RESEARCH PAPER

Identification of genes related to the development of bamboo rhizome bud

Kuihong Wang1,2,†, Huazheng Peng1,2,†, Erpei Lin1, Qunying Jin2, Xiqi Hua2, Sheng Yao1, Hongwu Bian1,*, Ning Han1, Jianwei Pan1, Junhui Wang1, Mingjuan Deng1 and Muyuan Zhu1,*

1 State Key Laboratory of Plant Physiology and Biochemistry, Key Laboratory for Cell and Gene Engineering of Zhejiang Province, College of Life Sciences, Zhejiang University, Hangzhou 310058, China
2 Zhejiang Provincial Key Laboratory of Bamboo Research, Zhejiang Forestry Academy, Hangzhou 310023, China
† These authors contributed equally to this work.
* To whom correspondence should be addressed. E-mail: hwbian@zju.edu.cn

Received 22 May 2009; Revised 18 September 2009; Accepted 20 October 2009

Abstract

Bamboo (Phyllostachys praecox) is one of the largest members of the grass family Poaceae, and is one of the most economically important crops in Asia. However, complete knowledge of bamboo development and its molecular mechanisms is still lacking. In the present study, the differences in anatomical structure among rhizome buds, rhizome shoots, and bamboo shoots were compared, and several genes related to the development of the bamboo rhizome bud were identified. The rice cross-species microarray hybridization showed a total of 318 up-regulated and 339 down-regulated genes, including those involved in regulation and signalling, metabolism, and stress, and also cell wall-related genes, in the bamboo rhizome buds versus the leaves. By referring to the functional dissection of the homologous genes from Arabidopsis and rice, the putative functions of the 52 up-regulated genes in the bamboo rhizome bud were described. Six genes related to the development of the bamboo rhizome bud were further cloned and sequenced. These show 66–90% nucleotide identity and 68–98% amino acid identity with the homologous rice genes. The expression patterns of these genes revealed significant differences in rhizome shoots, rhizome buds, bamboo shoots, leaves, and young florets. Furthermore, in situ hybridization showed that the PpRLK1 gene is expressed in the procambium and is closely related to meristem development of bamboo shoots. The PpHB1 gene is expressed at the tips of bamboo shoots and procambium, and is closely related to rhizome bud formation and procambial development. To our knowledge, this is the first report that uses rice cross-species hybridization to identify genes related to bamboo rhizome bud development, and thereby contributes to the further understanding of the molecular mechanism involved in bamboo rhizome bud development.

Key words: Bamboo, bamboo shoot, cross-species microarray, development, gene identification, rhizome bud, rhizome shoot.

Introduction

Bamboo (Bambusoideae) is one of the largest members of the grass family Poaceae, which includes >1500 species which are vital to the economy of many tropical and subtropical countries of the world. Most cultured bamboos are perennial woody evergreens and are basically reproduced by the rhizome. According to the type of the rhizome, bamboos have been divided into three groups: scattered bamboos with a monopodial rhizome, caespitose bamboos with a sympodial rhizome, and pluricaespitose bamboos with a monopodial and sympodial rhizome. The rhizome bud can either develop into a bamboo shoot which will grow into a bamboo culm in a very short period, or develop into a new rhizome which will enable the sustainable production of the bamboo grove. Therefore, the development of bamboo rhizome is fairly complex and distinct from that of ordinary grasses.

Several efforts have been made to reveal the anatomical and physiological mechanisms of rhizome bud development in bamboo. The morphological and structural analyses show
that the developmental course of the bamboo shoot is divided into six stages: dormancy, germination, development stage I, II, and III, and shoot stage (Zhang et al., 1996). Hu et al. (1996) observed high concentrations of gibberellin 3 (GA3), zeatin (ZT), and indole acetic acid (IAA) in the rhizome bud before bamboo shoot formation. Furthermore, precise detection by enzyme-linked immunosorbent assay (ELISA) showed that a high concentration of IAA correlated with the outgrowth of the rhizome bud before the formation of the new rhizome and bamboo shoot, while a high concentration of ZT corresponded only to the formation of the bamboo shoot from the rhizome bud (Huang et al., 2002). However, the molecular bases for the development of the bamboo rhizome bud are largely unknown.

DNA microarray technology has facilitated the identification of a large number of genes involved in a particular biological process. Due to the scarce genome resources available for bamboo, it is expensive and time-consuming to develop a microarray in bamboo. Cross-species microarray hybridization is a potentially useful technique for applying model organism genomics in related species for which few functional genomics resources are available (Chismar et al., 2002; Becher et al., 2004; Lee et al., 2004; Nieto-Díaz et al., 2007). Genome sequencing and functional analysis of rice which belongs to the Poaceae have been completed. Zhu (2001) has used an oligonucleotide microarray designed for the rice genome (Oryza L. ssp. japonica) to analyse the barley (Hordeum vulgare L.) transcriptome, and the results have been confirmed by RNA gel blot analysis. Based on these promising results, in the present study the anatomical structures of the rhizome bud, rhizome shoot (early form of the rhizome), and bamboo shoot (early form of the bamboo culm), are compared and then the gene expression of the rhizome bud versus the leaf in Phyllostachys praecox is examined by using a cross-species microarray with 7500 rice unigenes. A total of 318 up-regulated and 339 down-regulated genes were observed. Among them, 52 up-regulated genes with putative functions in the rhizome bud were identified and described. Furthermore, six genes related to bamboo rhizome bud development were cloned and sequenced, and their expression patterns and potential functions were analysed.

