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FLOWERING NEWSLETTER REVIEW

Long-distance regulation of flowering time

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Abstract

One of the great mysteries of plant science appears to have been resolved with the discovery that the protein FT can act as a phloem-mobile florigen hormone. The collective evidence from several laboratories, many from studies on photoperiod response, indicates that FT and its homologues are universal signalling molecules for flowering plants. Duplication and divergence of FT-like proteins reveals an increased complexity of function in certain taxonomic groups including grasses and legumes. There are additional components of long-distance flowering time control, such as a role for gibberellins in some species but probably not others. Cytokinins and sugars are further putative signals. Vernalization processes and responses are generally considered to occur in shoot meristems, but systemic responses to cold have been reported several times. Finally, there is increasing evidence that FT does not act purely to switch on flowering, but in addition, has broader roles in seasonal developmental switches such as bud dormancy and tuberization, and in the regulation of meristem determinacy and compound leaf development. This review seeks to highlight recent progress in systemic floral signalling, and to indicate areas in need of further research.

Key words: Cytokinin, florigen, flowering time, FT protein, gibberellin, graft, phloem, systemic signal.

Introduction: the need for control of flowering time

The onset of reproductive development is a pivotal switch in the life of plants, with optimal timing being especially crucial for species with monocarpic habits. Reproductive success is associated with floral development, pollination, fertilization, and seed development all occurring under appropriate environmental conditions and, in many cases, anthesis needs to coincide with the presence of pollinators. It is therefore no surprise that flowering plants have evolved the means to sense and respond to a number of different environmental cues. The most important and prevalent are responses to reliable seasonal signals: daylength (photoperiodism) and winter cold (vernalization). The former is a stable cue at all latitudes, whereas the latter can vary substantially from year to year, and is likely to be affected by global climate change. In addition, flowering time in many species can be influenced by environmental stresses, including drought, flood, salinity, nutrient deprivation, and shade. In the absence of any effective environmental signal, endogenous processes may predominate and result in flowering by default, often described as autonomous flowering. The broad topic of flowering time control has attracted much attention recently, and many comprehensive review articles are available (Giakountis and Coupland, 2008; Turck *et al.*, 2008; Zeevaart, 2008 Greenup *et al.*, 2009; Michaels, 2009; Amasino, 2010). This article, however, focuses specifically on the role of long-distance signals in the regulation of flowering time.

Long-distance signalling and co-ordination

Co-ordination of many developmental processes involves communication between different locations in the plant, one of the simplest concepts being the need to balance root and shoot growth. In relation to long-distance signalling in flowering time control, the preferred photoperiod sensing organ (leaf) is spatially separated from the site of response (shoot apical meristem, SAM). The sensing of cold has more options. Although classic vernalization models

propose that cold perception occurs locally in the meristem that itself switches into floral development (Dennis *et al.*, 1996; Amasino, 2010), other parts of the plant are capable of sensing cold and acting to regulate flowering time at a distance (Reid and Murfet, 1975; Searle *et al.*, 2006).

There is an extensive and well-documented history of experiments demonstrating long-distance regulation of flowering time, almost all of which point to a signalling component travelling in the phloem. The identity of the signal or signals, however, proved elusive for decades, a period that coincided with the plant hormone research world being dominated by the classic small molecule signals. It is now known that small RNA, larger RNA, proteins, and peptides can all convey information in a non-cell autonomous or systemic fashion (Ruiz-Medrano *et al.*, 2001; Ding *et al.*, 2003; Lough and Lucas, 2006; Kehr and Buhtz, 2008). Out of these, the protein FT has emerged as the clearest candidate for a universal florigen, and features extensively in this review. However, that discovery does not preclude additional or alternate signals. In particular, there is persuasive evidence that gibberellins can, in some circumstances, act as mobile florigen signals. Cytokinins and sucrose may be associated with some components of the inductive process (Corbesier *et al.*, 1998, 2003; Bonhomme *et al.*, 2000; King *et al.*, 2008*a*), and other hormones such as ethylene may have taxonomically restricted florigenic function, with the bromeliads being a prime example (Min and Bartholomew, 1996).

Experimental demonstration of long-distance signalling

Unambiguous evidence for signal transmission can derive from a range of different experimental approaches. The main strategies aim to detect (i) movement of the putative signal molecule, (ii) a phenotypic change, and/or (iii) altered expression of a molecular target. To distinguish local signalling in the SAM from signals arriving from distant sources, one of the most powerful tools is grafting, which combines donor and receiver tissues either differing genetically (An *et al.*, 2004; Ayre and Turgeon, 2004) or in prior treatment such as inductive photoperiod (King and Zeevaart, 1973; Lang *et al.*, 1977). Alternatively, spatially regulated gene expression can be tested, for example by employing the phloem-specific *SUC2* or minor vein phloem-specific *GAS1* promoters (Truernit and Sauer, 1995; Haritatos *et al.*, 2000). Ectopic expression approaches such as these must be viewed with some caution, because the levels, precise sites, and timing of expression are often very different from that in normal plants, and may result in atypical responses.

The evidence for floral signal transport in the phloem is based both on signal velocity and directionality (King and Zeevaart, 1973). In this context, it is important to define what is meant by 'delivery'. In the sense of molecules with signalling functions, it is essential first for the signal to be loaded into the phloem stream via companion cells and then move from this site towards the SAM. Arrival at its final destination within the SAM requires post-phloem transport, involving local cell-to-cell migration within the shoot meristem. The simple presence of a particular RNA, protein or small molecule hormone in sampled phloem sap is insufficient on its own to assess functional delivery.

A further caveat concerns interpretation of negative data from grafting experiments, which frequently may not be reported in the literature and, in some instances, may reflect particular elements of the experimental design. If vascular transport is moving a putative signal molecule from donor to receiver, it is essential to demonstrate that the flow direction is as intended. Where both scion and rootstock carry leaves, prediction of phloem flow is not straightforward and is best tested by labelled CO_2 or sugars to the donor side and confirming that label is detectable in the receiver tissues (King and Zeevaart, 1973). In one recent case testing graft transmission of FT effects in poplar, the lack of response could be due to incongruence between source–sink flow and donor–receiver directionality (Zhang *et al.*, 2010*a*). Frequently, receiver shoot defoliation is included as part of the experimental manipulation: this will indeed enhance donor–receiver flow, but may also have local effects on the receiver itself, from causing stress-induced flowering to the removal of other signalling molecules.

