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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 *SUPRESSOR 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

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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 florogenesis; temperatures >30 °C delay the subsequent inflorescence differentiation, and low temperatures (12 °C) completely inhibit all stages of florogenesis (Noy-Porat *et al.*, 2009). The *N. tazetta LFY* homologue, *NLF*, correlates with intrabulb florogenesis (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 florogenesis, 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 overexpression 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 *AP1* (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 (Lifschitz 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-1* 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.ims.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,

immediately dissected, and the following organs were collected for molecular analysis: green foliage leaves and their bases, roots, mature scales, young scales, basal plate, leaf primordia, and apical meristem in the central renewal bud (Fig. 2).

Morphological changes in the apical meristem were monitored from February to August with a stereoscope (Stemi 2000-C, Zeiss) and light microscope (Leica DM LB). Histological sections were prepared as described previously (Noy-Porat *et al.*, 2010). The meristem dimensions were measured on histological sections from the centre of the meristem, under the microscope.

For molecular analyses, plant tissues were collected in liquid nitrogen, sorted by developmental stage according to morphological characteristics (Noy-Porat *et al.*, 2009), and stored at –80 °C. A total of ~2000 bulbs were dissected during the whole period and their organs collected.

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′-ATGGTRGAYCCDGATGYWCCRAG-3′ and reverse, 5′-RTTRAARTTYTGNCGCCANC-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 unshiu*), AAW23034 (*Triticum aestivum*), NP_001056860 (*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′- TTGGGCTTGTTGATGTAGCTT-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.*, 2010).

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 RQ1-DNase (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 volume 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′-AGAGATAGTGTG TTATGAAAGTCC-3′ (forward), 5′-TGCCTACCCAATTAGCGA AA-3′ (reverse), and 5′-FAM-CCACCAAAACAAAGCGATGAAT CCCCTTGGTGG-3′ (probe); for *NLF*: 5′-GACGCTTCGAG TCCCTTAACAA-3′ and 5′-TTCGCCTCCGCTTTCATG-3′; for *ACTIN*: 5′-ATCAAGGAGAAACTGGCTTATGTTG-3′(forward) and 5′-CCATCAGGAAGTTCGTAGCTCTTC-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 Applied Science, 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

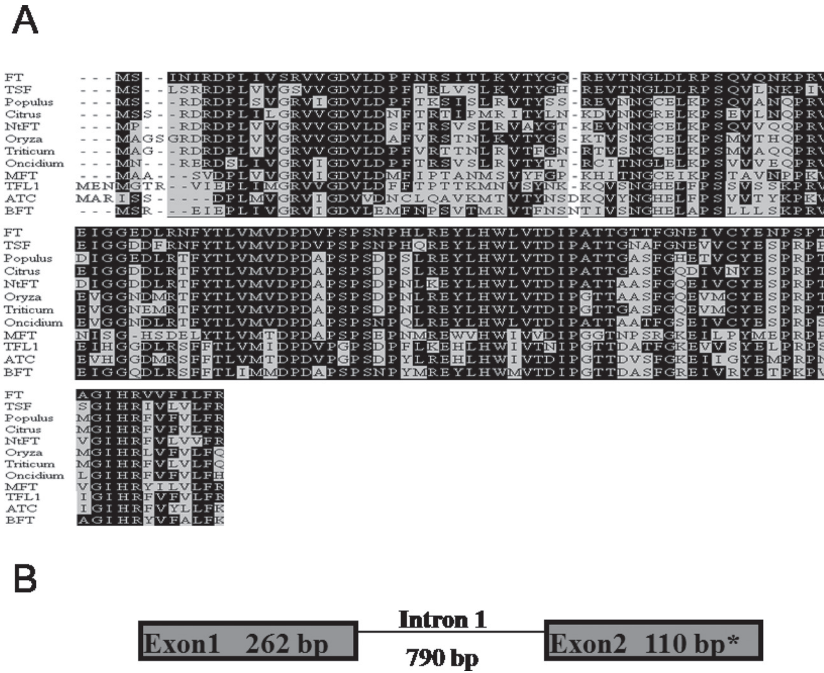

Fig. 1. *NtFT* gene structure. (A) Multiple alignment of the NtFT protein deduced from the cDNA sequence with FT homologues from different plant species. Accession numbers are listed in the Materials and methods. Similar amino acids were identified using BoxShade version 3.2. Black shading, identical 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.

