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Differential Expression of Genes Encoding Acid Invertases in Multiple Shoots of Bamboo in Response to Various Phytohormones and Environmental Factors

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Supporting Information

ABSTRACT: The promoter regions of two cell wall invertase genes, *Boβfruct1* and *Boβfruct2*, and a vacuolar invertase gene, *Boβfruct3*, in *Bambusa oldhamii* were cloned, and putative regulatory *cis*-elements were identified. The expression of these three genes in multiple shoots of bamboo that were cultured *in vitro* under different conditions was analyzed by real-time PCR. The two cell wall invertase genes were upregulated by indole-3-acetic acid and cytokinins but responded differently to other phytohormones and different temperatures. *Boβfruct1* was also upregulated by sucrose and glucose. In contrast, the *Boβfruct2* expression was induced by the depletion of sucrose, and this induction could be suppressed by glucose and sucrose. The expression of *Boβfruct3* was light-dependent; however, abscisic acid (ABA) could induce its expression in the dark. ABA and light exhibited an additive effect on the expression of *Boβfruct3*. Our results suggest that these three *Boβfruct* genes have individual roles in the adaption of the plant to environmental changes. *Boβfruct2* might also have an essential role in the immediate response of cells to sucrose availability and in the maintenance of sink activity. Moreover, *Boβfruct3* might be one of the interacting nodes of the light and ABA signaling pathways.

KEYWORDS: *Bambusa oldhamii, cell wall invertase, vacuolar invertase, promoter region, gene expression patterns*

INTRODUCTION

In higher plants, sucrose is a major transport form of carbohydrates and an important substrate for growth. For use as carbon and energy sources in sink organs, sucrose is hydrolyzed by invertase into glucose and fructose or transformed into UDP-glucose and fructose by sucrose synthase in the presence of UDP. The products of sucrose cleavage can be channeled into various metabolic pathways. Sucrose, glucose, and fructose can also act as signal molecules that modulate the expression of diverse genes.[1,2]

Depending on their subcellular localization, plant invertases are categorized as vacuolar, cell wall, and cytoplasmic invertases. Vacuolar and cell wall invertases belong to the acid invertase subfamily due to their optimum pH for sucrose hydrolysis.[3,4] High vacuolar invertase activity and gene expression levels have been associated with expanding and actively growing tissues, including maize ovaries,[5] carrot taproot,[6] sugar beet petioles,[7] cotton fiber, and *Arabidopsis* root.[8] The proposed functions of vacuolar invertases include osmoregulation, cell expansion, and sugar storage.[4,9] Cell wall invertases are thought to be involved in assimilate partitioning, the regulation of sink strength, and plant development.[9,10] The pivotal role of cell wall invertases in reproductive and vegetative development has been supported by a number of molecular genetic studies (refs 10 and 11 and references therein). Moreover, both the vacuolar and cell wall invertases have important roles in the responses of plants to different forms of stress. The enzyme activities and gene expression levels of these invertases have been shown to be regulated by a variety of internal and environmental stimuli, including carbohydrates, phytohormones, light, temperature, wounding, and pathogen infection.[10−14]

Bamboos, which belong to the family Poaceae, typically form clumps or spread via subterranean stems. Bamboo shoots that emerge from buds on subterranean stems usually grow rapidly and can reach their full height within a single growth season. To support the growth, sucrose is translocated from the mature plants to the developing shoots through the subterranean stems. The sucrose-cleavage enzymes, sucrose synthase and acid invertases, have been shown to be associated with the rapid growth of bamboo shoots.[15,16] However, the different genes that encode isoforms of these enzymes exhibit differential expression patterns in the various tissues of bamboo shoots at the different growth stages, which implies that each individual isoform has a specific function.[15,16] To gain more insight into the functions of the different acid invertases in bamboo, we cloned the promoter regions of two cell wall invertase genes, *Boβfruct1* and *Boβfruct2*, and a vacuolar invertase gene, *Boβfruct3*, from *Bambusa oldhamii* and analyzed the putative *cis*-elements *in silico*. We also examined the expression patterns of these invertase genes in multiple shoots of bamboo that were cultured *in vitro* and treated with different sugars, phytohormones, and abiotic factors. On the basis of the results, which show that the three invertase genes are differentially regulated

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Table 1. Putative *cis*-Elements in the Promoter Region of *Boβfruct1*

motif	sequence	PLACE *cis*-element	location	PLACE function and identity or references	factor involved
ARR	AGATT CGATT GGATT CGATT	NGATT	−745 to −741, −709 to −705, −438 to −434, −184 to −180	ARR1 binding element (ARR1AT)	cytokinin
ASF1	TGACG	TGACG	−415 to −419	ASF-1 binding site (ASF1MOTIFCAMV)	auxin, light salicylic,
CE1	CGGTG	CGGTG	−428 to −424	putative ABI4 binding element [58]	ABA
CGCG box	ACGCGG GCGCGT ACGCGG GCGCGT CCGCGC CCGCGC GCGCGC ACGCGC	(A/C/G) CGCG(G/T/C)	−567 to −562, −551 to −546, −535 to −530, −422 to −417, −340 to −335, −322 to −317, −320 to −315, −55 to −50	CGCG box recognized by CAMTAs (CGCGBOXAT)	ABA, ethylene, light
DRE/CRT	GCCGAC	(G/A) CCGAC	−589 to −584, −336 to −331	core motif of DRE/CRT (DRECRTCOREAT)	salt, drought, cold
ERE	AATTCAAA	A(A/T) TTCAAA	−124 to −117	ethylene-responsive element (ERELEE4)	ethylene
GCC box	GCCGCC	GCCGCC	−316 to −311	core motif of GCC box (GCCCORE)	ethylene, jasmonate
GT-1	GAAAAA	GAAAAA	−690 to −685, −620 to −615, −143 to −138, −127 to −132	GT-1 motif (GT1GMSCAM4)	pathogen, salt
LTRE-1	CCGAAA	CCGAAA	−514 to −519	low temperature responsive element-1 (LTRE1HVBLT49)	cold
LTRE-2	CCGAC	CCGAC	−588 to −584, −355 to −331	low temperature responsive element-2 (LTRECOREATCOR15)	cold
MYB	AAACCA TAACCA	(A/T) AACCA	−604 to −599, −649 to −654	MYB recognition site (MYB1AT)	ABA
MYC	CACATG	CACATG	−416 to −411	MYC recognition site (MYCATRD22)	drought, ABA
pyrimidine box	CCTTTT	(T/C) CTTTT	−101 to −96	DOF transcription factor (BPBF) binding site (PYRIMIDINEBOXOSRAMY1A)	GA
SORLIP1	GCCAC	GCCAC	−109 to −113	light-induced *cis*-element (SORLIP1AT)	light
W box	TGACT	TGACT	−470 to −466, −206 to −210	novel WRKY transcription factor (SUSIBA2) binding site (WBOXHVISO1)	sugar

by different internal and environmental factors, we proposed possible roles that are played by these invertases.

MATERIALS AND METHODS

Plant Materials. Multiple shoots of *B. oldhamii* cultured *in vitro* were maintained in MS medium containing 0.45 μM thidiazuron (TDZ) and 15% sucrose at 25 °C, as previously described.[17] To study the effects of different factors on the expression of the three *Boβfruct* genes, the shoots were transferred to fresh media and cultured at 25 °C for 14 d and then incubated under various conditions or treated with different factors, which were performed as follows.

Illumination: The multiple shoots were incubated at 25 °C either in the dark for 12 h and then in the light or in the light for 12 h and then in the dark.

