RESEARCH PAPER

Turned on by heat: differential expression of FT and LFY-like genes in Narcissus tazetta during floral transition

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Abstract

In Narcissus tazetta, a monocotyledonous bulbous geophyte, floral initiation and differentiation occur within the bulb during the quiescent period in summer, when ambient temperatures are relatively high and the bulb is located underground with no foliage or roots. In many plant species, FLOWERING LOCUS T (FT) and its homologues are considered powerful promoters of flowering. The Narcissus FT gene homologue (NtFT) was isolated, and organ-specific expression patterns of NtFT during the annual cycle and reproductive development under different temperature regimes were analysed using quantitative reverse transcription–PCR (qRT–PCR) and RNA in situ hybridization. During floral induction, NtFT was not expressed in bulb scales, roots, or foliage leaves, but it was detected inside the bulb in the apical meristem and leaf primordia. The expression of another key flowering gene, NLF, the LEAFY homologue in N. tazetta, was also observed only in meristem and leaf primordia within the bulbs; however, its expression did not coincide with that of NtFT during meristem transition to reproductive stage. Under high temperatures (25–30 °C) in the dark, NtFT expression occurred simultaneously with floral induction timing, indicating that floral induction is affected by high temperatures but not by photoperiod or vernalization. Monitoring the apical meristem of Narcissus in February–August of two growing seasons under ambient and controlled storage conditions showed that transition to flowering is temperature dependent and varies between years. Lack of NtFT and NLF expression in foliage leaves suggests that flower initiation control in Narcissus differs from that in common model plants.

Key words: Ambient temperature, flowering control, FLOWERING LOCUS T, LEAFY, Narcissus, NLF, NtFT.

Introduction

Transition of the shoot apical meristem from vegetative to reproductive phase is regulated by a network of signalling pathways responding to both endogenous and environmental cues. The paradigm from model plants, for example Arabidopsis, suggests that these pathways consist of a large group of flowering time genes (Henderson and Dean, 2004; Corbesier and Coupland, 2006; Kanno et al., 2007; Pin and Nilsson, 2012). The signals from the various flowering time pathways are integrated and lead to the activation of a small group of ‘floral integrator’ genes. These include, among others, FLOWERING LOCUS T (FT), LEAFY (LFY), and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1). Activation of these genes triggers the transition to flowering (Boss et al., 2004; Henderson and Dean, 2004; Lee et al., 2006; Tan and Swain, 2006; Kaufmann et al., 2010). At the shoot apices in Arabidopsis, FT and FD probably activate important regulators of floral fate (Xu et al., 2012). LFY appears to play an important role in specifying floral meristem
identity (Weigel and Nilsson, 1995), reproductive transition, flower development, and expression of floral organ identity genes (Benlloch et al., 2007).

Perennial plant species differ greatly from annuals in their life cycle and physiological requirements, and therefore might differ in the molecular mechanisms that control flowering (Tan and Swain, 2006; Townsend et al., 2006; Melzer et al., 2008; Jung and Muller, 2009). However, only limited information is available on the molecular aspects of reproductive development in perennial herbaceous plants, geophytes in particular (Kamenetsky et al., 2012).

The monocotyledonous geophyte Narcissus tazetta is one of the most popular ornamentals worldwide. Its annual cycle is naturally adapted to the environmental conditions in Mediterranean regions (Dulberger, 1967; Yahel and Sandler, 1986; Koike et al., 1994; Hanks, 2002). Following quiescence during the hot and dry summer, leaf elongation begins after the first rains in October–November, and flowering occurs in December–January. Foliage leaves remain green until senescence in April–May, and bulbing occurs in May–June. The bulb consists of true scales and leaf bases—the lower parts of the foliage leaves. Flower initiation and differentiation occur within the bulb during the summer, when the underground bulb remains underground with no live roots or foliage leaves. In previous studies, it has been shown that high temperatures (25–30 °C) in late spring (May–June) promote intrabulb florescence; temperatures >30 °C delay the subsequent inflorescence differentiation, and low temperatures (12 °C) completely inhibit all stages of florescence (Noy-Porat et al., 2009). The N. tazetta LFY homologue, NLF, correlates with intrabulb florescence (Noy-Porat et al., 2010). A dramatic increase in NLF expression was observed during floral initiation under ambient summer conditions and at a constant 30 °C, as well as during differentiation of flower primordia. When stored at 12 °C, meristems remained morphologically vegetative, but high NLF expression was observed in these non-differentiated meristems. It was suggested that temperature does not affect NLF expression directly, but might regulate other flower-related genes that are involved in floral transition.

