Eurasscience Journals

Eurasian Journal of Forest Science (2015) 3(1): 22 - 28

PHYSIOLOGICAL METABOLIC ANALYSIS AND KEY FACTORS DURING *Phyllostachys heteroclada* Olive AND *Neosinocalamus affinis* King FLOWERING

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Abstract

Phyllostachys heteroclada olive (*P. Het)* and *Neosinocalamus affinis* King (*N. Aff)* flowered from 2003 to 2007 in Ya'an district in China. We determined metabolism indices of these two bamboo species, and analyzed the key flowering metabolism factors by PCA (principal component analysis). The result showed that GA (Gibberellin), GA/ABA (Gibberellin/Abscisic Acid) were the most important factors of bamboo flowering, followed by starch, amylase activity and soluble sugar, all of which were positively correlated with bamboo flowering. ZT (Zeatin) was negatively correlated to bamboo flowering, it obstructed bamboo flowering and contributed to bamboo flower reversion. Based on the above and former research, we built a bamboo flowering model which showed that bamboo flowering is a long and complicated process. External factors, especially harsh environments such as droughts and high temperatures and internal mechanisms such as GA, GA/ABA, starch, amylase and soluble sugar would possibly accelerate or terminate growth and florescence, making bamboo florescence highly variable.

Keywords: Bamboo; flowering; physiological mechanism; PCA.

Özet

Phyllostachys heteroclada zeytin bambu (*P. Het*) ve *Neosinocalamus affinis* kral bambu (*N. Aff)* 2003-2007 yılları arasında Çin'in Ya'an bölgesinde vejetatif faaliyet göstermiştir. Bu çalışmada bu iki bambu türünün metabolik göstergeleri belirlenmiş ve çiçeklenmeyi en belirleyici faktör temel bileşenler analizi (PCA) ile tespit edilmiştir. Elde edilen sonuçlar GA (Gibberellin), GA/ABA (Gibberellin/Abscisic Asit) bambuların çiçek açmasında en belirleyici metabolitler olduğu, bunu nişasta, amilaz faaliyeti ve çözülebilen şekerlerin izlediği ve tümüne ait değerlerin çiçeklenme aktivitesi ile pozitif korelasyon gösterdiği belirlenmiştir. ZT (Zeatin) maddesi ile bambuların çiçek açması arasında negatif korelasyonun bulunduğu, bu maddenin çiçek açmayı engellediği ve faaliyeti durdurmaya çalıştığı tespit edilmiştir. Bugüne kadar yapılan çalışmalardan ve bu çalışmadan elde edilen sonuçlara dayanarak çok karmaşık bir süreç olan bambuların çiçek açması sürecini açıklayan bir model oluşturulmuştur. Kuraklık ve yüksek sıcaklıklar gibi sert iklim koşulları gibi dış faktörler ile GA, GA/ABA, nişasta, amilaz faaliyeti ve çözülebilen şekerlerin bambuların çiçek açması üzerinde pozitif yönde teşvik edici veya tersi yönde etkilerde bulunarak bambuların çiçek açması olgusunda değişik durumlara yol açmaktadır.

Anahtar kelimeler: Bambu, Çiçeklenme, Fizyolojik mekanizma, PCA.

INTRODUCTION

Flowering of a plant involves the transition from vegetative growth to reproductive growth, a process which serves as a means of survival and evolutionary strategy. Most plant flowering processes are activated by specific triggers, such as low temperature vernalization and photoperiodic induction (Zhang and Liu 2003). Bamboo is a typical one-time flowering perennial plant. There are no signs that bamboo flowering is associated with photoperiod. But all plants must have some trigger for flowering (Zhang et al. 2003), and bamboo is no exception. Bamboo belongs to the grass family, but has a much longer juvenile and vegetative growth stage than normal grass, and bamboo always blooms and dies at the same time in a large area (Janzen1976, Sharma 1994, Li 1997).