Sugar treatment: The multiple shoots were incubated with fresh media devoid of sucrose but containing 100 mM mannitol at 25 °C for 48 h in the dark. The shoots were then transferred to fresh media containing 100 mM glucose, sucrose, or mannitol.

H_2O_2 treatment: H_2O_2 (10 mM) was added to the culture medium of the multiple shoots, and the shoots were then incubated at 25 °C in the dark. The controls (without H_2O_2) were incubated under the same conditions.

Temperature effect: The multiple shoots were incubated at 4, 25, 37, or 42 °C in the dark.

Phytohormone treatment: Before treatment, the multiple shoots were transferred to fresh media without TDZ for 14 d. Indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BA), kinetin, 1-aminocyclopropane-1-carboxylic acid (ACC), jasmonic acid (JA), abscisic acid (ABA), or gibberellic acid (GA) was then added to the media at various concentrations. The shoots were incubated at 25 °C in the dark for 12 h.

Real-Time PCR. The total RNA was isolated from the various multiple-shoot samples using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The cDNA was synthesized as previously described[18] and used as a template for real-time PCR reactions with gene-specific primer pairs (Table S1). The real-time PCR reactions were performed using the SYBR FAST qPCR kit (KAPA Biosystems, Boston, MA, USA) in a Bio-Rad CFX96 real-time PCR thermal cycler. The specificity of the amplification was confirmed as previously described.[18] *BohUBQ5*, which encodes a ubiquitin in bamboo, was used as the reference gene.[19] The transcript levels of the target gene in each sample were normalized with respect to those of *BohUBQ5*. The fold changes in the abundance of the target transcript in the different samples were determined using the ΔΔCt method.[20]

Cloning of the Promoter Regions of the Three Invertase Genes. The genomic DNA was isolated from etiolated bamboo shoots using the Plant Genomic DNA Isolation Kit (Geneaid, Taipei, Taiwan). GenomeWalker libraries were constructed using a GenomeWalker kit (Clontech, Mountain View, CA, USA) according to the manufacturer's instructions. Primary and nested PCRs were performed using the adaptor forward primers (AP1 and AP2) that were provided in the kit and invertase gene-specific antisense primers (Table S1). The PCR products were separated by agarose gel

Table 2. Putative *cis*-Elements in the Promoter Region of *Boβfruct2*

motif	sequence	PLACE *cis*-element	location	PLACE function and identity or references	factor involved
ARR	TGATT CGATT TGATT TGATT TGATT TGATT AGATT AGATT CGATT AGATT TGATT TGATT GGATT	NGATT	−980 to −976, −853 to −857, −835 to −831, −776 to −772, −717 to −713, −681 to −677, −637 to −633, −593 to −589, −533 to −529, −525 to −521, −463 to −459, −253 to −257, −125 to −129	ARR1 binding element (ARR1AT)	cytokinin
CARE	CAACTC	CAACTC	−691 to −696, −551 to −556	CAACTC regulatory elements (CAREOSREP1)	GA
GARE	TAACAAA	TAACA(A/G)A	−356 to −350	amylase box (AMYBOX1)	GA
G box	CACGTG	CACGTG	−93 to −88	GBF binding site (CACGTGMOTIF)	ABA, sugar
GT-1	GAAAAA	GAAAAA	−362 to −367	GT-1 motif (GT1GMSCAM4)	pathogen, salt
I-box	GATAAG	GATAAG	−442 to −437, −183 to −188	I- box (IBOX)	light
LTRE-1	CCGAAA	CCGAAA	−971 to −976	low temperature responsive element-1 (LTRE1HVBLT49)	cold
LTRE-2	CCGAC	CCGAC	−797 to −801	low temperature responsive element-2 (LTRECOREATCOR15)	cold
MYB	AAACCA	(A/T)AACCA	−699 to −704	MYB recognition site (MYB1AT)	ABA
pyrimidine box	CCTTTT TCTTTT CCTTTT	(T/C)CTTTT	−801 to −806, −373 to −368, −97 to −102	DOF transcription factor (BPBF) binding site (PYRIMIDINEBOX-OSRAMY1A)	GA
SORLIP1	GCCAC	GCCAC	−237 to −233	light-induced *cis*-element (SORLIP1AT)	light
SP8b	TACTATT	TACTATT	−430 to −424, −511 to −517	SPBF binding site (SP8BFIBSP8BIB)	sugar
SURE-1	AATAGAAAA	AATAGAAAA	−342 to −334	sucrose-responsive element (SURE1STPAT21)	sugar
W box	TGACT	TGACT	−561 to −565, −398 to −402	novel WRKY transcription factor (SUSIBA2) binding site (WBOXHVISO1)	sugar

electrophoresis, and the major bands were isolated from the gel. The isolated DNA fragments were then cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA) and subjected to sequencing.

Sequence Analysis. The potential transcription initiation site and the putative regulatory *cis*-elements and conserved motifs were analyzed using the online TSSP program (http://linux1.softberry.com/berry.phtml?topic=tssp&group=programs&subgroup=promoter) and the PLACE database (http://www.dna.affrc.go.jp/PLACE/).

RESULTS

Cloning of the Promoter Regions of the Three Bamboo *Boβfruct* Genes. The sequences upstream of the open reading frames of *Boβfruct1*, *Boβfruct2*, and *Boβfruct3* (Figures S1, S2, and S3) were cloned using a genome-walking approach. The overall similarities between the three sequences were low (data not shown). The transcription initiation sites were predicted to be located 53 bp, 49 bp, and 82 bp upstream of the translation start sites of these three *Boβfruct* genes, respectively. Putative TATA boxes were also identified for these three genes (Figures S1, S2, and S3). The sequences upstream of the transcription initiation sites (designated +1) were hereafter assigned to the promoter regions of the three invertase genes. Although these sequences might be part of the regulatory sequences that direct and regulate the expression of these *Boβfruct* genes, a number of putative *cis*-elements that have been associated with the responses to physiological and environmental factors in plant genes were found in these sequences (Tables 1, 2, and 3).

Expression of the Three *Boβfruct* Genes in Multiple Shoots of *B. oldhamii* Cultured *in Vitro* under Different Conditions. The presence of multiple putative *cis*-elements in the promoter regions of the three *Boβfruct* genes suggests that these genes might be regulated by different physiological and environmental factors. Therefore, we examined their expressions under different conditions using real-time PCR. However, it is difficult to change the growth conditions of bamboo shoots because they are connected to a clump of mature bamboo plants. As a result, multiple shoots of bamboo that were cultured *in vitro* were used to investigate the expression of these three invertase genes. To determine the effect of light on the expression of these *Boβfruct* genes, multiple shoots were either cultured in the dark for 12 h and then continuously illuminated for 36 h or first cultured in the light for 12 h and then continuously incubated in the dark. The analysis of the transcript levels of each *Boβfruct* gene by real-time PCR showed that the expressions of *Boβfruct1* and *Boβfruct2* were not regulated by light (data not shown), whereas the expression of *Boβfruct3* was significantly regulated by light (Figure 1). The incubation of multiple shoots in the dark and then in the light

