

Pyroligneous Acid Improves In Vitro Rooting of Japanese Pear Cultivars

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Abstract. Effects of PA on in vitro shoot proliferation and root formation were investigated using shoot cultures of three Japanese pear (*Pyrus pyrifolia* Nakai) cultivars. PA inhibited shoot multiplication and promoted initiation and development of roots in the cultured shoots of three cultivars, resulting in increasing the proportion of rooted shoots. Chemical name used: pyroligneous acid (PA).

Pyroligneous acid (PA) (wood vinegar) is a dark brown solution obtained as a by-product of wood carbonization. It contains over 200 components, such as acids, alcohols, phenols, and neutrals (Jodai et al., 1989; Shirakawa et al., 1995b; Yatagai et al., 1988). PA accelerates seed germination of garland chrysanthemum (*Chrysanthemum coronarium* L.), honewort (*Cryptotaenia japonica* Hassk.) and lettuce (*Lactuca sativa* L.) (Uehara et al., 1993), seed germination and plantlet growth of chinese cabbage (*Brassica campestris* L.) and rice (*Oryza sativa* L.) (Yatagai and Unrinin, 1987), root (Tsuzuki et al., 1989) and plant (Ichikawa and Ota, 1982; Shirakawa et al., 1995a) growth of rice seedlings, and root formation of sweet potato (*Ipomoea batatas* Poir.) plantlets (Du et al., 1998). This study investigated PA influences on shoot proliferation and root formation of in vitro shoots of Japanese pear (*Pyrus pyrifolia* N.) cultivars.

Materials and Methods

Plant materials. Shoots of Japanese pear 'Hosui', 'Kosui', and 'Shinko' were subcultured monthly for 12 months on a shoot proliferation medium consisting of half-strength MS (Murashige and Skoog, 1962) with 0.5 μM indole-3-butyric acid (IBA), 4.4 μM N6-benzyladenine, 58.5 μM sucrose and 0.8% powder agar (Wako Co., Tokyo). They were incubated at a 16-h photoperiod illuminated by a cool-white fluorescent light (50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at 25 °C in a tube (25 \times 100 mm) with 10 mL of gelled medium.

Preparation of PA. The PA used in this study was manufactured at Toyama City Agricultural Center. Pruned branches of Japanese pear were cut during winter, were put in a greenhouse and were dried by natural wind for 2–3 weeks. Then, they were placed inside an iron kiln (0.75 m² \times 2.5 m) and were dried

by distillation. The distillation duration was \approx 10 h and the rate of temperature increase was 2 °C per min., with maximum temperature of \approx 500 °C. Crude PA was obtained as a dripping solution from the base of an opening of a gas spillage and was kept for 6 months in a dark, cool room. The top clear layer was removed from the deposited tar by filtering through a coffee filter paper (Karita Co., Tokyo) and was used in the following experiment.

Shoot proliferation and rooting investigation. To investigate the effect of PA on shoot proliferation, shoot tips were transferred to a proliferation medium containing 0%, 0.001%, 0.01%, or 0.1% (v/v) PA. The number of new shoots formed from each initial shoot and the rate of fresh weight increase after 30 d were recorded. To investigate the effect of PA on rooting, 10 mm shoot tips were cut and transferred to a root initiation medium consisting of half-strength MS with 4.9 μM IBA, 58.5 μM sucrose, 1.35 mM phloroglucinol (1,3,5-trihydroxybenzene), 0.8% powder agar and 0%, 0.001%, 0.01% or 0.1% (v/v) PA and were kept in the dark at 25 °C. After 5 d, they were transferred to the same medium without IBA (root development medium). Rooting frequency, the average root length and survival rate were recorded after 45 d. All media were adjusted to pH 5.7 and were autoclaved at 121 °C for 20 min. The PA solution was adjusted to pH 5.7 using 1.0 N NaOH, and the PA solution and phloroglucinol were filter-sterilized and were added to the medium after autoclaving. All cultures were incubated at a 16-h photoperiod illuminated by a cool-white fluorescent light (50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at 25 °C in a plastic jar (5 \times 15 cm) with 30 mL of gelled medium.

