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Original Paper

Occurrence of hydroxycinnamoylputrescines in xylogenic bamboo suspension cells

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Abstract To explore the chemical basis of xylogenesis in bamboo cells, we compared secondary metabolite compositions between xylogenic and non-xylogenic suspension cell cultures of bamboo (*Phyllostachys nigra*), which we developed previously. Two compounds, one major and one minor, showed large-scale increases in the cells cultured under two lignification (xylogenic) conditions, compared with cells cultured under proliferation (non-xylogenic) conditions. Based on spectroscopic analyses, the major compound was identified as feruloylputrescine (FP) and the minor compound was identified as *p*-coumaroylputrescine (pCP). We examined the accumulation profiles of these hydroxycinnamic acid amides during a 16-day culture period. When cells were kept in proliferation conditions for 16 days, the contents of FP and pCP peaked at 2 days (0.32 and 0.25 nmol mg^{-1} fresh weight, respectively) and decreased to trace levels thereafter. In contrast, the FP content increased throughout the 16-day culture period, reaching maximum levels of 4.3 and 6.8 nmol mg^{-1} fresh weight in the two xylogenic conditions. The pCP content was lower than that of FP under both xylogenic conditions. The pattern of FP accumulation resembled that of lignin accumulation, as monitored by phloroglucinol-HCl staining. It is likely that FP plays a role in xylogenesis in suspension-cultured bamboo cells.

Key words: Bamboo, hydroxycinnamic acid amide, lignification, secondary metabolite, suspension culture.

Bamboos are perennial lignified plants belonging to the Bambusoideae, a subfamily of the family Poaceae. They have attracted much interest because of their rapid growth and their characteristic flowering behavior. The growth features of bamboo culms have been analyzed in many studies, which have focused on their anatomical, physical, chemical, and mechanical properties (Fengel and Shao 1985; Liese and Weiner 1996; Suzuki and Itoh 2001). However, the use of bamboo plants as experimental materials prevents detailed biochemical and physiological characterization of some growth features such as cell division and cell wall development, including lignification, because of the non-uniform nature of the tissues, as well as their size, hardness, and life cycle in the field environment.

To avoid these difficulties, we previously established an efficient callus and suspension culture system for *Phyllostachys* bamboos, including *Phyllostachys nigra* Munro var. *Henonis* (hachiku-bamboo) and *Phyllostachys bambusoides* Sieb. Et Zucc. (madake-bamboo). Cells in these cultures proliferate in a highly synchronous manner and show uniform morphological features (Ogita 2005; Ogita et al. 2012c). We also established highly efficient particle bombardment transformation protocols for suspension-cultured cells of *P. nigra* and *P. bambusoides* (Ogita et al. 2011, 2012a). To study the sequential biological events in xylogenesis in living bamboo cells, we developed a culture system that promotes a high degree of lignification in *P. nigra* suspension cells (Ogita et al. 2012b). In parallel, we established culture conditions that promote rapid cell proliferation without lignin deposition (Ogita et al. 2011). Those culture systems enabled us to explore the mechanisms of xylogenesis in bamboo cells by comparative analyses of cells in distinct developmental phases, i.e., proliferation and lignification. We demonstrated that transcript levels of some key genes associated with early stages of lignin biosynthesis, such as *phenylalanine ammonia-lyase* (*PAL*), *cinnamate 4-hydroxylase* (*C4H*), *caffeoyl-CoA 3-O-methyltransferase* (*CCoAOMT*), and *cinnamoyl-CoA reductase* (*CCR*), were enhanced under lignification conditions (Ogita et al.

Abbreviations: BA, 6-benzyladenine; pCP, *p*-coumaroylputrescine; FP, feruloylputrescine; LG, lignification condition; PR, proliferation condition; Rt, retention time; SCV, sedimented cell volume.
The suspension-cultured bamboo cells used in this study are currently available as Pn (rpc00047) from the RIKEN Bioresource Center (http://www.brc.riken.jp/lab/epd/Eng).
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2012b). Although changes in the levels of some chemical components, such as sugars, amino acids, cellulose and lignin, have been analyzed during growth of bamboo plants (Fengel and Shao 1985; Fujii et al. 1993, 1996; Itoh 1990), there is little information about the biosynthesis of lignin and related secondary metabolites during xylogenesis. In this study, we used the suspension-culture models to examine the composition of secondary metabolites in xylogenic and non-xylogenic bamboo cells. We identified hydroxycinnamoylputrescines as major secondary compounds in xylogenic cells. Their involvement in xylogenesis in bamboo cells is discussed.