Table 3. Putative *cis*-Elements in the Promoter Region of *Boβfruct3*

motif	sequence	PLACE *cis*-element	location	PLACE function and identity or references	factor involved
ABRE	ACGTGGCC	ACGT(C/G)(C/G)(C/G)C	−166 to −159	ABA-responsive element (ABREOSRAB21)	ABA
ARR	CGATT TGATT TGATT	NGATT	−376 to −372, −355 to −351, −335 to −339	ARR1 binding element (ARR1AT)	cytokinin
ASF1	TGACG	TGACG	−502 to −498, −316 to −320	ASF-1 binding site (ASF1MOTIFCAMV)	auxin, light salicylic,
CE1	CGGTG	CGGTG	−447 to −443, −601 to −605	putative ABI4 binding element[58]	ABA
CGCG box	ACGCGC	(A/C/G)CGCG(G/T/C)	−619 to −614	CGCG box recognized by CAMTAs (CGCGBOXAT)	ABA, light ethylene
CMSRE-1	TGGACGG	TGGACGG	−814 to −808	carbohydrate metabolite signal responsive element 1 (CMSRE1IBSPOA)	sugar
DPBF-1,2	ACACAAG ACACCCG	ACACNNG	−483 to −477, −661 to −667	novel bZIP transcription factors (DPBF-1 and -2) binding core sequence (DPBFCOREDCDC3)	ABA
ERE	AATTCAAA	A(A/T)TTCAAA	−285 to −278	ethylene responsive element (ERELEE4)	ethylene
GARE	TAACAAA	TAACAA(A/G)	−740 to −746	GA-responsive element (GAREAT)	GA
GCC box	GCCGCC	GCCGCC	−493 to −498	core motif of GCC box (GCCCORE)	ethylene, jasmonate
GT-1	GAAAAA	GAAAAA	−429 to −434	GT-1 motif (GT1GMSCAM4)	pathogen, salt
LTRE-1	CCGAAA	CCGAAA	−603 to −598	low temperature responsive element-2 (LTRE1HVBLT49)	cold
MYB	AAACCA	(A/T)AACCA	−439 to −444	MYB recognition site (MYB1AT)	ABA
MYC	CACATG	CACATG	−246 to −241, −98 to −103	MYC recognition site (MYCATRD22)	drought, ABA
PRE	ACTCAT	ACTCAT	−239 to −234	pro- or hypoosmolarity-responsive element (PREATPRODH)	osmolarity
pyrimidine box	TCTTTT	(T/C)CTTTT	−695 to −700	DOF transcription factor (BPBF) binding site (PYRIMIDINEBOXOSRAMY1A)	GA
S3S1	AAATCA(N)xATAGAAA	AAATCA(N)xATAGAAA	−350 to −432	combination of sucrose box3 and SURE1 element[59]	ABA, sugar
SORLIP1	GCCAC	GCCAC	−607 to −603, −160 to −164	light-induced *cis*-element (SORLIP1AT)	light
SORLIP2	GGGCC	GGGCC	−98 to −94	light-induced *cis*-element (SORLIP2AT)	light
SORLREP3	TGTATATAT	TGTATATAT	−733 to −725, −682 to −690	light-repressed *cis*-element (SORLREP3AT)	light
SP8b	TACTATT	TACTATT	−753 to −747	SPBF binding site (SP8BFIBSP8BIB)	sugar
SRE	TTATCC	TTATCC	−166 to −171	sugar-repressive element (SREATMSD)	sugar
TATTCT motif	TATTCT	TATTCT	−231 to −226	light-reponsive element (−10PEHVPSBD)	light
W box	TGACT	TGACT	−590 to −594, −556 to −560, −514 to −518	novel WRKY transcription factor (SUSIBA2) binding site (WBOXHVISO1)	sugar

showed that the transcript level of *Boβfruct3* increased significantly after the first 12 h of illumination, peaked at 24 h, and decreased slightly thereafter (Figure 1A). In contrast, the incubation of multiple shoots in the light and then in the dark showed that the transcript levels of *Boβfruct3* significantly decreased after 1 h of dark incubation (Figure 1B). Thus, the results indicate that the expression of *Boβfruct3* is light-dependent.

In addition to light, we also investigated the effects of other factors. To avoid the complexity of photosynthesis, the shoots were cultured in the dark during their treatment with the different factors. Because the expression of *Boβfruct3* was downregulated in the dark, only the expressions of *Boβfruct1* and *Boβfruct2* are reported in the following investigations, with the exception of the ABA treatment. To examine the effect of exogenous sugars, multiple shoots were cultured in a medium containing 100 mM mannitol for 48 h to decrease the levels of the endogenous metabolizable sugars and were then exposed to 100 mM sucrose, glucose, or mannitol. As shown in Figure 2A, the levels of *Boβfruct1* mRNA remained constant in the mannitol-containing medium but increased after 6 h of glucose incubation, reached a maximum level after 12 h, and then decreased. In the presence of sucrose, the levels of *Boβfruct1* mRNA also increased. In contrast, the levels of *Boβfruct2* mRNA increased significantly after 48 h of mannitol incubation but decreased gradually after the shoots were transferred to media containing glucose or sucrose (Figure 2B). These results show that the expression of *Boβfruct1* was induced by sucrose/glucose, whereas that of *Boβfruct2* was induced by sucrose/glucose depletion.

Figure 3 shows the effects of H_2O_2 on the transcript levels of *Boβfruct1* and *Boβfruct2*. An increase in the *Boβfruct1* mRNA levels was observed after 1 h of treatment with 10 mM H_2O_2; after this 1 h period, a gradual decline was observed. Under the same conditions, the transcript levels of *Boβfruct2* increased within 6 h of H_2O_2 treatment and then decreased to a level close to that observed in the control. Multiple shoots were also incubated at 4, 25, 37, and 42 °C to examine the effects of temperature on the transcript levels of *Boβfruct1* and *Boβfruct2*. As shown in Figure 4A, the cold (4 °C) and hot (42 °C)

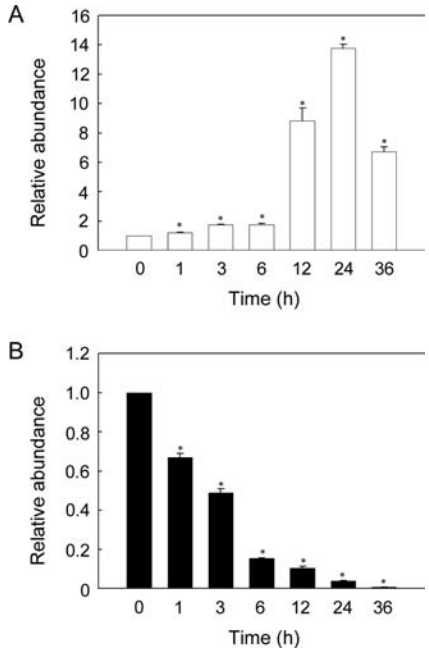

Figure 1. Effect of light on *Boβfruct3* expression in multiple shoots of bamboo that were cultured *in vitro*. The multiple shoots were either precultured in the dark for 12 h and then transferred to the light (A) or precultured in the light for 12 h and then transferred to the dark (B). The levels of *Boβfruct3* mRNA in samples collected at different time points, which were determined by real-time PCR, were calculated relative to the control (0 h), which was set to 1. The mean values ± SD of three independent experiments are shown. The asterisks indicate significant differences ($p < 0.001$) between the control and a subsequent time point.