Materials and methods

Sampling

Rhizome buds, rhizome shoots, and bamboo shoots (development stage I and shoot stage) of P. praecox (Chu and Chao), which is a typical scattered bamboo with high economic value in the east of China, were collected for anatomical comparison during early spring. Rhizome buds, bamboo shoots, rhizome shoots, leaves, and young florets were collected and prepared for gene expression analysis during early summer in the Bamboo Botanical Garden of Zhejiang Forestry Academy, PR China. All the samplings were repeated three times from different bamboos.

Histological analysis and in situ hybridization

The apical parts of the rhizome bud, rhizome shoot, and bamboo shoot were cut (~3–5 mm) and fixed overnight in 4% paraformaldehyde in a phosphate buffer, pH 7.0, at 4 °C. The fixed tissues were dehydrated five times in a graded ethanol series, replaced twice with xylene, and embedded in paraffin. The samples were sectioned at 5 μm on a rotary microtome (Leica RM2135). Sections were stained with Ehrlich’s haematoxylin and observed under a Nikon4500 digital camera.

RNA isolation

Tissues were ground in liquid nitrogen and the RNA was extracted using TRIZOL Reagent (BBI) according to the manufacturer’s instructions, and then treated with proportional DNase I at 37 °C for 30 min. The quality of the RNA was measured by both electrophoresis and optical absorbance. Only RNA samples with an A260/A280 >2.0 were used for RT-PCR. The extracted RNAs were stored in liquid nitrogen.

Detection of gene expression by using the rice gene chip

The rice gene chip (BioStar Genechip Inc., Shanghai, China) contained 10 369 spots comprising 500 bp sequences from a unigene set of 7500 clones assembled by a mixed rice cDNA library (not published). The extracted RNAs were purified with RNeasy columns according to the manufacturer’s instructions. First-strand cDNAs labelled with Cy5 or Cy3 were synthesized from ~100 μg of total RNA by reverse transcription, with an oligo(dT)14 primer, which were then further purified and fragmented by using a column.

Two samples were prepared from the leaves, designated as A1 and A2, and one from the rhizome lateral buds, designated as B1. cDNAs of either A2 or B1 was labelled with Cy5, while that of A1 was labelled with Cy3. The Cy5-labelled cDNA from B1 was hybridized against the Cy3-labelled cDNA from A1. Hybridization of A2 against A1 was considered as a control parameter to check the up- or down-regulated genes in the hybridization of B1 against A1. According to the results obtained from the three replicated experiments, the genes with a Cy5/Cy3 ratio of >2.0 or <0.5 have been defined as up- and down-regulated, respectively. For statistical analysis, a quality filter was applied to all the spots as follows: (i) spots with a high background, uneven morphology, or dust specks were manually flagged as ‘bad’ and removed; and (ii) the foreground intensity must be higher than twice the background standard deviation in both the channels or four times the background standard deviation in one channel. To balance the effects of different RNA treatment and dye bias, a normalization parameter was estimated by EXP(R). Hereby, R was the average of ln(Cy5/Cy3) for effective spots. Data were normalized as the ratio Cy5/B1/Cy3/A1. Arithmetic means of expression values were calculated from the three replicated experiments. The software Origin 6.1 was used to analyse the data from the gene chip.

Gene cloning and sequencing

3′ RACE (rapid amplification of cDNA ends) was carried out with 1 μg of the total RNA in a 10 μl reaction using a BD SMART™ RACE cDNA Amplification Kit according to the manufacturer’s instructions. A 1 μl aliquot of the diluted cDNA mix was used as a template in the 25 μl PCR of the BD Advantage™ 2 PCR Kit. The amplification was performed for 25 cycles at 94 °C for 30 s, 68 °C for 30 s, and 72 °C for 3 min. The specific primers (Table 1) of the target genes were designed using the Omega2.0 program based on the conserved regions of rice homologous genes detected by the gene chip. The PCR products were isolated by gel electrophoresis,
purified by a NucleoTrap® Gel Extraction Kit, and cloned by T-easy™. At least three positive clones, tested by PCR, were selected and sequenced using the DNA Analyzer. 5′ RACE was conducted using the same kit as above, and specific primers were designed according to the known sequences (Table 1). The sequences were assembled by ContigExpress. Sequence alignments were conducted using AlignX of Vector NTI suite 6.0 with the Dayhoff amino acids distance matrix. The phylogenetic tree of homologous genes was constructed using the Neighbor–Joining algorithm of MEGA3.1, with the multiple alignment parameters gap opening penalty 8, gap extension penalty 2, and PAM protein weight matrix (Kumar et al., 2004).