Materials and methods

General procedures

NMR spectra were recorded on a Bruker AVANCE 400 spectrometer (Bruker, Karlsruhe, Germany). UV spectra were measured on an Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan). Ion-spray mass spectra were measured on a Bruker microTOF focus spectrometer.

Cell cultures

Bamboo (*P. nigra*) suspension cells (Ogita 2005) were maintained in modified Murashige and Skoog (MS) liquid medium (Murashige and Skoog 1962) supplemented with 680 mg l^{-1} KH_2PO_4, 10 μM 4-amino-3,5,6-trichloropyridine-2-carboxylic acid (Picloram), and 3% (w/v) sucrose. This medium strongly promotes the proliferation of bamboo cells without lignification (Ogita et al. 2011), and is hereafter referred to as proliferation (PR) conditions. The cells were subcultured in 100 ml liquid medium in a 300-ml flask and maintained on a rotary shaker (110 rpm) in the dark at 25°C. To maintain stable morphology and synchronous growth of the cells, the sedimented cell volume (SCV) was adjusted to 2.5% every 2 weeks as described by Ogita et al. (2011). To promote lignification in the cells, 2-week-old cells cultured under PR conditions were transferred to the following fresh liquid media and cultured as described above: half-strength MS medium containing 3% (w/v) sucrose (lignification-1; LG1 conditions) and half-strength MS medium supplemented with 10 μM 6-benzyladenine (BA) and 3% (w/v) sucrose (lignification-2; LG2 conditions) (Ogita et al. 2012b).

Analysis of secondary metabolites in bamboo cell extracts

Cells (8-days old) cultured under PR, LG1, and LG2 conditions were collected onto filter paper by vacuum filtration. A portion of the cells obtained (100–150 mg) was extracted with 10 vol. MeOH containing 2% AcOH under ultrasonication for 10 min at room temperature. After centrifugation (21,500×g, 10 min, 4°C), the supernatant was analyzed by reversed-phase HPLC (column, Mightysil RP-18 GP Aqua, 5 μm, 4.6×150 mm, Kanto Chemical Co. Inc., Tokyo, Japan; solvent, 14% acetonitrile containing 0.1% trifluoroacetic acid; flow rate, 0.8 ml min^{-1}; detection, 280 nm; column temperature, 35°C). The HPLC peaks corresponding to *p*-coumaroylputrescine (pCP) and feruloylputrescine (FP) were identified by comparing their HPLC retention times, positive ion-spray mass spectra, and UV spectra with those of synthetic standards (see below for preparation). To identify FP, the compound was isolated from cell extracts and its NMR spectrum was compared with that of a synthetic standard.