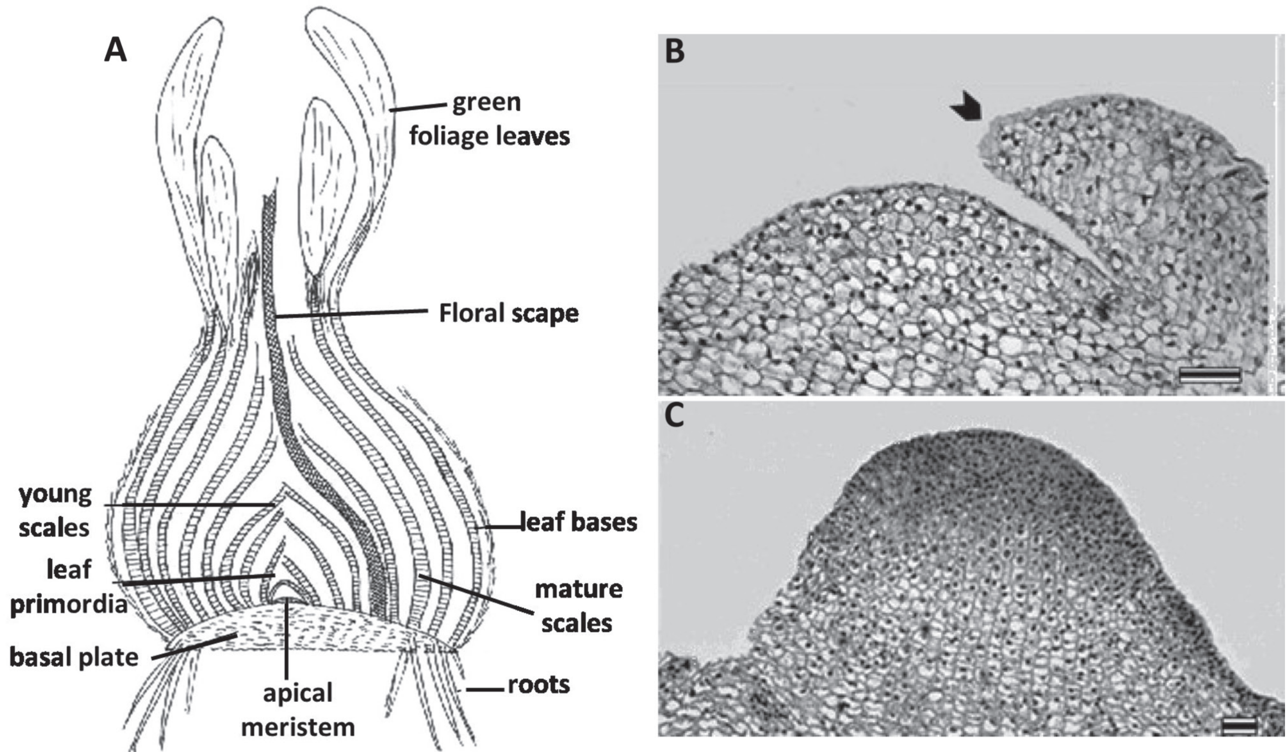

Fig. 2. Morphological analysis of *N. tazetta*. (A) Schematic representation of bulb structure in April–May. Note leaf bases of the foliage leaves, functioning as storage scales after leaf blade senescence. Mature scales were differentiated directly as storage organs within the bulb. The indicated organs 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

