J Wood Sci (2006) 52:367–371
DOI 10.1007/s10086-005-0768-x

NOTE

Jun Mu · Tohru Uehara · Takeshi Furuno

Regulation effect of *Phyllostachys pubescens* methanol extractives on growth of seed plants

Received: June 20, 2005 / Accepted: September 30, 2005 / Published online: March 2, 2006

Abstract Methanol extractives from moso bamboo (*Phyllostachys pubescens*) culms were found to have some regulation effects on the growth of the seed plants tested. They showed inhibition or promotion of hypocotyl and radicle growth of lettuce, watercress, and chrysanthemum. The methanol extractives were fractionated with *n*-hexane, diethyl ether, and ethyl acetate. The diethyl ether-soluble fraction contained more phenolic substances than the other fractions and showed strong inhibition of growth in lettuce seeds. The water-soluble fraction showed a promotion effect on the hypocotyl growth of lettuce. The water-soluble fraction was heated at 200°C for 8h, and the inhibition effect on the radicle growth of lettuce decreased and the promotion effect on the hypocotyl growth increased at 0.1% concentration.

Key words Bamboo extractives · Regulation effect · Plant seeds · Phenolic substance · Heat treatment

Introduction

Bamboo and its application are attracting more and more attention because of its fast growth and strong regeneration ability. Bamboo grows from buds of rhizomes in the soil. New shoots grow rapidly during both the day and night and reach full height within only 2–3 months. When it matures, the height and diameter of bamboo does not change.[1] Bamboo is not only a good construction material but is also an excellent pharmacological resource. Studies on bamboo extractives have mostly focused on roots, shoots, and leaves for the identification of bioactive components with antioxidant activity, antimicrobial activity, and others.[2–7] However, the regulation effect of bamboo extractives on the growth of seed plants has been reported in a limited number of studies.

As for regulation effect of extractives from trees, phenolic substances, especially phenolic acids such as benzoic acids, cinnamic acids, flavonoids, and lactones have a strong inhibition effect on growth of plants.[8] Lignans extracted from wood also showed an inhibition effect on plants.[9] Promotion effects of extractives came from plant hormone substances or their precursors and derivatives. Shimizu et al.[10] showed that cell wall polysaccharides had a promotion effect on elongation of pea stem. The extractives from trees with regulation effects on the growth of plants also had other biological activities.[11,12] Previously, we have studied the regulation effect of bamboo vinegar, a by-product of bamboo charcoal, on the growth of seed plants and found some promotion effects on the germination and radicle growth of seeds tested at appropriate concentration.[13] In the present study, we investigated the regulation effect of extractives from bamboo culms on the growth of seed plants.

J. Mu · T. Uehara (✉) · T. Furuno
Faculty of Science and Engineering, Shimane University, Matsue 690-8504, Japan
Tel. +81-852-32-6565; Fax +81-852-32-6123
e-mail: uehara@riko.shimane-u.ac.jp

J. Mu
College of Material Science and Technology, Beijing Forestry University, Beijing 100083, China

Experimental

Plant materials

The culms of 3-year-old moso bamboo (*Phyllostachys pubescens* Mazel) were collected in December 2001 in Aichi Prefecture, Japan. The culms were cleaned and dried in the dark. The culms were divided into the parts of nodes and internodes. The two part types were ground into powder in a Wiley mill and kept in a refrigerator before use.

Extraction and fractionation

The node and internode samples (50 g × 4) were extracted in Soxhlet apparatus for 24 h with methanol (400 ml × 4). The

Table 1. Yield of methanol extractives of bamboo culms and fractions from the methanol extractives

Moso bamboo	Methanol extractives[a]	*n*-Hexane fraction[b]	Diethyl ether fraction[b]	Ethyl acetate fraction[b]	Water-soluble fraction[b]
Nodes	6.53	5.37	2.98	6.71	48.02
Internodes	5.97	7.72	2.26	5.59	44.57

[a] Figures show weight percent of methanol extractives based on the oven-dried samples
[b] Figures show weight percent of the fractions based on the methanol extractives

concentrates of the methanol extractives were suspended in water (200 ml) and then fractionated with *n*-hexane, diethyl ether, and ethyl acetate to give the respective fractions and a water-soluble fraction. The fractions were concentrated in vacuo below 40°C.

Thin-layer chromatography

Thin-layer chromatography (TLC) analysis of the fractions was performed on silica-gel $60F_{254}$ plates. The developing solvent was a mixture of benzene/acetone (1:1). Spots were made visible using diazotized sulfanilic acid and 2,4-dinitrophenylhydrazine to detect phenolic compounds.

