**FT-Like NFT1 Gene May Play a Role in Flower Transition Induced by Heat Accumulation in Narcissus tazetta var. chinensis**

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The low-temperature flowering-response pathway, used as an inductive stimulus to induce flowering in plant species from temperate regions in response to cold temperature, has been extensively studied. However, limited information is available on the flower transition of several bulbous species, which require high temperature for flower differentiation. *Narcissus tazetta var. chinensis* (Chinese narcissus) exhibits a 2-year juvenile phase, and flower initiation within its bulbs occurs during summer dormancy. The genetic factors that control flower initiation are mostly unknown in Chinese narcissus. In the present study, we found that a high storage temperature is necessary for flower initiation. Flower initiation was advanced in bulbs previously exposed to extended high temperature. The heat accumulation required for flower transition was also determined. High temperature treatment rescued the low flower percentage resulting from short storage duration under natural conditions. In addition, extended high storage temperature was found to increase the flowering percentage of 2-year-old plants, which can be applied in breeding. *Narcissus FLOWERING LOCUS T1* (NFT1), a homolog of the *Arabidopsis thaliana* gene FLOWERING LOCUS T, was isolated in this study. NFT1 transcripts were abundant during flower initiation in mature bulbs and were up-regulated by high temperature. The genetic experiments, coupled with an expression profiling assay, suggest that NFT1 possibly takes part in flower transition control in response to high temperature.

**Keywords**: Flower initiation • *Narcissus tazetta* • var. chinensis • NFT1 • Temperature.

**Abbreviations**: CO, CONSTANS; FD, Flowering Locus D; FLC, FLOWERING LOCUS C; FT, FLOWERING LOCUS T; qRT-PCR, quantitative real-time PCR; RACE, rapid amplification of cDNA ends; SEM, scanning electron microscopy; SM, shoot meristem; SOC1, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1; TFL1, TERMINAL FLOWER 1; WT, wild type.

The nucleotide sequence of NFT1 reported in this paper has been submitted to NCBI under accession numbers JX316221 for cDNA and JX316222 for genomic DNA.

**Introduction**


Seasonal temperature changes elicit seasonal flowering responses that allow the synchronization of flowering with optimal conditions (King and Heide 2009, Hemming and Trevaskis 2011). Several plant species from temperate regions use cold
temperature signaling for reproduction (Wilkie et al. 2008, Hemming and Trevaskis 2011), a phenomenon known as ‘vernalization’. The MADS-box gene FLOWERING LOCUS C (FLC) is a key regulator of vernalization-induced flowering in A. thaliana and related species (Sheldon et al. 2008, Sheldon et al. 2009, Wang et al. 2009b). FLC inhibits flowering by repressing the genes that promote flowering, such as FT and the SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) (Helliwell et al. 2006, Searle et al. 2006). Long durations of cold temperatures suppress FLC transcription quantitatively, thereby allowing rapid flowering (Sheldon et al. 2009). In A. thaliana, FLC transcription is stably repressed by vernalization, and FLC expression remained low after the plants returned to warm conditions (Sheldon et al. 2000, Sheldon et al. 2009). This effect provides a molecular ‘memory’ of winter and allows rapid flowering as temperature and daylength increase during spring. The vernalization response in cereals is controlled by another MADS-box gene, namely VERNALIZATION1 (VRN1) (Trevaskis et al. 2007). In contrast to FLC, VRN1 promotes flowering, and its transcript levels are low in plants that have not been vernalized. Exposure to cold temperatures increases VRN1 transcript levels quantitatively, thereby accelerating transition to reproductive growth at the shoot apex, and also makes plants respond to long days by de-repressing the long-day flowering-response pathway in the leaves (Yan et al. 2003, von Zitzewitz et al. 2005, Sasani et al. 2009). In addition, the different roles of FT-like proteins in response to temperature, which regulates the reproductive transition in some biennials and perennials, were revealed (Lifschitz et al. 2006, Pin et al. 2010, Hsu et al. 2011). Two paralogues of the FT gene (BvFT1 and BvFT2) have evolved antagonistic functions in biennial sugar beets (Beta vulgaris ssp. vulgaris). BvFT2 is functionally conserved with FT and is essential to flowering. In contrast, BvFT1 represses flowering, and its down-regulation is crucial for the vernalization response in beets. In the woody perennial poplar (Populus spp.), the FLOWERING LOCUS T1 (FT1) and FLOWERING LOCUS T2 (FT2) paralogs coordinate repeated cycles of vegetative and reproductive growth. Reproductive onset is determined by FT1 in response to winter temperatures, whereas vegetative growth and inhibition of bud set are promoted by FT2 in response to warm temperatures and long days during the growing season (Hsu et al. 2011). These advances suggest that the duplication or changes in FT genes contributed to the evolution of plant adaptation to environmental cues.