The mechanics and molecular rules of phloem transmission

Phloem translocation systems centre on companion cells (CC) and sieve elements (SE), and can be divided into three functional components: loading (entry to CC and then CC→SE); long-distance transport in SE; and unloading (SE→CC and beyond). Although many signals are synthesized in companion cells, there are both apoplastic and symplastic routes into CC–SE complexes, best studied in relation to sugar transport. Between cells such as CC and SE, most macromolecules will move symplastically through plasmodesmata (PD), although protein secretion and reuptake may occur. Small molecules such as hormone signals often exist in the apoplastic space, so transmembrane and plasmodesmal routes both need to be considered.

Many unknowns remain about exactly how macromolecules traverse PD pores and several reviews are available on the complexities of PD structure and function (Maule, 2008; Lucas *et al.*, 2009; Xu and Jackson, 2010). These intercellular bridging organelles represent potential control points for long-distance signals including floral regulators, at the point of entry into the phloem transport system (mostly in leaves), at the exit site below the SAM, and during local migration through the SAM. The dynamic nature of PD is very evident. Whereas a typical size exclusion limit might be ~1 kDa, this limit can increase massively at certain cell junctions and/or at certain developmental stages and/or in response to certain viral challenges (Oparka and Roberts, 2001). Tracing protein movement with GFP translational fusions suggests an upper limit of at least 67 kDa for

phloem loading (Stadler *et al.*, 2005), and full-length mRNA species are also present. In some cases, a size-dependent lack of unloading beyond the phloem may attenuate the effectiveness of the signalling molecule (Stadler *et al.*, 2005). Proteins targeted to PD include PLDP1 (Thomas *et al.*, 2008) and there are several RNA-binding proteins found in phloem, such as PSRP-1 and CmPP-16, which may have essential functions in enabling RNA movement (Aoki *et al.*, 2005; Yoo *et al.*, 2004).

The large numbers of protein and RNA species detected in the best studied phloem transcriptomes (Omid *et al.*, 2007; Deeken *et al.*, 2008) and proteomes (Giavalisco *et al.*, 2006; Lin *et al.*, 2009) suggest that macromolecule passage from CC to SE in many instances may not be tightly regulated. However, phloem sampling methods may often introduce material from adjacent cells, especially CC, that would not be components of the moving sap and, similarly, the disruption due to incision or use of chelating agents (King and Zeevaart, 1974) may dislodge normally immobile components of the parietal layer of the sieve elements (Atkins *et al.*, 2011). Some phloem sap macromolecules may be left over from synthesis during SE differentiation prior to nuclear and ribosomal loss. Other routes into phloem transport systems involve access via CC from adjoining phloem parenchyma, bundle sheath or mesophyll cells. One example where a leaf signal is initiated from outside the phloem is deduced from the effectiveness of PHYB-GFP expressed in the mesophyll compared with a lack of response from expression in vascular tissue (Endo *et al.*, 2005). This strongly implicates a specific PHYB-dependent intercellular signal, the identity of which remains unknown. By contrast, similar approaches with CRY2 showed that only phloem expression was effective at regulating *FT* (Endo *et al.*, 2007).

Photoperiod signalling

Following some early reports on daylength responses in plants by Tournois (1912) and Klebs (1913), Garner and Allard (1920) provided arguably the first clearcut experimental demonstration of flowering control by photoperiod. Subsequent signalling studies indicated that the phloem-borne photoperiodic floral stimulus might be universal, based on grafts between species and between different photoperiod response classes: long-day (LD), short-day (SD) and day-neutral (Zeevaart 1958, 1976; Lang *et al.*, 1977).

Genetic, molecular, and physiological evidence from *Arabidopsis* and several other species has now filled in much of the detail of what has emerged as a likely generic mechanism for the regulation of flowering time. The photoperiodic signalling system comprises (i) daylength and light-sensing in leaves leading to (ii) synthesis, phloem loading, and long-distance transport of active signalling molecules, and (iii) final delivery to the SAM where detection of incoming signalling molecules causes activation of transcriptional cascades that specify the transition from vegetative to reproductive state (Fig. 1).

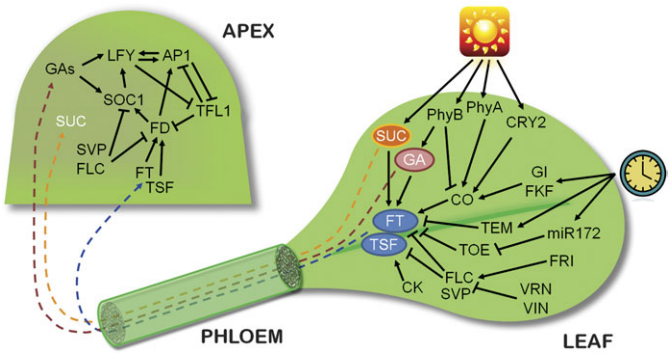

Fig. 1. Model depicting major known long-distance florigenic signals, together with their main regulators in the leaf and their main targets and co-regulators in the shoot apex. For visual clarity, not all known relationships are shown, and others are excluded where lines of evidence are equivocal or contradictory. Gene names are for *Arabidopsis*, but orthologous functions for most of these have been established in other species. Normal arrows represent positive regulatory relationships, and T-arrows represent negative regulatory relationships. Mobile signals are outlined with ovals. Pathways of phloem mobility are shown as dashed arrows, blue for FT and homologues, purple for GAs, and orange for sucrose. Further details of gene functions and relationships can be found in recent reviews (e.g. Giakountis and Coupland, 2008; Turck *et al.*, 2008; Zeevaart, 2008; Greenup *et al.*, 2009; Michaels, 2009; Amasino, 2010).

Signal induction

The sensing of photoperiod depends on the combined action of photoreceptors and the circadian clock systems. Based on the almost complete loss of photoperiodic flowering response in *constans* (*co*) mutants, much of the photoperiodic regulation in *Arabidopsis* and other species appears to be channelled through CO. Upstream processes associated with *CO* expression relate mainly to a combination of circadian clock control of transcription via *GI*, and photoreceptor effects (PHYA, PHYB, and CRY) on CO protein stability (Valverde *et al.*, 2004). In long-day plants (LDP) such as *Arabidopsis*, CO protein only accumulates late in the LD when two phenomena coincide: (i) the circadian peak of *CO* transcript abundance and (ii) the suppression of CO the protein degradation system by PHYA and CRYs. Protein levels then decay again during the night. By contrast, much of the *CO* transcript peak under SD occurs in the dark phase. During most of the SD photoperiod and the first part of a LD, CO protein is destabilized due the predominant effects of PHYB (Turck *et al.*, 2008).

