

Unique Orchid *Arundina graminifolia*

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Although the regulatory conduits for continuous flowering are basically connected to dormancy and bud release, *A. graminifolia* is unique in the aspect that it escapes dormancy and rapidly completes the flowering initiation and development. Therefore, the molecular patterning of continuous flowering is an interesting subject to study

Keywords: bamboo orchid ; flowering ; hormonal regulation

1. Introduction

Orchidaceae, one of the largest family of angiosperms, contains ornamental orchids ^{[1][2]}. More than 0.1 million orchid species are cultivated worldwide due to their immense horticultural importance. The most popular orchid species, such as *Cymbidium* and *Phalaenopsis*, flower in specific times of the year ^[3]. However, the bamboo orchid blooms year round and produces peak flowering from September to January. It is mainly found in sub-tropical and tropical areas of Asia ^{[4][5][6][7]}. It can grow on a variety of land and environmental conditions up to 2800 m altitudes, which shows its strong adaptability towards changing environment ^[7]. As a medicinal plant, it is a rich source of phenols, stilbenoids, bibenzyls and flavonoids, possessing antioxidant, anti-tumor and anti-viral properties ^[8]. The chemical constituents and medicinal compounds have been discussed in numerous studies, although the molecular patterning of floral regulation remains elusive for *A. graminifolia*.

Since antiquity, the plant hormones have been studied as important regulators of flowering in orchids ^[9]. Auxin is a morphogen and signalizes tissue specification through its concentration gradients ^{[10][11][12][13][14]}. Application of BA (6-benzylaminopurine) promotes flowering in orchids, such as *Dendrobium* and *Phalaenopsis*, but auxin counteracts this influence. However, BA is more effective in flowering regulation when applied with GA₃ (gibberellic acid) ^[15]. Gibberellins play important roles in the regulation of stem elongation ^{[16][17][18]} and flowering time ^{[19][20]}. Absciscic acid (ABA) emerges as an important hormone regulating flowering time and bud break ^{[21][22]}. However, the exact role of ABA in flowering is not clear, as it exerts both positive and negative effects ^{[21][22][23][24]}. Environmental changes may be the driving force behind these contradictory effects ^{[24][25]}. Strigolactones are another class of hormones and their cross-talk with GA, ethylene, cytokinin and auxin influences plant growth and development through various pathways ^{[26][27][28]}. Despite the extensive involvement, the genetic underpinnings of hormonal effects on orchid flowering remain poorly understood.

2. A General Sketch of *A. graminifolia*

The juvenile phase of bamboo orchid completes in six months, which is quite fast as compared to other orchids, such as *Cymbidium* and *Phalaenopsis*, taking 2–3 years for vegetative growth. It flowers years round with vigorous flowering period between September and January. The whole reproductive period is 32.3 days and the ornamental duration of inflorescence is 188.2 days. On an average, a single plant bears 6.1 flowers.

Flower develops in six stages, including Stage 0 to Stage 5. The development starts with flattened primordia (Stage 0), followed by appearance of floral buttress and undifferentiated division of primordia into different organ primordia (Stage 1). In the second stage a typical floral zygomorphy is established and floral organs differentiate. Then, sepals overlap the petals, showing an inverted triangle of floral apex (Stage 3). Rapid elongation of the gynostemium with immature pollinia represents Stage 4. In the last stage (Stage 5), the flowers open with a central column and four pollinia arranged around it.

3. Flower regulation

We found candidate genes from multiple regulatory conduits: photoperiod, circadian clock, vernalization, hormonal, and autonomous pathways. These pathways interact with floral integrators to regulate flowering time. FT1/FD may act as a central receiver of signals along with APETALA (AP1, AP2 and AP3) and SOC1. Moreover, ABA may control bud break

through SVP at low temperature during short days. In addition, candidate genes were studied for biosynthesis routes of flavonoids and bibenzyls. However, extended research is required to reveal these hypotheses in *A. graminifolia*.

We identified a large number of TFs through RNA-seq, which may contribute important roles in different regulatory pathways of *A. graminifolia* flowering. These pathways include photoperiod, vernalization, hormone and circadian clock pathways. The identification of key TFs involved in these pathways can do a great deal to reveal the genetic regulatory network that drives continuous flowering specifically in *A. graminifolia*, and seasonal flowering in other orchids, in general.

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