Quantification of total sugars and phenols

Sample solutions were prepared at a concentration of 50 μg/ml in distilled water. Total phenols was determined by the Folin-Dennis assay[14] with tanninic acid as a standard at 725 nm. Total sugars of the water-soluble fraction was determined by the phenol/ sulfuric acid method with glucose as a standard at 490 nm.[15] Absorbance was measured with a Jasco V-560 UV/VIS spectrophotometer.

Heat treatment of water-soluble fractions

Water-soluble fraction (0.1 g) was put into a 50-ml Erlenmeyer flask and treated with nitrogen gas before sealing. The samples was heated at 200°C in an oven box for 8 h and then cooled in a vacuum desicator.

Plant growth test

The methanol extractives from bamboo culms and the respective fractions were used as solutions at concentrations of 0.1%, 0.01%, and 0.001%. Distilled water was used as the control. Twenty seeds were scattered on two pieces of filter paper (Advantec no. 2) moistened with 10 ml of the test solution in a 9-cm petri dish and were allowed to stand in a dark room at 20°C and 60% relative humidity (RH). The radicle and hypocotyl growth were measured and compared with the controls after 4 days. The bioassay test was repeated three times for each sample. The tested seeds were lettuce (*Lactuca sativa* L.), watercress (*Rorippa nasturtium-aquaticum* Hayek), and chrysanthemum (*Chrysanthemum coronarium* L.).

Results and discussions

Yield of extractives

Being different from wood, bamboo grows not only from the top of the culm but also from each node simultaneously. Nodes of bamboo culms might contain some growth-regulation substances that are different from those found in the internodes. Therefore, we divided the culms into samples of nodes and internodes.

Nodes and internodes were extracted with methanol and the methanol extractives were further fractionated with *n*-hexane, diethyl ether, and ethyl acetate. Table 1 shows the yield of each fraction from methanol extractives of moso bamboo culms. The content of methanol extractives from nodes (6.53%) was more than that from the internodes (5.97%). It is possible that more substance was stored in the nodes during the growth process. The water-soluble fraction was the largest of the fractions separated from the methanol extractives, and the weight of the fraction made up about 50% of that of the methanol extractives.

Regulation effect of methanol extractives from moso bamboo on growth of three seed types

The regulation effects of methanol extractives from moso bamboo culms on three seed types are shown in Table 2. The methanol extractives showed little effect on germination for the three seeds. However, the radicle and hypocotyle growth were obviously affected by the extractives. For lettuce, radicle growth inhibition by methanol extractives from nodes was higher than that from internodes. The hypocotyl growth of the three seeds was promoted remarkably with internode methanol extractives at 0.1% concentration; the promotion effects were 145.1% for lettuce, 128.6% for watercress, and 138.7% for chrysanthemum based on the control.

The difference of regulation effect on seeds might come from the reaction of seeds with the different methanol extractives from nodes and internodes.

Table 2. Effect of methanol extractives from moso bamboo on lettuce, watercress, and chrysanthemum

Seeds	Concentration (%)	Based on the control (%)[a]		
		Germination	Radicles	Hypocotyls
Nodes				
Lettuce	0.1	96.7	44.5 ± 1.30**	112.4 ± 3.35*
	0.01	96.7	67.1 ± 3.32**	105.1 ± 1.67
	0.001	98.3	74.6 ± 0.72**	97.9 ± 2.92
Watercress	0.1	111.8	54.5 ± 2.74**	92.8 ± 3.36*
	0.01	115.7*	91.1 ± 4.52*	101.7 ± 3.97
	0.001	111.8	109.4 ± 4.76	100.1 ± 4.11
Chrysanthemum	0.1	79.5*	105.3 ± 6.75	96.5 ± 9.36
	0.01	107.7	90.7 ± 2.18	111.7 ± 6.89
	0.001	107.7	103.2 ± 9.82	107.6 ± 8.83
Internodes				
Lettuce	0.1	103.7	97.6 ± 1.97	145.1 ± 6.09**
	0.01	102.0	129.5 ± 4.23**	108.2 ± 8.01*
	0.001	102.0	138.4 ± 0.32**	102.3 ± 7.90
Watercress	0.1	103.9	51.0 ± 1.76**	128.6 ± 5.49**
	0.01	102.0	66.5 ± 3.58**	99.5 ± 4.15
	0.001	102.0	74.1 ± 1.44**	98.5 ± 8.76
Chrysanthemum	0.1	102.8	144.7 ± 8.43**	138.7 ± 2.17**
	0.01	106.5	114.6 ± 9.30	110.2 ± 3.63
	0.001	92.6	124.2 ± 11.82	114.5 ± 4.39

* $P < 0.05$; ** $P < 0.01$

[a] Values are percentages based on the control

Regulation of the growth of lettuce seeds with fractions of methanol extractives

Methanol extractives from nodes showed a strong inhibition effect on the radicle growth of lettuce, while methanol extractives from internodes appeared to have a promotional effect on hypocotyl growth. The methanol extractives were further fractionated with different solvents. Table 3 showed the effects of these fractions on lettuce seeds.