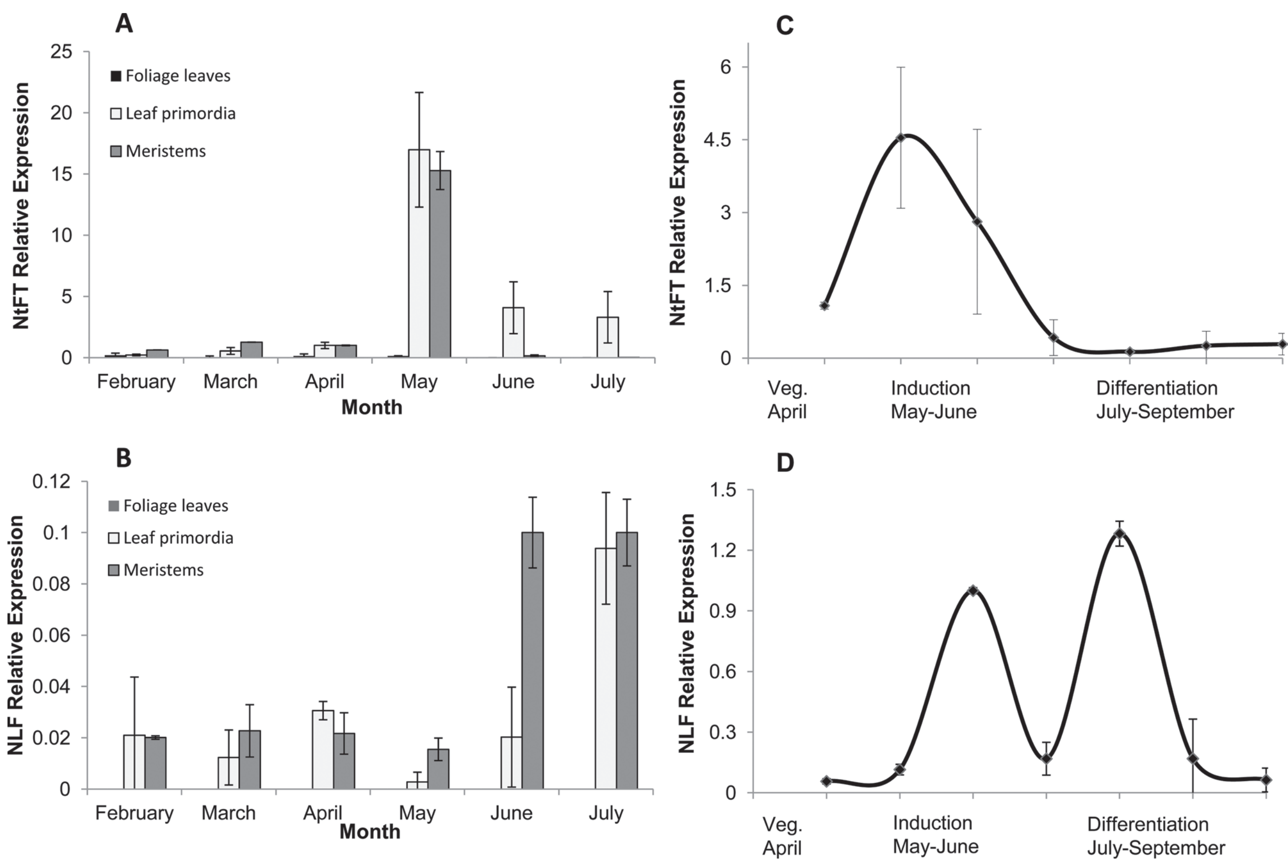

Fig. 3. Differential expression of *NtFT* and *NLF* during florogenesis of *Narcissus tazetta* cv. Ziva. Samples were normalized against β-actin. (A) Relative expression of *NtFT* in apical meristems, morphologically defined as vegetative, leaf primordia, and foliage leaves under ambient growth conditions 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).

Expression of NtFT and NLF under storage at different temperatures in the dark

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 ºC day and 16–22 ºC 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

