Purification of Protein AP-Toxin from *Arthrinium phaeospermum* Causing Blight in *Bambusa pervariabilis* × *Dendrocalamopsis grandis* and Its Metabolic Effects on Four Bamboo Varieties

Shu-Jiang Li, Tian-Hui Zhu, Han-Ming-Yue Zhu, Man Liang, Tian-Min Qiao, Shan Han, and Guan-Nan Che

First, second, fourth, fifth, and seventh authors: College of Forestry, Sichuan Agricultural University, Ya'an 625014, China; second and sixth authors: Key Laboratory of Forest Protection of Sichuan Province, Sichuan Agricultural University, Ya'an 625014, China; and third author: Department of Foreign Affairs Administration, Chengdu Institute, Sichuan International Studies University, Chengdu 611731, China. Accepted for publication 15 October 2012.

ABSTRACT

Li, S.-J., Zhu, T.-H., Zhu, H.-M.-Y., Liang, M., Qiao, T.-M., Han, S., and Che, G.-N. 2013. Purification of protein AP-toxin from *Arthrinium phaeospermum* causing blight in *Bambusa pervariabilis* × *Dendrocalamopsis grandis* and its metabolic effects on four bamboo varieties. *Phytopathology* 103:135-145.

Bambusa pervariabilis × *Dendrocalamopsis grandis* blight is caused by a toxin produced by the fungus *Arthrinium phaeospermum*. In this study, a toxin fraction (P1-2-2) with an estimated molecular mass of 31 kDa was purified from a culture filtrate of this fungus by ammonium sulfate precipitation, Sephadex G-50 gel chromatography, Q Sepharose Fast Flow anion exchange resin, and Sephadex G-75 chromatography. The N-terminal amino acid sequence (i.e., H₂N-Gln-Val-Arg-Asp-Arg-Leu-Glu-Ser-Thr) determined by Edman degradation showed homology

to known serine alkaline proteases. The purified protein was named AP-toxin. Effects of the purified protein toxin on total phenol, flavonoid, total nucleic acid, DNA, RNA, soluble protein, and soluble sugar content, as well as DNase and RNase activities and disease index, were analyzed in different bamboo varieties by the impregnation method. The toxin had a significant effect on each parameter tested. In addition, a significant correlation was observed among the metabolic index, treatment time, bamboo resistance, and disease index. These data suggest that AP-toxin plays an important role in mediating the phytotoxic activities of *A. phaeospermum*. This study also indicates that metabolic indices could reflect the resistance indices of hybrid bamboo to blight.

Additional keywords: disease resistance, phytotoxic activity.

Bambusa pervariabilis × *Dendrocalamopsis grandis* is an improved hybrid bamboo species derived from inbred parental lines *B. pervariabilis* McClure and *D. daii* Keng f. This bamboo is cultivated in southern China for paper production, and bamboo forests form a natural barrier against soil erosion in the Changjiang River basin. In forest reclamation regions (converted farmlands) in the middle and upper reaches of the river, covering an area of >3,000 hm² (70), a pathogenic fungus, *Arthrinium phaeospermum* (Corda) M. B. Ellis, was discovered to cause wilting and death of hybrid bamboo. Studies (70,71) have reported that *A. phaeospermum* conidiospores are the primary source of infection that occurs primarily in April to May, leading to an epidemic in August to September, with an overwintering stage in October. This shows regularity in the timing of infection and disease progression. Despite the importance of this disease, little is known about the infection mechanism of this fungus.

Phytotoxins are, in general, recognized as major pathogenic factors in host plant infection (3). Previous studies have demonstrated the damage caused by fungal toxins on the structure and, therefore, the functions of plasmalemma, cell nucleus, mitochondria, chloroplasts, ribosomes, and so on. (8,22,32,42,65). Helminthosporal toxin produced by *Helminthosporium sativum* was shown to inhibit electrical conductivity and oxidative phos-

phorylation in the mitochondria of *Bipolaris sorokiniana* (13). In addition, tentoxin from *Alternaria alternata* was also reported to inhibit the end reaction of photophosphorylation and electrical conductivity in chloroplasts (25). Pathogenic fungi can produce various toxins with unique chemical structures and physiological activities, and a number of studies have also reported their effects on plants at the molecular level (47,53). For example, reduced ATP synthesis was due to the combined effect of phytotoxins and coupling factor in chloroplasts (24); DNA strand breakage by aflatoxins and trichodermins (16,66); and disruption in the regulation of peroxidase, superoxide dismutase, and phenylalanine ammonia-lyase activities by helminthosporal toxin from *B. sorokiniana* (13). Most studies on fungal toxins have focused primarily on alkaloids (12,28), polysaccharides (including glycoproteins) (1), reductones (46,63), and terpenoid compounds (48). In particular, protein toxins such as Ap-toxin (45), Vd-toxin (39), Ptr ToxA (50), and Ptr ToxB (6) have been studied in depth. Previously, we showed that the symptoms caused by *Arthrinium phaeospermum* protein toxins are similar to blight disease in hybrid bamboo (27). However, characteristics of *A. phaeospermum* phytotoxins that contribute to bamboo wilting disease are not known yet.

Many plant-pathogen interactions are initiated at the cell surface by molecular recognition, followed by signal transduction into cells and induction of plant metabolic responses (49), which include changes in (i) water metabolism, causing plant wilting (38); (ii) phenolic metabolism, resulting in necrosis or browning (29); (iii) nucleic acid metabolism, disrupting specific gene expression (40); (iv) protein synthesis (34); and (v) fat and sugar metabolism. Previous studies on *Phyllostachys heterocycla* (67) and *P. prominens* wilting (23) caused by *Ceratosphaeria phyllo-*

Corresponding author: T.-H. Zhu; E-mail address: zhutianhui@yahoo.cn

*The e-Xtra logo stands for "electronic extra" and indicates the online version contains one supplemental figure.

<http://dx.doi.org/10.1094/PHYTO-07-12-0164-R>
© 2013 The American Phytopathological Society

stachydis and *A. phaeospermum*, respectively, revealed that infection with bamboo-wilt-causing pathogen induces typical host responses such as chlorosis, browning, and wilting of shoots; increased respiration of bamboo; decreased photosynthesis; and destruction of cell structure. However, it is not known how phytotoxins from *A. phaeospermum* cause bamboo wilt symptoms. As a source of phytotoxic or toxic factors, phytotoxins can be used as a selected stress to plant pathogens instead of the whole plant or its organs, cells, and protoplasts. Plant resistance to infection causing pathogens or their toxins is the result of both constitutive and inducible reactions which could be nonspecific and target a broad spectrum of pathogens (44). Engelhardt et al. (11) reported that the protein toxin HrpZ1 from *Pseudomonas syringae* pv. *phaseolicola* could infect parsley membrane and induce resistance in some plants. Such toxins could be used to determine the mechanisms involved in plant resistance. Nevertheless, reports on the effect of *A. phaeospermum* toxin on *Bambusa pervariabilis* × *D. grandis* physiological and biochemical metabolisms are lacking.

The primary objective of this study was to isolate and purify protein toxins produced by *A. phaeospermum* in liquid culture media. Once fungal protein toxin was confirmed, the second objective was to assess the role of this purified toxin in the tender shoots and leaves of four bamboo varieties, as well as their metabolic responses. Identification of the protein toxin from *A. phaeospermum* and understanding the mechanism that causes blight symptoms may aid in the development of resistant *B. pervariabilis* × *D. grandis* varieties.

MATERIALS AND METHODS

Fungal and plant material. *Plant samples.* Four varieties (resistant varieties number 3 and 6 and susceptible varieties number 8 and 30) of 1-year-old *B. pervariabilis* McClure × *D. grandis* Keng f. shoots were planted in the bamboo-growing areas of a reclaimed farmland (103°01'N, 29°54'E) in Sichuan, China. The study area was at an altitude of 515.98 m, with annual temperature of 6.1 to 25.4°C and annual precipitation of 1,400 to 1,700 mm. All plant samples were improved varieties selected from a number of hybrid bamboo lines based on a variety introduction experiment (64).

Fungal culture. The fungus *A. phaeospermum* was provided by the Key Laboratory of Forest Protection in Sichuan Province and was originally isolated from diseased *B. pervariabilis* × *D. grandis* (70). It was revived from storage at -20°C by pipetting a thawed suspension onto potato dextrose agar plates and grown at 25°C for 5 days.