Purification of FP from bamboo cell extracts

Bamboo cells (6-days old) were collected from 1 l culture onto filter paper by vacuum filtration. The collected cells (97.4 g) were extracted with 1 l MeOH containing 2% AcOH under ultrasonication for 1 h at room temperature. The cell residues were removed by filtration, and then the extract was concentrated in vacuo and washed three times with hexane. The MeOH layer was concentrated to a small volume, passed through a membrane filter (Millex-HV, 0.45 μm, Millipore), and subjected to reversed-phase preparative HPLC (column, TSKgel ODS-80Ts, 5 μm, 20×250 mm, Tosoh Corp., Tokyo, Japan; solvent, 14% acetonitrile containing 0.1% trifluoroacetic acid; flow rate, 5 ml min^{-1}; detection, 280 nm). The collected fraction was concentrated and lyophilized to yield 58.5 mg FP (trifluoroacetic acid salt): UV λ_{max} (MeOH): nm (relative intensity): 217 (83), 233 (77), 294 (78), 319 (100); ion-spray MS (positive ion mode), m/z (relative intensity): 265 $[M+H]^+$ (100), 177 $[M-C_4H_{11}N_2]^+$ (14); ^{1}H-NMR (400 MHz, CD_3OD): δ (ppm) 1.61–1.75 (4H, m, H-2, 3), 2.97 (2H, t, J=7.2 Hz, H-4), 3.34 (2H, t, J=6.6 Hz, H-1), 3.89 (3H, s, $-OCH_3$), 6.43 (1H, d, J=15.7 Hz, H-8′), 6.80 (1H, d, J=8.2 Hz, H-5′), 7.03 (1H, dd, J=8.2 Hz, 1.9 Hz, H-6′), 7.12 (1H, d, J=1.9 Hz, H-2′), 7.45 (1H, d, J=15.7 Hz, H-7′); ^{13}C-NMR (100 MHz, CD_3OD): δ (ppm) 25.9 (C-2 or 3), 27.6 (C-2 or 3), 39.6 (C-1 or 4), 40.4 (C-1 or 4), 56.4 ($-OCH_3$), 111.6 (C-2′), 116.5 (C-8′), 118.6 (C-5′), 123.3 (C-6′), 128.2 (C-1′), 142.3 (C-7′), 149.4 (C-3′), 150.0 (C-4′), 169.4 (C-9′).

Preparation of synthetic FP and pCP

FP and pCP were synthesized according to Matsuda et al. (2005) with some modifications. *N*,*N*′-Dicyclohexylcarbodiimide (255 mg, 1.24 mmol) was added to an ice-cold solution of ferulic acid (194 mg, 1 mmol) and *N*-(*tert*-butoxycarbonyl)-1,4-butanediamine (Boc-putrescine, 188 mg, 1 mmol) in 10 ml pyridine. The mixture was stirred in the dark at room temperature for 24 h. After evaporation of pyridine, the residue was suspended in MeOH (10 ml), and dicyclohexylurea was removed by filtration. The solvent was removed by evaporation and the residue was dissolved in $CHCl_3$ (10 ml), washed with aqueous $NaHCO_3$, and the organic layer was dried over anhydrous sodium sulfate. The concentrated residue was dissolved in AcOEt and passed through a silica gel short column to remove polar impurities. Further purification by silica gel column chromatography using hexane : AcOEt (1 : 2) as eluent yielded Boc-FP as a yellowish syrup (202.9 mg, yield 55.7%). To a solution of Boc-FP (202.9 mg, 0.557 mmol) in

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MeOH (1.25 ml) was added 4 N HCl/AcOEt (10 ml, 40 mmol), and the mixture was stirred overnight in the dark at room temperature. After evaporation of the solvent, the residue was dissolved in water, washed with AcOEt, concentrated in vacuo, and lyophilized to yield FP (HCl salt) as a pale yellow powder (161.7 mg, yield 53.8%): UV λ_{max} (MeOH): nm (relative intensity): 218 (79), 234 (77), 293 (78), 319 (100); ion-spray MS (positive ion mode), *m/z* (relative intensity): 265 $[M+H]^+$ (100), 177 $[M\text{-}C_4H_{11}N_2]^+$ (13); ^{1}H-NMR (400 MHz, CD_3OD): δ (ppm) 1.68–1.76 (4H, m, H-2, 3), 2.96 (2H, t, *J*=7.2 Hz, H-4), 3.35–3.38 (2H, m, H-1), 3.89 (3H, s, $-OCH_3$), 6.48 (1H, d, *J*=15.7 Hz, H-8′), 6.81 (1H, d, *J*=8.2 Hz, H-5′), 7.04 (1H, dd, *J*=8.2 Hz, 1.8 Hz, H-6′), 7.14 (1H, d, *J*=1.7 Hz, H-2′), 7.47 (1H, d, *J*=15.7 Hz, H-7′); ^{13}C-NMR (100 MHz, CD_3OD): δ (ppm) 25.9 (C-2 or 3), 27.5 (C-2 or 3), 39.8 (C-1 or 4), 40.4 (C-1 or 4), 56.5 ($-OCH_3$), 111.6 (C-2′), 116.5 (C-8′), 118.1 (C-5′), 123.5 (C-6′), 128.1 (C-1′), 142.7 (C-7′), 149.4 (C-3′), 150.1 (C-4′), 169.6 (C-9′).

