

A New Outlook on Sporadic Flowering of Bamboo

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Abstract

Moso bamboo (*Phyllostachys edulis*) is a temperate grass species with a tree-like habitus and an unusual reproduction strategy. While flowering is irregular and infrequent, new clonal bamboo shoots are established from an underground rhizome network during the spring season. In our previous study, we performed transcriptome analyses using bamboo shoot buds to understand the initiation of bamboo stem elongation. Interestingly, the expression profile in the shoot apical meristem (SAM) region of young bamboo shoots is similar to that of other plants. Specifically, some of the genes that control the timing of flowering and floral development are active in the SAM region. This data raises the question of how bamboo shoots start to elongate, and why they do not proceed to a seasonal cycle of flowering. Our analyses of the activation of shoot buds and subsequent rapid stem elongation provide new hints to unravel the unpredictable flowering pattern of bamboo. In this short communication, we discuss how bamboo might coordinate and integrate the vegetative and reproductive phases in relation to shoot emergence and stem elongation.

TEXT

Moso bamboo (*Phyllostachys edulis*) is considered one of the tallest grass species in the world. It is characterized by a seasonal rapid stem growth phase, vegetative propagation through an underground network of rhizomes, and the near-absence of sexual reproduction. Despite its commercial importance and its relatively close phylogenetic relationship with the very-well studied annual *Oryza* genus, very little is known about the factors that control its unique growth behavior. With the increased availability of ‘omics’ technology, several recent studies focused on those unusual traits. A major advance in understanding the bamboo plant was the release of a scaffold-based reference genome¹. In addition, several quantitative ‘omics’ approaches were

performed on a diverse set of tissues², internodes from elongating shoots³, internodes from different regions of shoots at various developmental stages⁴, and leaves treated with high light exposure⁵. Selected articles even reported floral transcriptomes, which are difficult to obtain given the infrequent and unpredictable flowering of bamboo plants.^{1,6-8}

Gene expression in young bamboo shoots

In our recent transcriptomics study⁹, we focused on the processes that occur in the different tissue regions of young bamboo shoots just prior to elongation (Fig. 1 A). Among other findings, we learned that in the SAM region of the young bamboo shoots transcripts that encode regulatory proteins were significantly enriched. In our dataset, we detected the expression of genes homologous to known regulators of the timing of flowering and floral development such as *FLOWERING-LOCUS T (FT)*, *VERNALIZATION 1 (VRN1)*, *LATE MERISTEM IDENTITY 2 (LMI2)*, *APETALA2 (AP2)*, *ANTINTEGUMENTA (ANT)*, *ABNORMAL FLORAL ORGANS (AFO)*, *INDETERMINATE1 (ID1)*, *EARLY BOLTING IN SHORT DAYS (EBS)* and *AGAMOUS (AGO)*. This finding might be a first hint as to how bamboo achieves and maintains its mostly vegetative reproduction. In agreement with¹⁰, we identified PH01002288G050 as the protein most similar to *Arabidopsis* FT and the rice homologue Hd3a (Fig. 1B), however there might be other loci in bamboo which have not been recognized in the reference assembly. The *PH01002288G050* transcript was found predominantly in the SAM-region of young bamboo shoot (Fig. 1C). It is tempting to speculate regarding the role of the presence of an FT-encoding transcript in the initiation of bamboo shoot growth, promoting rapid stem elongation, or vegetative to reproductive phase transition.

Regulation of bud growth and shoot elongation

In many grass species the main shoot axis initially consists of a stack of flattened discs from which the leaves originate. With the onset of the reproductive phase this shoot axis starts to elongate. This is usually followed by the development of flowers at the apical end of the shoot axis, which concludes the life cycle of annual species. However, bamboo (a member of the *Poaceae* family) deviates from this pattern in important aspects. In the spring season, new bamboo shoots develop from an underground rhizome network and quickly elongate to the height of several meters. However, fully mature bamboo shoots only flower sporadically. Instead, the time between two flowering events can reach up to several decades, which is one of the longest observed flowering cycles in the whole plant kingdom. Our tissue-specific transcriptomes from young bamboo shoots might provide first insights into how this unusual trait is achieved.