Fermentation liquid of toxin. Mycelial discs (5 mm) were grown in bottles containing 100 ml of modified Fries nutrient solution for liquid culturing (41). The solution contained dextrose at 20 g/liter, tartaric acid ammonium at 5 g/liter, NH₄NO₃ at 1 g/liter, KH₂PO₄ at 1 g/liter, MgSO₄ · 7H₂O at 0.5 g/liter, NaCl at 0.1 g/liter, CaCl₂ · 2H₂O at 0.1 g/liter, and yeast extract at 1 g/liter. After 15 days of growth in a rotary shaker (140 rpm) in dark at 26°C, cultures were filtered over double gauze to collect the supernatant.

Purification of protein toxin and activity assay. *Purification.* (i) Ammonium sulfate precipitation: fermentation liquid of toxin (2,000 ml) was centrifuged at 6,000 × *g* for 10 min. The clarified supernatant was then mixed with solid ammonium sulfate (20% saturation). After being left undisturbed for 2 h, the mixture was centrifuged at 8,000 × *g* for 15 min and the supernatant and precipitate were collected separately. Supernatant was then divided into two equal parts (milliliters). One was used for activity assays and the other for further precipitation as described earlier, with varying amounts of ammonium sulfate (30, 40, 50, 60, 70, 80, and 90% saturations). In each scale of saturation, supernatant and precipitate were collected separately, and all precipitates were

dissolved in phosphate-buffered saline (PBS) (pH 6.5). Optimal saturation varied depending on the impregnation method (20). (ii) Dialysis and concentration: toxin solution from optimal saturation was dialyzed overnight using 36-mm dialysis bags (8 to 14 kDa) (Boster Corp., Wuhan, China) filled two-thirds with the solution. Ratio of toxin solution to PBS (pH 6.5) was 1:10. After removal of ammonium sulfate, polyethylene glycol 60000 was added at a concentration of 20% of the original volume of toxin solution in the dialysis bag. (iii) The BioLogic LP system (Bio-Rad, Hercules, CA) was used for isolation and purification of the toxin. Sephadex G-50 gel chromatography was used as described by Wu (61). Sample (2 ml) was injected into the LP system with a flow rate of 0.5 ml/min and assessed at the 280-nm wavelength. Each flow peak was collected to assay phytotoxic activity. Q Sepharose Fast Flow anion exchange resin (60) was used for further purification. Conditions were the same as for Sephadex G-50 gel chromatography, and each flow peak was collected to assay phytotoxic activity. Sephadex G-75 gel chromatography was performed as described by Wu (61).

An impregnation method (20) was used to assay phytotoxic activity of each fraction obtained from the purification process described above. Clean and healthy shoots (each shoot with five leaves) of number 3 bamboo were cultured in centrifuge tubes with 1 ml of solution containing each purified fraction at 100 µg/ml. Sterile water, sterile Fries solution, and *A. phaeospermum* suspension (3.5 × 10⁶ spores/ml) served as three controls. Symptoms (wilting and brown discoloration) of branches and leaves were surveyed after culturing at 25°C for 24, 48, and 96 h (under 12 h of light each day). Each treatment was repeated six times.

Determination of molecular mass and N-terminal amino acid sequencing of the protein toxin. Molecular mass of the toxin was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (60). Protein toxin was mixed with an equal volume of buffer and the mixture was heated for 5 min in boiling water and centrifuged at 10,000 × *g* for 5 min at 4°C. A protein molecular mass marker of 20.1 to 97.2 kDa was used to determine the protein size (Boster Corp., Wuhan, China). Purity was evaluated using reversed-phase high-performance liquid chromatography (RP-HPLC) (ÄKTA avant 25) (GE Healthcare Life Sciences, Beijing) with a column containing Zorbax 300SBC-18 (4.6 by 250 mm, 5 µm) (Agilent Technologies, Santa Clara, CA). Both purity and N-terminal amino acid sequencing of the toxin were determined by the Agricultural Testing Center in China (Beijing). Homology of AP-toxin amino acid sequence was analyzed using the National Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov>).

Assessment of four *B. pervariabilis* × *D. grandis* varieties inoculated with AP-toxin. An impregnation method (20) was used as described earlier. Four treatments were performed to determine the indices: (i) 1 ml of protein toxin (40 µg/ml) for variety number 3, (ii) 1 ml of protein toxin (40 µg/ml) for variety number 6, (iii) 1 ml of protein toxin (40 µg/ml) for variety number 8, and (iv) 1 ml of protein toxin (40 µg/ml) for variety number 30. Controls comprised four varieties of bamboo with or without inoculation with *A. phaeospermum* suspension (1 ml, 3.5 × 10⁶ spores/ml). Each treatment was performed with six shoots (each shoot with five leaves).

Phenolic metabolism. Leaves with different treatments were randomly selected 0, 36, 72, 96, 120, 240, and 360 h after treatment for assessing total phenol (TP) and flavonoid contents. TP content was determined as described by Djeridane et al. (10). Bamboo shoot extract (1 ml) was added to 1 ml of Folin Ciocalteu's reagent followed by addition of 2 ml of sodium carbonate. After shaking, the mixture was maintained at 25°C for 60 min and absorption value was determined at 765 nm. A calibration curve was established using gallic acid as standard, which

has a correlation coefficient of $r = 0.9959$ and regression equation $y = 0.0077x - 0.0092$. Gallic acid content was used as the TP content (in milligrams per gram). Flavonoid content was determined as described by Xu et al. (62). Toxin-treated and untreated control samples of fresh bamboo shoots (0.5 g) were homogenized in liquid nitrogen and mixed with 10 ml of 70% ethanol for ultrasound oscillating (MECA2000; Beijing Meacsys. Co., Ltd., Beijing) extraction at 40°C for 12 h. The extract was then brought up to 4 ml with distilled water, and 1 ml of diluents was added to 0.3 ml of 0.5% NaNO₂, 0.3 ml of AlCl₃, and 2 ml of 4% NaOH. Total volume of the mixture was brought up to 10 ml with 60% ethanol, shaken, and then maintained for 15 min before determining the absorption value at 510 nm. A calibration curve was established using rutin as standard, which has a correlation coefficient of $r = 0.9998$ and regression equation $y = 0.0945x + 0.0012$. Flavonoid content (in OD/g) was determined based on rutin measurements.

Nucleic acid metabolism. Bamboo shoots (5 g) were homogenized in 20 ml of distilled water using a mortar and pestle. Filtrate was then separated from the residue and used in the study. Total nucleic acid content was assessed by UV spectrophotometry (69). The sample was mixed with 5 to 6% ammonia water until pH of the solution was 7.0, and the volume was brought up to 50 ml with distilled water. The mixture (2 ml) was centrifuged at $3,000 \times g$ for 10 min, and 0.5 ml of the supernatant was brought up to 50 ml with distilled water. Total nucleic acid content in the solution was determined at 260 nm. DNA content was determined at 595 nm after mixing 1 ml of the supernatant with 2 ml of diphenylamine reagent (1.5 g of diphenylamine was dissolved in 100 ml of glacial acetic acid and 1.5 ml of concentrated sulfuric acid, and 10 ml of this mixture was added to 0.1 ml of 0.2% acetaldehyde). RNA content was determined by subtracting the amount of DNA from total nucleic acid content. DNase activity was analyzed using the method described by Kunitz (26). Briefly, 0.5 ml of sample was mixed with 2.5 ml of substrate buffer (pH 5.0), and centrifuged at $3,000 \times g$ for 5 or 10 min at 260 nm was measured against a blank. Activity (U) was measured using the formula $(3.0 \times 1,000)/0.5 \times \Delta E$ (units per milliliter of sample). In all, ≈ 0.2 ml of the sample was mixed with 0.5 ml of yeast sodium RNA solution (30 ml of yeast sodium RNA dissolved in 100 ml of sodium acetate buffer, pH 5.0, at 0.1 mmol/liter), maintained at 37°C for 30 min, cooled in ice water, and then diluted 10 times with chilled distilled water. Absorption value was determined at 260 nm against a blank. Leaves under different treatments were randomly selected at 36, 72, 120, and 240 h to assess total nucleic acid, DNA and RNA contents, and DNase and RNase activities.

Soluble protein content was determined using Coomassie brilliant blue G250, with bovine serum albumin as control (72). Soluble sugar content was assessed by the anthrone method (72). Leaves under different treatments were randomly selected at 0, 36, 72, 96, 120, 240, and 360 h to assess the indices.

Calculation of disease index. Treatments of bamboo shoots were the same as those used to assess metabolic changes. Grading standards to rate disease were (i) no disease observed on the leaf, (ii) disease spots accounting for <30% of the total area, (iii) disease spots accounting for 30 to 50% of the total area, (iv) disease spots accounting for >50% of the total area, and (v) withered leaves. The formula to calculate disease index (57) was disease index (%) = $[\Sigma(\text{numerical value of each disease rating} \times \text{number of leaves in each disease rating})/(\text{total leaves} \times \text{most serious disease rating})] \times 100$.