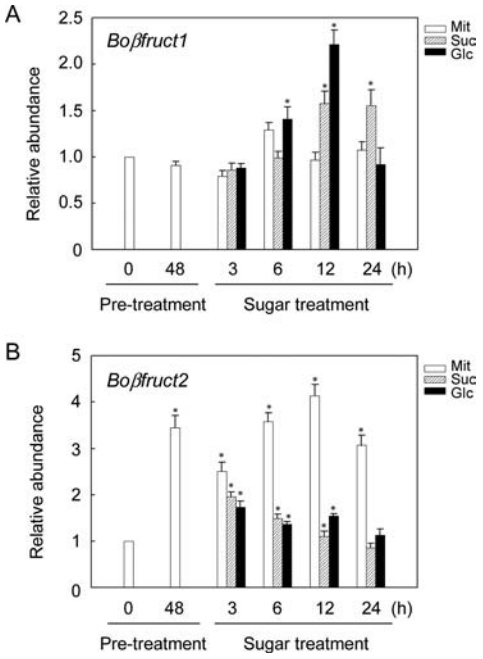

Figure 2. Effect of sugars on *Boβfruct1* and *Boβfruct2* expression in multiple shoots of bamboo that were cultured *in vitro*. The multiple shoots were incubated with fresh media devoid of sucrose but containing 100 mM mannitol at 25 °C for 48 h in the dark (Pre-treatment). The shoots were then transferred to fresh media containing 100 mM glucose, sucrose, or mannitol (Sugar treatment). The levels of *Boβfruct1* (A) and *Boβfruct2* (B) mRNA in the multiple shoots that were cultured in the different media for different time intervals were analyzed by real-time PCR. The gene transcript levels were calculated relative to the levels of the corresponding genes in the control (0 h), which were set to 1. The mean values ± SD of three independent experiments are shown. The asterisks indicate significant differences ($p < 0.05$) between a specific point and the control.

conditions significantly decreased the *Boβfruct1* mRNA levels after 12 h of incubation at these temperatures. At 37 °C, the *Boβfruct1* mRNA levels increased; however, a significant decrease was observed after a longer incubation period. The temperature 37 °C is close to the average temperature at which bamboo plants grow in the field during summer but is not the optimal condition for the *in vitro* growth of multiple shoots. A prolonged incubation at 37 °C might turn out to be stressful for multiple shoots cultured *in vitro*. The levels of *Boβfruct2* mRNA significantly increased after 48 h of incubation at 4 °C (Figure 4B), whereas significant decreases were observed after 24 h of incubation at either 37 or 42 °C.

The effects of different phytohormones on the expression of *Boβfruct1* and *Boβfruct2* are shown in Figure 5. The auxin IAA increased the levels of *Boβfruct1* mRNA, but no effect was observed when multiple shoots were incubated with 2,4-D (Figure 5A). The application of cytokinins (BA and kinetin) also increased the *Boβfruct1* transcript levels. In contrast to the effects of IAA and cytokinins, ethylene, JA, and ABA had negative effects, whereas GA had no effect on the expression of *Boβfruct1*. The transcript levels of *Boβfruct2* were also increased by auxins and cytokinins (Figure 5B). The addition of GA resulted in a 3-fold induction of *Boβfruct2* mRNA. Moreover, JA slightly increased the transcript levels of *Boβfruct2*, and ethylene and ABA had no effect (Figure 5B). The application of auxin, cytokinin, ethylene, and JA did not cause significant changes in the levels of *Boβfruct3* mRNA (data not shown). It was unclear whether this gene did not respond to these phytohormones or whether it was affected by these phytohormones but its mRNA was unstable in the dark. However, ABA had a significantly positive effect on the expression of *Boβfruct3* (Figure 6). When multiple shoots that were precultured in the light for 12 h were incubated in the dark for 12 h, the transcript levels of *Boβfruct3* were remarkably reduced in the absence of ABA, as expected; however, the presence of 5 or 30 μM ABA restored and even significantly increased the transcript levels of *Boβfruct3* (Figure 6A). The effect of ABA on the *Boβfruct3* transcript levels was more significant when the multiple shoots were continuously cultured in the light, and a dose-dependent pattern was also observed (Figure 6B).

DISCUSSION

Differential Expression of the *Boβfruct1* and *Boβfruct2* Genes in Response to Different Factors Suggests That These Genes Have Specific Roles in Bamboo. We have shown the effects of environmental factors and phytohormones on the transcript levels of the three acid invertase genes in multiple shoots of bamboo cultured *in vitro*. The differential expression patterns are summarized in Table S2. Both *Boβfruct1* and *Boβfruct2* encode cell wall invertases. Most of the known cell wall invertases are induced by sugars, which is consistent with their known functions in the mediation of assimilate partitioning and source−sink relation.[10,12,21−24] In contrast, the rice cell wall invertase *OsCIN5* is repressed by sucrose.[23] In this study, we found that the expression of *Boβfruct1* was induced by exogenous glucose and sucrose,

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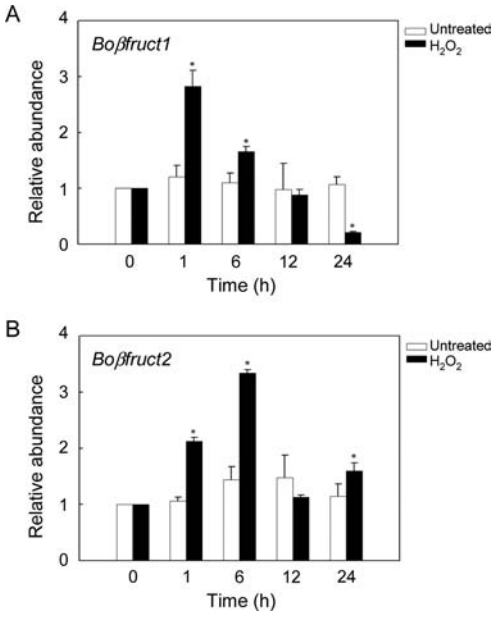

Figure 3. Effect of hydrogen peroxide on *Boβfruct1* and *Boβfruct2* expression in multiple shoots of bamboo cultured *in vitro*. Multiple shoots were treated with 10 mM H_2O_2 for the indicated time intervals (black column). Untreated samples were collected in parallel (white column). The levels of *Boβfruct1* (A) and *Boβfruct2* (B) mRNA at different time intervals were analyzed by real-time PCR. The gene transcript levels in the different samples were calculated relative to the levels of the corresponding genes in the control (0 h), which were set to 1. The mean values ± SD of three independent experiments are shown. The asterisks indicate significant differences ($p < 0.01$) between a specific point and the control.

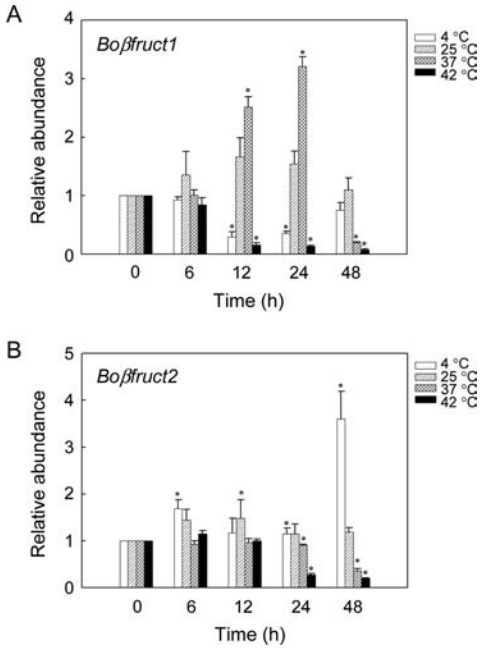

Figure 4. Effect of temperature on *Boβfruct1* and *Boβfruct2* expression in multiple shoots of bamboo cultured *in vitro*. The levels of *Boβfruct1* (A) and *Boβfruct2* (B) mRNA in multiple shoots cultured at various temperatures for different time intervals were analyzed by real-time PCR. The gene transcript levels in the different samples were calculated relative to the levels of the corresponding genes in the control (0 h), which were set to 1. The mean values ± SD of three independent experiments are shown. The asterisks indicate significant differences ($p < 0.01$) between a specific point and the control.