Although bamboo is a typical asexual reproduction plant, flowering is still an important breeding method for its survival, and most bamboo species flowers at periodic intervals and dies in large areas. In China, bamboo is not only important forestry resource but also panda's only food in wild, and bamboo flowering is always unexpected and needs at least eight to ten years to recover. So, bamboo flowering is an important natural factor that affects the survival of panda (Li 1997, Du et al. 2000).

From 2003 to 2007, *P. Het* bloomed extensively in Ya'an district which is renowned for being "panda's hometown" because the first panda was discovered there and it still is panda's habitat up to now, and *N. Aff* began to fragmentarily flower in this area in 2006. *P. Het* and *N. Aff* are not only dominant bamboos in the Yangzi River Valley in China, *P. Het* also is one of diet bamboo for giant panda, these two bamboo species flowering effected local obviously. So, study and analysis these two bamboo flowering mechanism and find solution is necessary (Picture 1 and 2).

Many research results have shown that the primary cause of bamboo flowering is its established growth cycle as well as its genetic factors, but the mechanism causing bamboo flowering can't be explained solely according to cycle theory. Therefore, it is important to identify the key factors responsible and characterize the detailed physiological process of bamboo flowering while considering both metabolic and environmental factors.

This paper characterizes hormones and metabolic substances of *P. Het* and *N. Aff* during their flowering period. We combined the results with data on climatic factors surveyed in former research to build a bamboo flowering model. Our results have significant contributions to research on bamboo flowering predictions and control.

Picture 1. Inflorescence of *P. Het*

Picture 2. Inflorescence of *N. Aff*

MATERIALS AND METHODS

The study was carried out in Ya'an Bi Feng Xia Base of CCRCGP (The China Conservation and Research Centre for the Giant Panda) and surrounding areas (Map 1). The area is characterized by yellow soils and a subtropical monsoon climate, which is wet and mildly humid with abundant rainfall. Annual rainfall is l, 250 mm – l, 800 mm, and annual temperature is 10.1-16.8 °C. The frost-free period lasts 240-280 days per year and sunlight is present for about 1,000 hours throughout the entire year (Ya'an Weather Bureau 2008).

Map 1. Bamboo flowering and research areas of this paper (Ya'an district)

The research was carried out when bamboo began to bloom in 2003. After two years of observation, we found that flowering reversion occurred in the *P. Het* forest. We noticed that in some areas, this bamboo species stopped flowering and returned to normal vegetable growth after some buds appeared (usually on the top of the plant). We took advantage of this opportunity to study the inner mechanisms contributing to bamboo flowering. We conducted our research in natural flowering and non-flowering bamboo forest in the study area. The non-flowering *P. het* forest was about 10 ha. Here, bamboo grew well and there was no sign of flowering. The flowering *P. het* forest was about 12 ha. Here, bamboo began to flower in 2004, and the percentage of the area undergoing flowering increased to 90% by 2005. The flowering *N. Aff* forest was about 20 ha. Flowering occurred in scattered locations throughout the area in March 2006, with the flowering area making up about 10% of the total forest. All samples, including flowering reversion, were taken from these sites.

From October 2006 to May 2007, we collected the top leaves of one year branches from flowering, non-flowering and flowering reversion bamboo as samples. We placed some samples into an ultra cold storage freezer at -70 °C after freezing with liquid nitrogen to reserve for enzyme and hormonal determination. We killed off other samples using an oven set at 105 °C and subsequently oven-dried at 75 °C for determination of solubility sugar, starch and protein content.

We took 0.1g of the oven-dried leaf samples to characterize the solubility of sugar, and the residue for starch determination, using methods outlined in the Plant Physiology Experiments Manual (Shanghai Society of Plant Physiology 1999). We took 0.2g of the oven-dried leaf samples and used a Kjeldahl Apparatus for total protein determination.

We determined solubility protein, amylase, invertase and GS (Glutamine synthesize) activity, and hormone content including GA (Gibberellin), ZT (Zeatin), ABA (Abscisic Acid) and IAA (Indole Acetic Acid) using a 1.0g sample of frozen leaves grinded in an ice-bath after being centrifuged (4500*5 min). We then used CBB (Coomassie brilliant blue) to determine soluble protein, the method proposed by Xiong (2003) to determine amylase activity, the method proposed by Zhao (2003) to determine GS activity, the method involving use of invertase kits (made in Jiancheng Biological engineering research institute in Nanjing, China) to determine invertase activity, and the method proposed by Wang (2002) to determine hormones including GA, ZT, ABA and IAA.

