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A comprehensive analysis of flowering transition in *Agapanthus praecox* ssp. *orientalis* (Leighton) Leighton by using transcriptomic and proteomic techniques

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ABSTRACT

Comprehensive transcriptomic and proteomic analyses were performed to gain further understanding of the molecular mechanisms of floral initiation in *Agapanthus praecox* ssp. *orientalis*. Samples of stem apexes were collected at three different time points including the vegetative, induced, and reproductive period. A total of 374 transcript-derived fragments and 72 proteins showed significant differential expression between the samples. The largest proportion of the identified genes and proteins are involved in metabolism, followed by signal transduction, protein fate, cellular transport, and biogenesis of cellular components. A large number of these genes and proteins were upregulated during the induced and reproductive stages. Their expression profiles demonstrate that carbohydrate metabolism provides nutrients foundation for floral initiation in *Agapanthus*. Furthermore, a transcription factors GAI (GA insensitive protein) that negatively regulates gibberellin signaling, auxin receptor protein TIR1 (Transport inhibitor response 1), a key enzyme of ethylene biosynthesis SAMS (S-adenosylmethionine synthase), and ethylene receptor protein ETR were isolated and identified. Expression patterns of these proteins, in combination with the results of quantitative phytohormone and immunolocalization analyses, indicated that GA, indole-acetic acid (IAA), and ethylene regulate floral morphogenesis and flowering. In conclusion, these data provide novel insight into the early regulatory steps of flowering in *Agapanthus*.

1. Introduction

The development of flowering plants is an orderly progression from the embryo to the mature plant involving continuous organ formation from the meristem. The transition from vegetative growth to reproductive growth is a major phase change in angiosperms. In this process, the shoot apical meristem (SAM) initiates leaf primordia on its flanks in an organized pattern. After a period of vegetative growth, a combination of endogenous and environmental cues induces floral development [1]; these induction factors include light (day length or light quality), ambient temperature, nutrients, hormones, and some molecular genetic pathways [2].

All higher plants share some common mechanisms that control this important switch from vegetative growth to reproductive growth [3,4]. First, the SAM gives rise to both

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vegetative and reproductive structures, and is the part of the plant where the actual transition occurs. Second, the SAM must be competent to perceive inductive signals for inflorescence and floral meristems. Third, although the SAM is the target of floral inductive signals, the signals themselves, in most cases, originate in the vegetative tissues. Finally, the floral transition can be affected by signals that feed into both environmental and endogenous (or autonomous) pathways [5]. Environmental factors often exert inductive effects by evoking changes in hormone levels; consequently, flowering is usually associated with hormonal changes [6]. Phytohormones serve within the signal network leading to a reproductive phase transition, as both positive and negative elements, depending on the phytohormones and growth conditions [7].

In some crop plants, hormones are used to alter the timing of flowering. One example is the commercial use of ethephon, which is converted to ethylene, to prevent flowering in sugarcane and to increase sugar yields [8]. Moreover, *Arabidopsis* mutants with reduced gibberellic acid (GA) synthesis are late flowering in nature, leading to the inclusion of a separate GA pathway in its floral regulatory model. In ryegrass, GA appears to play a major role in the floral transition, and it has been suggested that it acts as a leaf-derived, long-distance signaling molecule [9]; whether GA has a similarly important role in other grasses has not yet been reported [5].

Great progress has been made in deciphering the molecular mechanisms that regulate flowering in *Arabidopsis* and agronomically significant grass species. The roles of several genes that regulate the spatial and temporal expression of the floral organ identity genes have been clarified [10]. The floral meristem identity genes ensure that the primordia initiated along the periphery of an inflorescence meristem adopt a flower fate. Mutations in these genes result in the partial conversion of flowers into shoot-like structures, whereas ectopic expression of these genes is sufficient to convert inflorescence meristems into flowers [11–14]. This genetic framework can explain the diversity of flowering time among *Arabidopsis* accessions and even in other species. The current research highlights the synergy obtained by simultaneously analysing several genetically tractable flowering plant species rather than relying on a single model organism. Broad-scale comparative studies such as the evolutionary genomics effort of the Floral Genome Project [15], will be extremely valuable in probing the induction of flowering. Understanding the evolution of flowering mechanisms in all plants is a long-standing and central problem in evolutionary biology and botany [2].

Agapanthus (Agapanthaceae) is a monocotyledonous, herbaceous, and perennial plant. This species is endemic to southern Africa, and is characterized by a tuberous rootstock, fleshy tissues, long flowering duration, and large number of florets [16]. *Agapanthus* has become popular as a potted plant, cut flower, and landscape plant. We have investigated sporogenesis, gametogenesis, embryology, and flowering bud anatomy in *Agapanthus praecox* ssp. *orientalis* [16–18], and found that *Agapanthus* flowering is not sensitive to photoperiod; in contrast, temperature is the most important environmental factor for flower induction. There is abundant knowledge available regarding the characteristics of flower bud development and embryology. However, the molecular mechanisms regulating the floral transition require further clarification. Here, we selected various developmental buds and applied comparative transcriptomic and proteomic analyses to reveal the molecular genetic events related to flowering in *Agapanthus*.

2. Materials and methods

2.1. Plant materials and growth conditions

A. praecox ssp. *orientalis* were grown at the Seedlings Practice Training Base of the Shanghai Jiao Tong University in Shanghai, China (30°55′60″N, 121°71′46″′E). Annual precipitation is 1254.9 mm. The maximum, minimum and annual mean temperatures are 38.6 °C, −4.7 °C and 17.5 °C, respectively, and the annual sunshine duration is 1778.3 h [18]. Tests were performed on materials from 4-year-old plants. Three samples were collected from shoot tips or flower buds according to morphological differences.

2.2. Transcriptomic analysis

2.2.1. RNA extraction and cDNA synthesis

Total RNA was extracted using RNAiso Plus (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's instructions. The total RNA was purified using DNaseI and RNase inhibitor. Double-stranded cDNA was synthesized using M-MLV RTase cDNA Synthesis Kit (TaKaRa), according to the manufacturer's instructions.

2.2.2. Template preparation and cDNA-amplified fragment length polymorphism (AFLP) analysis

Templates for cDNA-AFLP were prepared by performing cDNA digestion and adaptor ligation. The cDNA was digested with restriction enzymes *Eco*RI and *Mse*I. The digested cDNA was connected with *Eco*RI and *Mse*I adaptors using T4 DNA Ligase (see the sequences in Table S1). Pre-amplification was carried out using 10 ng cDNA template and 10 pM primers of *Eco*RI-00 and *Mse*I-00 (Table S1). PCR amplifications were performed for 30 cycles (94 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min).

Diluted pre-amplification products were used as templates with 64 primer combinations for selective amplification (Table S1). The PCR reactions were incubated at 94 °C for 5 min, followed by 12 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 1 min, with the annealing temperature dropping by 0.7 °C per cycle. The reactions were completed by 23 additional cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. Selective amplification products were run on 6% polyacrylamide gels (30 cm × 40 cm) for 4 h at 40 mA, and visualized using silver staining procedure.

2.2.3. Transcript-derived fragment (TDF) extraction, verification and sequencing

Differentially expressed TDFs were recovered in 40 μL sterile H_2O at 95 °C for 10 min. Each fragment was re-amplified according to selective amplification. Resultant amplified fragments were cloned into pMD 18-T simple vector (TaKaRa), transformed into *Escherichia coli* DH5-α, and sequenced (three individual clones). The obtained sequences were compared with the nucleotide and translated sequences of the GenBank