### Semi-quantitative RT-PCR analysis

A two-step semi-quantitative RT-PCR method (Meadus, 2003) with some modifications was used to measure the gene expression in the rhizome shoot, rhizome bud, bamboo shoot, leaf, and young floret. The first-strand cDNA was synthesized from 5 mg of RNase-free DNase-treated total RNA using oligo(dT)15 and BD PowerScript™ Reverse Transcriptase (Clontech) as described by the manufacturer. An aliquot of 1/20 of the reverse-transcribed product was used as a template in the PCR amplification. The PCRs were conducted in a total volume of 25 μl containing 1× PCR buffer, 1.5 mmol l⁻¹ MgCl₂, 200 μmol l⁻¹ dNTPs, 1.5 U of Taq polymerase, and 40 μmol l⁻¹ gene-specific primers under the following conditions: 94 °C for 5 min, followed by 25–28 cycles each of 94 °C for 20 s, 58 °C for 30 s, 72 °C for 1 min. The β-actin homologous gene of *P. praecox*, named *PpACT1*, was cloned and used as an internal reference to determine the relative gene expression. The primers of *PpRLK1*, *PpHB1*, *PpSPY*, and *PpSINA* genes were designed for a semi-quantitative RT-PCR (Table 1). To ensure no false-positive PCR was generated from the genomic DNA, no transcriptional RNA template was chosen as the control. The PCR products were electrophoresed on 1.5% agarose gels. After electrophoresis, the gels were stained with 10 μg ml⁻¹ ethidium bromide and photographed under 280 nm UV light. The relative gene expression was assessed by densitometry based on the staining intensities of the corresponding bands using the ImageMaster VDS software program, version 2.0. Each experiment was repeated at least three times.

### Results

**The anatomical comparison of the rhizome bud, bamboo shoot, and rhizome shoot during development of *P. praecox***

Rhizome buds of *P. praecox* usually develop into bamboo culms in spring and form new rhizomes in summer. Rhizome shoots and bamboo shoots are quite different in their anatomical structure, although they both originate from rhizome buds (Fig. 1). Regular meristems were observed on the tip of the rhizome buds, and many isolated procambia were found at the front of the rhizome shoots, corresponding to their ability for unlimited growth (Fig. 1A–C). However, the meristems on the tip of bamboo shoots were irregular, and no procambia were observed at the front. Interestingly, many lateral buds appeared on the sides of the bamboo shoots, implying that the bamboo shoot was a micro-miniature of the bamboo (Fig. 1D–F). Simultaneously, a few lateral smaller buds were observed on the sides of the bamboo shoots, and many isolated procambia were found at the front of the rhizome shoots, corresponding to their ability for unlimited growth (Fig. 1A–C).

**Identification of genes related to the development of bamboo rhizome bud versus leaf by rice cross-species microarray analysis**

To investigate gene expression in the bamboo rhizome buds versus leaves, rice cross-species microarray analyses were performed. The microarray analysis was repeated at least three times.
carried out. Cy5-labelled cDNA from rhizome buds was hybridized against Cy3-labelled cDNA from the leaves on the same rice gene chip with 10,369 spots. The distribution of the average normalized hybridization intensity of the three measurements showed that a total of 8,795 spots were effective (Fig. 2A). The average hybridization efficiency is 84.82%. According to the criterion of the Cy5/Cy3 ratio being >2.0 or <0.5 for genes to be up- or down-regulated, respectively, a total of 318 up- and 339 down-regulated genes were identified. The reproducibility of the replicated hybridizations is 95%. By comparing the homologous genes from Arabidopsis and rice, the putative functions of these 318 up-regulated genes in the rhizome bud were classified into seven groups (Fig. 2B). Furthermore, except for the housekeeping genes, 52 of the 318 up-regulated genes with known functions in the rhizome bud are listed and described in Table 3.

Twenty-six genes related to signalling and biosynthesis of auxin are highly expressed in the rhizome buds. Their average Cy5/Cy3 ratios ranged from 2.0 to 5.5. Surprisingly, it was found that ethylene signalling-related genes were also highly expressed in the rhizome buds. In addition, some receptor-like kinase genes (such as leucine-rich repeat receptor, CLV1 receptor kinase, and adaptor protein SPIKE1) and some meristem initiation-related transcriptional factors (such as REVOLUTA) were detected with high expression levels in the rhizome buds.

A total of 11 metabolism-related genes with comparatively high expression levels were detected in the bamboo rhizome buds in comparison with the leaves. Their average Cy5/Cy3 ratios ranged from 3.0 to 8.0. In addition, the expression of starch accumulation-related genes, such as starch-branching enzyme class II (sbe2-1) and starch-branching enzyme I

Fig. 1. Anatomical comparison of the rhizome bud, bamboo shoot, and rhizome shoot during the development of Phyllostachys praecox. (A–C) Rhizome bud and its longitudinal section. (D–F) Bamboo shoot and its longitudinal section. (G–I) Rhizome shoot and its longitudinal section. Bar=100 μm.
(Rbe1), was notably high (average Cy5/Cy3 ratios were 5.5) in the rhizome buds.

Eleven stress response-related genes were up-regulated in the rhizome buds, most of which were involved in abiotic stress (e.g. ion homeostasis and temperature response). Expression of stress-related genes in the rhizome buds seemed to be parallel to the harsh growing conditions in early spring.

Four genes related to the synthesis and repair of the cell wall were also up-regulated in the rhizome buds. For example, the xyloglucan endotransglycosylase/hydrolase (XTH) gene was highly expressed, and the average Cy5/Cy3 ratio was 13.