The *CO* gene is expressed in both leaf phloem and the SAM (Simon *et al.*, 1996). However, ectopic *CO* expression restricted to the SAM does not result in flowering (An *et al.*, 2004), and *co* mutant receiver scions are complemented by grafts to *CO*-expressing donors (An *et al.*, 2004; Ayre and Turgeon, 2004). The logical conclusion is that CO, at least in terms of regulating flowering time, acts in leaves and specifically in the phloem. Following this discovery,

attention moved to possible mobile molecules that would convey the florigenic signal from leaf to SAM. The *Arabidopsis FT* gene, a known target of CO, together with homologues in tomato, rice, and cucurbits, rapidly emerged as the missing element in the story (Lifschitz *et al.*, 2006; Corbesier *et al.*, 2007; Jaeger and Wigge, 2007; Lin *et al.*, 2007; Mathieu *et al.*, 2007; Tamaki *et al.*, 2007). Genes in the *FT* family encode small soluble proteins of ~20 kDa, well within the size exclusion limit previously described for phloem mobility. Some other family members, notably *TFL1* and its homologues, were already known for their inhibitory effects on flowering and are described later.

Signal transmission

Expression of *FT* is strongest in leaf phloem, with transcripts and promoter activity typically below detection limits in the SAM (Kardailsky *et al.*, 1999; Takada and Goto, 2003). However, ectopic *FT* expression in the SAM does induce flowering (Corbesier *et al.*, 2007). Given that the targets of FT are found in the meristem, it was very likely that products of the *FT* gene itself represented a mobile florigenic signal, moving either as mRNA and/or as protein. Alternatively, other mobile signals regulated by FT could have florigenic activity.

Long-distance RNA signals and flowering

There are presently only a few confirmed examples of effective long-distance signalling conveyed by phloem RNA. These include shoot-to-root translocation of *miR399* during phosphate starvation (Lin *et al.*, 2008; Pant *et al.*, 2008) and movement of *GAI* mRNA to regulate leaf development (Haywood *et al.*, 2005). Systemic tuberization signals in potato, discussed later, may include *mir172* and *StBEL5* mRNA (Banerjee *et al.*, 2006; Martin *et al.*, 2009).

Phloem movement of *FT* transcripts remains a formal possibility. although several studies have failed to demonstrate graft transmission of *FT* mRNA (Lifschitz *et al.*, 2006; Corbesier *et al.*, 2007; Lin *et al.*, 2007; Notaguchi *et al.*, 2008). However, a recent report indicates that part of the *FT* transcript structure can confer non-cell autonomous properties (Li *et al.*, 2009), based on elegant experiments where a non-translatable version of *FT* was fused with exogenous viral or GFP sequences. What is needed next is evidence that mobility is associated with function, and that the transported mRNA leads to translation into FT protein at the meristem or acts to regulate some other specific target.

The florigenic function of FT protein

Conclusions drawn from across several species all point to FT protein acting as a mobile florigen in *Arabidopsis* (Corbesier *et al.*, 2007; Jaeger and Wigge, 2007; Mathieu *et al.*, 2007), rice (Tamaki *et al.*, 2007), tomato (Lifschitz *et al.*, 2006), and cucurbits (Lin *et al.*, 2007). Many subsequent reports have strengthened this view.

If FT is a phloem-mobile signal, any factor that regulates its expression, transport or activity in leaves could systemically influence flowering time. In addition to the well-established function of CO, there are CO-independent regulators of *FT* expression (Fig. 1). These include the negative effects of FLC via binding to an *FT* intron in an *SVP*-dependent manner (Searle *et al.*, 2006), the repressive effects of *AP2*-like genes which are themselves negatively regulated by *miR172* (Jung *et al.*, 2007; Yant *et al.*, 2009), and negative regulation by *TEM1/2* (Castillejo and Pelaz, 2008). There are also opportunities for deliberate manipulation of flowering time, for example via artificial microRNA, where *amiR-FT*, based on *miR172* as it happens, was able to suppress flowering but only when expressed from the leaf and not the SAM (Mathieu *et al.*, 2007). This reinforces the conclusion that *FT* mRNA does not significantly accumulate in the SAM.

Graft transmission of FT proteins has been directly demonstrated for FT:GFP and FT-T7 in *Arabidopsis* (Corbesier *et al.*, 2007; Notaguchi *et al.*, 2008), and for FT-like proteins in cucurbits (Lin *et al.*, 2007). However, there is presently little evidence to suggest that FT protein moves in a selective manner, nor that it requires a chaperone or other partner. One suggestion is that FT interactions with other proteins may restrict movement, although this was based on constitutive over-expression of a 14-3-3 protein that bound to the rice FT orthologue, Hd3a (Purwestri *et al.*, 2009). Other reports show the importance of mass or size in conferring or restricting bioactivity. For example, FT-GFP expressed under the generic phloem promoter *SUC2* is mobile and bioactive in *Arabidopsis*, although less so than FT on its own (Corbesier *et al.*, 2007). By contrast, FT fused to 2×GFP (net 74 kDa) is immobile and florally inactive. A more elegant demonstration used 3×YFP fused to FT via a TEV protease recognition site linker (Mathieu *et al.*, 2007). The intact FT:3×YFP protein was similarly immobile and inactive whereas, after cleavage, the released FT was able to rescue the late flowering phenotype of an *ft* mutant. It can also be inferred that PD pore size associated with SE access varies with cell location: in *Arabidopsis*, *FT:GFP* expression under the minor vein phloem-specific *CmGAS1* promoter completely lacked the mobility and systemic bioactivity seen with *SUC2::FT:GFP* which is more extensively expressed through the phloem. Expression and local function of *GAS1::FT:GFP* was confirmed via activation of the *FUL* gene in leaves (Corbesier *et al.*, 2007).

The collective evidence strongly indicates FT movement in the phloem (Table 1) yet, paradoxically, attempts to complement *ft* mutants across a graft union have not given very convincing effects, even when strong constitutive (*CaMV35S*) or phloem-specific (*AtSUC2; SULTR2;1*) promoters were employed (Corbesier *et al.*, 2007; Notaguchi *et al.*, 2008, 2009). Where the native expressed gene was tested, a marginal graft transmissible effect was noted in *Arabidopsis* (Notaguchi *et al.*, 2008; Turnbull and Justin, 2004) and none at all in tomato (Lifschitz *et al.*, 2006; Lifschitz and Eshed, 2006). The variable success may reflect technical limitations in delivering sufficient FT signal, or may indicate the need for an additional factor.