Statistical analyses. All data were subjected to one-way analysis of variance to determine the significance of individual differences at the $P < 0.05$ level. Significant means were compared using the least significant differences test. All statistical analyses were conducted using the SPSS commercial statistical package (SPSS, version 17.0 for Windows; SPSS Inc., Chicago).

RESULTS

Crude toxin component and optimal saturation of ammonium sulfate. Supernatants did not exhibit any phytotoxic activity after precipitation using ammonium sulfate but the precipitations showed some phytotoxic activity at different degrees of saturation (Table 1). Quantities of the active precipitation fractions gradually increased with increasing saturation of ammonium sulfate. The fraction from 50% ammonium sulfate saturation was the most active. However, quantities of active fractions reduced gradually as the saturation level continued to increase. These results indicated that the active component may be proteins and that the optimal saturation is 50%.

Purified toxin and its activity. Three peaks were obtained after the crude toxin was purified by Sephadex G-50 gel chromatography. Peak 1 (P1) was clearly separated but peaks 2 (P2) and 3 (P3) showed some overlap (Fig. 1A). Among the three peaks (Table 2), P1 exhibited phytotoxic activity while the others did not. Further separation and purification of P1 through High Q Sepharose Fast Flow anion exchange (Fig. 1B) showed one peak in the flow-through (P1-1) with no phytotoxic activity and one elution peak (P1-2) with phytotoxic activity. P1-2 was further purified by Sephadex G-75 gel chromatography, which yielded three peaks (Fig. 1C), and only the second peak (P1-2-2) exhibited some activity while the others had none. Results of SDS-PAGE (Fig. 2) indicated that P1-2-2 was a single band with an estimated molecular mass of 31 kDa compared with protein standards.

N-terminal amino acid sequencing of protein toxin P1-2-2. RP-HPLC C18 (Supplemental Figure 1) revealed three peaks with retention times of 14, 18, and 21 min, respectively, and only the last fraction had phytotoxic activity (Table 2). This fraction was freeze dried, which resulted in a yellow powder that was named AP-toxin. The N terminus of the protein was sequenced by Edman degradation which revealed a single sequence with nine amino acids (i.e., H₂N-Gln-Val-Arg-Asp-Arg-Leu-Glu-Ser-Thr). Comparison of this amino acid sequence to known proteins in the NCBI protein database revealed 100% homology with the amino acid sequence (sites 1 to 9 and 351 to 359) of serine alkaline protease (subtilisin E) (ID: ZP10513563.1).

TABLE 1. Phytotoxic activity of ammonium sulfate fractions derived from liquid cultures of *Arthrinium phaeospermum*

Saturation (%) ^y	Fraction	Time (h) ^z		
		24	48	96
20	Supernatant	–	–	–
	Precipitation	–	–	+
30	Supernatant	–	–	–
	Precipitation	–	+	+
40	Supernatant	–	–	–
	Precipitation	–	+	++
50	Supernatant	–	–	–
	Precipitation	+	+++	+++
60	Supernatant	–	–	–
	Precipitation	+	++	++
70	Supernatant	–	–	–
	Precipitation	+	+	++
80	Supernatant	–	–	–
	Precipitation	–	–	+
90	Supernatant	–	–	–
	Precipitation	–	–	+
FL	...	+	++	++
CK1	...	–	–	–
CK2	...	–	–	–

^y Saturation of ammonium sulfate. FL = fermentation liquid, CK1 = sterile water, and CK2 = sterile Fries solution.

^z Six shoots (each shoot with five leaves) per treatment were used for the assessment; – = no effect, + = light wilting, ++ = moderate wilting, and +++ = serious wilting.

Metabolic changes of four *B. pervariabilis* × *D. grandis* varieties inoculated with AP-toxin. In all four bamboo varieties, the TP content (Fig. 3A) increased after treatment with AP-toxin and *A. phaeospermum* suspension from 0 to 36 h, and the changes were similar in both treatments. TP contents of the number 3 and 6 bamboo varieties were significantly higher than those in number 8 and 30 during the entire testing period. The highest TP content was observed in number 6 followed by numbers 3 and 30, and the least amount in number 8. In addition, TP contents in the resistant varieties (numbers 3 and 6) remained high in the later period (96 to 360 h) whereas those in the susceptible varieties (number 8

and 30) were lower. TP content peaked at 72 h in the resistant varieties and was almost two times higher than the control group of numbers 3 and 6. In the non-toxin treatments, TP content did not change throughout the entire testing period. Flavonoid contents in the four bamboo varieties increased after treatments with AP-toxin and *A. phaeospermum* suspension (Fig. 3B). However, the increased flavonoid content in the resistant varieties was significantly greater than in the susceptible varieties. In the non-toxin treatments, no significant difference was observed in the data at ≈ 115 OD/g during the entire testing period. In addition, peak values of the toxin-treated resistant varieties (numbers 3 and 6) appeared at 36 and 96 h, with the values of number 6 higher than number 3.

Total nucleic acid, DNA, and RNA contents decreased after the shoots were treated with AP-toxin. However, those in the non-toxin treatments remained stable from 36 to 240 h (Table 3). Both the AP-toxin and *A. phaeospermum* suspension affected the nucleic metabolism of hybrid bamboo, though there was no significant difference between these treatments. However, significant differences were observed between the resistant and susceptible varieties. The three indices for numbers 3 and 6 (resistant varieties) showed no significant differences from 36 to 120 h. In particular, number 6 showed no significant difference until 240 h. In contrast, number 8 (susceptible variety) demonstrated significant differences at 36, 72, 120, and 240 h. Moreover, the total nucleic acid, DNA, and RNA contents in this variety decreased by 21.79, 31.21, and 19.13%, respectively. Number 30 exhibited significant differences until 120 or 240 h. Thus, the effect of AP-toxin on number 30 was less than on number 8.

Both the DNase and RNase activities in the four bamboo varieties increased after AP-toxin and *A. phaeospermum* suspension treatments (Table 4). No significant difference was observed in number 6 bamboo leaves. However, other varieties (in particular, numbers 8 and 30) showed a significant difference from 36 to 240 h. In contrast to DNase, all RNase activities were significantly higher in all four bamboo varieties. Correlation analysis

Fig. 1. Separation and purification of crude protein toxin from *Arthrinium phaeospermum* by chromatography. **A**, Crude toxin was extracted by Sephadex G-50 gel chromatography and 264 fractions were obtained; peak number 65, 147, and 202 were named P1, P2, and P3, respectively. **B**, Toxin P1 was extracted by High Q Sepharose Fast Flow Chromatography and 302 fractions were obtained; peak numbers 55 and 229 were named P1-1 and P1-2, respectively. **C**, Toxin P1-2 was extracted by Sephadex G-75 gel chromatography and 120 fractions were obtained; peak numbers 38, 60, and 83 were named P1-2-1, P1-2-2, and P1-2-3, respectively.

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of P1-2-2 protein toxin purified by three types of chromatography methods. **A**, Protein toxin P1-2-2 and **B**, standard marker protein with a molecular mass of 20.1 to 97.2 kDa. The gel was run at an initial voltage of 80 V. When the sample migrated in the separation gel (10% acrylamide), the voltage was increased to 130 V. Electrophoresis was stopped when the sample moved to 0.5 to 1.0 cm from the indicator.

revealed the regression equations of y (DNA) = $-0.2479x$ (DNase) + 0.6578 (correlation coefficient $r = -0.9878$) and y (RNA) = $-3.3565x$ (RNase) + 11.5323 (correlation coefficient $r = -0.9341$). Thus, AP-toxin treatment decreased the DNA and RNA contents in hybrid bamboo leaves, which is consistent with the increased DNase and RNase activities.

Soluble protein content in the leaves of the four bamboo varieties initially increased and then decreased after AP-toxin and *A. phaeospermum* suspension treatments (Fig. 4A). Soluble

protein content in the resistant varieties (numbers 3 and 6) increased from 0 to 72 h, reaching a peak at 72 h before decreasing. However, the values were still higher in the later period than those at 0 h. Although soluble protein content increased during the initial period (0 to 36 h) in the susceptible varieties (numbers 8 and 30), it rapidly decreased 36 h after AP-toxin treatment. Throughout the entire testing period, contents in the resistant varieties were significantly higher compared with their non-toxin controls. However, contents in the susceptible varieties were

TABLE 2. Phytotoxic activity of toxin fractions derived from liquid cultures of *Arthrinium phaeospermum* in the purification process

Process ^y	Fraction	Time (h) ^z		
		24	48	96
Sephadex G-50 gel chromatography	P1	+	++	+++
	P2	—	—	—
	P3	—	—	—
High Q Sepharose Fast Flow Anion exchange resin	P1-1	—	—	—
	P1-2	+	++	+++
	P1-2-1	—	—	—
Sephadex G-75 gel chromatography	P1-2-2	++	+++	+++
	P1-2-3	—	—	—
RP-HPLC	Peak at 14 min	—	—	—
	Peak at 18 min	—	—	—
	Peak at 21 min	++	+++	+++
Crude protein	...	—	+++	+++
CK1	...	—	—	+
CK2	...	—	—	—

^y RP-HPLC = reversed-phase high-performance liquid chromatography, CK1 = sterile water, and CK2 = sterile Fries solution.