Cloning and sequencing of six genes from the bamboo rhizome bud

In order to characterize selected genes related to the development of bamboo rhizome buds, genes for cloning were chosen based on three criteria: (i) cDNAs with a high signal intensity and up-regulation ratio >3.0 in the array analysis; (ii) homologous rice sequences of these 500 bp cDNAs known to be involved in meristem development; and (iii) that degenerate primers corresponding to the conserved region of homologous genes were designed. Six up-regulated genes, homologous to REVOLUTA, CLAVATA1, SPINDLY, SINAT5, ARF1, and AHK4 in rice, were cloned and sequenced from the rhizome buds, and were named as PpHB1, PpRLK1, PpSPY, PpSINA, PpARF1, and PpHK1, respectively (Table 2). The nucleotides and deduced amino acids of the six genes were compared with their homologous genes in rice, sharing 66–90% identity at the nucleotide level, and 68–98% identity at the amino acid level. Among them, PpSINA and PpHB1 displayed the highest degree of identity (97.8% and 93.2%, respectively), PpSPY and PpARF1 had a medium level of identity (87.9% and 84%, respectively), and PpRLK1 and PpHK1 shared a relatively low level of identity (74.6% and 67.7%, respectively).

The sequences of these six genes were further analysed by alignment and phylogeny reconstruction. Interestingly, these bamboo genes (PpHB1, PpRLK1, PpSINA, PpARF1, and PpHK1) have closer relationships with their homologous genes from rice (REVOLUTA, CLAVATA1, SINAT5, ARF, and AHK4) than those from other species (Fig. 3, Supplementary Figs S2–S6 available at JXB online).

To find out whether the cloned genes correspond to those analysed by microarray, Southern analyses were performed against bamboo genomic DNA. Three and four major bands were obtained for PpRLK1 and PpHB1, respectively, when blots of XbaI-digested genomic DNA were hybridized with a gene-specific probe for PpRLK1 and PpHB1 (Supplementary Fig. S7 at JXB online), suggesting that the bamboo genome contains at least three and four copies of PpRLK1 and PpHB1, respectively.

Expression analysis of four genes in different tissues and organs of bamboo

To verify the microarray results, semi-quantitative RT-PCR assays were carried out for the four genes (PpRLK1, PpHB1, PpSPY, and PpSINA) in different tissues and organs of bamboo.

Table 2. Six genes cloned in bamboo according to gene chip detection

| Gene  | PpHB1 | PpRLK1 | PpSPY | PpSINA | PpARF1 | PpHK1 |
|-------|-------|--------|-------|--------|--------|-------|
| Length (kb) | 2.6   | 2.7    | 2.7   | 1.0    | 1.1    | 1.2   |
| Identity (protein) | 93.2% (OsREV) | 74.6% (OsLRK1) | 89.2% (OsSPY) | 97.8% (OsSINA) | 84.0% (OsARF1) | 67.7% (OsHK1) |

Fig. 2. Rice cross-species microarray analysis of the bamboo rhizome bud versus leaves. (A) The distribution of the average normalized hybridization intensity (Cy5, rhizome bud; Cy3, leaf) of three measurements. Grey points indicate data with a ratio >0.5 and <2, while black points indicate data that are out of the range (>2.0 for up-regulated genes and <0.5 for down-regulated genes). (B) The putative functions of 318 up-regulated genes (U, unknown genes; N, not found in Arabidopsis; H, housekeeping genes; R, regulation and signalling-related genes; M, metabolism-related genes; S, stress-related genes; C, cell wall-related genes). The number of genes is indicated.
bamboo. As shown in Fig. 4a, expression of PpRLK1, PpHB1, and PpSPY was significantly higher in young florets than in other organs. In the rhizome buds, the expression of PpSINA and PpSPY was markedly higher than that of PpHB1 and PpRLK1. No difference was observed for the expression of these four genes in the rhizome shoots and bamboo shoots. In leaves, only the expression of PpSINA was detectable, but not those of the other three genes. PpHB1, PpRLK1, and PpSPY were highly expressed in the rhizome shoots, rhizome buds, bamboo shoots, and young florets. In situ hybridization further confirmed the observations that PpRLK1 was expressed in the developing procambium (Fig. 4b), but not in the tips of the bamboo shoots (Fig. 4e), suggesting a close relationship of PpRLK1
to the meristem development of bamboo shoots. However, PpHB1 was expressed not only in the developing procambium (Fig. 4d), but also in the tips of the bamboo shoots (Fig. 4f), indicating its close correlation to rhizome bud formation as well as procambial development.

Discussion
Rice cross-species microarray hybridization identifies homologous genes involved in the development of rhizome buds

DNA microarray technology is a basic tool to measure genome-wide changes in gene expression, and is helpful for understanding molecular mechanisms and functions. However, it is currently restricted to a few model species such as Arabidopsis and rice, due to lack of sequence information from other species. Recently, cross-species microarray analysis has been successfully established (Becher et al., 2004). This technique is promising and has been widely used in the identification of genes in non-model animal and plant species (Moody et al., 2002; Hudson et al., 2007; Nieto-Diaz et al., 2007). In the present study, a cross-species microarray with 7500 rice cDNAs 500 bp in length was used to identify the genes related to the development of bamboo buds. The data demonstrated that cross-species analyses do not significantly affect the hybridization reproducibility of gene chips. The average hybridization efficiency is 84.82%, and the reproducibility of replicate hybridizations is 95%, suggesting that the microarray chips containing rice cDNAs of 500 bp in length can be effectively applied for bamboo heterologous hybridization.