pCP was synthesized from *p*-coumaric acid by the same procedure. Deprotection of Boc-pCP (202.4 mg, 0.606 mmol) yielded pCP (HCl salt) as a pale yellow powder (148.2 mg, yield 54.8%): UV λ_{max} (MeOH): nm (relative intensity): 212 (58), 225 (64), 298 (99), 308 (100); ion-spray MS (positive ion mode), *m/z* (relative intensity): 235 $[M+H]^+$ (100), 147 $[M\text{-}C_4H_{11}N_2]^+$ (17); ^{1}H-NMR (400 MHz, CD_3OD): δ (ppm) 1.67–1.76 (4H, m, H-2, 3), 2.97 (2H, t, *J*=7.0 Hz, H-4), 3.35–3.38 (2H, m, H-1), 6.46 (1H, d, *J*=15.7 Hz, H-8′), 6.81 (2H, d, *J*=8.6 Hz, H-3′, 5′), 7.42 (2H, d, *J*=8.6 Hz, H-2′, 6′), 7.49 (1H, d, *J*=15.7 Hz, H-7′); ^{13}C-NMR (100 MHz, CD_3OD): δ (ppm) 25.9 (C-2 or 3), 27.4 (C-2 or 3), 39.9 (C-1 or 4), 40.4 (C-1 or 4), 116.8 (C-3′, 5′), 117.4 (C-8′), 127.4 (C-1′), 130.8 (C-2′, 6′), 142.8 (C-7′), 160.9 (C-4′), 169.7 (C-9′).

Time-course analysis of hydroxycinnamoylputrescine content and lignification profile

Subcultured suspension cells (2-weeks old) were collected and the cell density was adjusted to 25% SCV using each of the PR, LG1, and LG2 media. Each suspension (10 ml) was transferred to a 300-ml flask containing 90 ml of the corresponding fresh medium. This procedure set the initial cell density to 2.5% SCV. Nine flasks were prepared for each condition. The cultures were maintained on a rotary shaker (110 rpm) in the dark at 25°C. The cells from one flask were collected every other day until 16 days. After measuring the SCV, the cells were extracted and analyzed by HPLC as described above. To monitor the lignification profile of the cells, a portion of the collected cells was fixed and stained with phloroglucinol-HCl reagent (Siegel 1953) in a 96-well microplate as described previously (Ogita et al. 2012c).

Results and discussion

Identification of FP and pCP in bamboo cell extracts

Bamboo suspension cells were cultured under three different conditions; PR, LG1, and LG2. The PR conditions promote cell proliferation (Ogita et al. 2011) while the LG1 and LG2 conditions promote lignification (Ogita et al. 2012b). The effects of these culture conditions on the composition of secondary metabolites were examined using 8-day-old cells. When peaks in the HPLC chromatograms were compared among PR, LG1, and LG2 cell extracts, those at Rt 4.7 and Rt 5.6 min showed marked increases in the LG1 and LG2 cell extracts (Figure 1). In addition, a peak at Rt 7.0 min was detected only in cells cultured in LG2 conditions