similar to many cell wall invertases in other plant species. However, the expression of *Boβruct2* was enhanced when the sucrose in the media was replaced by mannitol (Figure 2); to the best of our knowledge, this effect has not previously been reported for other cell wall invertases. Moreover, the transfer of the shoots from a sucrose-depleted medium to sucrose- or glucose-abundant conditions induced a decrease in the *Boβfruct2* mRNA level. Therefore, sucrose and/or glucose might mediate the suppression of the sucrose-depletion-induced transcription of *Boβfruct2* and/or the instability of the *Boβfruct2* mRNA. Transcriptional regulation involves *cis*-elements and *trans*-acting factors. We did not observe any conserved sugar-responsive elements that are involved in the induction of gene expression within the 0.8 kb promoter region of *Boβfruct1*. The element(s) required for the sucrose/glucose-induced *Boβfruct1* expression may be unknown or may be present in the unidentified upstream promoter region. However, several elements involved in sugar-induced or repressed gene expression, which included the SURE-1,[25,26] SP8b,[27] GARE, and a pyrimidine box,[28] were found in the promoter region of *Boβfruct2*. Although our data indicate that sucrose and glucose downregulate the sucrose-depletion-induced gene expression of *Boβfruct2*, it should be noted that the levels of *Boβfruct2* mRNA remained at a threshold level in the presence of sucrose or glucose (Figure 2B), which suggests that the transcription of *Boβfruct2* still occurs under sucrose/glucose-abundant conditions and that other mechanisms are involved in the sugar-mediated regulation of this gene. Nevertheless, why does bamboo require *Boβfruct2* to be upregulated under sucrose-depleted conditions in which the substrate of its encoding enzyme is limited? We propose that *Boβfruct2* has an essential role in this plant. The expression of this gene under sucrose-depleted conditions could allow the sink cells to respond immediately when sucrose is available. Moreover, the *Boβfruct2*-encoded enzyme has a low K_m value for sucrose.[16] Therefore, the enzyme can bind to and act on sucrose under conditions in which low concentrations of sucrose are present, which could sustain the sink activity.

In addition to sugars, the cell wall invertases are known to be regulated by various phytohormones, by which these enzymes link the hormone response to the primary metabolism.[4,10] For example, many studies have reported that the growth-promoting phytohormones, such as cytokinins, auxin, and GA, upregulate the cell wall invertases in growing tissues. The increased invertase levels can not only support the increased carbohydrate demand for active growth and enhance the sink strength but also amplify the signaling molecules for the sugar-modulated gene expression (ref 10 and references therein). In a previous study, we showed that the transcript levels of *Boβfruct1* and *Boβfruct2* paralleled the growth of bamboo shoots despite the differential expression of these genes in the tissues.[16] In this study, we observed that these two genes were upregulated by auxin and cytokinins in bamboo shoots that were cultured *in vitro*, which supports the previously proposed roles of these invertases in bamboo growth.[16] We also observed that there are 4 and 13 copies of ARR-binding elements within the identified promoter regions of *Boβfruct1* and *Boβfruct2*, respectively (Tables 1 and 2). ARRs are a family of transcription factors that are involved in cytokinin signaling and plant development.[29−33] Whether the ARR-binding elements in the promoters of *Boβfruct1* and *Boβfruct2* have

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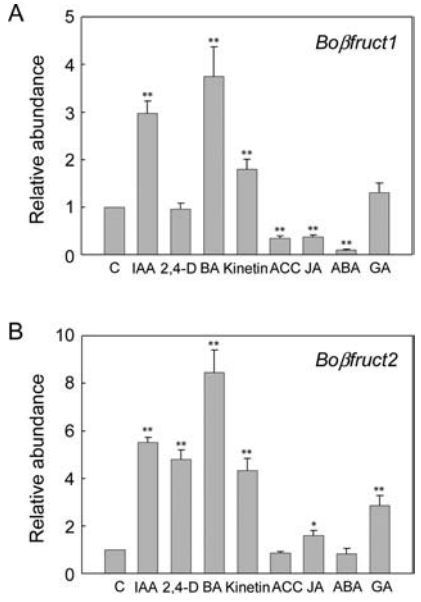

Figure 5. Effect of phytohormones on *Boβfruct1* and *Boβfruct2* expression in multiple shoots of bamboo cultured *in vitro*. Multiple shoots were incubated with 100 μM auxins (IAA and 2,4-D), 100 μM cytokinins (BA and kinetin), 100 μM ACC, 50 μM JA, 30 μM ABA, or 30 μM GA. The levels of *Boβfruct1* (A) and *Boβfruct2* (B) mRNA in each sample were analyzed by real-time PCR. The gene transcript levels in shoots that were cultured in the absence of phytohormones (control, C) were set to 1 to calculate the relative levels of the corresponding gene transcripts in the phytohormone-treated samples. The mean values ± SD of three independent experiments are shown. The asterisks indicate significant differences (*$p < 0.01$, **$p < 0.001$) between a treated sample and the control.

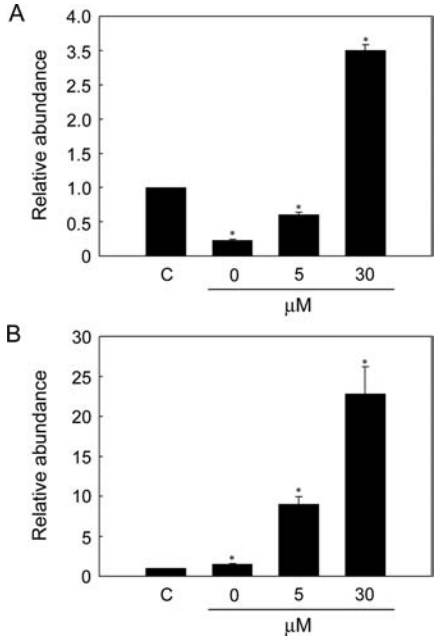

Figure 6. Effect of ABA on *Boβfruct3* expression in multiple shoots of bamboo cultured *in vitro*. Multiple shoots were precultured in the light for 12 h and then treated with 5 or 30 μM ABA in the dark (A) or the light (B) for 12 h. The levels of *Boβfruct3* mRNA in each sample were analyzed by real-time PCR. The level of *Boβfruct3* mRNA in the shoots precultured in the absence of ABA (control, C) was set to 1 to calculate the relative levels of the *Boβfruct3* mRNA in the ABA-treated samples. The mean values ± SD of three independent experiments are shown. The asterisks indicate significant differences ($p < 0.001$) between a treated sample and the control.

significant roles in the response to cytokinin requires further investigation.

Despite their upregulation by auxin and cytokinin, these two *Boβfruct* genes exhibited different responses to GA, ethylene, JA, and ABA. GAs promote many aspects of plant development, including cell elongation, stem and leaf growth, flowering time, and the development of seeds and fruits.[34−36] The differential responses of these two *Boβfruct* genes to GA correlate well with their differential expression patterns in growing bamboo shoots: *Boβfruct1*, which was not affected by GA, was predominantly expressed in the base culm of the shoot, whereas *Boβfruct2*, which was upregulated by GA, was more ubiquitously expressed in different regions of the shoot, including the elongating internodes and in both sink and source leaves.[16] The GA response complex, which is composed of a pyrimidine box and the GA-response element GARE, and CARE, which is also a GA-responsive element that is involved in the GA-inducible expression of genes,[37−39] were found within the promoter region of *Boβfruct2* (Table 2), which suggests that these elements might be involved in the GA-induced upregulation of *Boβfruct2*.