Flower transition requires different temperature conditions, depending on the ecological origin of the bulbous geophyte species (Haley 1990, Flaishman and Kamenskyy 2006). Species from temperate zones (e.g. Lilium, Galtonia and Allium cepa) usually require low temperatures for flower differentiation, such as vernalization in the winter annual model plant Arabidopsis. Species with thermoperiodic cycles (such as Tulipa, Narcissus and Hyacinthus), from the Irano-Turanian and Mediterranean regions, require relatively high temperatures for flower differentiation inside the bulb, as well as a period of low temperatures to allow floral stem elongation and anthesis (Flaishman and Kamenskyy 2006). Species from arid areas (e.g. Cyclamen, Pancratium and Bellevalia) require high summer temperatures for flower transition within the bulb. No cold induction is required for floral development and stalk elongation (Kamenetsky and Fritsch 2002, Kamenskyy and Rabinowitch 2002, Flaishman and Kamenskyy 2006). However, limited information on the molecular mechanisms that regulate flower initiation in response to temperature in bulbous geophytes is available to date.

A high summer temperature signal is often used to advance narcissus flowering. Chinese narcissus (Narcissus tazetta var. chinensis) is a plant from the Amaryllidaceae family that exhibits summer dormancy. Its bulbs sprout in October to November (when soil temperature drops), grow throughout the winter and flower in January to February. The above-ground parts of the plants begin to senescence in late spring. Chinese narcissus exhibits a 2 year juvenile phase. Florogenesis is initiated within large-sized bulbs during summer dormancy. Timing of flower initiation in its dormant bulbs varies, depending on where they were cultivated. Flower initiation occurs in early June in Guangzhou, early July in Zhangzhou and late July in Shanghai (Zhong 1984, Li et al. 1987, Zhang and Yang 1987, Li et al. 2012). Noy-Porata et al. (2009) showed that floral initiation and reproductive development in ‘Galilee’ (N. tazetta) cultivated in Israel is promoted by high temperature at an optimum of 25°C, whereas low temperatures (12°C) inhibit florogenesis completely. The floral transition in Chinese narcissus in response to environmental conditions and the molecular mechanisms that regulate these responses remain unknown.

In this study, different temperature regimes were designed and different planting dates were employed for >3 years to determine the right inductive stimuli that will predict the reproductive development in the bulb of Chinese narcissus. The reproductive organogenesis in 3-year-old bulbs and flowering percentage were assayed. Different storage temperature regimes were also designed and performed on 2-year-old bulbs to address the relationship between juvenile–adult phase change and temperature. One FT homolog, Narcissus Flowering Locus T1 (NFT1), was also isolated from Chinese narcissus. Its function was assayed to determine whether the genes shown previously in A. thaliana also regulate flowering. This study showed that extended high temperature exposure not only triggers the transition of the bulb shoot meristem (SM) from the vegetative stage to the reproductive stage, but also shortens the juvenile phase. In addition, NFT1 was shown to mediate flower transition in response to high temperature.