The activation of dormant, rhizome-derived shoots buds appears to be linked to environmental cues, such as day length, temperature and rainfall (Fig 2). The day length is likely perceived in the leaves of mature bamboo shoots which are all connected by the underground rhizome network. An unidentified signal then activates the dormant buds, which results in their enlargement and breaking through the soil surface. This event maybe coinciding with the expression of the bamboo FT homologue and several other putative regulatory genes that were highly expressed observed in the SAM region.

It may be speculated on how the altered flowering routine of bamboo relative to rice or *Arabidopsis* is controlled. The expression of flowering-related genes in elongating bamboo shoots may suggest that bamboo shoots always have the potential for flowering. The sporadic

flowering of bamboo may then be explained by the insensitivity of bamboo to flowering-inducing cues, or a specific environmental cue to promote bamboo flowering is missing. Also, the expression of flowering-related genes may instead promote shoot elongation or even suppress the flowering pathway. Thus, bamboo might have evolved similarly to potato, where a neofunctionalization of FT proteins led to two distinct control mechanisms, one controlling flowering and one controlling the development of tubers¹¹.

Outlook

Until now the reproductive habits of bamboo and its difficulties as an experimental system prevented more targeted approaches. Since both forward- and reverse genetic approaches in bamboo are not feasible for now, we are restricted to descriptive experiments and any other advanced experiments need to be carried out in related model species. Since flowering is such a rare event in bamboo, compared to the regular emergence of new shoots, the study of bud activation and subsequent elongation might present a way to perform regular experiments under natural conditions. For example, using sensitive transcriptomics or proteomics time-course experiments it might be possible to identify candidates for the signal that initiates shoot development. Such studies could approach the long-standing question of how bamboo coordinates clonal and sexual reproduction from a new perspective.

References

1. Peng Z, Lu Y, Li L, Zhao Q, Feng Q, Gao Z et al. The draft genome of the fast-growing non-timber forest species moso bamboo (*Phyllostachys heterocycla*). Nat Genet 2013; 45:456–61; PMID:23435089; <http://dx.doi.org/10.1038/ng.2569>.
2. Liu M, Qiao G, Jiang J, Yang H, Xie L, Xie J et al. Transcriptome sequencing and *De Novo* analysis for Ma bamboo (*Dendrocalamus latiflorus* Munro) using the Illumina platform. PLoS ONE 2012; 7:e46766; PMID:23056442; <http://dx.doi.org/10.1371/journal.pone.0046766>.
3. Cui K, He C-y, Zhang J-g, Duan A-g, Zeng Y-f. Temporal and spatial profiling of internode elongation-associated protein expression in rapidly growing culms of bamboo. J Proteome Res 2012; 11:2492–507; PMID:22397471; <http://dx.doi.org/10.1021/pr2011878>.
4. He C-y, Cui K, Zhang J-g, Duan A-g, Zeng Y-f. Next-generation sequencing-based mRNA and microRNA expression profiling analysis revealed pathways involved in the rapid growth of developing culms in Moso bamboo. BMC Plant Biol 2013; 13:119; PMID:23964682; <http://dx.doi.org/10.1186/1471-2229-13-119>.
5. Zhao H, Lou Y, Sun H, Li L, Wang L, Dong L et al. Transcriptome and comparative gene expression analysis of *Phyllostachys edulis* in response to high light. BMC Plant Biol 2016; 16:; PMID:26822690; <http://dx.doi.org/10.1186/s12870-016-0720-9>.
6. Wysocki WP, Ruiz-Sanchez E, Yin Y, Duvall MR. The floral transcriptomes of four bamboo species (Bambusoideae; Poaceae): support for common ancestry among woody bamboos. BMC Genomics 2016; 17:384; PMID:27206631; <http://dx.doi.org/10.1186/s12864-016-2707-1>.
7. Zhao H, Dong L, Sun H, Li L, Lou Y, Wang L et al. Comprehensive analysis of multi-tissue transcriptome data and the genome-wide investigation of GRAS family in *Phyllostachys edulis*. Sci Rep 2016; 6:27640; PMID:27325361; <http://dx.doi.org/10.1038/srep27640>.
8. Gao J, Zhang Y, Zhang C, Qi F, Li X, Mu S et al. Characterization of the floral transcriptome of Moso bamboo (*Phyllostachys edulis*) at different flowering developmental stages by transcriptome sequencing and RNA-seq analysis. PLoS ONE 2014; 9:e98910; PMID:24915141; <http://dx.doi.org/10.1371/journal.pone.0098910>.
9. Gamuyao R, Nagai K, Ayano M, Mori Y, Minami A, Kojima M et al. Hormone Distribution and Transcriptome Profiles in Bamboo Shoots Provide Insights on Bamboo Stem Emergence and Growth. Plant Cell Physiol 2017; 58:702–16; PMID:28204696; <http://dx.doi.org/10.1093/pcp/pcx023>.