We used the software program SPSS to perform PCA (principal component analysis) on the metabolic indices of flowering *P. Het* and *N. Aff*. We subscribed to the standard of using an accumulative contribution rate ≥85 % to conduct the principal screening and subsequently calculating the characteristic root of the correlation matrix and to obtain the key metabolic factors of the flowering bamboos.

RESULTS

PCA results for flowering *P. Het*

The 17 metabolic indices of flowering *P. Het* were analyzed by PCA (Table 1). The top three principal components contributed 63%, 20% and 10% of the variability, and the cumulative contribution rate was 93% (Table 2). According to the standards of PCA that cumulative contribution rate should be more than 85%, the top three components represented the most relevant information of the 17 metabolic indices during the flowering period. We therefore selected them as comprehensive indices.

For flowering *P. Het,* GA had the maximum positive load of the first principal component, followed by soluble sugar content. Starch content had the maximum positive load of the second principal

component, and ZT had the maximum negative load. GA/ABA had the maximum positive load of the third principal component. The result showed that GA, GA/ABA, starch, soluble sugar, and ZT content were more closely correlated with *P. Het* flowering compared to other metabolic indices. Among these five factors, all except ZT were positively correlated to bamboo flowering (Table 1).

PCA results for flowering *N. Aff*

The 17 metabolic indexes of flowering *N. Aff* were analyzed by PCA (Table 1). The top three principal components together were accounted for 97% of the variability in the data (Table 1).

For flowering *N. Aff,* GA had the maximum positive load of the first principal component, followed by GA/ABA. Amylase activity had the maximum positive load of the second principal component, followed by starch content. Soluble sugar/soluble protein had the maximum positive load of the third principal component, followed by GS activity. The result showed that GA, GA/ABA, amylase activity, starch content, soluble sugar/soluble and GS activity were more closely correlated with *N. Aff* flowering than other indices, and they all positively contributed to bamboo flowering (Table 1)

PCA results for flowering reversion of *P. Het*

The 17 metabolic indices of flowering reversion *P. Het* were analyzed by PCA (Table 1). The top two principal components together accounted for 90% of the variability (Table 1)

For flowering reversion *P. Het,* ZT/ABA had the maximum positive load of the first principal component, followed by ZT, which had nearly an equal absolute eigenvector to ZT/ABA. Total amount of sugar content had the maximum negative load of the second principal component, followed by amylase activity. The result showed that ZT, ZT/ABA, amylase activity and the total amount of sugar content were more closely correlated with *P. Het* flowering reversion, with ZT, ZT/ABA, amylase activity contributing positively and total amount of sugar content contributing negatively to flowering (Table 1)

Table 1. Characteristics of metabolic indices of *P. Het* and *N. Aff* determined by PCA