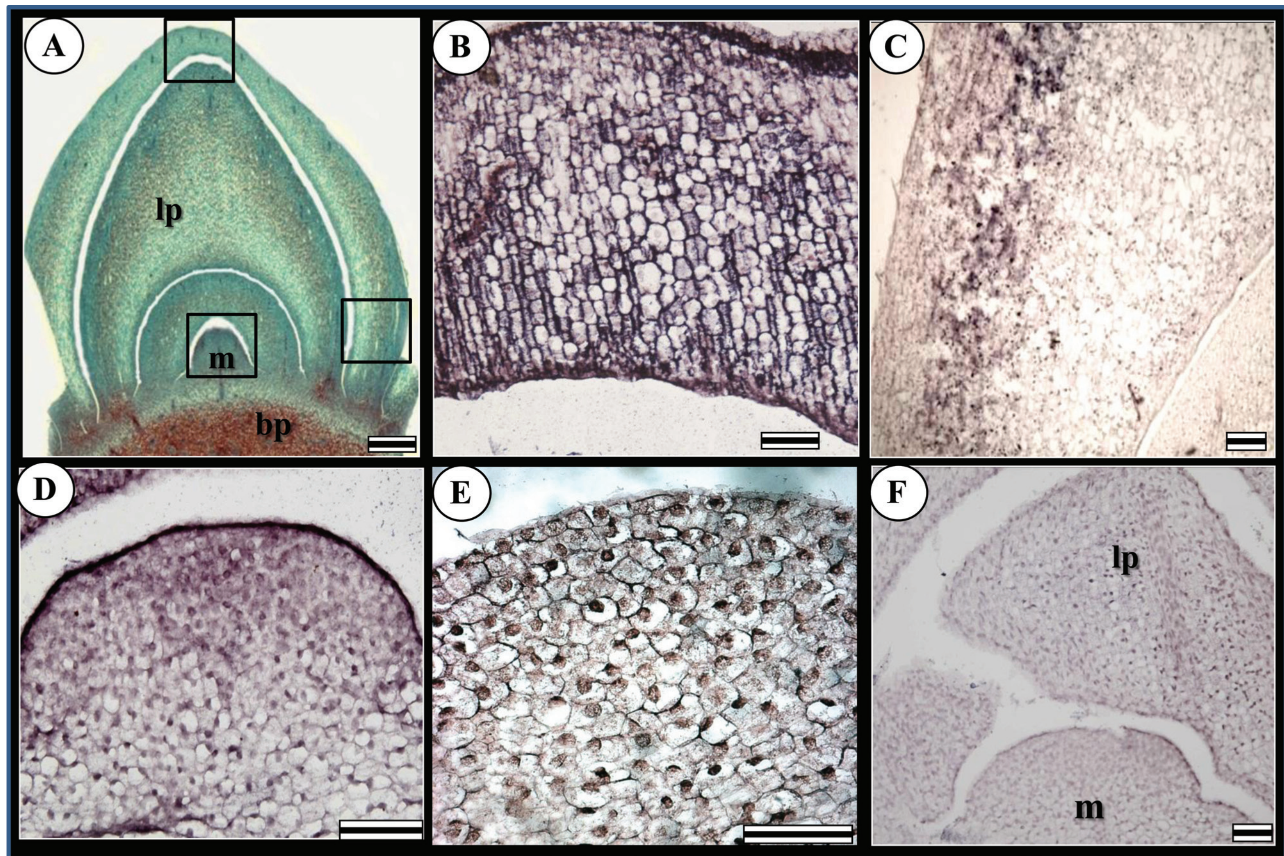

Fig. 4. *In situ* hybridization of *NtFT* and *NLF* in leaf primordia and apical meristems during transition from vegetative to reproductive development in *Narcissus tazetta* cv. Ziva; m, meristem; lp, leaf primordia; bp, basal plate. (A) Developing bud inside the bulb consisting of an apical meristem 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