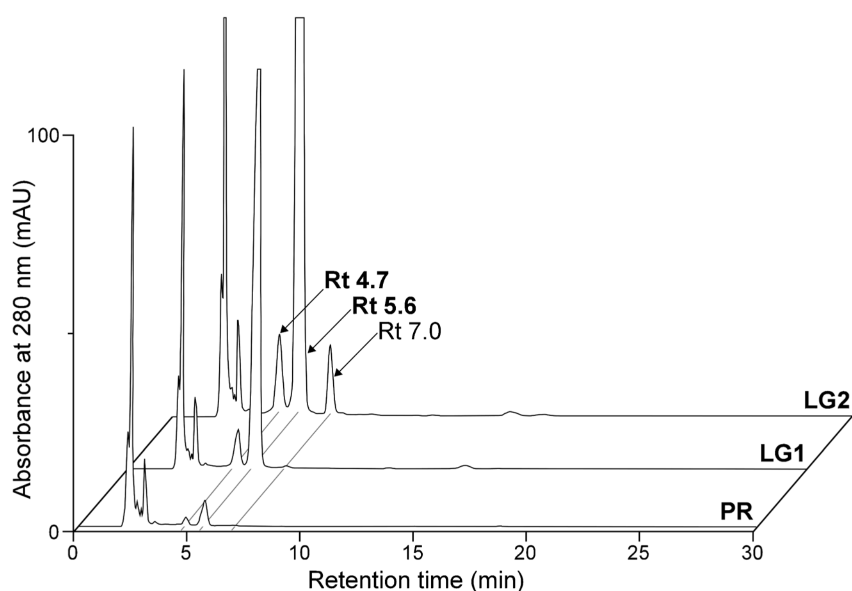

Figure 1. HPLC chromatograms of extracts from 8-day-old bamboo suspension cells cultured under PR, LG1, and LG2 conditions. Peaks at Rt 4.7 and Rt 5.6 min were identified as pCP and FP, respectively. The peak at Rt 7.0 min was predicted to be a metabolite of BA, and was not further characterized in this study.

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(BA-containing medium). Since the peak at Rt 5.6 min showed the greatest increase in both LG1 and LG2 cell extracts, the compound was isolated and its structure was elucidated. Ion-spray MS of the purified compound gave an ion at *m*/*z* 265 as the protonated molecule $[M+H]^+$, and a diagnostic ion at *m*/*z* 177 (Onkokesung et al. 2012), suggesting that this compound has a feruloyl moiety. In the ^{1}H-NMR spectrum, the presence of a *trans* double bond and a 1,3,4-trisubstituted benzene ring was assigned to the feruloyl moiety. The UV absorption spectrum of this compound was almost the same as that of free ferulic acid. Therefore, the rest of the structure was predicted to have no chromophore. ^{1}H-NMR signals of four protons observed at δ 1.61–1.75 ppm indicated the presence of two adjacent methylene groups. Two triplet signals (both for two protons) observed at δ 2.97 and 3.34 ppm were predicted to correspond to other methylene groups, each of which was connected to a methylene group and a nitrogen atom: the former was connected to the nitrogen of a primary amino group, the latter to that of an amide group. Based on these data, the compound eluting at Rt 5.6 min was suggested to be FP (Figure 2). Final confirmation of the structure was obtained by comparing all spectroscopic data and the HPLC retention time with those of synthetic FP.

The compound eluting at Rt 4.7 min gave an ion at *m*/*z* 235 as the protonated molecule $[M+H]^+$, and a diagnostic ion at *m*/*z* 147 (Onkokesung et al. 2012) in the ion-spray MS analysis. It exhibited almost the same UV absorption spectrum as that of free *p*-coumaric acid. Consequently, it was predicted to be pCP (Figure 2). Based on comparisons of ion-spray MS data, the UV absorption spectrum, and the HPLC retention time with those of the synthetic standard, this compound was confirmed to be pCP. The peak at Rt 7.0 min that appeared only in the cells cultured under BA-containing LG2 conditions showed an UV absorption spectrum different from those of hydroxycinnamoyl compounds, but similar to that of BA, although the retention time differed from that of BA (Rt 13.4 min; data not shown). Since exogenously applied BA is known to be metabolized into various conjugated forms, such as nucleosides, nucleotides, and glycosides (Hou et al. 2004; Mok and Mok 2001; Sakakibara 2006), this compound likely corresponds to one of such BA metabolites. Because the focus of our study was on secondary metabolites that appear under both LG conditions, this compound was not further characterized.