^z Six shoots (each shoot with five leaves) per treatment were used for the assessment; — = no effect, + = light wilting, ++ = moderate wilting, and +++ = serious wilting.

Fig. 3. Dynamic responses of phenol substances (total phenols and flavonoids) in the leaf samples of four *Bambusa pervariabilis* × *Dendrocalamopsis grandis* varieties inoculated with AP-toxin. **A**, Total phenol (TP) content and **B**, flavonoid content. Shoots of four *B. pervariabilis* × *D. grandis* varieties (numbers 3, 6, 8, and 30) were treated with AP-toxin at 40 µg/ml. CK1-3, CK1-6, CK1-8, and CK1-30 are bamboo varieties number 3, 6, 8, and 30, respectively, treated with an *Arthrinium phaeospermum* suspension at 3.5×10^6 spores/ml; CK2-3, CK2-6, CK2-8, and CK2-30 are bamboo varieties number 3, 6, 8, and 30, respectively, without toxin treatment. Six shoots (each shoot with five leaves) were used per treatment for assessment at 0, 36, 72, 96, 120, 240, and 360 h.

TABLE 3. Changes in total nucleic acid contents in the leaves of four *Bambusa pervariabilis* × *Dendrocalamopsis grandis* varieties

Variety, treatment ^v	Time (h) ^w											
	Total nucleic acid content (µg/ml) ^x				DNA content (µg/ml) ^y				RNA content (µg/ml) ^z			
	36	72	120	240	36	72	120	240	36	72	120	240
Number 3												
Toxin	165.25 ± 2.19 Aa	163.11 ± 2.25 Aab	160.30 ± 2.90 Bb	155.22 ± 2.36 Bc	32.25 ± 1.41 Aa	32.01 ± 1.10 Aa	31.54 ± 0.99 Aa	30.19 ± 1.07 Aa	133.00 ± 2.85 Aa	131.10 ± 2.44 Aa	128.76 ± 2.35 Ba	125.03 ± 2.67 Bb
CK1-3	165.13 ± 1.76 Aa	164.90 ± 1.50 Aa	162.87 ± 1.91 ABa	158.19 ± 2.02 Bb	32.55 ± 0.80 Aa	32.37 ± 0.74 Aa	31.96 ± 0.72 Aa	31.58 ± 0.85 Aa	132.58 ± 1.34 Aa	132.53 ± 1.69 Aa	130.91 ± 2.00 ABa	126.61 ± 2.10 Bb
CK2-3	165.34 ± 1.88 Aa	165.30 ± 1.60 Aa	165.28 ± 1.79 Aa	165.09 ± 1.33 Aa	32.38 ± 0.76 Aa	32.15 ± 0.80 Aa	31.67 ± 0.81 Aa	32.33 ± 0.90 Aa	132.96 ± 1.99 Aa	133.15 ± 1.77 Aa	133.61 ± 1.40 Aa	132.76 ± 1.58 Aa
Number 6												
Toxin	161.30 ± 2.17 Aa	160.02 ± 2.50 Aa	157.88 ± 1.97 Aa	156.94 ± 2.00 Aa	34.80 ± 2.22 Aa	33.87 ± 1.75 Aa	32.14 ± 1.90 Aa	31.97 ± 1.94 Aa	126.50 ± 2.87 Aa	126.15 ± 2.35 Aa	125.74 ± 2.44 Aa	124.97 ± 2.50 Ba
CK1-6	161.11 ± 2.25 Aa	160.95 ± 2.61 Aa	158.24 ± 2.00 Aa	157.33 ± 1.98 Aa	34.45 ± 1.03 Aa	33.75 ± 1.26 Aa	32.08 ± 1.65 Aa	31.86 ± 1.55 Aa	126.66 ± 2.24 Aa	127.20 ± 2.05 Aa	126.16 ± 2.38 Aa	125.47 ± 2.33 Ba
CK2-6	161.09 ± 1.40 Aa	161.55 ± 1.00 Aa	161.20 ± 1.19 Aa	162.04 ± 0.98 Aa	34.32 ± 1.19 Aa	34.22 ± 1.00 Aa	33.16 ± 1.03 Aa	33.43 ± 1.14 Aa	126.77 ± 1.25 Aa	127.33 ± 1.51 Aa	128.04 ± 1.31 Aa	128.61 ± 1.20 Aa
Number 8												
Toxin	169.18 ± 2.99 Aa	155.37 ± 2.79 Bb	149.28 ± 2.15 Bc	132.33 ± 1.90 Bd	37.14 ± 1.04 Aa	34.10 ± 1.30 Bb	31.44 ± 1.14 Bc	25.55 ± 0.87 Bd	132.04 ± 2.18 Aa	121.27 ± 2.10 Bb	117.84 ± 1.14 Bc	106.78 ± 1.21 Bd
CK1-8	169.00 ± 1.80 Aa	157.24 ± 2.05 Bb	151.44 ± 1.97 Bc	133.04 ± 1.88 Bd	37.03 ± 0.55 Aa	35.11 ± 0.64 Bb	32.32 ± 0.79 Bc	26.09 ± 0.52 Bd	131.97 ± 1.48 Aa	122.13 ± 1.74 Bb	119.12 ± 1.22 Bc	106.95 ± 1.35 Bd
CK2-8	169.01 ± 1.76 Aa	168.72 ± 1.14 Aa	167.63 ± 2.00 Aa	167.70 ± 2.18 Aa	37.00 ± 0.80 Aa	37.58 ± 0.38 Aa	37.26 ± 0.47 Aa	36.60 ± 0.90 Aa	132.01 ± 2.12 Aa	131.14 ± 1.66 Aa	130.37 ± 2.59 Aa	131.10 ± 1.80 Aa
Number 30												
Toxin	162.14 ± 3.01 Aa	160.80 ± 2.01 Aab	157.57 ± 1.01 Bb	145.18 ± 1.33 Bc	37.53 ± 1.12 Aa	35.60 ± 1.21 Aa	33.50 ± 0.99 Bb	28.38 ± 1.40 Bc	124.61 ± 0.97 Aa	125.20 ± 2.15 Aa	124.07 ± 1.43 Aa	116.80 ± 0.96 Bb
CK1-30	162.25 ± 1.79 Aa	161.14 ± 1.80 Aab	158.09 ± 1.33 Bb	147.76 ± 1.35 Bc	37.47 ± 1.00 Ba	36.08 ± 1.04 Ba	34.02 ± 0.65 Bb	28.78 ± 0.44 Bc	124.78 ± 0.82 Ba	125.06 ± 1.25 Aa	124.07 ± 1.22 Aa	118.98 ± 1.00 Bb
CK2-30	162.33 ± 1.58 Aa	162.35 ± 1.44 Aa	163.48 ± 1.50 Aa	161.89 ± 1.63 Aa	37.34 ± 0.30 Aa	37.28 ± 0.55 Aa	37.56 ± 0.49 Aa	37.07 ± 0.48 Aa	124.99 ± 1.00 Aa	125.07 ± 0.76 Aa	125.92 ± 0.88 Aa	124.82 ± 0.61 Aa

^v Variety and treatment. Shoots with leaves treated with (CK1-3, CK1-6, CK1-8, and CK1-30) and without (CK2-3, CK2-6, CK2-8, and CK2-30) AP-toxin (40 µg/ml) or *Arthrrium phaeospermum* suspension (3.5 × 10⁶ spores/ml).

^w Data in the same row followed by different lowercase letters indicate significant differences among different treatment times of one bamboo variety determined by least significant difference (LSD) test ($P < 0.05$). Data in the same column followed by different capital letters indicate significant differences between treatment with and without toxin per bamboo variety determined by LSD test ($P < 0.05$). Six shoots (each shoot with five leaves) were used per treatment for assessment at 36, 72, 120, and 240 h.

^x Data were assessed by UV spectrophotometry at 260 nm.

^y Determination of DNA content at 595 nm after diphenylamine reaction.

^z RNA content was determined by subtracting DNA content from total nucleic acid content.