In this context, FT and its homologues are considered to be involved in florescence, and their function appears to be remarkably conserved in all species tested (Turck et al., 2008). The role of FT and its homologue Hd3a in the photoperiod induction pathway has been extensively studied in Arabidopsis and rice, respectively. In Arabidopsis, FT was found to be regulated directly by CONSTANS (CO) under a long-day photoperiod perceived by the leaves (Abe et al., 2005; Wigge et al., 2005; Corbesier et al., 2007). Tissue-specific expression of FT in Arabidopsis caused early flowering when it occurred in the leaf phloem and in the shoot apex (An et al., 2004). CO regulates FT transcription in the leaf phloem; however, it was suggested that the FT protein is translocated from the leaves to the shoot apex and, therefore, FT is the long-sought mobile florigen signal (Kobayashi and Weigel, 2007; Zeevaart, 2007). At the shoot apex, FT interacts directly with the bZIP protein FD which seems to recruit FT to the promoter of API (Abe et al., 2005; Wigge et al., 2005; Kobayashi and Weigel, 2007; Kaufmann et al., 2010). Gene activation by the FT/FD complex is considered the earliest event in the floral transition to occur in the meristem itself (Turck et al., 2008). Recently, Li et al. (2009) showed that a cis-element in FT mRNA allows mobility of this RNA in the plant, suggesting that FT mRNA, along with its protein, may be involved in intraplant spread of the floral stimulus.

Overexpression of FT homologues causes early flowering in tomato and tobacco (Lišchtitz and Eshed, 2006), as well as in the monocotyledonous rice (Kojima et al., 2002), wheat, and barley (Yan et al., 2006); it also shortens the juvenile period in Populus (Hsu et al., 2006) and Citrus (Endo et al., 2005; Nishikawa et al., 2010). Ectopic expression of OnFT, an FT homologue from orchid, caused early flowering in Arabidopsis and partially restored the ft-I mutant phenotype (Hou and Yang, 2009). However, unlike FT in Arabidopsis, OnFT was highly expressed in the buds prior to floral transition, and also showed high expression at the beginning of flower differentiation, which then decreased during flower maturation (Hou and Yang, 2009).

In addition to photoperiod and vernalization pathways, FT has been suggested to be an important component of the ambient temperature signalling pathway in Arabidopsis (Blazquez et al., 2003; Halliday et al., 2003; Balasubramanian et al., 2006; Kumar and Wigge, 2010). However, the involvement of FT in other modes of flowering control has not been studied in detail.

In this report, data are provided, for the first time, on isolation and identification of the FT homologue in the Mediterranean geophyte N. tazetta. Spatial and temporal expression patterns of the FT and LFY homologues were examined in the plant organs during flower induction throughout the summer quiescence period and storage under various conditions, to reveal possible regulation of flower initiation by environmental signals.

Materials and methods

Plant material, growth and storage conditions, and sampling for histological and molecular analyses

Bulbs of N. tazetta cv. Ziva, 13–14 cm in circumference, were obtained from commercial producers in Israel. The plants were grown in local soil (Vertisol) in Beer-Tuvia and Bizaron, the southern coastal plain of Israel, and irrigated once every 10 d in October–November and May–June, in addition to natural rainfall (seasonal average of 400 mm, falling between October and April).

The effect of soil temperature on floral induction in bulbs was studied during two growing seasons, 2004/2005 and 2007/2008. Soil temperature was recorded at a depth of 10 cm at the Negba station, located in close proximity to the commercial fields (Israel Meteorological service http://www.imis.gov.il).

In 2005, bulbs were harvested in April, when the foliage leaves began to dry out. After sorting and cleaning, the bulbs were kept throughout the summer at ambient temperatures of 25–30/16–22 °C (day/night), or at a constant temperature of 12 °C. To avoid a possible effect of photoperiod, the bulbs were kept in complete darkness. For molecular analyses, 200 bulbs from each treatment were dissected every 3 weeks from April until October 2005, and meristems were collected according to their developmental stage.