Table 1. Summary of evidence for directly proven and indirectly deduced phloem-mobile florally active signalling molecules

Molecule	Plant	Evidence	Reference
FT	*Arabidopsis*	FT:GFP graft transmission into SAM	Corbesier *et al.*, 2007
		FT-T7 graft transmission	Notaguchi *et al.*, 2008
		FT graft rescue of *ft*	Notaguchi *et al.*, 2008
		FT:GFP graft rescue of *ft*	Corbesier *et al.*, 2007
Hd3a (OsFT)	Rice	Hd3a:GFP and RFT1:GFP	Tamaki *et al.*, 2007
and RFT1		accumulation in SAM	Komiya *et al.*, 2009
SFT (SlFT)	Tomato	35S::SFT graft rescue of *sft*	Lifschitz *et al.*, 2006
CmFTL1/2	Pumpkin	Direct detection in phloem sap	Lin *et al.*, 2007
		Interspecific graft floral induction	
PsFTa1 (GIGAS)	Pea	Graft rescue of *gigas* mutants	Beveridge and Murfet, 1996
			Hecht *et al.*, 2011
FT/TSF-like	Brassica	Direct detection in phloem sap	Giavalisco *et al.*, 2006
GA_5	*Lolium*	Translocation of leaf-applied GA to SAM	Hisamatsu and King, 2008
		Increased endogenous GA content in SAM by end of inductive LD	

Direct evidence for FT proteins in phloem sap

In addition to the imaging and biological evidence for FT protein movement, homologues have been directly detected in phloem sap of several plant groups including brassica, rice, and cucurbits (Giavalisco *et al.*, 2006; Lin *et al.*, 2007; Aki *et al.*, 2008), although cucurbit phloem sap has recently been shown to be derived from an atypical phloem (Zhang *et al.*, 2010*b*). Interestingly, the rice FT homologues found in phloem did not include Hd3a or RFT, the two major proven florigenic signals for this species: instead an FT-like homologue was found, along with two proteins in the TFL1 clade and one MFT-like member (Aki *et al.*, 2008). Although there is no published phloem proteome for *Arabidopsis*, recent results from our laboratory confirm the presence of FT protein in phloem (Z Rahmat, M Bennett, C Turnbull, unpublished data), and a T7 tagged version of FT was found to cross a graft union (Notaguchi *et al.*, 2008).

FT action in the shoot apical meristem

Unloading of FT protein from sub-apical phloem endings is predicted to be followed by local migration towards its final destination within cells of the SAM. In rice, FT:GFP is clearly visible throughout the meristem (Tamaki *et al.*, 2007), although FT:GFP and FT-myc in *Arabidopsis* SAMs appear largely restricted to the vicinity of the phloem (Corbesier *et al.*, 2007; Jaeger and Wigge, 2007), perhaps due to sensitivity limits of the methods used.

The transition to reproductive development requires a dramatic but highly co-ordinated switch in gene expression, initially to specify an inflorescence, then individual flowers, then the organ series within each flower. This is achieved through regulatory cascades that have been thoroughly reviewed over recent years (Soltis *et al.*, 2007; Causier *et al.*, 2010). Effective action of FT requires that it sits atop these cascades, activating transcription factors that act as master switches, including FD, SOC1, and AP1 (Fig. 1). Of these, FT directly interacts with FD, and this complex has floral promotive activity through direct or indirect transcriptional activation of *AP1, SOC1*, *and LEAFY* (Abe *et al.*, 2005; Wigge *et al.*, 2005; Mathieu *et al.*, 2007; Li and Dubcovsky, 2008; Meng *et al.*, 2011).

FT-like homologues are also systemic signals

In every species examined to date, there are additional FT-like genes, several of which appear likely to encode systemically mobile proteins. Some of the best supported examples are listed in Table 1.

The closest homologue of FT in *Arabidopsis* is TSF (twin sister of FT) but the late flowering of *ft* mutants and the relatively mild phenotype of *tsf* mutants suggest it does not provide complete photoperiodic signalling 'backup'. However, a positive function of TSF is clearly revealed from the extreme late flowering of *ft tsf* double mutants under LD, and there is also an important role in SD (Michaels *et al.*, 2005; Yamaguchi *et al.*, 2005). Similarly, over-expression of TSF results in precocious early flowering. Sites of TSF expression include phloem in the hypocotyl and sub-apical tissues, which partially overlaps with FT expression that is predominantly in leaf phloem companion cells, suggesting some divergence of regulation (Yamaguchi *et al.*, 2005). However, FT and TSF are regulated in a broadly similar manner by CO and SVP. It can be concluded that TSF is a phloem-expressed protein and most likely shares with FT the ability to convey long-distance florigenic signal activity (Jang *et al.*, 2009).

Rice is classed as a facultative short day plant (SDP) with Hd3a acting as the main promoting signal under inductive SD. RFT1 is the closest homologue to Hd3a in rice and is also a floral promoter. However, somewhat similar to *Arabidopsis* FT and TSF, regulation of *RFT1* expression has diverged and, instead, predominantly functions as a LD

signal (Komiya *et al.*, 2008, 2009). As with *Hd3a*, *RFT1* transcription occurs in leaves but is barely detectable in the SAM, yet RFT1:GFP fusion protein expressed under the native *RFT* promoter accumulates in the shoot tip in vegetative plants and also in the SAM following LD induction (Komiya *et al.*, 2009), indicating likely systemic movement. Expansion of the FT/TFL1 family in rice (Chardon and Damerval, 2005), maize (Danilevskaya *et al.*, 2008, 2010; Meng *et al.*, 2011), and other grasses may point to a more diverse range of functions, and it will be interesting to see how many members are mobile over long or short distances.