The growth and development of bamboo is fairly complex. The exploration of the molecular bases of genes in bamboo development has been intensified. Recently, Lin et al. (2006) made great efforts to identify bamboo genes differentially expressed in a bamboo albino mutant using suppressive subtractive hybridization (SSH) and microarray analysis. They prepared microarrays (18×27 mm) carrying 960 PCR-amplified cDNA clones hand picked from the bamboo SSH cDNA library on nylon membranes. Ten differentially expressed ESTs (expressed sequence tags) were isolated and sequenced. RT-PCR analysis of the ESTs supported the results of the microarray analysis. The combined use of this SSH library with microarray analysis will provide a powerful analytical tool for future studies of the bamboo genome. In the present study, rice cross-species microarray analysis was performed in bamboo for the first time. A total of 318 up- and 339 down-regulated genes were successfully identified. Among them, six up-regulated genes in rhizome bud were successfully cloned. Sequence analysis further indicated that these genes in bamboo shared medium to high identity with those in rice (66–90% identity at the nucleotide level, and 68–98% at the amino acid level). Thus, rice cross-species hybridization provides a useful tool to identify the conserved genes involved in the development of rhizome buds.

However, the cross-species microarray does raise some concerns about signal reduction and data analysis. Moreover, hybridization with imperfectly complementary nucleic acids would lead to severe bias because of the high number of interactions among probes and templates (Pariset et al.,
2009). As is known, there might be several homologous cDNAs contributing to one high signal intensity in a sample. It is quite difficult to determine the specificity of cross-species hybridizations, which is a main limitation of cross-species microarray. Therefore, the candidate genes identified from the cross-species microarray require further validation. For the validation, the genes with high signal intensity and an up-regulation ratio >3.0, and the corresponding rice sequences known to be involved in meristem development, were selected. Also degenerate primers corresponding to the conserved region of the homologous genes, mainly based on sequence alignment from *Oryza sativa*, *Zea mays*, *Hordeum vulgare*, and *Arabidopsis thaliana*, were also designed in order to amplify as many homologous genes as possible. However, only one fragment was cloned with cDNA templates from bamboo rhizome buds. No other sequences were obtained despite repetitive PCR tests being performed. It is suggested that the cloned fragment was, at least, a main member among the targeted gene family expressed in rhizome buds.

It is noteworthy that the degenerate primers designed from the conserved region of homologous genes of rice and other species do not guarantee amplification of the true orthologue of bamboo. Sequence divergence and the complexity of the targeted gene family will influence the outcome of the PCR amplification. The highly variable degree of sequence similarity indicates that members of large gene families were cloned in a somewhat random fashion (Hudson, 2007). It could not be extrapolated that the cloned genes from bamboo are putative orthologues in rice without functional analysis and genomic alignment.

**Rice cross-species microarray hybridization provides useful gene expression data for understanding the development of bamboo rhizome buds**

Cross-species microarray hybridization has been considered as one potentially useful technique to provide gene expression data and deduce gene function for applying model organism genomics in related species for which few functional genomics resources are available (Chismar et al., 2002; Becher et al., 2004; Lee et al., 2004; Hudson et al., 2007; Nieto-Díaz et al., 2007). In the present study, 26 genes related to signalling and biosynthesis of auxin are highly expressed in the rhizome buds of bamboo using rice cross-species microarray (Table 3). The results suggested that these genes might play important roles in the development of bamboo shoot. High concentrations of hormones were proved to be closely related to the formation of the bamboo shoot (Huang et al., 2002). The function of ethylene in the development of the rhizome bud has not been reported to be associated with bamboo shoot formation so far. In addition, the high expression of some receptor-like kinase genes and meristem initiation-related transcriptional factors was also observed in the rhizome buds, implying that these genes might be related to the meristem characteristics during rhizome bud development.

In addition, it is reasonable for the bamboo meristem bud to accumulate plenty of starch after the cool autumn and cold winter, which could provide both energy and gravising for the burst in growth in the spring. The starch–statolith hypothesis, proposed by Haberlandt and Némec, was widely used to explain how plants perceive gravity, and how the rhizome buds grow upward into bamboo shoot and in a horizontal stretch into rhizome (Audus, 1962). In the present study, starch synthesis- and cell wall synthesis-related genes were found to be highly expressed in the bamboo meristem (Table 3). The high expression levels of metabolism-related genes, especially starch-branching enzyme class II (sbe2-1) and starch-branching enzyme I (Rbe1), in the bamboo rhizome buds imply high metabolism intensity in the rhizome buds. It is thus conceivable that the meristem development of the rhizome bud is different from that of the ordinary axillary bud in plants. Similarly, the high expression of the cell wall synthesis-related genes, such as the XTH gene, suggests that the expression of XTH-homologous genes in bamboo might be necessary for cell replication and growth during meristem development. Most members of the rice XTH family exhibited organ- and growth stage-specific expression patterns, although their exact functions have not been revealed yet (Yokoyama et al., 2004). A recent report demonstrated that sucrose synthase isoforms play a variety of roles in bamboo shoots, directing translocated carbon towards both the polysaccharide biosynthesis and energy production necessary to sustain the remarkably rapid growth of bamboo (Chiu et al., 2006).