For the analysis of the early stages of floral transition, bulbs were harvested from the field once a month from February to July 2008,
Nucleic acid isolation
RNA from all collected plant parts was isolated according to Jaakola et al. (2001). DNA was isolated from green foliage leaves by a CTAB (cetyltrimethyl ammonium bromide)-based method (Noy-Porat et al., 2009). For vegetative and reproductive meristems and leaf primordia, each sample consisted of RNA isolated from ~100 bulbs. For all other plant parts (Fig. 2), each sample consisted of RNA isolated from 10 bulbs.

Gene identification
The Narcissus homologue of FT (NtFT) was amplified using degenerate primers designed according to FT homologues from various plant species. The following primers were used: forward, 5′-ATGATGACGATGTGGWCRCRAG-3′ and reverse, 5′-RTTRAARTTYTGNCGGCANC-3′.

Alignment of the partial amino acid sequence of the Narcissus (NtFT) cDNA with those of FT homologues from various species was performed using ClustalW. The accession numbers of the homologues were as follows: Arabidopsis NP_176726 (FT), NP_193770 (TSF), NP_196004 (TFL1), NP_201010 (BFT), NP_173250 (MFT), NP_180324 (ATC); BAG12904 (Populus nigra), BAF96645 (Citrus sinensis), AAW23034 (Triticum aestivum), NP_00105680 (Oryza sativa), and ACC59806 (Oncidium ‘Gower Ramsey’).

The Narcissus homologue of LFY (NLF) was amplified by standard reverse transcription–PCR (RT–PCR) using the following primers: forward, 5′-TTGGGCTTGTTGATGACATCGT-3′ and reverse, 5′-GAGCTCGACGACATGATG-3′. The PCR products were analysed on an agarose gel and cloned into a pGEM-T easy vector (Promega, Madison, WI, USA) for sequencing (Noy-Porat et al., 2009).

Quantitative RT–PCR (qRT–PCR)
Spatial and temporal analyses of the expression of genes during florogenesis were performed using qRT–PCR of all sampled plant tissues (Fig. 2). For reverse transcription, 1 μg of total RNA from each sample was digested with RNasin (Promega). cDNA first-strand synthesis was performed using the Verso cDNA kit (ABgene, Surrey, UK). For NtFT, qRT–PCR was carried out in a 20 μl reaction using the AbsoluteBlue QPCR mix (ABgene) and the PerfectProbe gene detection mix ( PrimerDesign, Southampton, UK) containing the probe and primers at a final concentration of 0.2 μM. The cDNA was diluted 1:5, and 5 μl were added to the reaction. For NLF, quantitative real-time PCR was carried out in a 20 μl reaction volume using Absolute QPCR SYBR Green mix (ABgene). The reaction mixture included a final primer concentration of 0.3 μM, and 2 μl of cDNA. Actin was used as an internal control (using SYBR Green chemistry).

Primers and probe used for NtFT were: 5′-AGAGATAGTGTGT TTATGAAAGTCC-3′ (forward), 5′-TGCTTACCCAATTAGCGA AA-3′ (reverse), and 5′-CCACCAAAACAAAGCGATGAT CCCCCGTGGTG-3′ (probe); for NLF: 5′-GAGCTCGACGACATGATG-3′ and 5′-TTGGGCTTGTTGATGACATCGT-3′; for ACTIN: 5′-ATCAAGGAGAACYCTGCTATGTTT-3′ (forward) and 5′-CCACTACGAATTCTGCTACT-3′ (reverse).

The qRT–PCR was performed in a Rotor-Gene 6000 apparatus (Corbett Life Science, Germantown, MD, USA). Each result is the mean of three biological replicates, each with two technical repeats.

In situ hybridization
Tissues were fixed in FAA (formaldehyde:acetic acid:alcohol, 5:5:90, v/v/v) for at least 2 d and then embedded in Paraplast. Tissue sections (10 μm) were mounted on SuperFrost Plus slides (Menzel-Glaser, Braunschweig, Germany) and left for 2 h on a 40 °C hot plate. A probe was designed based on areas of the gene that are unique to NtFT and are much less conserved in other genes of the family. The 240 bp segment of NtFT was cloned into a StrataClone vector (Stratagene, La Jolla, CA, USA), with a T7 promoter sequence attached to the 3′ end of the gene and a T3 promoter sequence attached to its 5′ end. Digoxigenin (DIG)-labelled RNA sense and antisense probes were then generated using the MEGAscript kit (Ambion, Austin, TX, USA) and DIG RNA labelling mix (Roche Diagnostics, Indianapolis, IN, USA). The probes were later purified using the MEGAClear kit (Ambion), and quantified by running 1 μl on an agarose gel and measuring the concentration in a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE, USA). The specificity of the probe was tested on a sense control as well as on tissues that do not express NtFT. In situ hybridization was performed as described previously (Noy-Porat et al., 2009).