A recent report by Hecht *et al.* (2011) has uncovered new dimensions in the functioning of multiple *FT*-like genes in pea (*Pisum sativum*). This species has a sub-clade of five *FT*-like homologues. Similar *FT* clade expansions are evident in other legumes such as *Medicago truncatula* and soybean (*Glycine max*). What is most intriguing are the divergent spatial expression patterns and photoperiod regulation of each *FT*-like gene. Two are highlighted as almost certainly carrying information systemically from leaves to SAM: *FTa1*, which represents the previously characterized *gigas* mutant; and *FTb2*. Even more revealing is the fact that FTa1 appears to be a true florigenic signal, whereas FTb2 affects a broader set of developmental responses to photoperiod including the control of axillary bud dormancy release. In addition, unlike FT homologues in *Arabidopsis* and other species, some of the pea genes (*FTa1, FTc* and, probably, *FTa2*) are expressed in the shoot tip and exhibit up-regulation under inductive LD conditions (Hecht *et al.*, 2011). Because *FTc* is not expressed in leaves, it cannot be part of the systemic signal, but its early expression in shoot tips under inductive LD indicates that it may be an immediate target of the incoming florigenic signal(s). It is important to note that, in this work, the shoot tips sampled represent the terminal 2–3 mm of apical tissue, rather than the much smaller true meristematic region. Nonetheless, the differential spatial regulation of each *PsFT* homologue presents a valuable perspective on the evolution of complexity overlaid on the basic model of FT function. It is intriguing that pea also has increased numbers of *TFL1* homologues with divergent functions between *PsTFL1a/DET* and *PsTFL1c/LF* (Foucher *et al.*, 2003).

Mobile floral inhibitors

Evidence across several species points towards possible systemic inhibitors of flowering (Lang *et al.*, 1977). However, the nature of the supporting data often allows alternative interpretations, particularly where recessive early flowering mutants have been examined. The clearest example of this is in pea, where mutants are abundant and grafting is straightforward. Whereas early flowering might be due to lack of an inhibitor, as originally proposed for *sn*, *dne*, and *ppd* mutants (reviewed by Reid *et al.*, 1996; Weller *et al.*, 1997), it now appears more likely to represent a state where a floral promoter is de-repressed or over-produced (Weller, 2005; Weller *et al.*, 2009). Although the molecular picture is not yet complete, the regulation of FT and its homologues can account for many of the results of physiological experiments. In particular, it was recently shown that the early flowering mutant *dne* has precocious early onset of *FTa1* and *FTb2* expression (Hecht *et al.*, 2011).

There are other species where graft-transmissible inhibition has been demonstrated. In cucurbits, a squash (*Cucurbita maxima*×*C. moschata*) rootstock dramatically inhibited flowering in cucumber (*Cucumis sativus*) (Satoh, 1996) although reciprocal interspecific grafts were not conducted, and the data are open to the alternative interpretation that the rootstock failed to provide a floral promoter. More robust evidence for transmissible floral inhibition comes from grafts among several near-isogenic lines of soybean with allelic variation at several flowering time loci (Cober and Curtis, 2003). Here, late-flowering genotypes used as leafy rootstocks appeared to have caused delayed flowering in early flowering genotype scions. Positive effects on flowering were noted in other combinations. The genes underlying these loci have not yet been clarified.

Systemic cold regulation

Cold-induced flowering relates largely to adaptations to extended periods of low temperatures during winter, when reproduction is unlikely to succeed. Most research has been on temperate species where a lengthy cold exposure, typically a few degrees above freezing, leads subsequently to prompt flowering when temperatures increase in spring. This process is commonly known as vernalization, although there is still debate as to whether the term should be applied to the environmental exposure (vernalization treatment), and/or the subsequent molecular and developmental changes (vernalization response). The site of cold perception is generally accepted as being principally in the vicinity of the shoot apex, obviating the need for systemic signal transmission (Sung and Amasino, 2004; Wellensiek, 1962). However, some evidence for systemic cold signalling has come from pea and sweet pea (*Lathyrus odoratus*) grafting experiments using vernalized and non-vernalized graft combinations (Reid and Murfet, 1975; Ross and Murfet, 1986). The molecular basis of these responses is not yet clear. In *Arabidopsis*, a systemic signalling component is associated with leaf expression of the *FLC* gene (Searle *et al.*, 2006), described further below.

In addition, many tropical and sub-tropical species respond to low temperature by initiating reproductive development. The temperature range is higher, often around 10–15 °C, and is sometimes referred to a cool temperature response (Wilkie *et al.*, 2008). At present, because little detail exists on the molecular basis of tropical species responses, it is not known to what extent the mechanisms are similar to vernalization. However, there is accumulating evidence that at least some components of cool temperature responses involve perception outside the SAM, i.e. in leaves,

and thus include a systemic signalling component. A clear demonstration of the importance of leaves comes from the cool temperature induction of mango (Davenport *et al.*, 2006; Ramirez *et al.*, 2010) where defoliation of girdled shoots prevented flowering but as little as a single leaf on a one-metre stem was sufficient to result in substantial floral induction. It will be interesting to ascertain whether FT mobilization from those leaves is the causative signal, and whether altered photosynthate transport in defoliated plants has a significant effect.

FLC as a systemic regulator

The *Arabidopsis* gene *FLC* is a major controller of flowering time. It is a member of the MADS box transcription factor family but, interestingly, appears not to have orthologues in grasses which use different genes for the analogous functions, especially in transducing cold signalling. FLC acts as a strong inhibitor of flowering, mainly through negative regulation of the genes *FT*, *SOC1*, and *FD* (Searle *et al.*, 2006), all of which represent positive components of the flowering time pathways. Thus *FLC* repression is a typical pre-condition for flowering to proceed, and indeed down-regulation of *FLC* expression occurs during the prolonged exposure to cold that occurs during experimental or natural vernalization treatments. Many of the popular laboratory ecotypes of *Arabidopsis* have minimal cold requirements because they have low expression of *FLC* due to mutations in upstream regulators (Sheldon *et al.*, 1999, 2000) or in *FLC* itself (Michaels *et al.*, 2003). The function of FLC in leaves represents a component of systemic action on flowering time because one main target here appears to be *FT* (Searle *et al.*, 2006), which is not significantly expressed in the SAM. Some cold-dependent regulation of flowering and *FT* expression is retained in *flc* null mutants (Moon *et al.*, 2003), implicating a wider set of genes including *MAF2*, another MADS member, in leaf-dependent vernalization responses (Alexandre and Hennig, 2008).