10. Biswas P, Chakraborty S, Dutta S, Pal A, Das M. Bamboo Flowering from the Perspective of Comparative Genomics and Transcriptomics. *Front Plant Sci* 2016; 7:1900; PMID:28018419; <http://dx.doi.org/10.3389/fpls.2016.01900>.
11. Navarro C, Abelenda JA, Cruz-Oró E, Cuéllar CA, Tamaki S, Silva J et al. Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. *Nature* 2011; 478:119–22; PMID:21947007; <http://dx.doi.org/10.1038/nature10431>.

Figure Legends

Figure 1

A bamboo homologue of *FLOWERING LOCUS T* is expressed in the SAM region.

(A) The sampled tissue regions. SAM, shoot apical meristem; YIN, young internodes; YNO, young nodes; MIN, mature internodes; MNO, mature nodes. (B) Amino acid sequence alignment of bamboo FT-like protein, rice Hd3a and *Arabidopsis thaliana* FT. Dark grey shading shows conserved amino acids and light grey shading shows similar amino acids. (C) Expression of the bamboo FT homologue shown in (B) is shown in five tissues from young bamboo shoots. Data is from four individual shoots and error bars are standard deviations (RPKM reads per kilobase of transcript per million library reads).

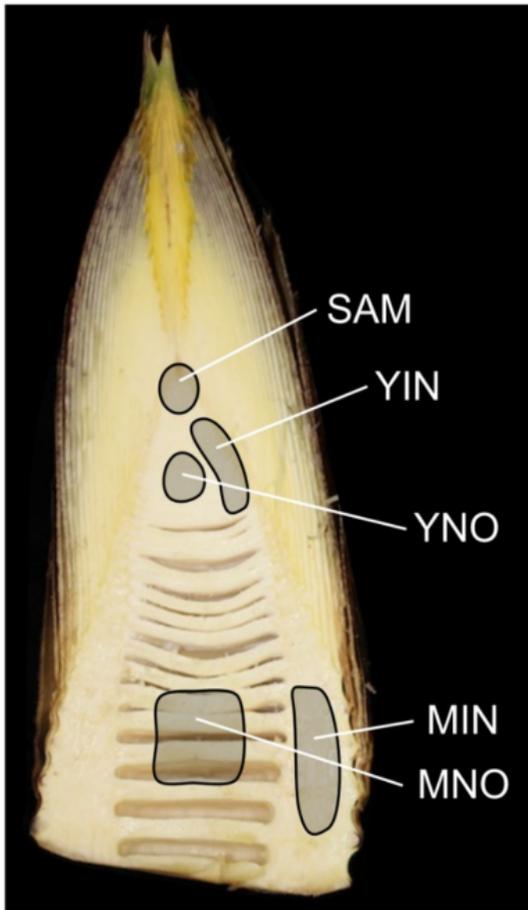
Figure 2

Bamboo and rice differ in their ability to switch from vegetative to reproductive growth.