Ethylene, JA, and ABA are involved in the regulation of diverse plant growth and development and also participate in the plant responses to biotic and abiotic stresses. Differences in the responses to these phytohormones could be related to the differential *Boβfruct1* and *Boβfruct2* expression in different tissues of bamboo. Both *Boβfruct1* and *Boβfruct2* are expressed in the culm base of growing bamboo shoots.[16] The down-regulation of *Boβfruct1* by ethylene and ABA and the unresponsiveness of *Boβfruct2* to these two phytohormones suggest that the unloading of sucrose in the culm base through the cell wall invertases was partly repressed, which might in turn affect the expression of the sugar-modulated genes to adjust the cellular metabolism for stress tolerance or defense. In addition to the culm base, the expression of *Boβfruct2* was also observed in different tissues of growing shoots and in leaves.[16] The upregulation of this gene by JA indicates that *Boβfruct2* might be involved in JA-dependent defense responses to biotic stresses in various tissues of bamboo. Moreover, *Boβfruct2* was upregulated under cold conditions, which suggests that *Boβfruct2* participates in the cold adaption of the bamboo plant.

The most striking feature within the 0.8 kb *Boβfruct1* promoter was the presence of eight copies of CGCG elements. The CGCG elements are present in the promoters of many genes that are related to various functions, including ABA signaling, ethylene signaling, and light perception.[40] In addition to the CGCG elements, other potential *cis*-elements that are associated with phytohormone-related responses, such as ERE (ethylene-responsive element), GCC box (involved in ethylene response), and CE1 (involved in ABA response), were also found in the promoter of *Boβfruct1* (Table 1) but not in the 1.1 kb promoter region of *Boβfruct2*. These elements might contribute to the corresponding downregulation of the expression of *Boβfruct1*.

Based on the expression patterns of the two *Boβfruct* genes that encode cell wall invertases, we conclude that both Boβfruct1 and Boβfruct2 participate in the unloading of sucrose that is translocated from the culture medium or source organs to maintain the sink strength. However, these invertases

also have individual roles. Their gene expressions differentially respond to different environmental factors, which might influence the availability of sucrose from the sources and, in turn, affect the primary metabolism required for the adaption to environmental changes.

Light and ABA Are Important Regulators for the Expression of the Vacuolar Invertase Gene *Boβfruct3*. Vacuolar invertases are known to be major regulators in cell expansion, and high expression levels of vacuolar invertases are associated with a wide range of expanding tissues,[5−8,41] including the elongating internodes of bamboo shoots.[16] In this study, we show that the expression of *Boβfruct3* is upregulated by light, which is one of the most important environmental factors that affect plant growth and development. The light-dependent expression pattern of *Boβfruct3* is consistent with the previous finding that showed that the mRNA levels of this gene in the internodes of etiolated bamboo shoots are much lower than the levels observed in green shoots.[16] The promoter region of *Boβfruct3* contains many light-response-related *cis*-elements, such as the SORLIP-1, SORLIP-2, SORLREP-3, and TATTCT motifs.[42,43] Although *Boβfruct1* and *Boβfruct2* share some light-response elements with *Boβfruct3*, only *Boβfruct3* was significantly regulated by light. This result indicates that the predicted light-response elements in *Boβfruct1* and *Boβfruct2* were not functional and that the light-induced expression of *Boβfruct3* might involve multiple *cis*-elements. Moreover, the rapid decrease (within one hour) in the *Boβfruct3* mRNA levels upon the transfer of the shoots from light to dark conditions suggests that the transcription of *Boβfruct3* was suppressed and that the stability of the transcripts was affected.

In addition to light, the expression of *Boβfruct3* is upregulated by ABA. ABA not only controls plant growth and development, such as seed maturation and dormancy, but also participates in plant adaption to environmental stresses, such as dehydration and other stresses that ultimately cause the desiccation of the cell and osmotic imbalance.[44] Many genes involved in the production of osmoprotectants, including those genes that encode vacuolar invertases, are induced by ABA.[45−47] The known promoters of the ABA-induced genes often contain the conserved ABA-responsive element ABRE. The pairing of ABRE−ABRE or the coupling of ABRE with other elements (such as CE1 and CE3) is required for successful ABA-induced gene expression (ref 48 and references therein). An ABRE and two copies of CE1 were found within the 0.9 kb promoter region of *Boβfruct3*. In addition, other elements involved in ABA-modulated gene expression, including the DPBF binding element and the MYB and MYC binding elements, were also found in the *Boβfruct3* promoter. The presence of these elements correlated well with the ABA-induced gene expression that was observed.

Although it is known that vacuolar invertases in some plant species are regulated by ABA,[45,46,49,50] new insights regarding this regulation were obtained in this study: 1. The transcription of *Boβfruct3* was induced in the dark upon the application of ABA, which suggests that ABA signaling can regulate the expression of *Boβfruct3* independent of light signaling. 2. ABA and light showed an additive effect on the expression of *Boβfruct3*, which might indicate the existence of interactions between the *trans*-acting factors that are responsible for light signaling and ABA signaling. These results indicate that *Boβfruct3* expression is finely tuned by light and ABA-related environmental changes and also support the existence of cross-talk between the signaling pathways that respond to light and ABA, which have been reported by a number of studies.[51−54]

In summary, we have shown that the transcript levels of the three acid invertase genes in bamboo are differentially regulated by phytohormones and environmental factors. These regulations might involve the putative *cis*-elements that are present in the promoter regions of these genes. However, it should be noted that the regulation of plant invertases can occur at multiple levels, including post-translational modifications and the regulation by protein factors.[3,55−57] Several putative glycosylation sites were found in each of the three bamboo acid invertase genes.[16] In addition, we also found conserved motifs for SUMOylation in the amino acid sequences of these three invertases (Figure S4). An *in vitro* SUMOylation assay of the recombinant Boβfruct2 proteins that were expressed and purified from *P. pastoris* showed that the recombinant proteins were able to be SUMOylated (data not shown); however, whether this modification occurs *in planta* remains unknown. Nevertheless, the results of this study show that the expressions of different isoforms of acid invertases in bamboo are under complex regulation, which highlights the importance of these invertases in the growth, development, metabolism, and stress responses of this plant. Moreover, the promoter sequences and expression data presented here also lay a foundation for future studies of the mechanisms involved in regulation of bamboo growth, which can be further applied in other agriculturally important plants to improve their growth and development.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table S1: Primers used in this study. Table S2: Summary of the effects of different environmental factors and phytohormones on the transcript levels of *Boβfruct1*, *Boβfruct2*, and *Boβfruct3* in multiple shoots of bamboo cultured *in vitro*. Figure S1: Nucleotide sequence of the promoter region of *Boβfruct1*. Figure S2: Nucleotide sequence of the promoter region of *Boβfruct2*. Figure S3: Nucleotide sequence of the promoter region of *Boβfruct3*. Figure S4: Prediction of possible SUMOylation sites in the deduced amino acid sequences of *Boβfruct1*, *Boβfruct2*, and *Boβfruct3*. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

ABA, abscisic acid; ABRE, ABA-responsive element; ACC, 1-aminocyclopropane-1-carboxylic acid; BA, 6-benzylaminopurine; 2,4-D, 2,4-dichlorophenoxyacetic acid; ERE, ethylene-responsive element; GA, gibberellic acid; GARE, GA-responsive element; IAA, indole-3-acetic acid; JA, jasmonic acid; UDP, uridine diphosphate; SURE, sucrose responsive element; TDZ, thidiazuron

REFERENCES

(1) Koch, K. E. Carbohydrate-modulated gene expression in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1996**, *47*, 509−540.

(2) Smeekens, S. Sugar-induced signal transduction in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2000**, *51*, 49−81.

(3) Sturm, A. Invertase. Primary structures, functions, and roles in plant development and sucrose partitioning. *Plant Physiol.* **1999**, *121*, 1−8.