Metabolic index for PCA	Flowering reversion *P. Het*		Flowering *P. Het*			Flowering *N.Aff*		
	Q1	Q2	Q1	Q2	Q3	Q1	Q2	Q3
Soluble sugar	0.156	-0.341	**0.291**	0.058	-0.118	0.284	-0.167	-0.203
Starch	-0.203	-0.313	-0.121	**0.477**	-0.132	-0.104	**0.448**	0.132
Total amount of sugar	-0.046	**-0.392**	0.204	0.364	-0.201	0.305	0.039	-0.186
Soluble protein	0.248	-0.193	0.288	-0.154	0.016	0.276	0.002	0.308
Total amount of protein	-0.270	0.197	-0.217	0.037	0.402	-0.016	-0.386	-0.295
Soluble guar/soluble protein	-0.293	0.090	-0.240	0.324	0.079	-0.242	0.027	**0.401**
Total amount of sugar/Total amount of protein	0.144	-0.365	0.237	0.139	-0.319	0.253	0.289	0.059
GS activity	0.137	-0.112	-0.251	0.218	0.185	-0.126	-0.212	**0.390**
Sucrose activity	-0.253	0.251	0.262	0.228	0.131	0.217	0.288	-0.226
Amylase activity	0.079	**0.391**	0.147	0.422	0.301	0.040	**0.469**	0.144
GA	0.305	0.139	**0.292**	0.026	0.218	**0.321**	0.018	0.044
IAA	0.150	0.330	0.287	0.124	0.149	-0.253	0.251	0.198
ABA	0.283	0.193	0.257	0.009	-0.117	0.306	0.012	0.059
ZT	**0.324**	0.031	0.172	**-0.434**	0.088	0.291	-0.113	0.205
GA/ABA	0.306	0.139	0.239	0.027	**0.537**	**0.315**	-0.004	-0.018
GA/ZT	-0.321	0.019	0.288	0.072	0.218	0.271	0.161	-0.240
ZT/ABA	**0.325**	0.021	0.243	-0.001	-0.126	0.185	-0.295	0.324

Q1: Contribution to the first principal component; Q2: Contribution to the second principal component; Q3: Contribution to the third principal component

The first and second highest contributors for each component are denoted in bold.

Table 2. Key metabolic factors of flowering *P. Het* and *N. Aff* determined by PCA

Principal component	Flowering reversion *P. Het*		Flowering *P. Het*			Flowering *N. Aff*		
	F1	F2	F1	F2	F3	F1	F2	F3
Key factor	ZT/ABA	Total amount of sugar	GA	Starch	GA/ ABA	GA	Amylase	Soluble sugar/soluble protein
Eigenvalue	9.269	6.034	10.746	3.474	1.645	9.622	4.156	2.683
Proportion of variance	54.52%	35.49%	63.21%	20.44 %	9.67%	56.60 %	24.45%	15.78%
Cumulative proportion of variance	54.52%	90.01%	63.21%	83.65 %	93.33 %	56.60 %	81.05%	96.83%

F1: The first principal component; F2: The second principal component; F3: The third principal component

DISCUSSION

Key flowering metabolic factors of *P. Het* and *N. Aff*

After synthesis of the PCA results on flowering *P. Het* and *N. Aff*, we can conclude that GA, GA/ABA were the most important factors producing bamboo flowering because they were the general key metabolic factors of these two flowering bamboos. These factors were followed by starch, amylase and soluble sugar, all of which were positively correlated with bamboo flowering. ZT was important too, but it was negatively correlated to flowering. ZT, ZT/ABA, amylase activity and total amount of sugar were important factors for bamboo flowering reversion, higher ZT, ZT/ABA, and amylase activity helped bamboo stop flowering and return to normal vegetable growth, and higher total amount of sugar contributed to bamboo flowering (Table 2).

The key flowering metabolic factors found in this study for *P. Het* and *N. Aff* are similar to results found by other researchers (Tanimoto and Harada 1981, Zemin et al. 2002). Early in 1937, Chailakhgan (1937) proposed the theory of florigen. He postulated that florigen was a mixture made of GA and flowering stimulating elements, and a plant cannot flower if it lacks any one of them. Although this matter had not been extracted and isolated for more than half a century, it still showed that GA was important for plant flowering. Later research showed that although hormones and their corresponding balance mechanisms are different among different plants, their importance to plant flowering is universal (Fu and Meng 1998). Sachs (1977) found that the main condition of apple (*Malus pumila* Mill) flower bud differentiation was abundant nutrients and a balance of hormones. Luckwill (1977)found that the variation in hormone balance can result in flowering genes being removed from repression. Zhou and Ma（1988） analyzed the variation of CTK and GA during flower bud differentiation and found that CTK／GA became higher in non bud organization compared to flowering transition. The former research also showed that carbohydrates are very important for bamboo flowering. Under photoinduction conditions, starch and other carbohydrates that are stored in stems and leaves can translate into sugar and accumulate in the apical stem meristem (Zhou and Xu 2002). Araki and Komeda (1993) showed that *Arabidopsis thaliana* Heynh could flower when sugar and glucose infected its above ground parts, even in darkness. Han (2003) summarized the mechanism of hormone to floral induction and flowering process, that is: illumination, humidity, fertilization and water → nutrient substance accumulated → hormones tend to balance → gene activation → metabolic direction changed → new protein synthesis (including enzyme) → flower formation state built. He considered that plant efflorescence is not the result of one substance action in isolation, but a process involving multiple factors, multi-step control, with each substance acting in a series of completed actions, with a transition from quantitative change to qualitative change. During this process, nutrition is the foundation; hormone