Autonomous pathway signalling

Flowering time regulators that are independent of environmental inputs, apart from those involving gibberellins, are normally grouped within the autonomous pathway. In dicots, *FLC* is again a key gene, opening up the possibility that there is a systemic component here too, although this has not been directly reported. In cereals, the *ID1* gene of maize is expressed mainly in leaves and has been suggested as an upstream element of systemic floral signal initiation (Colasanti *et al.*, 1998), although direct evidence for the activation of maize *FT* genes is presently lacking (Coneva *et al.*, 2007). However, the rice *ID1* homologue, *Ehd2*, is also expressed in leaves and acts via *Ehd1* to regulate the *FT* homologues *Hd3a* and *RFT* (Matsubara *et al.*, 2008). In tomato, a day neutral species with no known cold requirement, flowering time regulation is, in essence, autonomous and is controlled by the systemic movement of FT protein (Lifschitz *et al.*, 2006; Shalit *et al.*, 2009).

Gibberellins and flowering

Of all the small-molecule candidates for floral signals, there is the most substantial evidence of a role for gibberellins (GAs), at least in some species, although probably not in others (reviewed by Mutasa-Gottgens and Hedden, 2009). In pea, for example, many GA-deficient and GA-insensitive mutants are known, but they display little or no change in flowering time (Murfet and Reid, 1987). In many woody species, GAs may instead have an inhibitory role (reviewed by Wilkie *et al.*, 2008) possibly by promoting antagonistic vegetative growth.

Here the focus is on whether GAs themselves are mobile florigens and/or whether they control other mobile floral signals. Gibberellin levels are often regulated by photoperiod, typically elevated in plants such as spinach after the transfer from SD to florally inductive LD, due to altered expression of biosynthetic and catabolic genes (Lee and Zeevaart, 2005, 2007). Similar rapid LD responses are found in *Arabidopsis* (Gocal *et al.*, 2001), where GA may have a permissive role and GA addition generally accelerates flowering in LD (Hisamatsu and King, 2008). An FT-independent role for GAs in *Arabidopsis* flowering is revealed most clearly under SD when the CO/FT system is not activated. The independence of GA promotion and photoperiodic promotion in *Arabidopsis* was demonstrated by GA applications to *ft tsf* double null mutants which lack any detectable photoperiod flowering response (Jang *et al.*, 2009). Leaf-to-apex transmission of endogenous GAs has not been explicitly demonstrated in *Arabidopsis*. However, the accumulation of GAs at the apex prior to floral initiation points to a systemic signalling component of the GA flowering pathway, especially as this increase was not accompanied by the altered expression of GA biosynthetic genes in the apex (Eriksson *et al.*, 2006).

King, Evans, and others have used *Lolium temulentum* as a model species amenable to induction by a single LD (King *et al.*, 2001; King and Evans, 2003). Although lacking the genetic tools of *Arabidopsis*, *Lolium* has provided valuable insights into the detail of GA function in flowering. Certain endogenous GAs rapidly accumulate in the SAM following transfer to inductive LD, and this increased GA content precedes or coincides with the earliest detectable developmental changes. In addition, GA transport, almost certainly in the phloem, from leaves into SAMs is enhanced under inductive photoperiods: the amount of exogenous labelled GA_5 transported is proportional to the magnitude of the flowering response (King *et al.*, 2001).

There is also evidence that different GAs exhibit divergent bioactivity between internode growth promotion and floral promotion. In *Lolium* at least, a likely explanation for differences in floral activity has emerged that does not relate to intrinsic bioactivity or mobility. Instead, growth-promoting GAs such as GA_1 and GA_4 may be, at

best, weakly effective at promoting flowering because of high de-activating GA2oxidase activity in the apical and sub-apical meristematic regions but not in the main elongation zone (King *et al.*, 2006, 2008). By contrast, florally inductive GAs such as GA_5 have a double bond in the A-ring which prevents 2-oxidase action, thus they can remain bioactive within the SAM.

Local GA signalling in the SAM is also likely. GA biosynthesis genes are active in the shoot tip, show diurnal cycling and, in some cases (e.g. *AtGA20ox2*), show LD-dependent increases in transcript abundance (Hisamatsu and King, 2008). Increased imported and locally produced gibberellins in the SAM may activate LEAFY expression via effects on a GA-responsive MYB transcription factor that binds to the LEAFY promoter (Gocal *et al.*, 1999, 2001; Hisamatsu and King, 2008).

A second parallel GA-dependent systemic mechanism may act via GA regulation of FT expression in the leaf, with FT being the mobile factor. In *Arabidopsis*, added GA results in increased FT transcript levels under LD (Hisamatsu and King, 2008). This could be due to direct GA signalling effects on *FT*, as there are GA response elements in the *FT* promoter. Alternatively, GA may act via CO-dependent regulation of *FT*. Although there is a modest increase in *CO* transcripts in *Lolium* leaves in response to GA application (Hisamatsu and King, 2008), it is unknown whether CO protein levels are affected.

Cytokinins and flowering

Cytokinins (CKs) under some conditions may induce flowering. Very recently, D'Aloia *et al.* (2011) reported that cytokinin application can cause flowering via activation of the FT homologue, TSF. Genetic and molecular evidence strongly indicates that this response requires *TSF* and *SOC1* but not *FT*. It is likely that TSF, known also to be a mobile protein (Jang *et al.*, 2009), is the main long-distance signal agent in this case, and leads to gene activation in the shoot apex. The timing and nature of downstream events, including up-regulation of the receptor/transcription factor *FD* and the early target genes *SOC1* and *AP1*, are consistent with a normal but *FT*-independent progression towards flowering. It has not yet been resolved whether, in addition to cytokinin acting in the leaves via *TSF* activation, there may be direct action of systemically transported CK in the SAM. The latter remains a possibility because of the very rapid (30 min) up-regulation of the cytokinin response gene *ARR5* in the SAM following CK supply to the root system (D'Aloia *et al.*, 2011), and leads to the question of whether endogenous CKs show the same activating behaviour. Experiments with CK-defective genotypes may help to resolve the issue, but their late-flowering phenotypes may be more associated with impaired shoot growth rates (Werner *et al.*, 2003). Endogenous cytokinins themselves are highly mobile in vascular systems. Changes in xylem and phloem CK levels correlate with inductive photoperiods (Lejeune *et al.*, 1994; Havelange *et al.*, 2000; Corbesier *et al.*, 2003), but this hormone on its own is often insufficient to cause full progress to flowering (Bernier *et al.*, 1993; Bonhomme *et al.*, 2000). In addition to effects of cytokinin on meristem gene expression, cytokinin application modifies PD connections between meristem cells (Ormenese *et al.*, 2006) which probably influences the non-cell autonomous local movement of incoming signalling macromolecules such as FT and TSF, but also locally expressed TFL1 and early markers of the floral transition such as LEAFY (Sessions *et al.*, 2000). Somewhat similar changes in symplasmic size exclusion limits into and within the SAM are seen as part of the normal developmental progression from the vegetative to the reproductive state (Gisel *et al.*, 1999, 2002).