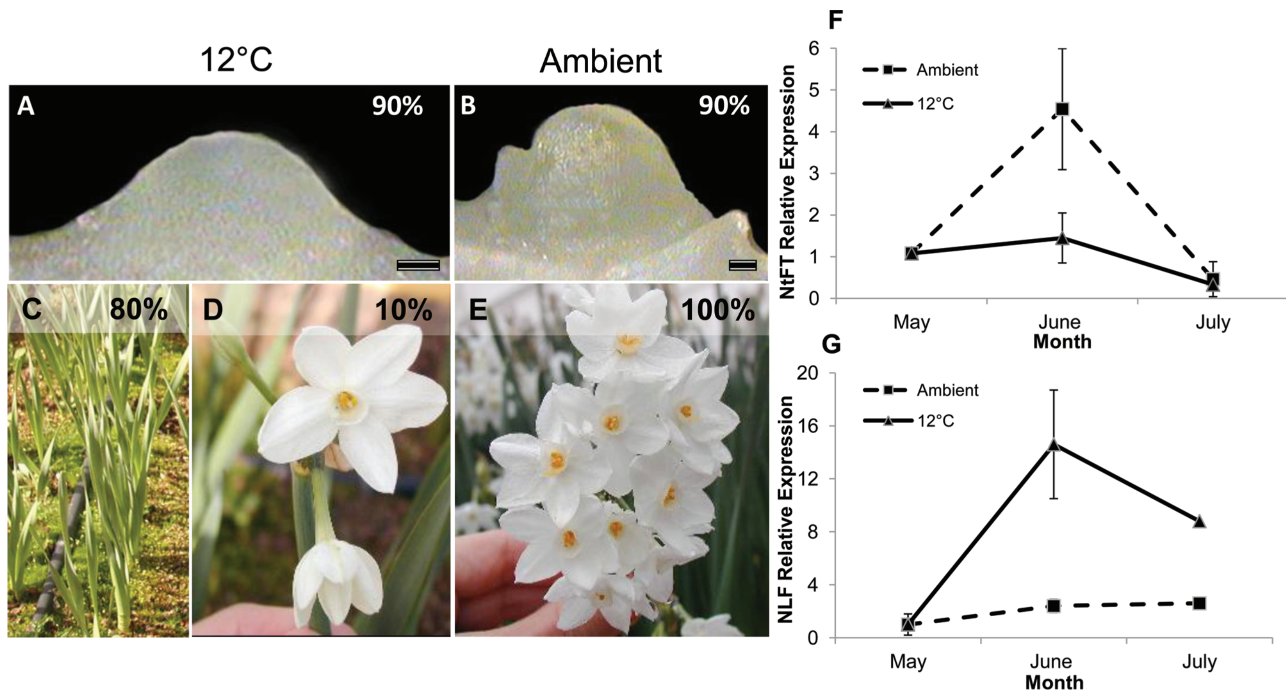

Fig. 5. The effect of high ambient versus low storage temperatures on floral transition and flowering in *Narcissus tazetta* cv. Ziva. Bulbs were stored in the dark at high ambient temperatures (25–30 ºC day, 16–22 ºC night) or a constant 12 °C between May and September 2005 and then transferred to ambient growth conditions in October. (A) Vegetative meristem. Under storage at 12 °C, 90% of the meristems were not induced to flower and remained vegetative. (B) Reproductive meristem. Under storage at ambient temperatures, 90% of the meristems became reproductive and developed inflorescences. (C) Foliage leaf development after storage at 12 °C and planting in the autumn. Only 80% of the bulbs showed leaf emergence, and this was extremely delayed compared with bulbs stored under ambient conditions. (D) Inflorescence development in bulbs stored at 12 °C. After planting in the autumn, only 10% of the plants developed inflorescences, with a significantly lower number of flowers per inflorescence, as compared with ambient temperatures, and flower abnormalities. (E) Inflorescence development after storage under ambient conditions. All bulbs developed leaves and 100% of the inflorescences were normal. (F) Relative expression of *NtFT* in meristems of bulbs stored at high ambient temperatures or constant 12 °C between May and July 2005. Samples were normalized against β-actin. (G) Relative expression of *NLF* in meristems of bulbs stored at high ambient temperatures or constant 12 °C between May and July 2005. Samples were normalized against β-actin.

