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Characterization of summer dormancy in *Narcissus tazetta* var. *Chinensis* and the role of *NtFTs* in summer dormancy and flower differentiation

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ABSTRACT

Summer dormancy is critical for plant survival under unfavorable conditions. However, unlike winter dormancy, summer dormancy is relatively uncharacterized, especially in *Narcissus tazetta* var. *Chinensis*. The aim of the present study was to characterize the summer dormancy of *N. tazetta* var. *Chinensis* and to assess the role of *NtFTs* in summer dormancy and flower differentiation. Bulbs were obtained every 7 days between April 14 and July 14, 2012. Bulbs were weighed and measured. Morphology and amyloplast properties of the main bud and the fourth layer scale were assessed by hematoxylin staining and periodic acid Schiff reaction, respectively. Relative mRNA expressions of *NtFTs* were determined by real-time RT-PCR. Morphological observation and amyloplast changes in main buds and the fourth layer scale revealed that summer dormancy is separated in the early and late dormancy stages. *NtFT1* expression was in opposition with *NtFT2* and *NtFT3* expressions during dormancy, which suggested that *NtFTs*, which are target genes of temperature-signal pathways, regulate the dormancy process. The expression levels of *NtFT1* gradually increased while *NtFT2* and *NtFT3* decreased during the bulb swelling stage, in the dormancy period (from May 5 to July 7), the expression levels of *NtFT1* changed with a inverse "V" pattern in the early dormancy stage, which is opposite with both the expression of *NtFT2* and *NtFT3*; the expression levels of *NtFT1* decreased while *NtFT2* and *NtFT3* showed an increasing pattern in the late dormancy stage. In conclusion, summer dormancy in *N. tazetta* var. *Chinensis* was separated in two distinct phases. *NtFTs* might be involved in summer dormancy of *N. tazetta* var. *Chinensis*.

1. Introduction

Summer dormancy is a means for plants to survive the hot and dry summer season, and happens under increasing photoperiods and temperatures (Norton et al., 2006a,b; Volaire and Lelievre, 2001). In *Narcissus tazetta* var. *Chinensis*, high temperature has been suggested necessary for endo-dormancy release (Li et al., 2012). Previous studies focused on water content (Norton et al., 2006a,b; Ofir and Kigel, 2010; Volaire et al., 2009), hormones (Bufler, 2009; Ofir, 1976; Ofir and Kigel, 2007; Suttle, 2009), environment cues (Kamenetsky and Rabinowitch, 2006; Ofir and Kigel, 2006; Phillips, 2010; Phillips et al., 2008), and expression of genes involved in dormancy release (Noy-Porat et al., 2013). However, it is still unclear how summer dormancy is regulated by environmental cues and genes.

N. tazetta var. *Chinensis* bulbs are usually planted in October and harvested in May or June in the next year. The annual growth cycle includes four phases: vegetative stage (October to November), flowering stage (December to January), bulb swelling stage (February to April or May), and dormancy stage (May or June to September). The dormancy phase is accompanied with florogenesis initiation after harvesting (Li et al., 2012), which is inconsistent with common dormancy as defined by Lang et al. (1987) and Volaire and Norton (2006). Therefore, it is imperative to assess when true dormancy starts in *N. tazetta*'s annual life cycle.

Flowering locus T (FT) is a member of gene families encoding phosphatidylethanolamine-binding proteins (PEBPs) and includes *TFL1*-like, *FT*-like, and *MFT*-like. *FT* is a multifunctional regulator playing important roles in flowering, photoperiod and vernalization (Baurle and Dean, 2006; Jaeger et al., 2006;

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Navarro et al., 2011). In addition, *FT* participates in the induction and release from dormancy (Bohlenius et al., 2006; Corbesier et al., 2007; Gyllenstrand et al., 2007; Hsu et al., 2011; Sreekantan et al., 2010). Under long sunshine conditions, *FT1* and *FT2* (a homologous gene of *FT1*) show high transcription levels during the flowering process of *Populus* spp., while low expression level was observed when growth stopped (Hsu et al., 2006). Studies of *FT* homologous gene in *Picea abies* indicated that, high expression levels of *PaFTL1* and *PaFTL2* in summer regulates the initiation and stop of growth in *P. abies* (Karlgren et al., 2013). *NFT1*, which was cloned from *N. tazetta* var. *Chinensis* for the first time by Li et al. (2012), was proved to play roles in the induction of flower buds.

However, there is no report about the regulation of dormancy by *FT* homologous genes in *N. tazetta* var. *Chinensis*. Therefore, the aim of the present study was to characterize *FT* gene expression in *N. tazetta* var. *Chinensis* during summer dormancy, which might provide more molecular basis for study of the mechanism under the dormancy of *N. tazetta* var. *Chinensis*.

2. Materials and methods

2.1. Plant materials

Three-year-old *N. tazetta* var. *Chinensis* plants were obtained from the same commercial producer (Zhangzhou, Fujian, China) every 7 days between April 14 and July 14, 2012. Plants were freshly uprooted. Leaves and roots of plants were cut off after cleaning and dried with aseptic paper to obtain bulbs (Fig. 1A). Then, the fourth layer scale was stripped from their interior bulbs, and the main buds were obtained after complete removal of scales (Fig. 1B). Twenty-five bulbs were obtained every 7 days between April 14 and July 14, 2012. Six main buds as well as its fourth layer scale at each time point were fixed in 70% alcohol-glacial acetic acid-formaldehyde (FAA, V:V:V = 90:5:5) for morphological and amyloplast analyses. Remaining bulbs were frozen in liquid nitrogen and stored at −80 °C for cloning (n = 1–2 each time point), real-time RT-PCR analysis (n = 3 each time point) and Illumina transcriptome sequencing (data not shown).