Statistical analysis. For each investigation, five jars, each with four shoots, were used and treatments were arranged as a randomized complete block. The data were analyzed using analysis of variance (ANOVA) with mean separation by least significant difference (LSD) at $P \leq 0.05$. For the experiment on shoot

proliferation the number of shoots formed by each explant and the increased in fresh weight were subjected to a two-way ANOVA with PA concentrations and cultivars. For the experiment on rooting, the rooting frequency, average root length and the survival rate of shoots were analyzed using two-way ANOVA with PA concentrations and cultivars. Also, all investigation items had regression curves drawn using the concentration of PA as the standard.

Results and Discussion

Shoot proliferation. Table 1 shows the effects of PA on shoot number and increased rate of shoots in fresh mass. Shoot proliferation and fresh weight of multiplied shoots increased in the proliferation medium without PA. All of the cultivars were inhibited by adding PA, but especially 'Hosui'. The inhibitory effect on fresh weight tended to increase with increasing PA concentration. Regression analysis indicated that the shoot number was related to increasing the PA concentration in 'Kosui' and the fresh weight increase was related to the PA concentration in 'Hosui' and 'Shinko'.

Our results disagreed with previous studies in which PA improved the growth of a few plants (Ichikawa and Ota, 1982; Shirakawa et al., 1995a; Yatagai and Unrinin, 1987). These results might be related to the effects of PA on plant growth, however, the effects differed depending on the plant species (Uehara et al., 1993; Yatagai and Unrinin, 1987). Perhaps the refining treatment of PA, such as activated charcoal, accelerates the growth (Yatagai and Unrinin, 1987).

Survival rate, rooting, and root length. Table 2 shows the effect of PA on the survival rate of shoots, number of shoots forming roots and the average length of roots in Japanese pear cultivars. The four PA concentrations had no effect on the survival of explants of the three cultivars. PA stimulated rooting, with optimum concentrations at 0.01% and 0.1% for 'Hosui' and 0.1% for 'Kosui' and 'Shinko', whereas PA had no effect on root growth. Regression analysis indicated that the rooting frequency was related to the PA concentration for 'Kosui' and 'Shinko'.

Shirakawa et al., (1995b) reported that the main components of organic acids and phenols contained in PA affect the growth of rice plants. They found that some organic acids, such as isocaproic acid (4-methylvaleric acid), caproic acid (n-caproic acid) and tiglic acid [(E)-2-methyl-2-butenic acid], and some phenols, such as 2,6-dimethoxyphenol, 4-ethylphenol, and guaiacol (o-methoxyphenol), accelerate root growth at each 10 ppm. Furthermore, they compared the effect of each organic acid or phenol with PA treatment and suggested that organic acids and phenols in PA are synergistic in PA treatment because the effects of organic acids and phenols treated alone were inferior to PA treatment. Many studies have been undertaken to improve rooting in *Pyrus* sp. (Bertazza et al., 1995; Reed, 1995; Rodríguez

Table 1. Effect of pyroligneous acid (PA) on shoot proliferation of Japanese pear^a

Concn. of PA (v/v) (%)	Mean no. shoots formed per explant			Fresh wt increase from initial wt (%)		
	Hosui	Kosui	Shinko	Hosui	Kosui	Shinko
0	2.3 a ^y	3.8 a	2.7 a	799 a	865 a	888 a
0.001	1.6 c	3.5 a	2.9 a	483 bc	781 a	901 a
0.01	1.7 bc	2.8 a	2.1 b	557 b	607 b	709 b
0.1	1.9 b	2.9 a	2.3 b	376 c	671 ab	661 b
Significance						
Concn.		NS			**	
Cultivar		**			**	
Concn. × cultivar		NS			**	
Regression analysis ^x						
	$y_1 = 4.7^{-3}x$	$y_1 = 3.4^{-2}x$	$y_1 = -1.6^{-2}x$	$y_2 = -2.7^{-4}x$	$y_2 = -1.1^{-4}x$	$y_2 = -2.1^{-4}x$
	$-1.2^{-3}x^2$	$-5.0^{-3}x^2$	$-1.5^{-3}x^2$	$+1.46x^2$	$-4.08x^2$	$+5.7x^2$
	$+2.4^{-2}$	-2.1^{-2}	-1.5^{-3}	$+1.3^{-1}$	$+0.8^{-1}$	$+1.6^{-1}$
	($r^2 = 0.02^{NS}$)	($r^2 = 0.32^{**}$)	($r^2 = 0.18^{NS}$)	($r^2 = 0.56^{**}$)	($r^2 = 0.23^{NS}$)	($r^2 = 0.50^{**}$)