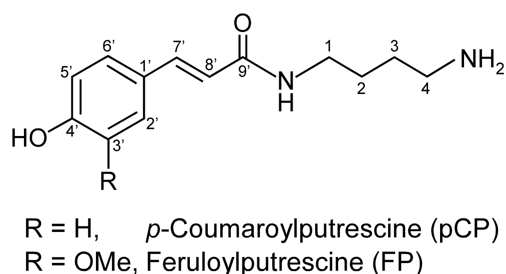

Figure 2. Chemical structures of pCP and FP.

Growth and lignification profiles of suspension cells

Changes in growth profiles of the cells cultured under PR, LG1, and LG2 conditions were monitored by measuring the SCV throughout the culture period (Figure 3A). The SCV of PR cells reached approximately 84% at 16 days, while those of LG1 and LG2 cells reached approximately 31% and 23%, respectively, at 16 days. These results were consistent with our previous findings (Ogita et al. 2011, 2012b).

The lignification profiles of the cells were monitored by staining the cells with phloroglucinol-HCl reagent. As shown in Figure 3B, red staining was observed in cells at later culture stages (12–16 days) under both LG conditions, while no staining was observed in PR cells throughout the 16-day culture period. These results confirmed that the lower cell proliferation rates in LG1 and LG2 conditions than that in PR conditions were because of lowered mitotic activity associated with promoted lignification in bamboo cells under LG1 and LG2 conditions (Ogita et al. 2012b). The lignification

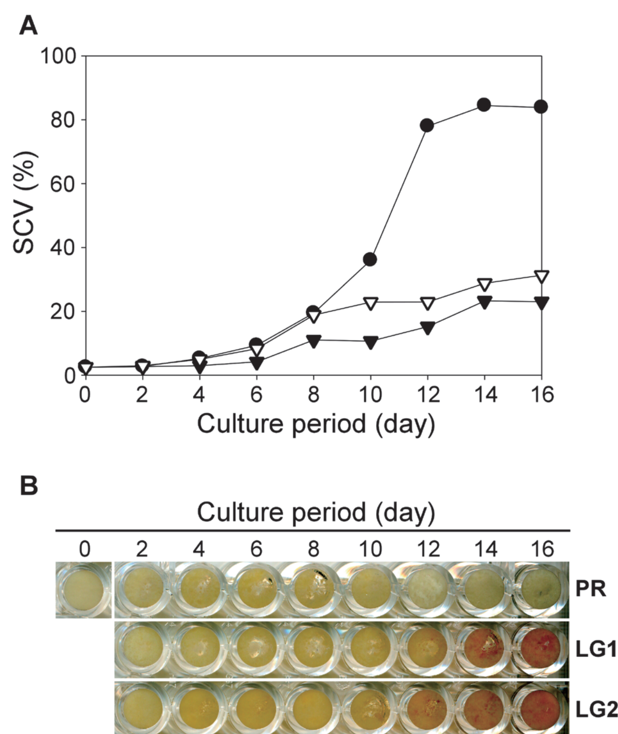

Figure 3. Growth and lignification of bamboo suspension cells. (A) Growth profiles of suspension cells starting from 2.5% SCV (sedimented cell volume per 100 ml medium) under PR (closed circle), LG1 (open triangle), and LG2 (closed triangle) conditions. (B) Lignification profiles of suspension cells cultured under PR, LG1, and LG2 conditions. Cells collected at each time point were stained with phloroglucinol-HCl reagent to detect lignin deposition.

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efficiency was slightly higher in LG2 (BA-containing) conditions than in LG1 conditions, because red staining became visible at earlier stages in cells cultured under LG2 conditions (10–12 days) than under LG1 conditions (12–14 days). These results were consistent with the lignin content of the suspension cells determined in our previous study: 2-week-old cells cultured in the presence and absence of BA showed a lignin content of approximately 25% and 20% of the cell dry weight, respectively (Ogita et al. 2012b).