Results

High storage temperature is essential to flower initiation in Chinese narcissus

The scanning electron microscopy (SEM) assay showed that flower initiation occurred earlier in 3-year-old bulbs stored at 30°C compared with those under natural conditions (Fig. 1). Flower transition began in late July under natural conditions,
which were temperatures of between 19 and 33°C in June, July and August in Shanghai (Fig. 1B–D). Before mid-July, the SM in the 3-year-old bulbs was sharp conical and only leaf primordia were differentiated around its edge (Fig. 1C, F0). In late July (i.e. 50–60 d post-harvest), some SMs began to flatten (F1 and F2 in Fig. 1C), spathe primordium began to initiate on the periphery of the flat inflorescence meristem (F3 in Fig. 1C), and the spathe enwrapped the inflorescence meristem (F4 in Fig. 1C) gradually. The flower meristems in the spathe began to initiate at about 70 d post-harvest (F5 in Fig. 1C, D). Afterwards, the floral organ primordia were soon initiated, and several fully developed flowers formed in one spathe inflorescence in late August. Early flat inflorescence meristems (F1 in Fig. 1C) appeared at about the 40th day (Fig. 1D), and the whole flower initiation process was advanced by about 10 d relative to that under natural conditions, when the bulbs were stored at 30°C (Fig. 1D). The SM was kept at its sharp conical shape and at the F0 stage when the 3-year-old bulbs were stored at 15°C. No flat inflorescence meristem was found throughout 1 year (Fig. 1D).

**High temperature can rescue the low flowering percentage due to short storage duration under natural conditions**

The flowering percentage of 3-year-old bulbs became higher as the planting date was delayed. Only 66.7% of the bulbs planted on July 25 flowered, which was significantly lower than those planted on August 15 and September 1 (87.7% and 94.1%, respectively). This preliminary test showed that long natural storage duration improves flowering percentage, while short storage duration causes a low flowering percentage. However, the flowering percentage of the bulbs treated at 30°C for 20 d, followed by short storage at natural temperature (No. 1 in Fig. 2A), increased to 92.3%, which was not significantly different from those planted on August 15 (Fig. 2A, B). These data suggested that a high percentage of flowering required certain high temperature accumulation during storage.
shown in (A).

The amino acid sequence encoded by NFT1 encodes a protein with a predicted length of 174 amino acids. NFT1 of the sequences showed that comprising the complete coding regions, was isolated. Analysis narcissus plants NFT1 cDNA ends) method, a full-length cDNA of the Using degenerate primers and the RACE (rapid amplification of

Isolation of NFT1, a homolog of FT, from Chinese narcissus plants

Using degenerate primers and the RACE (rapid amplification of cDNA ends) method, a full-length cDNA of the NFT1 clone, comprising the complete coding regions, was isolated. Analysis of the sequences showed that NFT1 cDNA was 816 bp long and encodes a protein with a predicted length of 174 amino acids. The amino acid sequence encoded by NFT1 was highly similar to that of the FT-like genes in the FT-TFL family genes, with an identity of 70% and 77% to Arabidopsis FT and rice Hd3a, respectively, as well as 52% identity to TFL in Arabidopsis. Sequence comparison between NFT1 and the FT-TFL family proteins showed that NFT1 carries the functionally important FT signatures Tyr85(Y) (Tyr79 in NFT1) and Gln140(Q) (Gln134 in NFT1) (Fig. 4A) (Hanzawa et al. 2005). In addition, the gene structure of NFT1 was similar to that of FT (Fig. 4B), and NFT1 exhibited a region identical to most other FT genes within segment B of the fourth exon (encoding an external loop of PEBP) (Fig. 4A; Supplementary Fig. S1), which is important to FT vs. TFL1 function in Arabidopsis (Ahn et al. 2006). Phylogeny reconstructions with other published FT-TFL genes clearly showed that NFT1 falls into the FT-like subfamily, rather than the TFL- and MFT-like subfamily (Supplementary Fig. S2).

Expression pattern of NFT1 in Chinese narcissus

The spatial expression pattern of NFT1 was detected through in situ hybridization analysis. Mature leaf blades, shoot apices from 3-year-old growing plants and flower buds were used for in situ hybridization. The transcripts of Arabidopsis FT, rice Hd3a and RFT1 were mainly detected in the leaf phloem (Tamaki et al. 2007, Komiya et al. 2009). Similarly, hybridization with NFT1 RNA antisense probe revealed signals over vascular bundles within transverse leaf sections (Fig. 5A, B). The signal was detected primarily in the phloem, xylem parenchyma and muscle cells at high magnification (Fig. 5B). NFT1 signal was too low to be detected in the shoot apices of the 3-year-old bulbs during endo-dormancy (i.e. from April to early July under natural conditions) (data not shown). However, signal was detected in the apices from late July (Fig. 5C) and in early flower buds. Gene-specific quantitative real-time PCR (qRT-PCR) analyses were performed on mRNA samples from flowers, leaves, shoot apices and bulbs during active growth. Strong expression levels of NFT1 were detected in the leaves and shoot apices, whereas low levels were detected in opening flowers and bulbs. The bulbs have several layers of scales, and the expression levels in scales at different locations did not show any significant difference (Fig. 5D).