TABLE 4. Changes in DNase and RNase activities in the leaves of four *Bambusa pervariabilis* × *Dendrocalamopsis grandis* varieties

Variety, treatment ^v	Time (h) ^z							
	DNase activity (U/ml)				RNase activity (U/ml)			
	36	72	120	240	36	72	120	240
Number 3								
Toxin	42.33 ± 2.24 Ab	44.14 ± 2.17 Aab	46.73 ± 2.23 Aa	47.90 ± 2.30 Aa	83.10 ± 2.12 Ac	85.55 ± 2.20 Abc	89.24 ± 2.25 Aab	91.39 ± 2.19 Aa
CK1-3	42.19 ± 0.55 Ac	43.56 ± 1.20 Abc	45.70 ± 1.35 Aab	46.88 ± 1.58 Aa	82.96 ± 0.90 Ab	83.68 ± 1.25 Ab	87.80 ± 1.45 Aa	89.21 ± 1.40 Aa
CK2-3	41.25 ± 0.92 Aa	40.12 ± 0.88 Ba	40.04 ± 0.91 Ba	39.89 ± 1.00 Ba	80.13 ± 0.79 Aa	80.02 ± 0.60 Ba	79.56 ± 0.55 Ba	79.19 ± 0.50 Ba
Number 6								
Toxin	41.25 ± 2.00 Aa	42.33 ± 2.12 Aa	42.67 ± 2.24 Aa	43.01 ± 2.24 Aa	83.48 ± 2.30 Aa	84.53 ± 2.41 Aa	85.17 ± 2.21 Aa	86.21 ± 2.09 Aa
CK1-6	40.96 ± 0.80 Aa	41.53 ± 1.21 Aa	41.90 ± 1.25 Aa	42.32 ± 1.40 Aa	82.50 ± 0.89 Aa	83.49 ± 1.33 Aa	84.20 ± 1.56 Aa	85.25 ± 1.34 Aa
CK2-6	40.02 ± 0.51 Aa	40.13 ± 0.40 Aa	39.91 ± 0.57 Aa	39.76 ± 0.50 Ba	82.16 ± 0.24 Aa	81.77 ± 0.21 Ba	81.64 ± 0.30 Ba	81.09 ± 0.33 Ba
Number 8								
Toxin	42.35 ± 1.36 Ad	50.19 ± 2.01 Ac	65.70 ± 1.49 Ab	80.18 ± 1.91 Aa	95.26 ± 2.13 Ac	109.95 ± 2.00 Ab	112.39 ± 1.56 Ab	126.27 ± 2.59 Aa
CK1-8	40.55 ± 1.59 Ad	47.24 ± 1.96 Ac	62.12 ± 2.04 Ab	77.75 ± 2.20 Aa	93.13 ± 2.00 Ac	105.20 ± 2.35 Bb	109.34 ± 2.14 Ab	123.19 ± 2.40 Aa
CK2-8	38.17 ± 0.45 Ba	38.25 ± 0.53 Ba	38.08 ± 0.32 Ba	37.94 ± 0.86 Ba	90.17 ± 0.60 Ba	90.90 ± 0.55 Ca	89.93 ± 0.34 Ba	89.20 ± 0.42 Ba
Number 30								
Toxin	40.24 ± 1.29 Ad	45.33 ± 1.50 Ac	48.19 ± 1.10 Ab	51.24 ± 1.33 Aa	92.33 ± 1.14 Ad	100.10 ± 1.31 Ac	106.69 ± 1.30 Ab	113.15 ± 1.27 Aa
CK1-30	38.99 ± 1.50 ABc	42.74 ± 1.78 Ab	45.59 ± 1.66 Ab	49.92 ± 1.70 Aa	92.00 ± 1.50 ABd	97.89 ± 1.55 Ac	103.50 ± 1.82 Bb	110.95 ± 1.94 Aa
CK2-30	38.95 ± 0.23 Ba	38.77 ± 0.70 Ba	38.39 ± 0.64 Ba	38.40 ± 0.21 Ba	90.22 ± 0.96 Ba	89.54 ± 0.70 Ba	89.38 ± 0.58 Ca	88.74 ± 1.00 Ba

^v Variety and treatment. Shoots with leaves treated with (CK1-3, CK1-6, CK1-8, and CK1-30) and without (CK2-3, CK2-6, CK2-8, and CK2-30) AP-toxin (40 µg/ml) or *Arthrrium phaeospermum* suspension (3.5 × 10⁶ spores/ml).

^z Data were assessed by UV spectrophotometry at 260 nm. Data in the same row followed by different lowercase letters indicate significant differences among different treatment times for one bamboo variety determined by least significant difference (LSD) test ($P < 0.05$). Data in the same column followed by different capital letters indicate a significant difference between the treatments with and without the toxin per bamboo variety determined by LSD test ($P < 0.05$). Six shoots (each shoot with five leaves) were used per treatment for assessment at 36, 72, 120, and 240 h.

lower compared with their non-toxin controls 240 h after AP-toxin treatment.

Soluble sugar content in the leaves of four bamboo varieties decreased throughout the entire testing period (Fig. 4B). At 36 h, these values were significantly lower than their controls (non-toxin treatment). However, a difference was observed between the resistant and susceptible varieties. At 120 h, values in number 3 and 6 bamboo rose again after the decrease. At 240 and 360 h, the values remained stable. The values in number 8 and 30 bamboo declined and were significantly lower than in numbers 3 and 6. Notably, soluble sugar content in variety number 8 decreased below $0.05 \mu\text{g/g}$ at 360 h. Soluble sugar content was the highest in number 6 followed by numbers 3 and 30, and the least amount was in number 8.

Disease index of four *B. pervariabilis* × *D. grandis* varieties inoculated with AP-toxin. Disease spots were the visible symptoms of bamboo shoots infected with AP-toxin. Changes in the disease indices are shown in Figure 5 and changes in the disease symptoms are shown in Figure 6. Disease index increased in both toxin and *A. phaeospermum* suspension treatments from 0 to 360 h, with small differences between the treatments that were not significant in each bamboo variety. Disease index sharply increased from 0 to 36 h. All disease indices in the four bamboo varieties showed significant differences after 36 h. Disease index for numbers 3 and 6 increased steadily after 96 h of treatment and later maintained at $<40\%$. However, the indices for number 8 and 30 increased until 120 h, and was $>50\%$ at 96 h. Finally, disease index for number 8 was $>70\%$ at 120 h compared with 0 h.

Correlation analysis among metabolic index, time, resistance, and disease index. Correlation coefficients are shown in Table 5. Except flavonoid content and time, DNase and RNase

activities and disease index showed high significance. Treatment time for AP-toxin negatively correlated with TP, total nucleic acid, DNA, RNA, soluble protein, and soluble sugar contents but positively correlated with DNase and RNase activities. Further-

Fig. 5. Changes in disease index in the leaves from four *Bambusa pervariabilis* × *Dendrocalamopsis grandis* varieties inoculated with AP-toxin. Shoots of four *B. pervariabilis* × *D. grandis* varieties (numbers 3, 6, 8, and 30) were treated with AP-toxin at $40 \mu\text{g/ml}$. CK1-3, CK1-6, CK1-8, and CK1-30 are bamboo varieties number 3, 6, 8, and 30, respectively, treated with an *Arthrinium phaeospermum* suspension at 3.5×10^6 spores/ml. Six shoots (each shoot with five leaves) were used per treatment for assessment at 0, 36, 72, 96, 120, 240, and 360 h.

Fig. 4. Dynamic responses of the soluble proteins and soluble sugars in the leaves of the four *Bambusa pervariabilis* × *Dendrocalamopsis grandis* varieties inoculated with AP-toxin. **A**, Soluble protein content and **B**, soluble sugar content. Shoots of four *B. pervariabilis* × *D. grandis* varieties (numbers 3, 6, 8, and 30) were treated with AP-toxin at $40 \mu\text{g/ml}$. CK1-3, CK1-6, CK1-8, and CK1-30 are bamboo varieties number 3, 6, 8, and 30, respectively, treated with an *Arthrinium phaeospermum* suspension at 3.5×10^6 spores/ml; CK2-3, CK2-6, CK2-8, and CK2-30 are bamboo varieties number 3, 6, 8, and 30, respectively, without toxin treatment. Six shoots (each shoot with five leaves) were used per treatment for assessment at 0, 36, 72, 96, 120, 240, and 360 h.

more, treatment time positively correlated with disease index. Resistance of the four *B. pervariabilis* × *D. grandis* varieties showed positive correlations with all indices except disease index and DNase and RNase activities. In addition, there was a negative correlation between the disease index and all physiological indices except DNA content and DNase and RNase activities.