Among the four fractions based on *n*-hexane, diethyl ether, ethyl acetate, and water, the diethyl ether-soluble fractions from nodes and internodes showed the strongest inhibition of germination and radicle and hypocotyl growths. As for the effect of the ethyl acetate fraction, both radicle and hypocotyl growths were inhibited. Radicle and hypocotyl growth with the *n*-hexane fraction were less inhibited than those with the diethyl ether and ethyl acetate fractions. Radicle growth with the water-soluble fraction was also inhibited at 0.1% and 0.01%, while the hypocotyl growth showed some promotion. From the regulation effect of these four fractions, inhibition components were present in the organic fractions, while the promotion effect on the hypocotyl growth appeared to come from only the water-soluble fraction.

To compare the composition of these four fractions, TLC was used. The observed inhibition by the *n*-hexane fraction may be due to terpenoids or wax, which are commonly found in these extracts, because no phenolic substance could be detected by the color reaction. The diethyl ether and ethyl acetate fractions were subjected to TLC and spots corresponding to phenolic acids were detected by the color reaction. TLC of the water-soluble fraction also showed the existence of phenolic substance. The quantity of phenolic substance were measured and the results are shown in Table 4. The diethyl ether fraction appeared to contain more phenolic substances than other fractions and appeared to have a stronger inhibition effect. Most phenolic acids and phenolic substances have been found to have inhibition effects on plant growth.[16] Therefore, the toxic constituents in these two fractions might due to phenolic acids and phenolic substances.

The water-soluble fraction showed a promotion effect on the hypocotyl growth of lettuce, shown in Fig. 1. Water-soluble extractives of bamboo culms usually comprise monosaccharides or oligosaccharides such as glucose and sucrose.[17] The sugar content in the water-soluble fractions of the methanol extractives for the node and internode samples were 16.2% and 19.7%, respectively (Table 4). The results of the color test with 10% α–naphthol in ethanol and Benedict's reagent also proved that water-soluble fractions contain sugars and reducing sugars. Therefore, we investigated the effect of glucose and sucrose on germination and the radicle and hypocotyl growths of lettuce. Although glucose and sucrose act as nutrient substances for plant growth, there was no promotion effect on germination and growth for lettuce seeds; rather, some inhibition appeared to have occurred (data not shown). These results suggested that the promotion effect on hypocotyl growth was not due to glucose and sucrose. The promotion effect might come from the polysaccharides or oligosaccharides stored in the bamboo culms. It was thought that a hormone-evoked loosen effect on cell wall would contribute to growth promotion.[10] Moreover, polysaccharides are known to affect the production and regulation of plant hormones.[18] Some water-soluble polysaccharides in the extractives might affect the

Table 3. Effect of fractions from methanol extractives of moso bamboo on lettuce

Fractions	Concentration (%)	Based on the control (%)[a]		
		Germination	Radicles	Hypocotyls
Nodes				
n-Hexane	0.1	102.0	91.5 ± 3.99*	90.6 ± 3.61**
	0.01	100.4	82.8 ± 7.96**	80.0 ± 6.49**
	0.001	103.7	96.1 ± 5.45	91.4 ± 2.81**
Diethyl ether	0.1	22.4**	–	–
	0.01	103.7	53.5 ± 2.69**	84.4 ± 3.48**
	0.001	103.7	73.7 ± 4.67**	88.0 ± 5.52**
Ethyl acetate	0.1	103.7	45.4 ± 1.99**	74.6 ± 1.43**
	0.01	102.0	71.8 ± 4.67**	83.6 ± 3.56**
	0.001	103.7	80.1 ± 3.83**	88.1 ± 2.72**
Water-soluble	0.1	100.4	57.4 ± 2.32**	121.9 ± 6.38**
		(101.6)	(74.2 ± 2.10)	(155.0 ± 1.42)**
	0.01	103.9	72.2 ± 2.41**	115.2 ± 4.32*
		(101.6)	(81.3 ± 2.75)**	(111.7 ± 1.89)**
	0.001	105.6	105.4 ± 4.94	111.4 ± 8.18
		(100.0)	(86.9 ± 3.51)**	(99.8 ± 0.71)
Internodes				
n-Hexane	0.1	102.1	100.2 ± 2.37	94.6 ± 3.91*
	0.01	98.7	117.2 ± 5.48**	95.5 ± 2.21
	0.001	100.3	107.0 ± 4.15	101.2 ± 3.55
Diethyl ether	0.1	45.0**	–	–
	0.01	100.5	81.3 ± 3.44**	96.2 ± 0.59
	0.001	102.1	94.0 ± 2.30	98.9 ± 1.84
Ethyl acetate	0.1	100.3	69.8 ± 1.05**	79.0 ± 1.64**
	0.01	102.2	90.6 ± 6.33*	94.6 ± 4.19*
	0.001	103.9	111.4 ± 7.17*	97.5 ± 3.86
Water-soluble	0.1	93.3	83.0 ± 2.26**	140.1 ± 2.96**
		(100.2)	(93.3 ± 4.18)*	(152.8 ± 4.11)**
	0.01	100.0	94.7 ± 3.14	118.1 ± 1.57**
		(98.5)	(99.1 ± 3.56)	(133.5 ± 1.19)**
	0.001	100.0	94.3 ± 3.24	101.0 ± 3.41
		(103.7)	(90.6 ± 1.12)	(106.0 ± 2.85)