(4) Roitsch, T.; Gonzalez, M. C. Function and regulation of plant invertases: sweet sensations. *Trends Plant Sci.* **2004**, *9*, 606−13.

(5) Andersen, M. N.; Asch, F.; Wu, Y.; Jensen, C. R.; Naested, H.; Mogensen, V. O.; Koch, K. E. Soluble invertase expression is an early target of drought stress during the critical, abortion-sensitive phase of young ovary development in maize. *Plant Physiol.* **2002**, *130*, 591−604.

(6) Tang, G. Q.; Luscher, M.; Sturm, A. Antisense repression of vacuolar and cell wall invertase in transgenic carrot alters early plant development and sucrose partitioning. *Plant Cell* **1999**, *11*, 177−189.

(7) Gonzalez, M. C.; Roitsch, T.; Cejudo, F. J. Circadian and developmental regulation of vacuolar invertase expression in petioles of sugar beet plants. *Planta* **2005**, *222*, 386−395.

(8) Wang, L.; Li, X. R.; Lian, H.; Ni, D. A.; He, Y. K.; Chen, X. Y.; Ruan, Y. L. Evidence that high activity of vacuolar invertase is required for cotton fiber and *Arabidopsis* root elongation through osmotic dependent and independent pathways, respectively. *Plant Physiol.* **2010**, *154*, 744−756.

(9) Sturm, A.; Tang, G. Q. The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. *Trends Plant Sci.* **1999**, *4*, 401−407.

(10) Roitsch, T.; Balibrea, M. E.; Hofmann, M.; Proels, R.; Sinha, A. K. Extracellular invertase: key metabolic enzyme and PR protein. *J. Exp. Bot.* **2003**, *54*, 513−524.

(11) Ruan, Y. L.; Jin, Y.; Yang, Y. J.; Li, G. J.; Boyer, J. S. Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. *Mol. Plant* **2010**, *3*, 942−955.

(12) Huang, L. F.; Bocock, P. N.; Davis, J. M.; Koch, K. E. Regulation of invertase: a 'suite' of transcriptional and post-transcriptional mechanisms. *Funct. Plant Biol.* **2007**, *34*, 499−507.

(13) Rabot, A.; Henry, C.; Ben Baaziz, K.; Mortreau, E.; Azri, W.; Lothier, J.; Hamama, L.; Boummaza, R.; Leduc, N.; Pelleschi-Travier, S.; Le Gourrierec, J.; Sakr, S. Insight into the role of sugars in bud burst under light in the rose. *Plant Cell Physiol.* **2012**, *53*, 1068−1082.

(14) Hyun, T. K.; Hoffmann, A.; Sinha, A. K.; Roitsch, T. Tomato mitogen activated protein kinases regulate the expression of extracellular invertase *Lin6* in response to stress related stimuli. *Funct. Plant Biol.* **2009**, *36*, 1088−1097.

(15) Chiu, W.-B.; Lin, C.-H.; Chang, C.-J.; Hsieh, M.-H.; Wang, A.-Y. Molecular characterization and expression of four cDNAs encoding sucrose synthase from green bamboo *Bambusa oldhamii*. *New Phytol.* **2006**, *170*, 53−63.

(16) Hsieh, C. W.; Liu, L. K.; Yeh, S. H.; Chen, C. F.; Lin, H. I.; Sung, H. Y.; Wang, A. Y. Molecular cloning and functional identification of invertase isozymes from green bamboo *Bambusa oldhamii*. *J. Agric. Food Chem.* **2006**, *54*, 3101−3107.

(17) Lin, C. S.; Kalpana, K.; Chang, W. C.; Lin, N. S. Improving mutiple shoot proliferation in bamboo mosaic virus-free *Bambusa oldhamii* Munro propagation by liquid culture. *Hortscience* **2007**, *42*, 1243−1246.

(18) Chen, C. Y.; Hsieh, M. H.; Yang, C. C.; Lin, C. S.; Wang, A. Y. Analysis of the cellulose synthase genes associated with primary cell wall synthesis in *Bambusa oldhamii*. *Phytochemistry* **2010**, *71*, 1270−1279.

(19) Yeh, S. H.; Lin, C. S.; Wu, F. H.; Wang, A. Y. Analysis of the expression of *BohLOL1*, which encodes an LSD1-like zinc finger protein in *Bambusa oldhamii*. *Planta* **2011**, *234*, 1179−1189.

(20) Bookout, A. L.; Cummins, C. L.; Mangelsdorf, D. J.; Pesola, J. M.; Kramer, M. F. High-throughput real-time quantitative reverse transcription PCR. *Curr. Protoc. Mol. Biol.* **2006**, Chapter 15, Unit 15 8.

(21) Godt, D. E.; Roitsch, T. Regulation and tissue-specific distribution of mRNAs for three extracellular invertase isoenzymes of tomato suggests an important function in establishing and maintaining sink metabolism. *Plant Physiol.* **1997**, *115*, 273−282.

(22) Tymowska-Lalanne, Z.; Kreis, M. Expression of the *Arabidopsis thaliana* invertase gene family. *Planta* **1998**, *207*, 259−265.

(23) Cho, J. I.; Lee, S. K.; Ko, S.; Kim, H. K.; Jun, S. H.; Lee, Y. H.; Bhoo, S. H.; Lee, K. W.; An, G.; Hahn, T. R.; Jeon, J. S. Molecular cloning and expression analysis of the cell-wall invertase gene family in rice (*Oryza sativa L.*). *Plant Cell Rep.* **2005**, *24*, 225−236.

(24) Proels, R. K.; Roitsch, T. Extracellular invertase *LIN6* of tomato: a pivotal enzyme for integration of metabolic, hormonal, and stress signals is regulated by a diurnal rhythm. *J. Exp. Bot.* **2009**, *60*, 1555−1567.

(25) Grierson, C.; Du, J. S.; de Torres Zabala, M.; Beggs, K.; Smith, C.; Holdsworth, M.; Bevan, M. Separate *cis* sequences and *trans* factors direct metabolic and developmental regulation of a potato tuber storage protein gene. *Plant J.* **1994**, *5*, 815−826.

(26) Sun, C.; Palmqvist, S.; Olsson, H.; Boren, M.; Ahlandsberg, S.; Jansson, C. A novel WRKY transcription factor, SUSIBA2, participates in sugar signaling in barley by binding to the sugar-responsive elements of the *iso1* promoter. *Plant Cell* **2003**, *15*, 2076−2092.

(27) Ishiguro, S.; Nakamura, K. Characterization of a cDNA encoding a novel DNA-binding protein, SPF1, that recognizes SP8 sequences in the 5′ upstream regions of genes coding for sporamin and beta-amylase from sweet potato. *Mol. Gen. Genet.* **1994**, *244*, 563−571.

(28) Morita, A.; Umemura, T.; Kuroyanagi, M.; Futsuhara, Y.; Perata, P.; Yamaguchi, J. Functional dissection of a sugar-repressed α-amylase gene (*RAmy1 A*) promoter in rice embryos. *FEBS Lett.* **1998**, *423*, 81−85.

(29) Sakai, H.; Aoyama, T.; Oka, A. *Arabidopsis* ARR1 and ARR2 response regulators operate as transcriptional activators. *Plant J.* **2000**, *24*, 703−711.

(30) Ross, E. J.; Stone, J. M.; Elowsky, C. G.; Arredondo-Peter, R.; Klucas, R. V.; Sarath, G. Activation of the *Oryza sativa* non-symbiotic haemoglobin-2 promoter by the cytokinin-regulated transcription factor, ARR1. *J. Exp. Bot.* **2004**, *55*, 1721−1731.