DISCUSSION

Phytotoxic activity of any toxin can have two possible effects. The purified toxin can cause disease symptoms in the host similar to the pathogen or can have an increased effect on host metabolism and growth (14). As shown in the present study, AP-toxin from *A. phaeospermum* caused metabolic changes and wilting in the shoots of *B. pervariabilis* × *D. grandis*. In addition, disease

spots significantly increased with treatment time, which correlated with an increase in disease indices in all four bamboo varieties. This indicates that AP-toxin may be the main phytotoxic factor causing *B. pervariabilis* × *D. grandis* blight.

Thus far, separation and purification of proteins have been performed using a combination of several methods, such as molecular sieve chromatography, ion exchange chromatography, hydrophobic interaction chromatography, affinity chromatography, and so on (60). In this study, a single protein with high purity and an estimated molecular mass of 31 kDa was obtained through Sephadex G-50 gel chromatography, Q Sepharose Fast Flow anion exchange resin, and Sephadex G-75 gel chromatography. This indicates that purification of this macromolecule is complicated. Unlike arthrictin or arthrinic acid from *A. phaeospermum* that cause human dermatosis (7,59), AP-toxin is a protein. This

Fig. 6. Changes in disease symptoms in the leaves from four *Bambusa pervariabilis* × *Dendrocalamopsis grandis* varieties inoculated with AP-toxin. Shoots of four *B. pervariabilis* × *D. grandis* varieties (numbers 3, 6, 8, and 30) were treated with AP-toxin at 40 µg/ml. **A**, AP-toxin treatment; **B**, *Arthrinium phaeospermum* suspension treatment at 3.5×10^6 spores/ml; and **C**, without treatment. Six shoots (each shoot with five leaves) were used per treatment for assessment at 0, 36, 72, 96, 120, 240, and 360 h.

indicates that the same fungal species can produce a variety of active phytotoxins depending on host type and environmental conditions. In addition, RP-HPLC was used to determine the purity and N-terminal amino acid sequence of AP-toxin. The resulting nine-amino-acid sequence was identical to serine alkaline protease in the NCBI protein database. Serine alkaline protease is an important industrial catalyst and is produced by a wide range of microorganisms, including bacteria and fungi, in addition to mammalian tissues (2). Serine alkaline protease with a molecular mass of 32 kDa was isolated using Sephadex G-100 column *Aspergillus clavatus* ES1 (17), and their homologs were isolated from *Ophiostoma floccosum* (21), *Trichoderma virens* (43), and *T. hamatum* (54). We report for the first time the serine alkaline protease from *Arthrinium phaeospermum*. After infection, plants accelerate phenolic compound synthesis and accumulation, which is an important mechanism in plant disease resistance (37). According to Matern and Kneusal (33), the first step in plant defense involves rapid accumulation of phenols at the infection site, which restricts or slows growth of the pathogen. Our analysis revealed that inoculation of AP-toxin increased TP content. A significant difference between the resistant and susceptible varieties was observed, indicating that the accumulation of TP was higher in resistant than susceptible varieties. Together with the reports on wheat (15) and sorghum (9), AP-toxin can induce *B. perversabilis* × *D. grandis* to produce large amounts of TP. However, phenolic compounds are very complex and the specific phenolic substance induced by AP-toxin is yet to be determined. Flavonoid is an important phenolic compound with a strong antifungal function in plant-pathogen interactions. It has been shown that flavonoid could be used for self-defense to provide protons that may reduce or remove excessive free radicals produced under toxin-stress conditions (18). Flavonoid contents in the four bamboo varieties increased after shoots were treated with AP-toxin. This indicates that AP-toxin may induce the production of flavonoids in hybrid bamboo for the protection of physiological metabolism. Nevertheless, changes in flavonoid content varied among the resistant and susceptible varieties inoculated with the toxin. Flavonoid accumulation was higher and faster and its duration was longer in the resistant than susceptible varieties. This finding is similar to the reports of Mabrouk et al. (30) and Treutter (58).

Environmental stress or toxins could disturb nucleic acid metabolism and inhibit growth inhibition (19). Previous studies have reported that many adverse conditions such as excessive water and abnormal metal content could impair the metabolism of plants and could directly or indirectly affect DNA and RNA (35,51). Similarly, phytotoxins can also disrupt nucleic acid metabolism of the host and induce active synthesis of nucleolytic enzymes. In addition, DNA content was higher than RNA, which may be attributed to their locations in the cell. DNA is mainly present in the cell nucleus and is less vulnerable than RNA to outside influences. Decline in the total nucleic acid content can be attributed to decreased RNA content. In addition, toxin-treated susceptible varieties (numbers 8 and 30) showed greater nucleic acid catabolism than anabolism, and associated disease symptoms

such as wilting. However, decline in the total nucleic acid and DNA and RNA contents, as well as the RNase and DNase activities of the resistant varieties (numbers 3 and 6), was not significant when compared with susceptible varieties. Thus, synthesis and decomposition in the resistant varieties were balanced by the plant's active defense system. However, these results should be further confirmed by studies on the nucleic acid synthesis.

Besides nucleic acids, previous studies have suggested that protein synthesis would be inhibited by stress such as drought, low temperature, or infection by pathogens (5,68). However, a new protein may be produced, or the normal protein content may increase significantly. In this study, we observed that soluble protein content in both susceptible and resistant varieties increased 36 h after inoculation. This finding is in agreement with the results of Ashfaq et al. (4) and Ashry and Mohamed (5). Together with the findings of others (55,56), our data indicate that the decrease in soluble protein content after 72 h may be attributed to the resistance reaction of hybrid bamboo. However, integrated function of the cells was damaged and protein synthesis was inhibited. Soluble sugars in plant tissue are mainly involved in photosynthesis and can act as gene regulation signals or synthesize defensive substances (36). Data of this study show a decrease in soluble sugar content after AP-toxin inoculation, which suggests a reduction in photosynthesis and destruction of the hybrid bamboo tissues. Thus, these findings may support the contention of previous studies (31,52). However, soluble sugar content increased at 120 h in resistant varieties, which indicates the rapid synthesis of defensive substances corresponding to the increase in soluble sugars. However, the compounds of soluble sugars are involved in complex functions such as photosynthesis, respiration, translocation, enzyme activity, and sugar utilization by toxins. These processes interact to influence sugar content (31).

Correlation analysis between the metabolic index and toxin treatment time indicate a significant correlation except flavonoid content, which was relatively stable during the treatments. No correlation was observed in RNase and DNase activities between metabolic index and disease index. However, a significant correlation was observed between RNA and DNA contents, which have indirect effects on the metabolic index. Moreover, all metabolic indices had significant correlation with the resistance of hybrid bamboo, which indicates that all nine metabolic indices presented in our study can be used as important biochemical indices to determine the resistance of *B. perversabilis* × *D. grandis* varieties. Furthermore, based on the effects of AP-toxin on the metabolism of *B. perversabilis* × *D. grandis* varieties, the purified toxin can be used to select resistant varieties or identify the resistance profile of resistant bamboo varieties, as well as to enhance disease resistance of hybrid bamboo blight by genetic engineering.

ACKNOWLEDGMENTS

This research was supported by the National Natural Science Foundation of China (31070578) and the National Natural Science and Technology Resources Sharing Platform of China (2005DKA21207-13).

TABLE 5. Correlation coefficients among metabolic index, time, resistance, and disease index

Item ^y	Metabolic index ^z								Disease index
	TP content	Flavonoid content	Total nucleic acid content	DNA content	RNA content	DNase activity	RNase activity	Soluble protein content	Soluble sugar content
Time	-0.7470*	0.3251	-0.7950*	-0.7922*	-0.8775*	0.6303*	0.7920*	-0.9044*	-0.9730**
Resistance	0.8332*	0.8830*	0.8574*	0.8543*	0.7473*	-0.6812*	-0.7205*	0.9581**	0.9552**
Disease index	-0.8015*	-0.8612*	-0.7040*	0.2177	-0.6545*	0.1938	0.3329	-0.9714**	-0.9653**

^y Time: shoots of *Bambusa perversabilis* × *Dendrocalamopsis grandis* varieties inoculated with AP-toxin for assessment at 0, 36, 72, 96, 120, 240, and 360 h. Resistance of the four bamboo varieties could be ranked as: number 6 > number 3 > number 30 > number 8.

^z TP = total phenol; * = significant correlation among metabolic index, time, resistance, and disease index at $P < 0.05$ using least significant difference (LSD) test; ** = remarkably significant correlation at $P < 0.05$ using LSD test.