**Potential functions of the genes identified from cross-species microarray hybridization**

Rhizome bud development is one of the most important problems in bamboo research. Previous studies have revealed some anatomical, physiological, and molecular mechanisms for bamboo growth (Hu et al., 1996; Zhang et al., 1996; Huang et al., 2002; Chiu et al., 2006; Hsieh et al., 2006). In the present study, six genes related to rhizome bud development were successfully cloned from rhizome buds. *PpSPY* may affect meristem initiation indirectly according to its expression pattern and the function of its homologous gene in *Arabidopsis*. Previous research revealed that SPINDLY, homologous to *PpSPY*, encoded tetrapeptide repeat proteins involved in gibberellin signal transduction in both monocotyledons (Robertson, 2004) and dicotyledons (Greenboim-Wainberg et al., 2005). The high expression of *PpSPY* may be helpful in elucidating the signal transduction of gibberellins in the formation and development of bamboo shoot, although it is still not clear whether the gibberellins play an important role during the transition of the rhizome bud to bamboo shoot (Huang et al., 2002). *PpSINA*, which is an *Arabidopsis* homologue of the RING-finger *Drosophila* protein, is expressed in the vascular tissues of mature roots and can target NAC1 to down-regulate auxin signals in the plant cells (Xie et al., 2002). However, *PpSINA* in bamboo is expressed in all the organs including leaves (Fig. 4), suggesting that the role of *PpSINA* in bamboo might be different from that in *Arabidopsis* and rice.
Table 3. Up-regulated genes excluding for housekeeping ones with possible known functions in rhizome bud

The rice genes on the chip were mainly described by the homologous genes from Arabidopsis since most sequences of rice had not been published or studied yet. Accession numbers and descriptions of the corresponding rice genes are indicated in bold.

| Gene_ID       | Accession | Annotation                                                                 | Average ratio | Function classification               |
|---------------|-----------|-----------------------------------------------------------------------------|---------------|---------------------------------------|
| R0067B07      | ATY16046  | Leucine-rich repeat protein (G8B7T7)                                        | 5.5           | Regulation and signal transduction    |
| R0354C06      | AF193835  | CLV1 receptor kinase (CLAVATA1); LRK1 protein                               | 4.5           |                                       |
| R0186A08      | AU086829  | Adaptor protein SPIKE1 (SPK1)                                                | 4.3           |                                       |
| R0181H01      | AY063945.1| Putative receptor kinase                                                     | 3.9           |                                       |
| R0201B09      | AU068950  | Ethylene-insensitive3 (EIN3); ethylene-insensitive-3-like protein (OsEIL1)   | 3.6           |                                       |
| R0210A11      | AB046871  | AHK4 mRNA for histidine kinase                                               | 3.5           |                                       |
| R0159E02      | AP002819  | Calcium-dependent protein kinase (CDPK6)                                    | 3.5           |                                       |
| R0156D04      | AU082872  | Auxin-induced protein IAA9                                                    | 3.4           |                                       |
| R0213E09      | AF141942  | MRP-like ABC transporter; phytochrome C (phyC)                              | 3.2           |                                       |
| R0500A07      | AF042196  | ARF6 (ARF6)                                                                  | 3.1           |                                       |
| R0008A01      | AB061407  | Phosphatidic acid phosphatase                                                 | 3.0           |                                       |
| R0383C08      | AU056864  | IA8                                                                         | 3.0           |                                       |
| R0200A06      | AY072205.1| Putative glucose-regulated repressor protein                                | 2.9           |                                       |
| R0125A05      | AY059850.1| Putative casein kinase I                                                     | 2.8           |                                       |
| R0440D02      | ATU62135  | Gibberellin signal transduction protein (SPINDLY)                           | 2.8           |                                       |
| R0151H01      | AP002860  | Putative transcriptional regulator protein                                 | 2.7           |                                       |
| R0605C09      | AY045777.1| Putative receptor serine/threonine protein kinase ARK2                       | 2.6           |                                       |
| R0464B02      | AC024594  | REVOLUTA                                                                    | 2.6           |                                       |
| R0008A08      | Y07748    | Protein kinase (TMK1) gene, complete cds; leucine-rich repeat receptor-like | 2.6           |                                       |
| R0014B03      | C22395    | CBL-interacting protein kinase 18 (CIPK18); protein kinase                  | 2.5           |                                       |
| R0099B10      | AY06239.1 | Calcium-dependent protein kinase                                             | 2.5           |                                       |
| R0022C09      | AC079936  | Putative leucine-rich repeat transmembrane protein kinase                    | 2.3           |                                       |
| R0101H03      | AF185577  | Chromatin remodelling factor CHD3 (PKL)                                     | 2.3           |                                       |
| R0389E08      | AY059785.1| Putative kinase                                                             | 2.2           |                                       |
| R0548E04      | AP002481  | Ring finger E3 ligase SINAT5 (SINAT5)                                        | 2.0           |                                       |
| R0081030      | BE607353  | Putative β-glucosidase protein; β-glucosidase                               | 8.0           | Metabolism                            |
| R0272A08      | LO3366    | Putative sucrose synthase; sucrose synthase 3 (RSs3)                         | 6.3           |                                       |
| R0338B11      | AA750532  | Starch-branching enzyme class II (sbe2-1); starch-branching enzyme I (Rbe1)| 5.8           |                                       |
| R0162H08      | AY072210.1| Putative trehalose-6-phosphate synthase                                      | 4.2           |                                       |
| R0082F06      | U95816    | HMG-CoA reductase (HMG1); 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR)   | 4.0           |                                       |
| R0184G05      | AY066285.1| Putative β-galactosidase                                                     | 3.5           |                                       |
| R0025C09      | AY074343.1| Putative N-carbamyl-L-amino acid amidohydrolase                             | 3.4           |                                       |
| R0185F03      | AY059821.1| α-Glucosidase-like protein                                                   | 3.3           |                                       |
| R0042H09      | ATUT9160  | HMG-CoA synthase (MVA1)                                                     | 3.3           |                                       |
| R0419G09      | AY064010.1| Putative trehalose-6-phosphate synthase                                      | 3.2           |                                       |
| R0038E06      | AY039932.1| Putative aldose 1-epimerase                                                  | 3.0           |                                       |
| R0215G08      | AY051033.1| Putative post-transcriptional gene silencing protein                        | 3.9           | Stress                                |
| R0180E10      | AC084218  | Cultivar Cv-0 RPM1 variant gene                                              | 3.3           |                                       |
| R0193H10      | AF400590.1| Na+/H+ exchanger 6 (NHX6)                                                   | 2.8           |                                       |
| R0170D08      | AF322255  | Magnesium transporter protein (GMN10)                                       | 2.8           |                                       |
| R0245D03      | AF213400  | SLT1 protein                                                                 | 2.7           |                                       |
| R0269D12      | AU182068  | Heat shock protein 70; LMW heat shock protein                               | 2.5           |                                       |
| R0026H01      | ATU60445  | 14-3-3 protein GF14 nu (GRF7)                                                | 2.3           |                                       |
| R0038B12      | AJ276594  | Putative 14-3-3 protein; 14-3-3-like protein (gf14-e gene)                   | 2.3           |                                       |
| R0018D01      | AF272747  | WRKY transcription factor 9 (WRKY9)                                          | 2.2           |                                       |
| R0509B06      | AF012660  | Putative potassium transporter AKT5p (AtKT5)                                 | 2.2           |                                       |
| R0177C01      | AY044049.1| Phytochelatin synthase (PCS2)                                                | 2.1           |                                       |
| R0245B06      | AF003674  | Xyloglucan endotransglycosylase (XTR9)                                       | 13.6          | Cell wall                             |
| R0160E08      | AY063117.1| Putative β-1,3-glucanase (MRP15.22)                                         | 2.8           |                                       |
| R0095C06      | AF237733  | Callose synthase 1 catalytic subunit (CalS1)                                 | 2.8           |                                       |
| R0098A10      | AU183649  | At2g21660/F2G1.7; glycine-rich protein (OSGRP1)                              | 2.2           |                                       |
RT-PCR analysis showed that *PpRLK1* was expressed in both the rhizome bud and bamboo shoot. *In situ* hybridization confirmed that *PpRLK1* was expressed in the developing procambium (Fig. 4b), but not in the tips of the bamboo shoots (Fig. 4e), suggesting that the function of *PpRLK1* might be necessary for the meristem development of the bamboo shoot. Previous research revealed that the homologous gene of *PpRLK1*, *CLV1*, in *Arabidopsis* can determine the size of the meristem (*Gross-Hardt et al.*, 2003), and *OsLrk1* in rice plays a critical role in meristem development (*Kim et al.*, 2000). However, both RT-PCR and *in situ* hybridization showed that *PpHB1* was strongly expressed in the developing procambium (Fig. 4d) and in the tips of the bamboo shoots (Fig. 4f), suggesting that *PpHB1* might play an important role in rhizome bud formation as well as procambial development.