* $P < 0.05$; ** $P < 0.01$

[a] Values are percentages based on the control. The values in parentheses are bioassay results with water-soluble fraction after heat treatment

Table 4. Quantity of phenolic substances and sugars in each fraction

Moso bamboo	Phenolic substances		Sugars	
	Et_2O	EtoAc	Ws	Ws
Nodes	38.3	25.6	12.3	16.2
Internodes	36.0	24.0	13.2	19.7

Values show contents percentage in each fraction

Et_2O, diethyl ether fraction; EtoAc, ethyl acetate fraction; Ws, water-soluble fraction

hormone to regulate the growth when they were absorbed in seeds.[19,20]

Heat treatment of water-soluble fraction and its effect on the growth of lettuce seeds

Compared with the effect of bamboo vinegar, bamboo methanol extractives showed more inhibition than promotion on the radicle growth of the seed plants tested. Bamboo vinegar that contain many low molecular weight compounds produced in pyrolysis that showed promotion

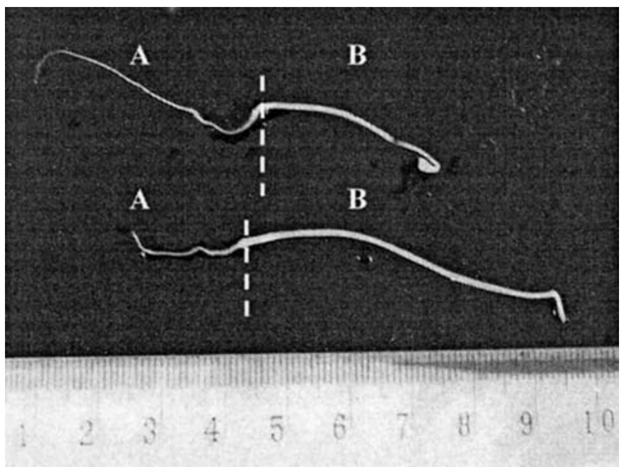

Fig. 1. Radicle (*A*) and hypocotyl (*B*) growths of lettuce with water-soluble fraction from moso bamboo methanol extractives at 0.1% concentration measured 4 days after germination. The *upper* shoot is the control with distilled water

effects on germination and radicle growth at appropriate dilution.[13] Because water-soluble fraction showed some regulation effects on radicle and hypocotyl growths, the water-soluble fraction was heat-treated and investigated with lettuce seeds. The effects of heat-treated extractives on the radicle and hypocotyl growths of lettuce seeds are shown in Table 2. Extractives with heat treatment showed remarkable promotion of hypocotyl growth. For the radicle growth, the inhibition effect was still present, but decreased in comparison with that without heat treatment. Germination was nearly the same as the control.

Pyrolytic components from bamboo extractives might be involved in the promotion effect on the growth of seeds. Hypocotyl growth is often regulated by hormones such as gibberellin. Dihydroconiferyl alcohol was identified as an active component in the hypocotyl growth of lettuce by inducing gibberellin to take effect.[8] The promotion effect found in this study might come from the water-soluble polysaccharides present in the extractives. After the heat treatment, these water-soluble polysaccharides would take good effect on the plant hormone such as gibberellin to regulate the growth of seeds. The variation of the regulation effect could be due to the change of water-soluble substances after heat treatment. Further studies are need to determine the relation between them.