LITERATURE CITED

- Abang, M. M., Abraham, W. R., Asiedu, R., Hoffmann, P., Wolf, G., and Winter, S. 2009. Secondary metabolite profile and phytotoxic activity of genetically distinct forms of *Colletotrichum gloeosporioides* from yam (*Dioscorea* spp.). *Mycol. Res.* 113:130-140.
- Anwar, A., and Saleemuddin, M. 1998. Alkaline proteases: A review. *Bioresour. Technol.* 64:175-183.
- Aremu, E. A., Tanaka, K., Akagi, Y., Maekama, N., Akamatsu, H., Kodama, M., and Otani, H. 2004. Detection of fungi producing infection-inhibiting metabolites against *Alternaria alternata* Japanese pear pathotype from fungi inhabiting internal tissues of Japanese pear shoots. *J. Gen. Plant Pathol.* 70:139-142.
- Ashfaq, M., Khan, M. A., Javed, N., Mughal S. M., Shahid, M., and Sahi, S. T. 2010. Effect of urdbean leaf crinkle virus infection on total soluble protein and antioxidant enzymes in blackgram plants. *Pak. J. Bot.* 42:447-454.
- Ashry, N. A., and Mohamed, H. I. 2011. Impact of secondary metabolites and related enzymes in flax resistance and or susceptibility to powdery mildew. *World J. Agric. Sci.* 7:78-85.
- Betts, M. F., Manning, V. A., Cardwell, K. B., Pandelova, I., and Ciuffetti, L. M. 2011. The importance of the N-terminus for activity of Ptr ToxB, a chlorosis-inducing host-selective toxin produced by *Pyrenophora tritici-repentis*. *Physiol. Mol. Plant Pathol.* 75:138-145.
- Bloor, S. 2008. Arthrinic acid, a novel antifungal polyhydroxyacid from *Arthrinium phaeospermum*. *J. Antibiot.* 61:515-517.
- Damann, K. E., Gardner, R. J., and Scheffer, R. P. 1974. An assay for *Helminthosporium victoriae* toxin based on induced leakage of electrolytes from oat tissue. *Phytopathology* 64:652-654.
- Dicko, M. H., Gruppen, H., Barro, C., Traore, A. S., van Berkel, W. J. H., and Voragen, A. G. J. 2005. Impact of phenolic compounds and related enzymes in sorghum varieties for resistance and susceptibility to biotic and abiotic stresses. *J. Chem. Ecol.* 31:2671-2688.
- Djeridane, A., Yousfi, M., and Nadjemi, B. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem.* 97:654-660.
- Engelhardt, S., Lee, J., Gäbler, Y., Kemmerling, B., Haapalainen, M. L., Li, C. M., Wei, Z. M., Keller, H., Joosten, M., Taira, S., and Nürnberger, T. 2009. Separable roles of the *Pseudomonas syringae* pv. *phaseolicola* accessory protein HrpZ1 in ion-conducting pore formation and activation of plant immunity. *Plant J.* 57:706-717.
- Fumiharu, H., Hiroyasu, N., and Hideo, H. 2006. Purification and structure determination of glucosides of capsaicin and dihydrocapsaicin from various *Capsicum* fruits. *J. Agric. Food Chem.* 54:5948-5953.
- Geimba, M. P., Corbellini, V. A., and Scroferneker, M. L. 2005. Chemical and immunological differentiation of exoantigens from four *Bipolaris sorokiniana* strains. *Process Biochem.* 40:2051-2057.
- Gill, M. A., Arshad, H. M., and Hamid, K. 2003. Effect of toxin produced by *Xanthomonas campestris* pv. *oryzae* on the seed germination and seedlings growth of rice. *J. Agric. Res.* 41:79-84.
- Gogoi, R., Singh, D. V., and Srivastava, K. D. 2001. Phenols as a biochemical basis of resistance in wheat against kamal bunt. *Plant Pathol.* 50:470-476.
- Goyer, C., Charest, P. M., Toussaint, V., and Beaulieu, C. 2000. Ultrastructural effects of thaxtomin A produced by *Streptomyces scabies* on mature potato tuber tissues. *Can. J. Bot.* 78:374-380.
- Hajji, M., Kanoun, S., Nasri, M., and Gharsallah, N. 2007. Purification and characterization of an alkaline serine-protease produced by a new isolated *Aspergillus clavatus* ES1. *Process Biochem.* 42:791-797.
- Hartwig, U. A., Joseph C. M., and Phillips D. A. 1991. Flavonoids released naturally from alfalfa seeds enhance growth rate of *Rhizobium meliloti*. *Plant Physiol.* 95:797-803.
- Heidari, M. 2010. Nucleic acid metabolism, proline concentration and antioxidants enzyme activity in canola (*Brassica nupus* L.) under salinity stress. *Agric. Sci. China* 9:504-511.
- Ho, S. H., Koh, L., Ma, Y., Huang, Y., and Sim, K. Y. 1996. The oil of garlic, *Allium sativum* L. (Amaryllidaceae), as a potential grain protectant against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *Postharvest Biol. Technol.* 9:41-48.
- Hoffman, B., and Breuil, C. 2002. Cloning and genetic analysis of subtilases in sapstaining fungi. *Curr. Genet.* 41:168-175.
- Holden, J. H. 1984. *Helminthosporium maydis* T toxin increased membrane permeability to Ca²⁺ in susceptible corn mitochondria. *Plant Physiol.* 75:225.
- Hu, G. L., Yu, C. Z., Lou, J. F., Zhao, Y. G., Zhou, P. S., and Zhan, G. H. 2005. Occurrence regularity and control experiment of shoot wilt in *Phyllostachys promineus*. *For. Pest Dis.* 24:38-41. (In Chinese)
- Jiang, S. J., and Qiang, S. 2005. The effect of the mycotoxin of α,β -dehydrocurvularin from *Curvularia eragrostidis* on PS II in *Digitaria sanguinalis*. *Sci. Agric. Sin.* 38:1373-1378. (In Chinese)
- Johnson, R. D., Johnson, L., Itoh, Y., Kodama, M., Otani, H., and Kohmoto, K. 2000. Cloning and characterization of a cyclic peptide synthetase gene from *Alternaria alternata* apple pathotype whose product is involved in AM-Toxin synthesis and pathogenicity. *Mol. Plant-Microbe Interact.* 13:742-753.
- Kunitz, M. 1950. Crystalline desoxyribonuclease. I. Isolation and general properties. *J. Gen. Physiol.* 33:349-362.
- Li, S. J., and Zhu, T. H. 2012. Binding site of toxic protein from *Arthrinium phaeospermum* on plasmalemma of hybrid bamboo. *J. Zhejiang Univ. (Agric. Life Sci.)* 38:355-361. (In Chinese)
- Lorenz, N., Haarmann, T., Pažoutová, S., Jung, M., and Tudzynski, P. 2009. The ergot alkaloid gene cluster: Functional analyses and evolutionary aspects. *Phytochemistry* 70:1822-1832.
- Lygin, A. V., Li, S. X., Vittal, R., Widholm, J. M., Hartman, G. L., and Lozovaya, V. V. 2009. The importance of phenolic metabolism to limit the growth of *Phakopsora pachyrhizi*. *Phytopathology* 99:1412-1420.
- Mabrouk, Y., Simier, P., Arfaoui, A., Sifi, B., Delavault, P., Zourgui, L., and Belhadj, O. 2007. Induction of phenolic compounds in pea (*Pisum sativum* L.) inoculated by *Rhizobium leguminosarum* and infected with *Orobanche crenata*. *J. Phytopathol.* 155:728-734.
- Madsen, J. P., and Hodges, C. F. 1983. Soluble sugar and free amino acids of *Poa pratensis* exposed to chlorophenoxy herbicides and pathogenesis by *Drechslera sorokiniana*. *Phytopathology* 73:737-740.
- Manning, V. A., and Ciuffetti, L. M. 2005. Localization of Ptr ToxA produced by *Pyrenophora tritici-repentis* reveals protein import into wheat mesophyll cells. *Plant Cell* 17:3203-3212.
- Matern, U., and Kneusal, R. E. 1988. Phenolic compounds in plant disease resistance. *Phytoparasitica* 16:153-170.
- Miller, J. D., and Ewen, M. A. 1997. Toxic effects of deoxynivalenol on ribosomes and tissues of the spring wheat cultivars *Frontana* and *Casavant*. *Nat. Toxins* 5:234-237.
- Morefield, H., Goodman, T. V., and Hamissou, M. 2005. The effects of aluminum toxicity on the protein expression of *Arabidopsis thaliana*. *BIOS* 76:84-88.
- Mullet, J. E., Klein, P. G., and Klein, R. R. 1990. Chlorophyll regulates accumulation of the plastid-encoded chlorophyll apoproteins CP43 and D1 by increasing apoprotein stability. *Proc. Natl. Acad. Sci. USA* 87:4038-4042.
- Nicholson, R. L., and Hammerschmidt, R. 1992. Phenolic compounds and their role in disease resistance. *Annu. Rev. Phytopathol.* 30:369-389.
- Nordin, J. H., and Strobel, G. A. 1981. Structural and immunochemical studies on the phytotoxic peptideboromannan of *Ceratocystis ulmi*. *Plant Physiol.* 67:1208-1213.
- Palmer, C. S., Saleeba, J. A., and Lyon, B. R. 2005. Phytotoxicity on cotton ex-plant of an 18.5 kDa protein from culture filtrates of *Verticillium dahliae*. *Physiol. Mol. Plant Pathol.* 67:308-318.
- Penner, D., James, E. D., and Backman, P. 1969. The influence of *Syringomycin* on ribonucleic acid synthesis. *Plant Physiol.* 44:806-808.
- Pestka, J. J., Bahrawy, A. E., and Hart, L. P. 1985. Deoxynivalenol and 15-monoacetyl deoxynivalenol production by *Fusarium graminearum* R6576 in liquid media. *Mycopathology* 91:23-28.
- Potrich, C., Bastiani, H., Colin, D. A., Huck, S., Prévost, G., and Serra, M. D. 2009. The influence of membrane lipids in *Staphylococcus aureus* Gamma-Hemolysins pore formation. *J. Membr. Biol.* 227:13-24.
- Pozo, M. J., Baek, J. M., Garcia, J. M., and Kenerley, C. M. 2004. Functional analysis of tvsp1, a serine protease-encoding gene in the biocontrol agent *Trichoderma virens*. *Fungal Genet. Biol.* 41:336-348.
- Prusky, D., and Keen, N. T. 1993. Involvement of preformed antifungal compounds in the resistance of subtropical fruits to fungal decay. *Plant Dis.* 77:114-118.
- Quayyum, H. A., Gijzen, M., and Traquair, J. A. 2003. Purification of a necrosis-inducing, host-specific protein toxin from spore germination fluid of *Alternaria panax*. *Phytopathology* 93:323-328.
- Raoudha, B. A. M., Samiha, S., and Lilia, F. B. F. 2006. Purification and structure determination of four bioactive molecules from a newly isolated *Streptomyces* sp. TN97 strain. *Process Biochem.* 41:1506-1513.
- Rasmussen, J. B., Kwon, C. Y., and Meinhardt, S. W. 2004. Requirement of host signaling mechanisms for the action of Ptr ToxA in wheat. *Eur. J. Plant Pathol.* 110:333-335.
- Rattan, R. S. 2010. Mechanism of action of insecticidal secondary metabolites of plant origin. *Crop Prot.* 29:913-920.
- Rodriguez, E. A. E., Jonkers, W., Corby, K. H., and May, G. 2012. Interactions between *Fusarium verticillioides*, *Ustilago maydis*, and *Zea mays*: An endophyte, a pathogen, and their shared plant host. *Fungal Genet. Biol.* 49:578-587.
- Sarma, G. N., Manning, V. A., Ciuffetti, L. M., and Karplus, P. A. 2005. Structure of Ptr ToxA: an RGD-containing host-selective toxin from *Pyrenophora tritici-repentis*. *Plant Cell* 17:3190-3202.
- Sasaki, T., Yamamoto, Y., Ezaki, B., Katsuhara, M., Ahn, S. J., Ryan, P. R., Delhaize, E., and Matsumoto, H. 2004. A wheat gene encoding an aluminum-activated malate transporter. *Plant J.* 37:645-653.