adjustment is the key and genetic expression is the means of floral induction.

An earlier study we did on the environmental factors that influenced *P. Het* and *N. Aff* flowering showed average temperatures in January and July in during flowering years were all higher than non-flowering years. There was an obvious relationship between higher temperature and bamboo flowering. The average annual precipitation in flowering years was less than non-flowering years and there were obvious drought in the years before bamboo flowering (Wang, 2009). Another former research on the ecological niche of flowering *P. Het* also found higher air temperature, humidity and sunshine could cause flowering (Wang and Zhou, 2006). Therefore, it can be concluded that extensive bamboo flowering might be a stress reaction to external environmental change. Such a process is necessary for a population's survival, since flowers and seeds are very important for reproducing in forthcoming disasters, especially drought (Campbell 1987, Wang 2010).

Based on the results of our and other scholars' research, we can draw some conclusions about *P. Het* and *N. Aff* flowering. After a period of growth, the physiology of bamboo is already mature and it can transition into reproductive growth. During this period, bad environmental factors (such as high temperature and drought) can stimulate a plant to undergo a stress reaction (Wang 2010). At first, hormone contents including GA、GA/ABA、ZT will change. At the same time, carbon and nitrogen metabolism will change, starch content will rise, amylase activity will be enhanced, and the amylolysis ability will increase and lead to an increase in soluble sugar content. When each substance reaches a certain level and becomes balanced, the plant will finish the flowering bud differentiation stage and transition into the generative stage, or undergo the flowering process. During this transition, if other factors are induced to cause significant changes in ZT content and ZT/ABA, flowering reversion will occur and the bamboo will stop flowering and return to vegetable growth (Table 1 and 2).

Based on the above research and analysis, we built a bamboo flowering model (Figure 1). From this model, we can determine that bamboo growth from the vegetable to reproductive stage is relatively long. If any link during this process is influenced or broken, this may accelerate or stop the growth and flowering process, which accounts for why bamboo flowering is so variable.

Of course, this model is simple, and just based on the results of physiological indices and environmental factors. In the future, we hope to conduct further research on other potential mechanisms including bamboo flowering genes and flowering semiochemicals and integrate this information into the model.

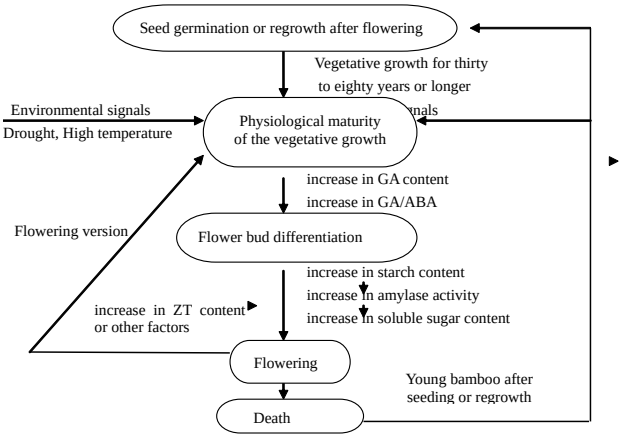

Figure 1. Bamboo flowering model

ACKNOWLEDGEMENTS

We are thankful to OPCF (Ocean Park Conservation Foundation, Hong Kong) Fund and Hong Kong SAR Government for their support, and also thank Vanessa Hull for his language correcting.

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Submitted: 08.05.2013

Accepted: 03.05.2015