Ectopic expression of NFT1 in A. thaliana advanced flowering

Ectopic expression through the 35S promoter provided evidence that NFT1 encodes a protein that acts as floral regulator in Arabidopsis. A total of nine lines with the 35S::NFT1 construct in Col plants [wild type (WT)] and five lines with transformation of the 35S::NFT1 construct directly into ft-3 mutant plants were selected for flowering time analysis. No developmental abnormality, except for flowering time, was caused in these lines. The flowering time of most lines was significantly earlier than that of WT or ft-3 mutant plants under inductive long-day conditions (Fig. 6A, B, F). The lines of the 35S::NFT1 construct in Col flowered 7–14 d earlier and made 5–7 leaves fewer than those of Col controls (Fig. 6B). Although the lines of
the 35S::NFT1 construct in ft-3 flowered later than the WT Ler plants, these lines flowered about 15 d earlier and made 3–10 leaves fewer than the ft-3 controls (Fig. 6F). Several transgenic lines were selected for the semi-quantitative or qRT-PCR analysis of the expression levels in mature leaves. All transgenic lines showed NFT1 expression (Fig. 6C, G; Supplementary Fig. S3).SOC1 is one of the targets of FT (Lee and Lee 2010), and the SOC1 transcript was up-regulated by the ectopic expression of NFT1 (Fig. 6D, H; Supplementary Fig. S3). SOC1 expression levels in the transgenic lines of ft-3 were higher than those of the parent line of ft-3. However, they were not consistently higher than that of WT Ler plants (Fig. 6H; Supplementary Fig. S3B). The expression of endogenous AtFT in the transgenic lines was also not consistently higher than in the parent lines (Fig. 6D, H; Supplementary Fig. S3).

Expression of NFT1 in Chinese narcissus induced by floral inductive treatment

The foregoing data showed that flowers were differentiated in the bulbs during dormancy, when the species has no leaf, and that flower initiation in Chinese narcissus was induced and advanced by high temperature. The change in transcription abundance in the bulb apices was assayed using RT-PCR and qRT-PCR during the entire storage time to determine whether flower transition is related to NFT1 expression. NFT1 gene transcription increased gradually at 1 month before the early flower transition (indicated by the triangle in Fig. 7A) under natural conditions, and reached its peak at the moment of flower transition (Fig. 7A, B). NFT1 gene transcription also increased gradually in the apices of the bulbs stored at high temperature (30°C). Moreover, NFT1 gene transcription in the bulb apices...

Fig. 3 High storage temperature increases the flowering percentage of 2-year-old bulbs. (A) Illustration of different storage treatments of narcissus bulbs, with temperature regimes shown below the corresponding date, and continuous lines representing periods between 22 and 25°C. (B) Effect of storage temperature on the percentage of bolting plants at different days from the planting date. Different lower case letters indicate significant differences between different treatments at a given time point (Pearson’s $\chi^2$, $p < 0.05$, $n = 30–0$). Detailed temperature regimes in (B) are shown in (A).
**Discussion**

**Heat accumulation is necessary for flower initiation in Chinese narcissus**

Flowering initiation of Chinese narcissus was induced gradually by summer heating. Flowering is initiated in the dormant bulbs during summer, wherein the reported critical growth temperature was 25°C, above which growth stops (Lin 2002). In Shanghai, summer usually starts in early June, with a mean temperature of 25.0–29.2°C; flowering initiation in Chinese narcissus occurs between late June and early August (Fig. 1). No flowering initiation was found in bulbs stored at 13–15°C, even after 1 year, whereas it was advanced in bulbs stored at high temperature (30°C) compared with those stored under natural conditions (Fig. 7A), which was consistent with the early flower transition in the bulbs stored at high temperature.