52. Scarpari, L. M., Meinhardt, L. W., Mazzafera, P., Pomella, A. W. V., Schiavinato, M. A., Cascardo, J. C. M., and Pereira, G. A. G. 2005. Biochemical changes during the development of witches' broom: the most important disease of cocoa in Brazil caused by *Crinipellis pernicioso*. J. Exp. Bot. 56:865-877.
53. Shimizu, N., Hosogi, N., and Hyon, G. S. 2006. Reactive oxygen species (ROS) generation and ROS-induced lipid peroxidation are associated with plasma membrane modifications in host cells in response to AK-toxin I from *Alternaria alternata* Japanese pear pathotype. J. Gen. Plant Pathol. 72:6-15.
54. Steyaert, J. M., Stewart, A., and Ridgway, H. J. 2004. Co-expression of two genes, chitinase (chit 42) and proteinase (prb 1), implicated in mycoparasitism by *Trichoderma Hamatum*. Mycologica 96:1245-1252.
55. Taiwo, M. A., and Akinjogunla, J. 2006. Cowpea viruses: Quantitative and qualitative effects of single and mixed viral infections. Afr. J. Biotechnol. 5:1749-1756.
56. Thind, S. K., Monga, P. K., Kaur, N., and Cheema, S. S. 1996. Analysis of some biochemical and micro-nutrient constituents of yellow mosaic virus infected moong. Indian J. Virol. 12:157-159.
57. Townsend, G. R., and Heuberger, I. W. 1943. Methods of estimating losses caused by diseases in fungicide experiments. Plant Dis. Rep. 27:340-343.
58. Treutter, D. 2006. Significance of flavonoids in plant resistance: A review. Environ. Chem. Lett. 4:147-157.
59. Vijayakumar, E. K., Roy, K., and Chatterjee, S. 1996. Arthrictin. A new cell wall active metabolite from *Arthrimum phaeospermum*. J. Org. Chem. 61:6591-6593.
60. Wang, J. Z., and Fan, M. 2001. Pages 77-100 in: Protein Technical Manual. Science Press, Beijing. (In Chinese)
61. Wu, S. L. 2004. Pages 20-24 in: Experiment Tutorial of Biochemistry and Molecular Biology. Science Press, Beijing. (In Chinese)
62. Xu, F., Zhu, J., Zhang, F. X., Wang, Y., Cheng, S. Y., and Cheng, S. H. 2008. Cloning of PAL gene from *Sophora japonica*, construction of anti-sense gene of *SjPAL* and its genetic transformation in *Arabidopsis*. For. Res. 21:611-618. (In Chinese)
63. Xu, L. S., Jia, J. G., Lv, J. E., Liang, X. F., Han, D. J., Huang, L. L., and Kang, Z. S. 2010. Characterization of the expression profile of a wheat aci-reductone-dioxygenase-like gene in response to stripe rust pathogen infection and abiotic stresses. Plant Physiol. Biochem. 48:461-468.
64. Yang, Q. 2007. Research status of *B. pervariabilis* × *D. grandis* and cost-benefit analysis. Shandong For. Sci. Technol. 37:101-103. (In Chinese)
65. Ye, J. R., Qiao, G. F., Bao, H., and Feng, W. Z. 2000. Studied on the role mechanisms of the brown spot needle blight fungus toxin to make host cell damages. Sci. Silv. Sin. 36:82-86. (In Chinese)
66. Zemanek, A. B., Ko, T. S., Thimmapuram, J., Hammerschlag, F. A., and Korban, S. S. 2002. Changes in β -1,3-glucanase mRNA levels in peach in response to treatment with pathogen culture filtrates, wounding, and other elicitors. J. Plant Physiol. 159:877-889.
67. Zhang, W. Q. 1998. Study on some physiological biochemical index of top blight of *Phyllostachys heterocycla*. J. Fujian Coll. For. 18:83-86. (In Chinese)
68. Zheng, H. X., and Wang, J. M. 2009. Toxic effects of *Fusarium oxysporum* extracts on the soluble protein content in watermelon seedling. J. Shanxi Agric. Sci. 37:13-15. (In Chinese)
69. Zhu, G. L., Zhong, H. W., and Zhang, A. Q. 1990. Pages 134-138 in: Plant Physiological Experiment. Peking University Press, Beijing. (In Chinese)
70. Zhu, T. H., Huang, Z. C., Gao, Q. Z., Li, F. L., Luo, L. J., and Li, X. D. 2009a. Pathogen and occurrence regularity of *Bambusa ervariabilis* × *Dendrocalamopsis daii* blight. For. Pest Dis. 28:10-12. (In Chinese)
71. Zhu, T. H., Huang, Z. C., Gao, Q. Z., Mu, Y. Q., Luo, Y. Q., Mao, X. T., and Li, X. D. 2009b. Research on survey and space distribution of *Bambusa ervariabilis* × *Dendrocalamopsis daii* blight. J. Sichuan For. Sci. Technol. 30:29-31, 37. (In Chinese)
72. Zou, Q. 2000. Pages 129-130 in: Plant Physiology Experiment Guide. China Agriculture Press, Beijing. (In Chinese)