Results
Isolation of the FT homologue in N. tazetta
RT–PCR with primers designed according to conserved domains of FT homologues from different species amplified a cDNA fragment of 372 bp in N. tazetta. Sequence analysis of the partial translation product revealed 75% similarity to FT and TSF from Arabidopsis, and 80–84% similarity to FT homologues from rice, wheat, Populus, Citrus, and orchid (Fig. 1A). The Narcissus gene was less similar to other genes of the FT family, showing 53–58% similarity to TFL1, MFT, BFT, and ATC from Arabidopsis (Fig. 1A). It was therefore classified as an FT homologue, named NtFT, and deposited in GenBank under accession no. HM537233. Using the identified NtFT sequence, a 1350 bp fragment was isolated from N. tazetta DNA by PCR. It contained a large intron with location and size similar to those of introns found in other FT homologues (Fig. 1B).

Anatomical and morphological observations of floral transition
During the growing season under ambient conditions, between October and April, new leaf primordia are produced inside the N. tazetta bulb by the vegetative apical meristem, which are 500 μm (±40) in diameter and 300 μm (±15) in height (Fig. 2B). In May–June, after the transition to reproductive development and inflorescence initiation, the
Fig. 1. *NtFT* gene structure. (A) Multiple alignment of the NtFT protein deduced from the cDNA sequence with FT homologues from different plant species. Accession numbers are listed in the Materials and methods. Similar amino acids were identified using BoxShade version 3.2. Black shading, identical residues; grey shading, similar residues. (B) Exon/intron arrangements and sizes (in bp) of NtFT. *Only part of exon 2 was identified and therefore its size is not complete.*

Fig. 2. Morphological analysis of *N. tazetta*. (A) Schematic representation of bulb structure in April–May. Note leaf bases of the foliage leaves, functioning as storage scales after leaf blade senescence. Mature scales were differentiated directly as storage organs within the bulb. The indicated organs were collected for the molecular analysis. (B) Vegetative meristem in April. Bar=100 μm. Arrowhead indicates new leaf primordium. (C) Reproductive meristem in June. Bar=100 μm.
meristem becomes dome-like and its size increases to 800 μm (±124) in diameter and 500 μm (±33) in height (Fig. 2C).

Organ-specific expression patterns of NtFT and NLF during floral transition

To examine the organ-specific expression patterns of NtFT and NLF in N. tazetta, qRT-PCR analysis was performed. During the observation period in February–July 2008, both NtFT and NLF expression was absent in the leaf bases, the mature scales, the basal plate, and the roots.

From February to May, NtFT was expressed at a constant basal level in the mature foliage leaves (Fig. 3A). By mid-May, the foliage leaves had dried up and therefore were not examined any further.

In the leaf primordia surrounding the apical meristem, NtFT showed a basal expression level from February to April similar to that observed in mature foliage leaves. A sharp increase (~17-fold) in NtFT expression in the leaf primordia was detected in May, followed by a decrease in June–July (Fig. 3A). A similar pattern was detected in the apical meristem: from February to April, only basal expression of NtFT was registered, with a sharp increase in expression (~15-fold) occurring in May, simultaneously with the increase in the leaf primordia (Fig. 3A). NtFT also showed very low basal expression in young scales, which was significantly lower than in foliage leaves and was stable throughout the observation period (data not shown).

NLF was not detected in mature green leaves or any other vegetative tissue at any time during the observation period. However, this gene was expressed at a relatively low level from February to May in vegetative non-differentiated meristems, with a significant increase (~6.5-fold) in June (Fig. 3B). In leaf primordia, NLF expression increased only in July, ~4.5-fold, a month later than in the apical meristems.