Conclusions

Methanol extractives from moso bamboo culms showed a regulation effect on the seeds tested. The regulation effect appeared as an inhibition of radicle growth and as promotion of hypocotyl growth at 0.1% concentration. The inhibition effect was thought to be due to phenolic acids and phenolic substances contained in the extractives. The promotion effect might be due to water-soluble polysaccharides. After treatment with heat, the water-soluble fraction showed more promotion of hypocotyl growth and less inhibition of radicle growth than that without heat treatment. The result suggested that heat treatment such as pyrolysis might induce some changes for regulation of bamboo extractives on seed plants. Heat treatment was thought to be a useful method for utilization of bamboo extractives.

References

1. Ikeshima Y (1999) Method of producing and using bamboo charcoal and bamboo vinegar (in Japanese). Nosan Gyoson Bunka Kyokai, Tokyo, pp 13–14
2. Chuyen NV, Kurata T, Kato H, Fujimaki M (1982) Antimicrobial activity of kumazasa (*Sasa albo-marginata*). Agric Biol Chem 46:971–978
3. Nishina A, Hasegawa K, Uchibori T, Seino H, Osawa T (1991) 2,6-Dimethoxy-*p*-benzoquinone as an antibacterial substance in the bark of *Phyllostachys heterocycla* var. Pubescence, a species of thick-stemmed bamboo. J Agric Food Chem 39:266–269
4. Vastano BC, Chen Y, Zhu NQ, Ho CT, Zhu ZY, Rober TR (2000) Isolation and identification of stilbenes in two varieties of *Polygonum cuspidatum*. J Agric Food Chem 48:253–256
5. Hu C, Zhang Y, David DK (2000) Evaluation of antioxidant and prooxidant activities of bamboo *Phyllostachys nigra* var. *Henonis* leaf extract in vitro. J Agric Food Chem 48:3170–3176
6. Kweon MH, Hwang HJ, Sung HC (2001) Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllostachys deulis*). J Agric Food Chem 49:4646–4655
7. Ding YQ, Chen CY, Elmahadi EA, Xu HB (1998) Chromatographic analysis of polysaccharides extracted from Chinese *Indocalamus Tesselatus*. Biomed Chromatogr 12:86–88
8. Takahashi N, Marumo S, Otake N (1981) Chemistry of natural substance with bioactivity. Tokyo University Publishing, Tokyo, pp 80–90
9. Umezawa T (1996) Biological activity and biosynthesis of lignans (in Japanese). Mokuzai Gakkashi 42:911–920
10. Shimizu Y, Hayashi T, Kawada T, Sakuno T (1997) Promotion of pea stem elongation by the fragments of plant cell wall polysaccharides (in Japanese). Mokuzai Gakkaishi 43:121–127
11. Ohara S, Suzki K, Ohira T (1994) Condensed tannins from *Acacia mearnsii* and their biological activities (in Japanese). Mokuzai Gakkaishi 40:1363–1374
12. Yatagai M, Ding Y (1996) Amounts of extractives from *Pinus massoniana*, their chemical compositions, miticidal activities, and growth regulation effects on radish seeds (in Japanese). Mokuzai Gakkaishi 42:1221–1227
13. Mu J, Uehara T, Furuno T (2003) Effect of bamboo vinegar on regulation of germination and radicle growth of seed plants. J Wood Sci 49:262–270
14. Swain T, Hills WE (1959) The phenolic constituents of *Prunus domestica* – the quantitative analysis of phenolic constituents. J Sci Food Agric 10:63–68
15. Dubois M, Gillies KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356
16. Ohira T, Yatagai M (1994) Allelopathic compounds produced by forest plants II. The relationships between the inhibition effects on plant growth and killing activities of brine shrimp of phenolic compounds. Mokuzai Gakkaishi 40:541–548
17. Yoshimoto T, Morita S (1985) Studies on hot-water extractives of bamboo stem-seasonal variation of the content of free sugars. Bull Tokyo Univ For 74:9–15
18. Harada T, Misaki A (1974) Comprehensive polysaccharide science (in Japanese). Kodansya, pp 625–635
19. Antoni F, Pablo GP, Susana S, Carmen R (2003) Effect of heat treatment and dehydration on bioactive polysaccharides acemannan and cell wall polymers from *Aloe barbadensis* Miller. Carbohydr Polym 51:397–405
20. Sun RC, Tomkinson J (2002) Comparative study of organic solvent-soluble and water-soluble lipophillic extractives from wheat straw 2: spectroscopic and thermal analysis. J Wood Sci 48:222–226