *veg2-2* plants (74 days after sowing). **(A)** and **(B)** show results from the same experiments shown in **Figure 2D** and **E**, respectively. Mean values ± standard error are shown for n = 2 to 3 biological replicates.

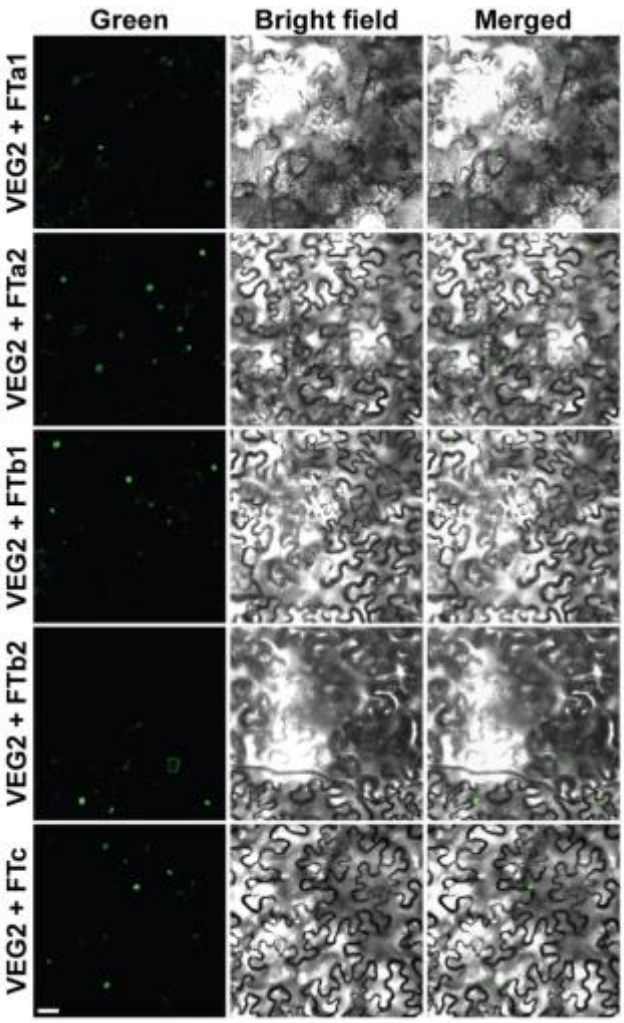

Figure 7. VEG2/FDa can interact with each pea FT protein *in planta*.
For each interaction, VEG2 fused to the N-terminal half of YFP (YFN) was co-expressed separately with the FT protein fused to the C-terminal half of YFP (YFC). Photographs from left to right comprise the green channel image showing fluorescence of YFP, the bright field image and the merged YFP fluorescence and bright field images. Scale bars indicate 40µm. Positive and negative controls are shown in **Supplemental Figure 11**.

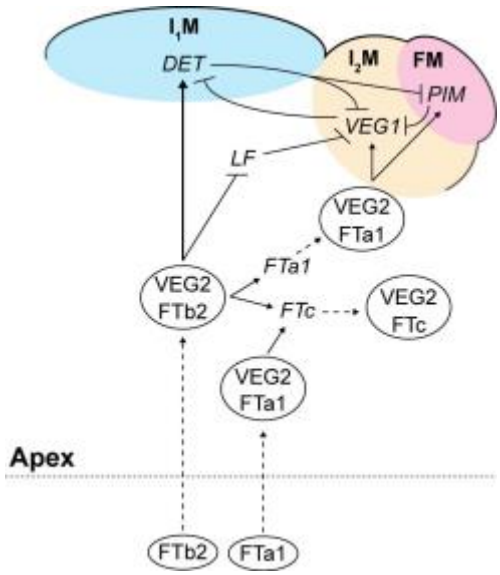

Figure 8. A model for the roles and interactions of *VEG2* during pea inflorescence development.
This model summarizes the main hypotheses derived from the major results of this study and previous studies. We propose that FTb2, the best candidate for the pea florigen signal, travels from the leaf to the shoot apex under LD and interacts within a florigen activation complex (FAC) with VEG2/FDa in the apex to promote primary inflorescence meristem (I_1M) identity, through upregulation of *DET*, *GIGAS*/*FTa1* and *FTc* and repression of the floral repressor *LF*. *FTa1* is expressed in the leaf and encodes a graft-transmissible floral stimulus, and is also expressed in the apex. We infer that FTa1 protein acts in a FAC with VEG2 in the apex to promote expression of *FTc* and induce *VEG1* for specification of secondary inflorescence meristem (I_2M) identity. Repressive interactions between *DET*, *VEG1* and *PIM* are based on a previous model (Berbel et al., 2012).
Proteins and protein complexes are shown as ovals and genes are shown in italics. Unbroken lines indicate inferred roles for genes/proteins as promoting (arrows) or repressing (blunt ends) expression of other genes, either directly or indirectly. Broken lines indicate movement of proteins or translation of genes into proteins. Coloured zones indicate specific meristems boundaries. Genes/proteins shown outside coloured meristem zones are not intended to represent spatial patterns of expression within the apex.