Changes in hydroxycinnamoylputrescine content under different culture conditions

Since hydroxycinnamic acid amides of putrescine (FP and pCP) were identified as major secondary metabolites in bamboo suspension cells, we next investigated changes in their contents during the 16-day culture period under PR, LG1, and LG2 conditions. Under PR conditions (Figure 4A), the contents of both amides increased at an early stage of culture, peaking at 2 days (0.32 and 0.25 nmol mg^{-1} fresh weight for FP and pCP, respectively), and decreased gradually to trace levels thereafter. Their accumulation profiles under LG1 and LG2 conditions were totally different from those under PR conditions. The FP content greatly increased through the culture period to peak at 4.3 and 6.8 nmol mg^{-1} fresh weight under LG1 and LG2 conditions, respectively (Figure 4B, C). In contrast, the pCP concentration remained low throughout the culture period under both LG conditions, although it was still higher than that under PR conditions. The patterns of FP accumulation under LG conditions were consistent with the lignification profiles of the cells (Figure 3B). In addition, the higher FP content under LG2 conditions than under LG1 conditions was consistent with the higher degree of lignification of LG2 cells. These data suggested that FP is implicated in xylogenesis in bamboo suspension cells.

Hydroxycinnamic acid amides of various amines are widespread throughout the plant kingdom, and are implicated in inducible defense systems because of their toxicity (e.g. Muroi et al. 2009, and references cited therein). In bamboo, it was reported that twigs infected by the witches' broom disease fungus, *Aciculosporium take*, accumulated antifungal *N-p*-coumaroylserotonin and less active *N*-feruloylserotonin, both of which are hydroxycinnamic acid amides of serotonin (Tanaka et al. 2003). In addition, it is generally accepted that hydroxycinnamic acid amides are important cell wall constituents and are involved in the physical reinforcement of cell walls, in which amides cross-link to each other and to polysaccharides and lignin by peroxidase-mediated oxidative coupling (Bassard et al. 2010; Kristensen et al. 2004). This was exemplified by the polymerization of various phenolic substrates, including hydroxycinnamoylputrescines, by a suberization-

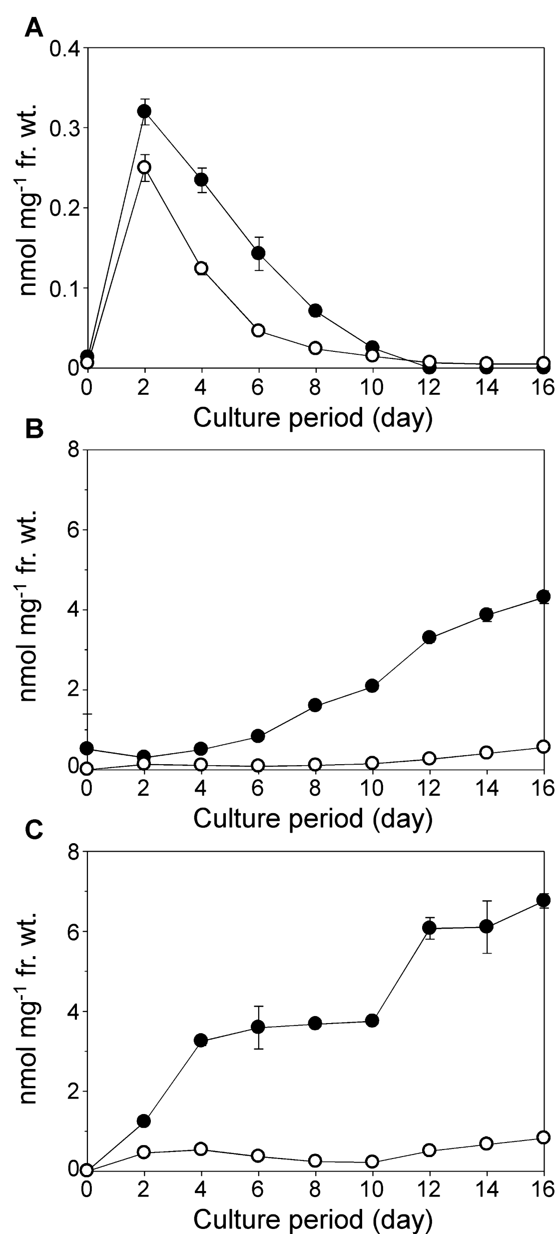

Figure 4. Changes in pCP (open circle) and FP (closed circle) contents in bamboo suspension cells cultured under PR (A), LG1 (B), and LG2 (C) conditions. Data are mean values from triplicate experiments with SD.