Sugars and flowering

Sugars such a sucrose are almost continuously moving in the phloem stream from source to sink, and in this regard could convey signalling information from the leaf to the SAM, in addition to their core functions in carbon metabolism and growth. Indeed there are several lines of evidence implicating roles for sugars in regulating flowering time.

Transient increases in leaf sugar export via phloem correlate with successful floral induction, and mutants impaired in carbon metabolism display late-flowering phenotypes (Corbesier *et al.*, 1998; Havelange *et al.*, 2000). Rapid quantitative increases in leaf and shoot apex sucrose content in *Arabidopsis* were detected during an inductive LD of relatively high intensity light (King *et al.*, 2008*a*) but this was not seen in low intensity red- or far red-rich LD, suggesting that sucrose may be an additional signal rather than an essential one. The role of sucrose under LD appears to be partly through the amplification of FT expression (King *et al.*, 2008*a*), consistent with the rescue of many late-flowering mutants, but not *ft*, when cultured on media with moderate levels of sucrose (Roldan *et al.*, 1999; Ohto *et al.*, 2001). Recently, the *Arabidopsis IDD8* gene was reported to have functions in FT-dependent flowering by mediating sugar responses via the regulation of sucrose synthase expression (Seo *et al.*, 2011).

Sucrose or other sugars on their own are not usually sufficient, as high intensity SD of equivalent light integrals to those used in LD induction, were ineffective. Nonetheless, acceleration of *Arabidopsis* flowering is seen in both wild-type and *ft* mutants under extended periods of high intensity SD, implicating an FT-independent component (King *et al.*, 2008*a*). If sucrose itself is a mobile signal, in addition to indirect effects via FT and other regulatory pathways, then there would need to be specific targets and sucrose-sensing components in the SAM (Francis and Halford, 2006). Although timely import and accumulation of sucrose in the SAM is well documented, the downstream consequences require elucidation.

FT and TFL1 relationships

TFL1, closely related to FT, is also a non-cell autonomous protein but does not act systemically. The main site of

expression of *TFL1* and orthologues in other species such as tomato *SP* is within shoot meristematic tissues (Bradley *et al.*, 1997; Pnueli *et al.*, 1998). Conti and Bradley (2007) demonstrated that TFL1 protein distribution within the SAM clearly extends beyond the sites of *TFL1* mRNA accumulation, and thus the protein has intercellular mobility similar to that predicted to be necessary for FT movement through the same region.

The structural similarities between FT and TFL1 proteins are clear, but a few small differences appear sufficient to confer their largely opposing functions, based on amino acid and exon swapping experiments (Hanzawa *et al.*, 2005; Ahn *et al.*, 2006). FT and TFL1 interact with at least some shared subsets of proteins, most notably the transcription factor FD and its homologues (Pnueli *et al.*, 2001; Abe *et al.*, 2005; Wigge *et al.*, 2005). Binding of TFL1 may maintain FD in an 'off' state. This condition sustains indeterminate meristem development and is essentially repressive for flowering, although it is too simplistic to suggest that TFL1 is purely a floral inhibitor. Nonetheless, FT arrival in the phloem and accumulation in SAM cells may compete with TFL1 for binding to FD, with floral activation occurring if sufficient FT molecules are present. Other interactors include NEK [NIMA-related kinase] and 14-3-3 proteins (Pnueli *et al.*, 2001; Abe *et al.*, 2005; Purwestri *et al.*, 2009), but their functions have not yet been fully resolved by genetic or molecular studies.

Other developmental switches with regulatory features in common with floral induction

Arabidopsis as a model for flowering time has been central to much of the recent impetus towards elucidation of the florigen concept. Other species share many elements of flowering control with *Arabidopsis*, but may feature additional developmental switching events. In particular, *Populus*, potato, and tomato have provided new insights into seasonal bud dormancy, tuberization, leaf development, and the sympodial habit (Table 2).

CO/FT in tree bud dormancy

Photoperiod-dependent switching between shoot growth and bud dormancy is typical of many temperate tree species, and appears to be highly adaptive for matching the onset of dormancy with the length of seasons at different latitudes. The control, at least in *Populus*, is associated with tight regulation of *FT* expression by *CO* and photoperiod, closely matching predictions of the external coincidence model (Bohlenius *et al.*, 2006). There is presently no direct evidence for systemic FT transmission in *Populus* (Zhang *et al.*, 2010*a*) but the regulation by CO is most likely to occur in leaves, from which a long-distance signalling component can be inferred. Flower induction occurs under long summer days, followed by the onset of bud dormancy as daylength shortens. The critical daylength shifts with latitude of origin, suggesting local ecotypic adaptation. Exit from dormancy depends largely on chilling, and may be mediated by elevated FT and GAs. An intriguing model was recently proposed in which intercellular and long-distance signal movement is restricted during winter by callose deposition at PD and on sieve plates (Rinne *et al.*, 2011). Chilling-induced FT expression is accompanied by the up-regulation of β-glucanases that hydrolyse the callose and re-open communications between undeveloped leaves and the SAM. A regulatory role is implicated for GAs because they can induce expression of these glucanases. Callose-based systemic restriction has not been reported in herbaceous species in relation to developmental control but, by analogy, any mechanism that reversibly prevents PD or phloem passage could affect signal movement in this way.

An FT-dependent dormancy mechanism also appears to operate in gymnosperms, but in this case increased FT expression was associated with dormancy and not growth (Gyllenstrand *et al.*, 2007).

Systemic signals in potato tuberization

In potato, tuberization is a switching event at the tips of stolons (underground lateral shoots), characterized by apical meristem arrest and the onset of radial growth. The regulatory processes are still not completely understood but there is a proven graft-transmissible element, a photoperiod-independent role for CO-dependent signals and systemic transmission of FT-like proteins to stolon tips (Martinez-Garcia *et al.*, 2002; Rodriguez-Falcon *et al.*, 2006; Abelenda *et al.*, 2011). Similar to control of flowering, altered expression of *CO* or *PHYB* both result in graft-transmissible effects on tuberization (Jackson *et al.*, 1998; Martinez-Garcia *et al.*, 2002; Jackson, 2009). Alternative or additional candidates for phloem-mobile signals or

Table 2. Summary of switching events in plant development that are influenced by FT and TFL1

Processes and regulatory relationships are described in more detail in the text.