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**Fig. 4** Chinese narcissus NFT1 belongs to the FT-like gene family. (A) Partial amino acid sequence alignment of FT-TFL family members. Black stars on the upper row indicate the Tyr85(Y)/His88(H) and Gln140(Q)/Asp144(D) residues distinguishing all FT-like and TFL1/CEN-like members. The homeotic subfamily gene groups are labeled at the right margin based on phylogeny. (B) Gene structures of NFT1 and FT. Boxes represent exons, whereas thin lines represent introns. The 5′- and 3′-untranslated regions are indicated by a shaded box.
flowering percentage. Based on our data, 40 d of constant high temperatures (30°C) without a day and night temperature difference, or 50–60 d of natural temperature (average temperature >25°C, Fig. 1B) were enough for flower transition. Consistently, flowering initiation in Chinese narcissus takes place in June in Guangzhou (Zhong 1984, Zhang and Yang 1987), which was earlier than that in Shanghai, because the average temperature above 25°C begins in mid-April. In agreement with our observations, high temperatures promotes floral initiation in 'Galilee', N. tazetta cultivated in Israel, while lower temperatures (12°C) inhibits florogenesis completely, which was reported previously (Noy-Porata et al. 2009). However, Noy et al showed that 30°C treatments delay flower initiation in N. tazetta, which is inconsistent with our results in this study showing that heat accumulation at 30°C promotes flowering in Chinese narcissus. One explanation might be that the temperature range for the flower initiation in 'Galilee' and Chinese narcissus is different. In fact, in the report of Noy-Porata et al. (2009), the percentage of flower transition in bulbs stored at 30 and 25°C was as not significantly different, although the former is lower than the latter (Noy-Porata et al. 2009). We also found that treatment at 26 and 30°C caused a similar flowering rate.

**NFT1 integrates endogenous and temperature signals for flowering**

FT1, an FT ortholog, might integrate endogenous and temperature signals for flowering in Chinese narcissus. Chinese narcissus is not transformable, and the function of NFT1 in inducing early flowering is conserved in transgenic Arabidopsis (Fig. 6) (Kardailsky et al. 1999). Although high signals of NFT1 transcription were detected in leaves (Fig. 5), floral initiation takes place in the bulbs when there is no leaf. Shoot tips from 3-year-old bulbs of Chinese narcissus were collected to test whether the NFT1 expression level is an important determinant of flowering time. NFT1 gene transcription increased gradually at 1 month before the early flowering transition under natural conditions; and it achieved its peak at the moment of flower transition (Fig. 7A, B), suggesting that a critical level of NFT1 expression was required to initiate flowering. Consistent with early flowering initiation under high temperature, NFT1 gene transcription in bulb apices was strongly up-regulated and the higher level
Fig. 6 Ectopic expression of NFT1 promoted flowering in transgenic Arabidopsis. (A) Growth of 35S::NFT1 in Col, and Col plants under long-day (LD) conditions after 28 d. (B) Effect of ectopic NFT1 expression in Col on the percentage of plants with a different number of rosette leaves when bolting under LD conditions. n = 62 and 20 for 35S::NFT1 in Col and Col, respectively. (C) and (D) NFT1, SOC1, and AtFT expression was assayed via qRT-PCR in the selected transgenic lines of 35S::NFT1 in Col. (E) Growth of 35S::NFT1 in ft-3 and ft-3 (in the Ler background) plants under LD conditions after 32 d. (F) Effect of ectopic NFT1 expression in ft-3 on the percentage of plants with a different number of rosette leaves when bolting under LD conditions. n = 20, 17 and 15 for 35S::NFT1 in ft-3 transgenic plants, ft-3 and Ler plants, respectively. (G) and (H) Ectopic expression of NFT1 increased SOC1 transcription in transgenic plants. Expression of NFT1, SOC1, and the Arabidopsis FT gene (At-FT) was assayed via qRT-PCR in the selected transgenic lines.
Similar to Arabidopsis FT, BvFT2 function is needed for flowering. The vernalization response suppresses BvFT1 expression and releases its inhibition against BvFT2 to induce flowering. FLC, other FT orthologs and more of the relevant genes in Chinese narcissus need to be characterized in order to elucidate the regulation of NFT1 expression in the SM.