Further analysis at the various stages of inflorescence development confirmed a transient increase in NtFT expression with the meristem shift from vegetative to reproductive stage (Fig. 3C). Following inflorescence induction, during its differentiation, NtFT expression in the meristem decreased to basal levels (Fig. 3C). On the other hand, NLF expression increased

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**Fig. 3.** Differential expression of NtFT and NLF during florogenesis of Narcissus tazetta cv. Ziva. Samples were normalized against β-actin. (A) Relative expression of NtFT in apical meristems, morphologically defined as vegetative, leaf primordia, and foliage leaves under ambient growth conditions between February and July 2008. In mid-May, the foliage leaves dried up and were not examined any further. (B) Relative expression of NLF in apical meristems, morphologically defined as vegetative, leaf primordia, and foliage leaves under ambient growth conditions between February and July 2008. In mid-May, the foliage leaves dried up and were not examined any further. (C) Relative expression of NtFT at the various stages of florogenesis. Samples of morphologically vegetative meristems, reproductive meristems, and differentiated inflorescences were analysed under ambient conditions in April–September 2005. (D) Relative expression of NLF at the various stages of florogenesis. Samples of morphologically vegetative meristems, reproductive meristems, and differentiated inflorescences were analysed under ambient conditions in April–September 2005.
during inflorescence initiation and again during differentiation. The second significant increase in NLF expression was observed during differentiation of the flower primordia (Fig. 3D).

**Spatial expression of NtFT and NLF in the apical meristem and leaf primordia**

Since the results from qRT–PCR indicated an increase in NtFT and NLF expression in both meristem and leaf primordia in May–June, spatial expression of the two genes in these organs was examined by in situ hybridization (Fig. 4). NtFT expression was observed in both apical meristem and leaf primordia collected in May (Fig. 4B–D). In the leaf primordia, NtFT expression was observed at the tip and in the spongy mesophyll (Fig. 4B, C). In the meristem, NtFT expression was weaker, and appeared mostly in the central zone and upper cell layers (Fig. 4D).

NLF showed weak expression in cells throughout the meristem (Fig. 4E). Similar expression was observed in the leaf primordia (data not shown).

To investigate the effect of light and temperature on NtFT and NLF expression in N. tazetta, the genes’ expression patterns in the meristems with a vegetative morphological appearance were investigated during bulb storage in May–July 2005. NtFT and NLF expression in the meristem was monitored under two temperature regimes: high ambient (25–30 °C day and 16–22 °C night) and constant (12 °C). In the bulbs stored at 12 °C, only 10% of the meristems progressed to reproductive development (Fig. 5A), but, after planting, only 80% of the plants showed leaf emergence and all inflorescences in these plants had a significantly lower number of flowers, which were malformed: flowers differentiated five tepals instead of six, and degenerated anthers (Fig. 5C, D). qRT–PCR analysis showed no NtFT expression in the meristems stored at 12 °C during the examination period (Fig. 5F), whereas NLF expression was high (Fig. 5G). In bulbs stored under ambient conditions, >90% of the meristems became reproductive (Fig. 5B), and all of them produced normal flowers after planting in

**Fig. 4.** In situ hybridization of NtFT and NLF in leaf primordia and apical meristems during transition from vegetative to reproductive development in Narcissus tazetta cv. Ziva; m, meristem; lp, leaf primordia; bp, basal plate. (A) Developing bud inside the bulb consisting of an apical meristem surrounded by three leaf primordia. The tip and side of the outer leaf are marked and shown in B and C, respectively. The meristem is marked and shown in D and E. Bar=500 μm. (B, C) Leaf primordia collected in May. Strong expression of NtFT is observed in all tissues, including epidermis and palisade tissue, and the expression is not restricted to the vascular tissue. Bar=100 μm. (D) Meristem collected in May and stained for NtFT. Expression is visible in the central zone of the meristem and in the upper cell layers. Bar=100 μm. (E) Meristem collected in June and stained for NLF. Expression is weakly observed in cells throughout the meristem. Bar = 100 μm. (F) Sense control of meristem and leaf primordia. Bar=100 μm.
Flower induction in *Narcissus tazetta*

In the autumn (Fig. 5E). In these plants, both *NtFT* and *NLF* expression increased significantly in June (Fig. 5F, G).

**Effect of seasonal ambient temperature regime on floral transition**

To investigate the effect of seasonal temperature on *NtFT* and *NLF* expression, the expression patterns of these genes during two different growing seasons were compared. It should be noted that in 2005, soil temperatures in April–June were close to the seasonal average for this location. In 2008, temperatures in March–May were well above average and, in fact, April 2008 was the hottest April since 1994 (Fig. 6A). Temperatures >25 °C in 2008 were therefore observed on 21 April, in comparison with 2005, when temperatures increased to this level on 19 May. Consequently, the morphological appearance of the reproductive meristem was registered in mid-June in 2008, at least 4 weeks earlier than in 2005.