^aFresh mass of shoot tips of each cultivars at the start of the experiment were 17.5 ± 0.8 mg in Hosui, 22.9 ± 0.9 mg in Kosui, and 20.1 ± 0.8 mg in Shinko.

^bMean separation with in the same cultivar by LSD at $P \leq 0.05$.

^x y_1 , y_2 , and x indicate no. of shoots formed by each explant, rate of fresh weight increase (%) and concn. of PA (%), respectively.

^{NS, **}Nonsignificant or significant at $P \leq 0.01$.

Table 2. Effect of pyroligneous acid (PA) on in vitro rooting of Japanese pear.

Concn of PA (v/v) (%)	Rooting frequency (%)			Avg root length (mm)			Survival (%)		
	Hosui	Kosui	Shinko	Hosui	Kosui	Shinko	Hosui	Kosui	Shinko
0	25 b ^z	30 b	30 b	11 a	20 a	26 a	100 a	90 a	95 a
0.001	30 b	20 b	20 b	27 a	22 a	22 a	100 a	70 a	95 a
0.01	50 a	25 b	30 b	24 a	21 a	22 a	95 a	85 a	95 a
0.1	55 a	70 a	70 a	19 a	20 a	23 a	95 a	75 a	100 a
Significance									
Concn.		**			NS			NS	
Cultivar		NS			NS			**	
Concn. × cultivar		**			NS			NS	
Regression analysis ^y									
	$y_1 = 1.4^{-3}x$	$y_1 = 0.7^{-3}x$	$y_1 = 0.5^{-3}x$	$y_2 = 3.3^{-3}x$	$y_2 = 1.3^{-3}x$	$y_2 = -3.5^{-3}x$	$y_3 = 1.2^{-3}x$	$y_3 = 0.7^{-3}x$	$y_3 = -0.7^{-3}x$
	$-2.84x^2$	$+2.66x^2$	$+2.43x^2$	$-6.32x^2$	$-2.42x^2$	$+5.7x^2$	$-2.2x^2$	$-1.1x^2$	$+2.7x^2$
	-2.3^{-2}	-0.5^{-2}	$+0.1^{-2}$	$+6.3^{-2}$	$+3.5^{-2}$	$+9.5^{-2}$	$+3.6^{-4}$	$+44^{-4}$	$+48^{-4}$
	($r^2 = 0.51^{NS}$)	($r^2 = 0.91^{**}$)	($r^2 = 0.91^{**}$)	($r^2 = 0.32^{NS}$)	($r^2 = 0.11^{NS}$)	($r^2 = 0.17^{NS}$)	($r^2 = 0.21^{NS}$)	($r^2 = 0.11^{NS}$)	($r^2 = 0.17^{NS}$)

^zMean separation with in the same cultivar by LSD at $P \leq 0.05$.

^y y_1 , y_2 , y_3 and x indicate rooting frequency (%), average root length, survival rate (%) and concn. of PA (%), respectively.

^{NS, **}Nonsignificant or significant at $P \leq 0.01$.

et al., 1991; Wang, 1991, 1992; Yeo and Reed, 1995), but most did not study Japanese pear. The results of this study clearly show that PA improved Japanese pear rooting in vitro. We are now studying the effect of PA on rooting in other woody rosaceous species and the specific components of PA that accelerate the rooting processes.

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