**Heat treatment is beneficial to the adjustment of the flowering time of Chinese narcissus**

Heat accumulation was not only necessary for flowering initiation in the 3-year-old bulbs, but also promoted flowering in the 2-year-old bulbs. Chinese narcissus is characterized by 2 year juvenile phases before the first flower is formed. During farming, 3-year-old bulbs were selected as flowering plants according to their sizes. Our data also showed that the flowering percentage in the 2-year-old bulbs increased with longer high-temperature treatment. In addition, treatment at 30°C for 80 d significantly increased flowering percentage (86.1% vs. 25% under natural temperature) (Fig. 3B). Setting the heat accumulation of one summer equal to the treatment at 30°C for 40 d was necessary to induce flowering initiation in the 3-year-old bulbs. The flowering capability in most 2-year-old bulbs treated at 30°C for 80 d was due to the heat accumulation, equivalent to two summer seasons. In addition to heat treatment combined with a short storage duration to improve the flowering percentage, these results may have interesting applications in farming to adjust flowering time.

**Materials and Methods**

**Plant materials and growth conditions**

*Narcissus tazetta var. chinensis* bulbs were commercially obtained from Shanghai, China. Healthy bulbs of similar sizes (2-year-old bulbs, each with a 10 ± 1 cm circumference, and 3-year-old bulbs, each with a 15 ± 1 cm circumference) were grouped and stored in natural or controlled conditions. A dry and ventilated warehouse with ambient light and temperature was used as the natural environment.

*Arabidopsis thaliana*, ecotype Col, Ler and ft-3 (SALK line cs185 in the Ler background) plants were grown in a greenhouse under constant illumination (approximately 80 μmol m⁻² s⁻¹) at 22 ± 2°C. Seeds were surface sterilized, stratified at 4°C for 3 d, plated on half-strength Murashige and Skoog (Murashige and Skoog 1962) medium containing 50 μg ml⁻¹ kanamycin, and grown under continuous fluorescent light at 22°C to screen the transgenic plants.

**Treatment method to affect flowering**

The flower differentiation during storage of the 3-year-old bulbs at natural temperature, high temperature (30°C) or low temperature (15°C; Nos. CK, 1 and 2 in Fig. 1A) was analyzed via SEM to determine whether high or low temperatures favor flowering initiation. The average natural temperatures in

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**Fig. 7** NFT1 expression in the bulb apices was induced by high temperature. (A) Effect of temperature on the NFT1 expression profile in the bulb apices shown via qRT-PCR. The upper part illustrates the different storage treatments of narcissus bulbs, with temperature regimes shown below the corresponding date, and continuous lines representing periods between 22 and 25°C. The two time points indicated by arrows are the beginning of flower transition in bulbs with relevant treatment. Relative expression levels indicate the data relative to those obtained on June 9 under natural temperatures. (B) Effect of temperature on the change of NFT1 transcripts in bulb apices during storage, shown via RT-PCR. Detailed temperature regimes are shown in (A).
Shanghai are 20.8, 25.0, 29.2, 28.5 and 25.1 °C in May, June, July, August and September, respectively. The highest and lowest temperatures are shown in Fig. 1B.

Three-year-old bulbs were stored under natural conditions and planted on different dates, specifically, July 25, August 15 and September 1 (Nos. CK1, CK2 and CK3 in Fig. 2A). The percentages of the flowering plants were recorded to analyze the effects of different planting dates on flowering. The percentages of the flowering plants treated at 30 °C for 20 d stored under natural conditions and planted on July 25 (No. 1 in Fig. 1A) were compared with those stored under natural conditions. The experiment was repeated three times: in 2006, 2007 and 2008.

Two-year-old bulbs were initially subjected to high temperature (30 °C) for 20, 40 and 80 d, and then stored between 22 and 25 °C until planting to determine the effects of different high temperature durations on flowering. The results for the 2-year-old bulbs stored at natural temperature and with treatment at 30 °C for 40 d, with storage from 22 to 25 °C (Nos. 1–4 in Fig. 3A), were also compared to test the effect of different temperatures on flowering. The experiment was conducted in triplicate with independent materials and was repeated twice, in 2010 and 2011.

Temperature-controlled incubators were used for different temperature treatments. The samples in each treatment consisted of at least 40 bulbs, unless otherwise stated. The detailed methods are illustrated in Figs. 1–3. After treatment, all bulbs were planted in a controlled greenhouse with a short photoperiod (10 h/14 h light/dark) at 18–20 °C. The growing conditions and planting order were previously described (Li et al. 2012).