(31) Argyros, R. D.; Mathews, D. E.; Chiang, Y. H.; Palmer, C. M.; Thibault, D. M.; Etheridge, N.; Argyros, D. A.; Mason, M. G.; Kieber, J. J.; Schaller, G. E. Type B response regulators of *Arabidopsis* play key roles in cytokinin signaling and plant development. *Plant Cell* **2008**, *20*, 2102−2116.

(32) Taniguchi, M.; Sasaki, N.; Tsuge, T.; Aoyama, T.; Oka, A. ARR1 directly activates cytokinin response genes that encode proteins with diverse regulatory functions. *Plant Cell Physiol.* **2007**, *48*, 263−277.

(33) Argueso, C. T.; Raines, T.; Kieber, J. J. Cytokinin signaling and transcriptional networks. *Curr. Opin. Plant Biol.* **2010**, *13*, 533−539.

(34) Richards, D. E.; King, K. E.; Ait-Ali, T.; Harberd, N. P. How gibberellin regulates plant growth and development: a molecular genetic analysis of gibberellin signaling. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2001**, *52*, 67−88.

(35) Sun, T. P.; Gubler, F. Molecular mechanism of gibberellin signaling in plants. *Annu. Rev. Plant Biol.* **2004**, *55*, 197−223.

(36) Swain, S. M.; Singh, D. P. Tall tales from sly dwarves: novel functions of gibberellins in plant development. *Trends Plant Sci.* **2005**, *10*, 123−129.

(37) Cercos, M.; Gomez-Cadenas, A.; Ho, T. H. Hormonal regulation of a cysteine proteinase gene, *EPB-1*, in barley aleurone layers: *cis*- and *trans*-acting elements involved in the co-ordinated gene expression regulated by gibberellins and abscisic acid. *Plant J.* **1999**, *19*, 107−118.

(38) Gubler, F.; Kalla, R.; Roberts, J. K.; Jacobsen, J. V. Gibberellin-regulated expression of a *myb* gene in barley aleurone cells: evidence for Myb transactivation of a high-pI α-amylase gene promoter. *Plant Cell* **1995**, *7*, 1879−1891.

(39) Sutoh, K.; Yamauchi, D. Two *cis*-acting elements necessary and sufficient for gibberellin-upregulated proteinase expression in rice seeds. *Plant J.* **2003**, *34*, 635−645.

(40) Yang, T.; Poovaiah, B. W. A calmodulin-binding/CGCG box DNA-binding protein family involved in multiple signaling pathways in plants. *J. Biol. Chem.* **2002**, *277*, 45049−45058.
(41) McLaughlin, J. E.; Boyer, J. S. Sugar-responsive gene expression, invertase activity, and senescence in aborting maize ovaries at low water potentials. *Ann. Bot.* **2004**, *94*, 675−689.
(42) Thum, K. E.; Kim, M.; Morishige, D. T.; Eibl, C.; Koop, H. U.; Mullet, J. E. Analysis of barley chloroplast *psbD* light-responsive promoter elements in transplastomic tobacco. *Plant Mol. Biol.* **2001**, *47*, 353−366.
(43) Hudson, M. E.; Quail, P. H. Identification of promoter motifs involved in the network of phytochrome A-regulated gene expression by combined analysis of genomic sequence and microarray data. *Plant Physiol.* **2003**, *133*, 1605−1616.
(44) Mahajan, S.; Tuteja, N. Cold, salinity and drought stresses: an overview. *Arch. Biochem. Biophys.* **2005**, *444*, 139−158.
(45) Trouverie, J.; Chateau-Joubert, S.; Thevenot, C.; Jacquemot, M. P.; Prioul, J. L. Regulation of vacuolar invertase by abscisic acid or glucose in leaves and roots from maize plantlets. *Planta* **2004**, *219*, 894−905.
(46) Trouverie, J.; Thevenot, C.; Rocher, J. P.; Sotta, B.; Prioul, J. L. The role of abscisic acid in the response of a specific vacuolar invertase to water stress in the adult maize leaf. *J. Exp. Bot.* **2003**, *54*, 2177−2186.
(47) Yamada, K.; Osakabe, Y.; Mizoi, J.; Nakashima, K.; Fujita, Y.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Functional analysis of an *Arabidopsis thaliana* abiotic stress-inducible facilitated diffusion transporter for monosaccharides. *J. Biol. Chem.* **2010**, *285*, 1138−1146.
(48) Fujita, Y.; Fujita, M.; Shinozaki, K.; Yamaguchi-Shinozaki, K. ABA-mediated transcriptional regulation in response to osmotic stress in plants. *J. Plant Res.* **2011**, *124*, 509−525.
(49) Greiner, S.; Rausch, T.; Sonnewald, U.; Herbers, K. Ectopic expression of a tobacco invertase inhibitor homolog prevents cold-induced sweetening of potato tubers. *Nat. Biotechnol.* **1999**, *17*, 708−711.
(50) Pelleschi, S.; Guy, S.; Kim, J. Y.; Pointe, C.; Mahe, A.; Barthes, L.; Leonardi, A.; Prioul, J. L. *Ivr2*, a candidate gene for a QTL of vacuolar invertase activity in maize leaves. Gene-specific expression under water stress. *Plant Mol. Biol.* **1999**, *39*, 373−380.
(51) Seung, D.; Risopatron, J. P.; Jones, B. J.; Marc, J. Circadian clock-dependent gating in ABA signalling networks. *Protoplasma* **2012**, *249*, 445−457.
(52) Moriwaki, T.; Miyazawa, Y.; Fujii, N.; Takahashi, H. Light and abscisic acid signalling are integrated by MIZ1 gene expression and regulate hydrotropic response in roots of Arabidopsis thaliana. *Plant Cell Environ.* **2012**, *35*, 1359−1368.
(53) Chen, H.; Zhang, J.; Neff, M. M.; Hong, S. W.; Zhang, H.; Deng, X. W.; Xiong, L. Integration of light and abscisic acid signaling during seed germination and early seedling development. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 4495−4500.
(54) Robertson, F. C.; Skeffington, A. W.; Gardner, M. J.; Webb, A. A. Interactions between circadian and hormonal signalling in plants. *Plant Mol. Biol.* **2009**, *69*, 419−427.
(55) Krausgrill, S.; Sander, A.; Greiner, S.; Weil, M.; Rausch, T. Regulation of cell wall invertase by a proteinaceous inhibitor. *J. Exp. Bot.* **1996**, *47* Spec No, 1193−1198
(56) Bonfig, K. B.; Gabler, A.; Simon, U. K.; Luschin-Ebengreuth, N.; Hatz, M.; Berger, S.; Muhammad, N.; Zeier, J.; Sinha, A. K.; Roitsch, T. Post-translational derepression of invertase activity in source leaves via down-regulation of invertase inhibitor expression is part of the plant defense response. *Mol. Plant* **2010**, *3*, 1037−1048.
(57) McKenzie, M. J.; Chen, R. K.; Harris, J. C.; Ashworth, M. J.; Brummell, D. A. Post-translational regulation of acid invertase activity by vacuolar invertase inhibitor affects resistance to cold-induced sweetening of potato tubers. *Plant Cell Environ.* **2013**, *36*, 176−185.
(58) Niu, X.; Helentjaris, T.; Bate, N. J. Maize ABI4 binds coupling element1 in abscisic acid and sugar response genes. *Plant Cell* **2002**, *14*, 2565−2575.
(59) Cakir, B.; Agasse, A.; Gaillard, C.; Saumonneau, A.; Delrot, S.; Atanassova, R. A grape ASR protein involved in sugar and abscisic acid signaling. *Plant Cell* **2003**, *15*, 2165−2180.