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

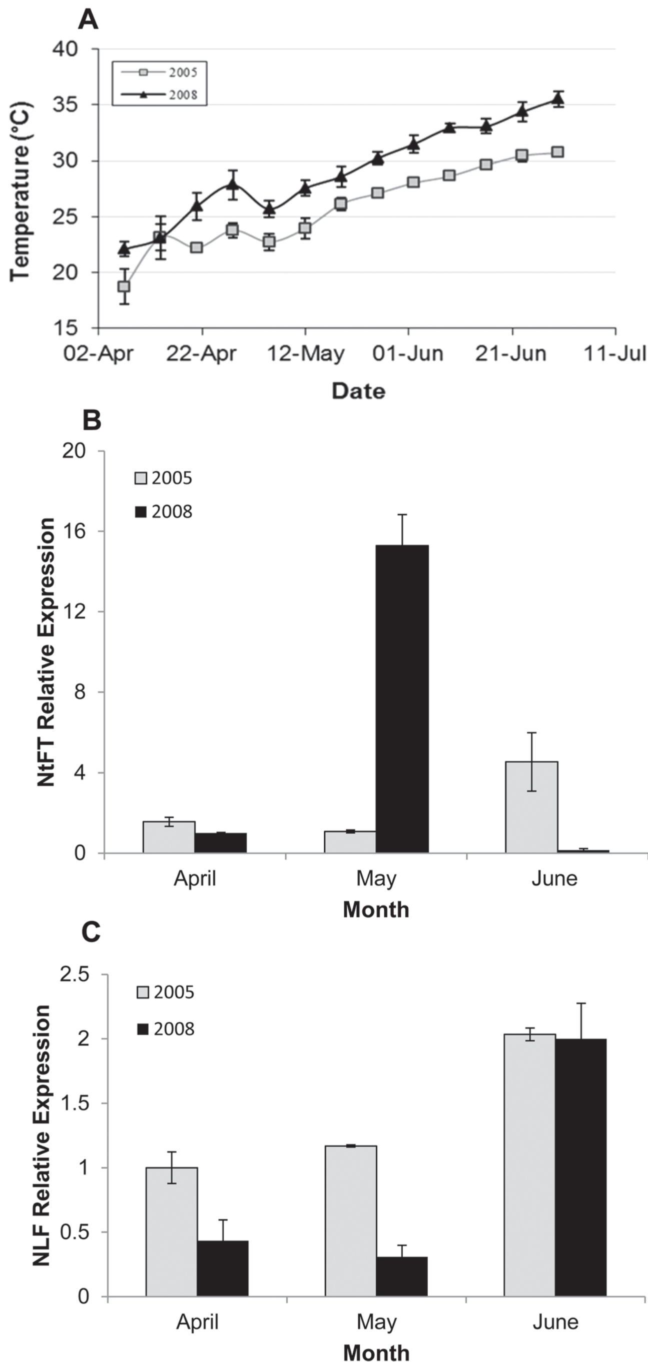

Fig. 6. Relative expression of *NtFT* and *NLF* under different temperature regimes recorded in April–July 2005 and 2008. Samples were normalized against β-actin. (A) Soil temperature recorded at 10 cm depth in the open field in April–July 2005 and 2008. (B) Relative expression of *NtFT* in apical meristems, morphologically defined as vegetative. (C) Relative expression of *NLF* in apical meristems, morphologically defined as vegetative.

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,

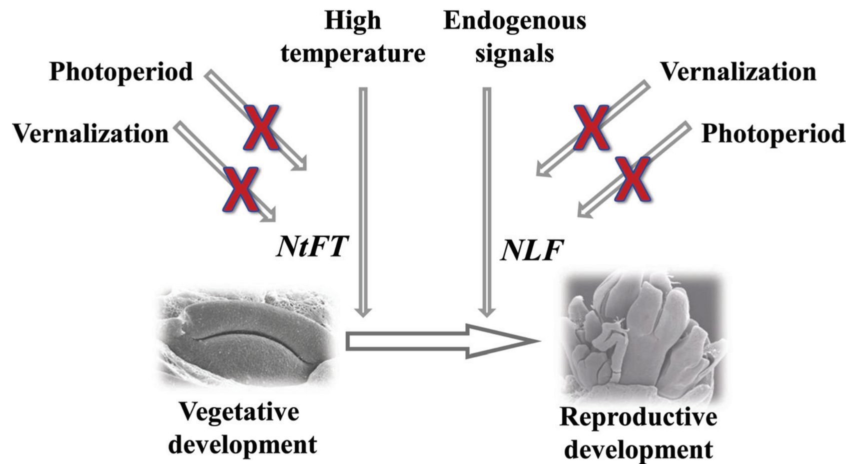

Fig. 7. Proposed scheme for environmental and molecular control of floral transition in *Narcissus tazetta*. Floral initiation is probably not stimulated by photoperiodic signal or low temperatures (vernalization). High temperatures at the end of the growth period induce expression of the *FT* homologue *NtFT* in leaf primordia and the apical meristem inside the bulb, followed by floral initiation and meristem transition to the reproductive stage. The *LFY* homologue *NLF* might be regulated differently from *NtFT*, and does not act in the same signalling cascade. *NLF* expression is not induced directly by ambient temperature, and under this pathway might not regulate floral transition but acts in later stages. However, it might regulate floral transition under an endogenous signalling cascade.

in *Arabidopsis*, *LFY* is known to be the target of several endogenous signals, such as age (Wang *et al.*, 2009) and gibberellin (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 *N. tazetta* 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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