In conclusion, the present results demonstrate that there were significant differences in the anatomical structures among rhizome buds, rhizome shoots, and bamboo shoots, and for the first time confirm that rice cross-species microarray hybridization can be successfully applied to identify genes in bamboo. According to the rice cross-species microarray hybridization, six genes related to the development of the bamboo rhizome bud were cloned, which contributed to a further understanding of the molecular mechanism of bamboo rhizome bud development.

**Supplementary data**

Supplementary data are available at *JXB* online.

**Figure S1** Microarray data of leaves (left) and rhizome lateral buds (right). White arrows indicate up-regulated genes and pink arrows indicate down-regulated genes in rhizome lateral buds.

**Figure S2.** Alignment of four CLAVATA homologues and their structure.

**Figure S3.** Alignment of four SPINDLY homologues and their structure.

**Figure S4.** Alignment of four SINAT5 homologues and their structure.

**Figure S5.** Alignment of the DNA-binding domain among *PpARF1*, *OsARF1*, and *ARF1*. The deduced amino acid sequences of *OsARF1* and *ARF1* were aligned with the Dayhoff amino acids distance matrix.

**Figure S6.** Phylogenetic analysis of the isolated or predicted *PpHK1*, *OsHK1* ZmHK1, and AHK1 homologues. The deduced amino acid sequences of *PpHK1*, *OsHK1*, ZmHK1, and AHK1 were aligned with the Dayhoff amino acids distance matrix.

**Figure S7.** Southern analysis of *PpRLK1* and *PpHB1* in genomic DNA from *Phyllostachys praecox*. DNA was digested with *XbaI*.

**Table S1.** The rice EST on the microarray and corresponding homologous genes for *PpRLK1* cloning.

**Table S2.** The rice ESTs on the microarray and corresponding homologous genes for *PpHB1* cloning.

**Table S3.** The rice EST on the microarray and corresponding homologous genes for *PpSPY* cloning.

**Table S4.** The rice EST on the microarray and corresponding homologous genes for *PpSINA* cloning.

**Table S5.** The rice EST on the microarray and corresponding homologous genes for *PpARF1* cloning.

**Table S6.** The rice EST on the microarray and corresponding homologous genes for *PpHK1* cloning.

**Acknowledgements**

We sincerely thank YJ Wang for critical reading of this manuscript, and LJ Fan for helpful suggestions. This work was supported by the Natural Science Foundation of China (grant nos 30700635 and 30771347), Zhejiang Provincial Natural Science Foundation of China (grant nos Y305317 and Y3080539), the program for science and technology of Zhejiang Province (grant no. 2009C32064), and the International Foundation for Science (grant no. D/4352-1).