### Scanning electron microscopy

SMs of 3-year-old bulbs were collected weekly after their harvest. Apices were dissected under an anatomical lens as rapidly as possible, and fixed in formaldehyde–acetic acid (composed of 63% ethanol, 5% formaldehyde and 6% acetic acid) at 4 °C overnight. Afterwards, the samples were dehydrated in an ethanol series, critical-point dried in liquid CO2, sputter-coated with gold–palladium, analyzed, and photographed with a Philips XL 30 FEG SEM. Five to 10 samples of each treatment were detected at each time point, and the number of samples at different development stages was analyzed.

### Cloning of NFT1 cDNAs

Partial cDNAs were isolated from younger inflorescences of Chinese narcissus using degenerate primers according to the consensus-degenerate hybrid oligonucleotide primer method (Rose et al. 1998, Rose et al. 2003, Staheli et al. 2011). The first PCR was conducted using a pair of degenerate primers, NFT-A64 (5'-GCCGGGGGGTANACNGTYG-3') and NFT-A20 (5'-GCACACTGCATGCAANGATAG-3'). Nested PCR was performed using another pair of degenerate primers, NFT-A65 (5'-CCAGGCCGGGGCRTANACNGTY-3') and NFT-A28 (5'-CTACACCCCTGATGATGTRGAYCCNGA-3'). A partial cDNA clone was isolated from Chinese narcissus. BLAST searches in public databases, with the conceptual amino acid sequence of the clone as query sequences, showed that the clone shared high sequence identity with FT-like genes from various plants, and thus was named NFT1 (Narcissus FT-1). The 3' and 5' regions corresponding to the cDNAs of NFT1 were isolated using the RACE method (Roche Diagnostics). cDNA and DNA clones with a complete open reading frame were isolated via PCR, with primers located in the 5' and 3' regions.

### Plant transformation and transgenic plant analysis

An EcoRI fragment containing the full-length cDNA for the NFT1 gene was introduced into the binary T-DNA vector pMon530 (Monsanto), driven by the 35S promoter. Genetic transformation lines were obtained, as previously reported (Li et al. 2009). The phenotypic effects of NFT1 in transgenic Col plants were analyzed in nine independent transgenic lines. Five independent lines were created by directly transforming the 35S:NFT1 construct into ft-3 mutant plants, and were selected for flowering time analysis.

### Sequence alignments

Predicted amino acid sequences were used for phylogenetic analysis. Protein sequences were aligned using Clustal W (http://www.clustal.org/), then manually adjusted.

### RT-PCR

Total RNA from different tissues was extracted from frozen tissue using TRIZOL reagent (Invitrogen) and purified on RNeasy columns (QIAGEN). The first cDNA strand was generated according to the instructions for the Superscript RT (Toyobo). PCR amplification was performed with gene-specific primers, NFT F2 (5'-TAGCCCAAGTACAAAACACT-3') and NFT R2 (5'-GGGATTCCACCCCCAATTATT-3'). ACTIN expression levels were monitored to serve as a quantifying control, and the experiments were repeated at least three times.

qRT-PCR was performed with 3–6 independent biological replicates and three technical replicates for each sample. Data were analyzed via the 2^-ΔΔCt method, as described by Schmitgen and Livak (2008). Statistical significance between any pair of relative expression means was determined by a t-test. The experiment was repeated with samples from another year with a similar result.

### In situ hybridization

Samples were fixed in 4% (v/v) paraformaldehyde and 0.5% (v/v) glutaraldehyde for 24 h at 4 °C. Then samples were dehydrated through an ethanol series, embedded in Histoplast and sectioned at 7 μm using a rotary microtome. A probe for NFT1 was amplified with primers 5’-gaattcATGATGAGAATCCTTTTGGT-3' and 5’-gaattcTCATCTAGGGTACATCCCT-3'. Digoxigenin-labeled sense and antisense RNA probes were synthesized.
according to the instructions of the manufacturer (Roche Applied Science). Hybridization detection of the signals with alkaline phosphatase was performed as described (Ludevid et al. 1992).

Supplementary data

Supplementary data are available at PCP online.

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