A schematic overview comparing the transition from vegetative to reproductive growth in rice and bamboo. Rice is an annual plant that switches to reproductive growth including stem elongation and flowering in response to changes in day length and other cues. *Heading date 3a* is

central regulator of flowering time. Bamboo activates dormant rhizome-derived buds in the spring season. Several bamboo homologues of genes regulating flowering time and floral organ development are expressed in young bamboo shoots, but flowering itself is a very rare event. Several alternative hypothesis explaining this behavior are given. Gene abbreviation are given in the main text.

A



B

PhFT-like PH01002288G0050
OsHd3a Os06g0157700
AtFT AT1G65480

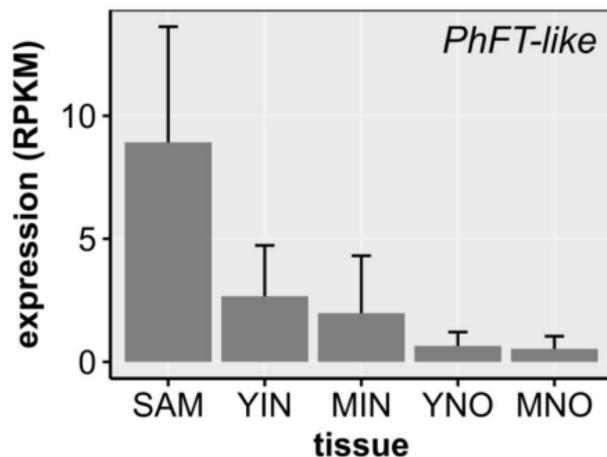
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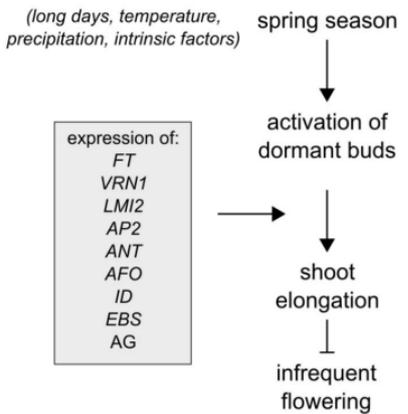
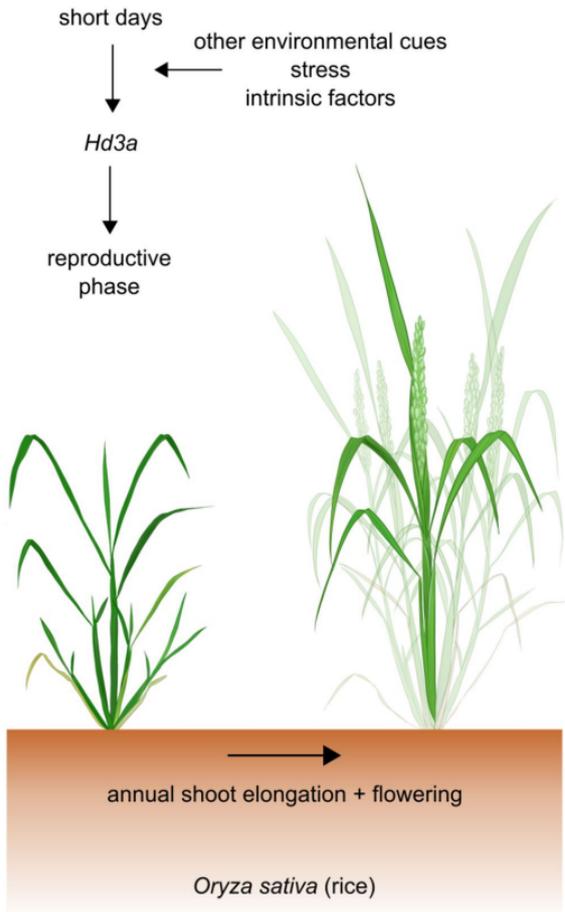
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C





- possible explanations:
- insensitivity to environmental cues
 - missing environmental cue
 - neo-functionalization