associated anionic peroxidase in potato (*Solanum tuberosum*) (Bernards et al. 1999) and incorporation of avenanthramide phytoalexin into the cell wall fraction by apoplastic basic peroxidases in oats (*Avena sativa*) (Okazaki et al. 2004). Muroi et al. (2009) suggested the possibility that hydroxycinnamoylputrescines are incorporated into cell walls of *Arabidopsis thaliana* in response to infection by the pathogenic fungus *Alternaria brassicicola*. Thus, it is likely that FP, which accumulated in parallel with xylogenesis in bamboo cells, also functions as a reinforcing agent for cell walls.

The bamboo suspension-cultured cells used in this study were originally developed from young edible

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shoots (Ogita 2005). Because we found FP and pCP in suspension-cultured cells, we analyzed bamboo shoot extracts for these compounds, but did not detect the corresponding HPLC peaks (data not shown). Presumably, this is attributable to the growth stage of shoots, in which cell proliferation, rather than xylogenesis, is active. Alternatively, it could simply be due to differences in secondary metabolic properties between plant tissues and cultured cells. To confirm that hydroxycinnamoylputrescines are involved in xylogenesis of bamboo culms in nature, analyses of sequential stages of bamboo materials, i.e., from young shoots to mature culms, should be conducted in the future.

Hydroxycinnamic acid amides are biosynthesized through the *N*-coupling reaction of (poly)amines to CoA thioesters of hydroxycinnamic acids. This reaction is catalyzed by enzymes in the BAHD acyltransferase family (D'Auria 2006), except for hydroxycinnamoyl-CoA:tyramine hydroxycinnamoyltransferase in *S. tuberosum* (Schmidt et al. 1999). The enzyme catalyzing the formation of hydroxycinnamic acid amides of putrescine in cultured cells of tobacco (*Nicotiana tabacum*) has been well characterized (Meurer-Grimes et al. 1989; Negrel 1989; Negrel et al. 1991, 1992). The gene encoding the enzyme remained unidentified for a long time; however, Onkokesung et al. (2012) recently reported a gene encoding hydroxycinnamoyl-CoA: putrescine hydroxycinnamoyltransferase, which belongs to the BAHD acyltransferase family, from tobacco. The enzyme, referred to as AT1 in the literature, accepts caffeoyl-CoA and putrescine as the best substrates for the acyl-donor and acyl-acceptor, respectively, which is consistent with the accumulation of caffeoylputrescine as the most abundant putrescine conjugate with hydroxycinnamic acid in tobacco cells and plant tissues (Kaur et al. 2010; Mizusaki et al. 1970, 1971; Negrel 1989; Onkokesung et al. 2012). In the bamboo suspension cells analyzed here, however, the putrescine conjugates were FP and pCP. We carried out analyses, using synthetic standards for comparison, to detect caffeoylputrescine and sinapoylputrescine in extracts from bamboo cells cultured under PR, LG1, and LG2 conditions. However, the corresponding HPLC peaks could not be detected (data not shown). It is still unclear whether FP is synthesized directly from feruloyl-CoA and putrescine or indirectly from caffeoyl-CoA and putrescine in bamboo cells. In the latter case, caffeoylputrescine formed via the activity of acyltransferase needs to be methylated by *O*-methyltransferase to form FP. Considering the fact that guaiacyl lignin accounted for approximately two-thirds of total lignin of bamboo suspension cells cultured under LG2 conditions (Ogita et al. 2012b), the most actively biosynthesized monolignol should be coniferyl alcohol. Thus, in bamboo cells, it seems likely that FP is biosynthesized directly from feruloyl-CoA, an intermediate of coniferyl alcohol biosynthesis, and putrescine. It is of great interest to determine whether the use of caffeoyl-CoA as the primary acyl-donor is a common feature among putrescine hydroxycinnamoyltransferases, or whether the bamboo enzyme utilizes feruloyl-CoA as the preferred substrate. An enzymatic characterization study is now in progress to address this question.

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