FT high and/orTFL1 low	←Switching event→	FT low and/or TFL1 high	Systemic FT effect
Promoted	Flower initiation (lateral meristem determinacy)	Delayed	Yes
Promoted	Inflorescence initiation	Delayed	Yes
Accelerated	Leaf meristem determinacy	Inhibited	Yes
Promoted	Tuber formation (stolon apical arrest)	Delayed?	Yes
Delayed	Tree bud dormancy (apical arrest)	Promoted	Inferred

regulators of tuberization signals include *miR172* (Martin *et al.*, 2009), *BEL5* mRNA (Banerjee *et al.*, 2006; Hannapel, 2010), and gibberellins (Martinez-Garcia *et al.*, 2001).

Graft experiments show that *miR172* expressed in donor scions can regulate tuberization, but not when expressed locally in receiver stolons/rootstocks (Martin *et al.*, 2009). Although *miR172* is one class of microRNA that has been detected as phloem-mobile in brassicas (Buhtz *et al.*, 2008), it is unlikely itself to be the mobile tuberization factor. The simplest conclusion instead is that *miR172* is sufficient to initiate the systemic signal, perhaps through targets such as *RAP1,* an *AP2*-like gene whose homologues regulate *FT* in other species (Jung *et al.*, 2007). *StBEL5* is one of the few examples of graft-transmitted mRNA that may have a developmental consequence in the destination tissues (Banerjee *et al.*, 2006; Hannapel, 2010). The strongest mRNA transmission and tuberization responses are seen under SD, yet *StBEL5* is expressed equally in leaves under LD and SD. This suggests that either additional photoperiod-dependent mechanisms are involved and/or there is specific control of *BEL5* movement rather than transcription. Both *miR172* and FT are likely candidates for the former, whereas the latter is supported by evidence that untranslated regions of the *BEL5* transcript are essential for mobility (Banerjee *et al.*, 2009). Whatever the tuberization signal(s) are, it is unlikely that they are only produced by tuberizing species, as potato rootstocks grafted to tomato scions were still stimulated to produce tubers (Peres *et al.*, 2005).

Systemic signals in tomato meristem determinacy and leaf development

Developmental studies, many from the Lifschitz laboratory and mainly based on the tomato model, have provided convincing evidence of further functions of FT and TFL1 beyond the florigen role (Lifschitz and Eshed, 2006; Lifschitz *et al.*, 2006; Lifschitz, 2008; Thouet *et al.*, 2008; Shalit *et al.*, 2009; Efroni *et al.*, 2010). There are substantial complexities in tomato development because of its early termination of apical meristem development linked to the transition to sympodial development, and its compound leaf morphology. It is also a day neutral species which precludes the photoperiod experiments that have been highly informative in most other flowering time models. Indeed, over-expression of *CO* in tomato and potato does not accelerate flowering (Ben-Naim *et al.*, 2006), yet it does affect graft-transmissible tuberization in potato (Martinez-Garcia *et al.*, 2002). In essence, Shalit *et al.* (2009) propose a model where SFT (the tomato FT orthologue) is a generic growth regulator acting to limit meristem activity and promote determinancy. This function is opposed by SP (the tomato TFL1 orthologue) which maintains indeterminacy and promotes continued development. In *sp* mutants, early shoot termination results from failure to sustain sympodial initiation, and *sft* mutants exhibit delayed onset of flowering and leafy inflorescences with single instead of multiple flowers (Shalit *et al.*, 2009). Grafting experiments showed that SFT donor shoots can systemically influence both flowering time and the sympodial patterns. SP and SFT also affect compound leaf development with SP sustaining lateral leaf meristem development and SFT having the opposite effect through lateral meristem arrest. As a consequence, artificially varying the SFT/SP ratio over a wide range results in diverse leaf morphologies, from highly simplified to over-complex (Shalit *et al.*, 2009), perhaps providing some insights into the evolution of leaf form. As with the other effects of SFT, regulation of leaf development was graft-transmissible, whereas SP appears to act over short distances within meristematic zones.

Conclusions and prospects

There is no doubt that the past few years have seen some of the greatest advances in our understanding of flowering time, especially with the discovery of FT as a systemic florigen hormone. Many further complexities and additional FT functions are now being revealed across a wide range of species (Bohlenius *et al.*, 2006; Shalit *et al.*, 2009; Abelenda *et al.*, 2011; Hecht *et al.*, 2011), making a compelling case for expansion of comparative studies that extend beyond the *Arabidopsis* model.

It is vital that the breakthroughs in fundamental science are applied to the pressing need for more efficient global production of plant-based resources for food, energy, materials, and medicines. In this context, better and novel means to control flowering will be highly valuable and, in some situations, essential. Although the photoperiod–latitude relationship remains a constant, climate change results in temperature and rainfall shifts that necessitate the rapid adaptation of most crop species. Targets might include earlier flowering to complete life cycles ahead of high summer temperatures or drought; and accelerated genetic improvement through inducing breeding lines to flower on demand without compromising their normal development. Further broad adaptation of crops to new locations may be possible, for example, to take rice into northern Europe.

Many different classes of flowering time gene may prove beneficial in agriculture depending on the crop type and context, but some well-established and more recent examples already highlight the remarkably powerful effects of changes in levels of FT and TFL1. In tomato, much of the modern mechanized industry was founded on self-pruning varieties carrying the *sp* mutation in the tomato *TFL1* homologue (Rick, 1978). More recently, also in tomato, a dramatic yield heterosis was uncovered in F_1 hybrids between genotypes carrying WT and mutant alleles of *SFT* (Krieger *et al.*, 2010). In soybean, where domestication has been associated with earlier and more efficient cropping resulting from a determinate habit, it has been shown that a *TFL1* orthologue (*GmTFL1b*) is the gene underlying the major *Dt1* (determinate stem) locus (Liu *et al.*, 2010; Tian *et al.*, 2010). Detailed SNP analysis has revealed the major involvement of four independent *TFL1* alleles at this locus, all with a single amino acid change, and all deduced to have

been selected independently during early domestication (Tian *et al.*, 2010).

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