2.2. Determination of the summer dormant stage in bulbs

Bulbs were measured in weight and size on every 7 days between April 14 and July 14, 2012. Changes in weight and size of bulbs were analyzed.

Morphological and amyloplast changes in the apical meristem of main buds were observed using an Axio Observer A1 inverted microscope (Carl Zeiss GmbH, Oberkochen, Germany) on every 7 days between April 14 and July 14, 2012. Morphological and amyloplast changes in the fourth layer scale were also observed by inverted microscopy (Li-Yun, 1990). Morphology was determined by Ehrlich's hematoxylin staining. Amyloplast changes were determined by periodic acid Schiff reaction.

2.3. Cloning of NtFTs genes

Total RNA was extracted from main buds at each time point (every 7 days from April 14 to July 14, 2012) using cetyltrimethylammonium bromide (CTAB; Sangon Biotech, Shanghai, China) in combination with Trizol (Invitrogen Inc., Carlsbad, CA, USA). RNA purity was determined by $OD_{260\,nm}/OD_{280\,nm}$. For reverse transcription, the first cDNA strand was obtained using the Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's instructions. Data was obtained from transcriptome libraries (data not shown) to design the primers. The 3′ and 5′ ends of the genes were amplified by nested PCR. Then, cDNA was amplified by PCR to verify the complete open reading frames. PCR was carried out with a 5-min denaturation at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s annealing at 57 °C, a 1-min extension at 72 °C, and a final extension period of 10 min at 72 °C. All primers were designed according to the transcriptome assembly library (data not shown) using the Oligo 6.0 software (LifeScience Software Resource, Long Lake, MN, USA).

Primers for *NtFT*1 were: 5′-TTT CCG CTT ATA TCT CTT CTG GGA C-3′ (forward) and 5′-TCG GGA AGT AGC AAG ACG ATC AAA C-3′ (reverse); for *NtFT*2: 5′-GCA TTC AAC CTC CTC CTA TAT TC-3′ (forward) and 5′-TTA CTC GTG CAT CCA TTA TTC C-3′ (reverse); and for *NtFT*3: 5′-AAG CAG TGG TAT CAA CGC AGA GT-3′ (forward) and 5′-GCT AAT CCT GTG CCG AAC TGA AC-3′ (reverse).

2.4. Sequence analysis

Sequences were analyzed using the DNAMAN software, and compared with the NCBI DNA or protein databases for similarity analysis and prediction of conserved domains in amino acid sequences using the Blastx and Blastp methods.

2.5. Quantitative real-time RT-PCR

Total RNA was extracted from main buds collected every 7 days from April 14 to July 14 using CTAB in combination with Trizol. Reverse transcription was carried out using the PrimeScript™ RT reagent Kit (Takara Bio, Otsu, Japan), according to the manufacturer's instructions. cDNA samples were diluted 10 times and 2 iL was used for qRT-PCR, in a total reaction volume of 20 iL containing 2× SYBR® Premix Ex Taq™ (Takara Bio, Otsu, Japan) and gene-specific primers in a LightCycler 480 Real-Time PCR System (Roche Applied Science, Penzberg, Germany). Actin expression levels were used as internal control. The experiments were carried out in triplicates and repeated two times.

Primers used for *NtFT1* were: 5′-CCA GCC AAA GGT TGA AGT CG-3′ (forward) and 5′-CCC TGT GGT TCC TGG TAT G-3′ (reverse); for *NtFT2*: 5′-TAG ATC CTG ATG CTC CAA GTC C-3′ (forward) and 5′-AGA GCA CGA GGA TGA ACC GAT G-3′ (reverse); for *NtFT3*: 5′-GTT ATG AGC CTC CGA AGC CAA CT-3′ (forward) and 5′-CCA GTT CCG GAT TCC CTC TGG CAA-3′ (reverse); and for actin: 5′-CTT GAC CTT GCT GGG AGA GAT-3′ (forward) and 5′-GAT GTC ACG GAC AAT TTC ACG C-3′ (reverse).

Data were analyzed by the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008).

2.6. Statistical analyses

The SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Continuous data are expressed as means ± standard deviation (SD). Statistical significance was evaluated by one-way analysis of variance (ANOVA) with Tukey's *post hoc* analysis. Differences were considered statistically significant and highly statistically significant at $P < 0.05$ and $P < 0.01$, respectively.

3. Results

3.1. External properties of bulbs

Bulb weights and sizes were determined every 7 days between April 14 and July 14, 2012. As shown in Fig. 2, bulb weights were high on April 28 (122 ± 30 g) and slightly increased on June 2 (127 ± 24 g), before dropping on June 9 (110 ± 13 g) and July 7 (100 ± 10 g) (Fig. 2A). The same trend was observed with bulb sizes, measured longitudinally and horizontally from bulb center (Fig. 2B and C).

Morphologically, bulb began to swell from February to April. During this period, leaves were green and internal scales swelled.