In agreement with morphological observations, the increase in *NtFT* expression in 2008 occurred at the beginning of May (Fig. 6B), whereas no difference was found in the temporal expression of *NLF*. In both bulb populations, sampled in 2005 and 2008, a marked increase in *NLF* was observed in meristems in June (Fig. 6C).

**Discussion**

Extensive development of geophytes as ornamental crops has led to the generation of a considerable amount of research data on their flowering physiology. However, only limited information on the genetic control of floral transition is available in herbaceous perennial plants in general, and geophytes in particular (Townsend *et al.*, 2006; Albani and Coupland, 2010; Kamenetsky *et al.*, 2012). Following major breakthroughs in understanding flowering biology in model species, the homologues of several key flowering genes have also been found in geophyte and herbaceous species. For example, *LFY* homologues have been isolated from *Allium sativum* (gaLFY; Rotem *et al.*, 2007, 2011), *N. tazetta* (NLF; Noy-Porat *et al.*, 2010), and *Aquilegia formosa* (AqLFY; Ballerini and Kramer, 2011). *FT*-like *AcFTL* has been found in onion (*Allium cepa*) (Taylor, 2009; Taylor *et al.*, 2010). In this report, first evidence is provided of the
expression of two key flowering genes, *NtFT* and *NLF*, in different plant organs of *N. tazetta* under various environmental conditions. A major distinction in flowering control in this geophyte from the known paradigms for model plants is presented in Fig. 7.

**High temperature provides a flowering signal in *N. tazetta***

The common paradigm of ‘florigen’ movement from foliage leaves to the apical meristem, developed for model plants in the context of the photoperiodic pathway (Kobayashi and Weigel, 2007; Turck et al., 2008; Xu et al., 2012), is not always confirmed in other species. *FT* expression following temperature signals may be different from that observed following photoperiodic signals in model plants. For example, *Citrus* flowering is induced by low ambient temperature and, during floral induction, mRNA levels of the *FT* homologue *CiFT3* increase in stems, paralleling the decrease in temperature (Nishikawa et al., 2007). In addition, in adult citrus under inductive temperatures, leaves are not necessary for floral initiation (Wilkie et al., 2008). In the perennial herbaceous *A. formosa*, the *FT* homologue *AqFT* is expressed before the transition to flowering under both long-day and short-day conditions. Although vernalization is critical to flowering in *Aquilegia*, low temperature is not strictly required for the transcriptional activation of *AqFT* (Ballerini and Kramer, 2011).

In the present experiments, floral induction in *N. tazetta* occurred either when bulbs remained underground with no foliage leaves or active roots, or during bulb storage at high temperatures in complete darkness. Therefore, the light signal was not perceived by the foliage leaves or other plant organs prior to meristem transition. Peak *NtFT* expression was recorded in the apical meristem in May, prior to visible morphological changes (Fig. 3A). The temporal analysis of gene expression implied that *NtFT* is regulated by temperature and its expression correlates with timing of floral induction. A comparison of flower initiation under different temperature regimes during two growing seasons (Fig. 6) showed that an earlier rise in temperature causes earlier *NtFT* up-regulation and floral transition. Therefore, it is argued that high soil temperatures at the end of the vegetative period (April–May) affect the expression of *NtFT* in the quiescent renewal bud of *N. tazetta* and that *NtFT* up-regulation marks the time point of floral induction within the bulb (Fig. 7).

Previous reports have suggested a possible role for ambient temperatures in flower transition. In *Arabidopsis*, elevated temperatures (>23 °C) were shown to induce flowering as efficiently as long days. The process can be regulated by genes belonging to the autonomous pathway, and perhaps by histone modification and microRNA abundance as well (Kumar and Wigge, 2010; Lee et al., 2010; McClung and Davis, 2010). Consistent with the present findings, most studies on ambient temperature signalling suggest that *FT* is this pathway’s target gene (Blazquez et al., 2003; Halliday et al., 2003; Samach and Wigge, 2005; Balasubramanian et al., 2006; Kumar and Wigge, 2010).