**References**

Audus LJ. 1962. The mechanism of the perception of gravity by plants. Symposia of the Society for Experimental Biology 16, 197–226.

Becher M, Talke IN, Krall L, Kramer U. 2004. Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyperaccumulator *Arabidopsis halleri*. The Plant Journal 37, 251–268.

Braissant O, Wahl W. 1998. A simplified in situ hybridization protocol using non-radioactively labeled probes to detect abundant and rare mRNAs on tissue sections. Biochemica 1, 10–16.

Chiu WB, Lin CH, Chang CJ, Hsieh MH, Wang AY. 2006. Molecular characterization and expression of four cDNAs encoding sucrose synthase from green bamboo *Bambusa oldhamii*. New Phytologist 170, 53–63.

Chismar JD, Mondala T, Fox HS, Roberts E, Langford D, Masliah E, Salomon DR. 2002. Analysis of result variability from high-density oligonucleotide arrays comparing same-species and cross-species hybridizations. Biotechniques 33, 516–518 520, 522 passim.

Greenboim-Wainberg Y, Maymon I, Borochov R, Alvarez J, Olszewski N, Ori N, Eshed Y, Weiss D. 2005. Cross talk between gibberellin and cytokinin: the Arabidopsis GA response inhibitor SPINDLY plays a positive role in cytokinin signaling. The Plant Cell 17, 92–102.

Gross-Hardt R, Laux T. 2003. Stem cell regulation in the shoot meristem. Journal of Cell Science 116, 1659–1666.

Hsieh CW, Liu LK, Yeh SH, Chen CF, Lin HI, Sung HY, Wang AY. 2006. Molecular cloning and functional identification of invertase isozymes from green bamboo *Bambusa oldhamii*. Journal of Agriculture and Food Chemistry 54, 3101–3107.

Hu C, Jin A, Zhang Z. 1996. Change of endohormone in mixed bud on Lei bamboo rhizome during differentiation. Journal of Zhejiang Forestry College 13, 1–4.

Huang J, Liu L, Zhang B, Qiu L. 2002. Dynamic changes of endophytotnemone in rhizomal buds of *Phyllostachys praecox*. Scientia Silvae Sinicae 38, 38–41.

Hudson ME, Bruggink T, Chang SH, Yu W, Han B, Wang X, van der Toorn P, Zhu T. 2007. Analysis of gene expression during
Brassica seed germination using a cross-species microarray platform. *Crop Science* **47**, S96–S112.

Kim C, Jeong DH, An G. 2000. Molecular cloning and characterization of OsLRK1 encoding a putative receptor-like protein kinase from Oryza sativa. *Plant Science* **152**, 17–26.

Kumar S, Tamura K, Nei M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* **5**, 150–163.

Lee HK, Hsu AK, Sajdak J, Qin J, Pavlidis P. 2004. Coexpression analysis of human genes across many microarray data sets. *Genome Research* **14**, 1085–1094.

Lin CS, Lai YH, Sun CW, Liu NT, Tsay HS, Chang WC, Jeremy JW. 2006. Identification of ESTs differentially expressed in green and albino mutant bamboo (Bambusa edulis) by suppressive subtractive hybridization (SSH) and microarray analysis, *Plant Cell, Tissue and Organ Culture*. **86**, 169–175.

Nieto-Díaz M, Pita-Thomas W, Nieto-Sampedro M. 2007. Cross-species analysis of gene expression in non-model mammals: reproducibility of hybridization on high density oligonucleotide microarrays, *BMC Genomics* **8**, 899.

Meadus WJ. 2003. A semi-quantitative RT-PCR method to measure the in vivo effect of dietary conjugated linoleic acid on porcine muscle PPAR gene expression *Biological Procedures Online* **5**, 20–28.

Moody DE, Zou Z, McIntyre L. 2002. Cross-species hybridisation of pig RNA to human nylon microarrays. *BMC Genomics* **3**, 27.

Pariset L, Chillemi G, Bongiorni S, Spica VR, Valentini A. 2009. Microarrays and high-throughput transcriptomic analysis in species with incomplete availability of genomic sequences. *New Biotechnology* **25**, 272–279.

Robertson M. 2004. Two transcription factors are negative regulators of gibberellin response in the HvSPY-signaling pathway in barley aleurone. *Plant Physiology* **136**, 2747–2761.

Xie Q, Guo HS, Dallman G, Fang S, Weissman AM, Chua NH. 2002. SINAT5 promotes ubiquitin-related degradation of NAC1 to attenuate auxin signals. *Nature* **419**, 167–170.

Yokoyama R, Rose JKC, Nishitani K. 2004. A surprising diversity and abundance of xyloglucan endotrans-glucosylase/hydrolases in rice. Classification and expression analysis. *Plant Physiology* **134**, 1088–1099.

Zhang Z, Hu C, Jin A. 1996. Observation of the morphology and the structure of Phyllostachys praecox rhizome lateral bud developing into shoot. *Journal of Bamboo Research* **15**, 60–66.

Zhu T, Chang HS, Schmeits J, Gil P, Shi L, Budworth P, Zou G, Chen X, Wang X. 2001. Gene expression microarrays, improvements, and applications towards agricultural gene discovery. *Journal of the Association for Laboratory Automation* **6**, 95–98.