On the other hand, the second key gene, *NLF*, might not be regulated directly by temperature, since its expression was registered in the renewal bud independent of the temperature regime (Fig. 6), and was not down-regulated during bulb storage at 12 °C (Fig. 5). It is proposed that *NLF* expression is not regulated by photoperiod or temperature, but might be affected by an endogenous signal (Fig. 7). For comparison,
in Arabidopsis, LFY is known to be the target of several endogenous signals, such as age (Wang et al., 2009) and giberellin (Blazquez and Weigel, 2000; Mutasa-Gottgens and Hedden, 2009). In agreement with the known functions of FT and LFY in Arabidopsis (Kobayashi and Weigel, 2007), it is argued that in Narcissus, NtFT and NLF might act in parallel signalling flows, rather than in a downstream cascade. However, the present results suggest that under ambient temperature NLF does not take part in the floral transition, but is up-regulated slightly later, at the initiation stage (see also Noy-Porat et al. 2010).

Organography and spatial patterns of NtFT and NLF expression

Numerous studies (Carmona et al., 2002; Wada et al., 2002; Hsu et al., 2006; Hattasch et al., 2008; Igasaki et al., 2008) have shown that transcription of FT and LFY homologues in perennial plants coincides with flower induction, and that these genes are involved in floral meristem formation. The ‘florigen’ theory states that the light signal is perceived in the leaves, leading to the formation of FT mRNA (Corbesier et al., 2007; Turck et al., 2008). Surprisingly, however, in Narcissus, both NtFT and NLF were expressed in meristems and leaf primordia within the bulb, but not in foliage leaves or other mature vegetative organs (Fig. 3; Noy-Porat et al., 2010). NtFT expression was found mainly in the central zone of the meristem, prior to its shift to reproductive development. At this stage, the vascular system of the reproductive organs is not differentiated. Therefore, NtFT is assumed to play a key role in the meristem transition to reproductive development, but NtFT mRNA is transcribed in the renewal bud inside the bulb and is not translocated from other organs. On the other hand, NLF is up-regulated in the apical meristem later than NtFT and might be involved in several stages of florogenesis, from the meristem transition to flower differentiation and gametogenesis (Noy-Porat et al., 2010; Fig. 3D). Similar activity has been demonstrated for LFY homologues in garlic (Rotem et al., 2011). In Arabidopsis, LFY is expressed throughout the development of floral meristems and also activates different floral organ identity genes in distinct patterns within the flower. This seems to result from interactions between the globally expressed LFY and cofactors expressed in more spatially restricted domains (Krizek and Fletcher, 2005; Moyroud et al., 2010).

In addition to florogenesis, FT and LFY homologues might be involved in a range of plant growth processes. Shalit et al. (2009) showed that in the perennial tomato, SFT, the respective orthologue of FT, regulates diverse growth processes, such as flowering, growth and termination of typical perennial plant cycles, leaf maturation, growth of stems, and the formation of abscission zones. The FT homologues have been suggested to control seasonal growth cessation as well as flowering in Populus and Norway spruce (Picea abies) (Bohlenius et al., 2006; Gyllenstrand et al., 2007; Olsen, 2010). FT-like proteins have also been suggested to regulate potato tuberization (Abelenda et al., 2011). In Narcissus, the elevated expression of NtFT in leaf primordia suggests a role in leaf development (Figs 3, 4). Storage at 12 °C prevented NtFT expression and also negatively affected leaf elongation after planting (Fig. 5). It is therefore possible that NtFT down-regulation at 12 °C inhibits both flower induction and leaf development. Further studies might also reveal a possible role for FT homologues in dormancy induction and the bulbing process (Okubo, 2012).

LFY homologues have been shown to play a significant role in compound leaf development of Medicago truncatula (Wang et al., 2008), and have also been detected in leaf primordia in Vitis (Carmona et al., 2002), tomato
(Molinero-Rosales et al., 1999), radish (Oshima and Nomura, 2008), Populus (Rottmann et al., 2000), and Eucalyptus (Southerton et al., 1998). Similarly, the present findings show that NLF might be involved in leaf or scale development within the bulb.

In conclusion, high temperature is required for floral induction inside the bulb of Nasturtium officinale during the summer quiescent period, while the photoperiodic signal is probably not essential for flower transition. These findings expand our understanding of the flowering process in various life forms, and open up the use of Narcissus as an alternative perennial plant model for studies of flowering control.

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