Technical paper

Banana Polyphenol Oxidase: Occurrence and Change of Polyphenol Oxidase Activity in Some Banana Cultivars during Fruit Development

Chang-Peng YANG,¹ Zhi-Rong NONG,¹ Jian-Lin LU,¹ Lu LU,¹ Jian-Shou XU,¹ Yun-Zhe HAN,² Ying-Jie Li² and Shuji FUJITA^{2*}

¹Guangxi Agricultural Vocation-Technical College, Nanning 530007, Guangxi, China

²Laboratory of Food Science, Faculty of Agriculture, Saga University, 1 Honjou-machi, Saga-shi, Saga 840-8502, Japan

Received August 18, 2003; Accepted October 24, 2003

Occurrence of polyphenol oxidase (PPO, *o*-diphenol: oxygen oxidoreductase, EC.1.10.3.1) in the fruits of nine cultivars of banana (*Musa* spp.) commercially cultivated in China was investigated. All banana fruits peel and pulp tested had PPO activity, and all PPO strongly oxidized dopamine and 3-hydroxytyramine (tyramine). However, very weak and/or no oxidative activity was recognized on such trihydroxybenzenes as phloroglucinol and gallic acid. Similar substrate specificity of PPOs toward phenolic compounds was detected in the pulp and peel of all banana cultivars tested. The specific PPO activities toward dopamine for pulp were between 5.11 and 25.72, and those of peel were between 6.75 and 26.86 units/mg protein. However, PPO activities toward dopamine in the pulp and peel of AAA and AA genome group bananas were higher than those of ABB and AAB groups. The changes of pulp and peel PPO activities during fruit development were also determined in the three banana cultivars. During this development a remarkable decrement in pulp and peel PPO activities was found in all banana cultivars used.

Keywords: banana (*Musa* spp.) cultivars, polyphenol oxidase, tyrosinase, development period

Polyphenol oxidase (PPO, *o*-diphenol: oxygen oxidoreductase, EC.1.10.3.1) is a copper-containing enzyme which oxidizes phenolic compounds to produce undesirable browning during storage and processing of fresh fruits and vegetables. PPO has been widely studied in many fruits and vegetables to prevent the browning which results in the loss of their marketability (Kahn, 1977; Park *et al.*, 1980; Park & Luh, 1985; Augustin *et al.*, 1985; Tono *et al.*, 1986; Fujita & Tono, 1979; Fujita & Tono, 1988; Fujita *et al.*, 1991; Murata *et al.*, 1992; Chilaka *et al.*, 1993; Fujita *et al.*, 1995; Castaner *et al.*, 1996; Fujita *et al.*, 1997; Ding *et al.*, 1998). The PPO in banana (*Musa sapientum* L.) had been characterized (Palmer, 1963; Galeazzi *et al.*, 1981; Galeazzi & Sgabieri, 1981; Thomas & Janave, 1986; Yang *et al.*, 2000; Yang *et al.*, 2001), because a remarkable browning occurred in the pulp section. Many banana cultivars which fall into several genome groups are being cultivated in the world, however, little is known about the occurrence of PPO in them or on the change of PPO activity during development of the fruit. In the present study, determinations were made on the occurrence of PPO in nine banana cultivars commercially cultivated in China, and on the change of PPO activity during fruit development.

Materials and Methods

Fruit materials Banana fruits of the nine cultivars at the mature stage for marketing were harvested from the experimental orchard of Guangxi Agricultural Vocation-Technical College and from a local farm in Nanning, China, placed in a plastic bag, and kept at room temperature to ripen the fruits for 2 to 3

days. The fruits at the same yellow-green and/or yellow-red stage were used as materials for our study.

The cultivars, scientific name and genomic groups of bananas used were: Brazil Jiao (*Musa cavendishii* L.; AAA genome group), Nalong Jiao (*Musa cavendishii* L.; AAA), Williams Jiao (*Musa cavendishii* L.; AAA), Red Dacca Jiao (*Musa sapientum* L.; AAA), Gong Jiao (*Musa acuminata* Colla; AA), Mi Jiao (*Musa acuminata* Colla; AA), Ji Jiao (*Musa paradisiaca* L.; AAB), Da Jiao (*Musa sapientum* L.; ABB), and Fen Jiao (*Musa paradisiaca* L.; ABB).

To determine the changes of PPO activity during fruit development, the fruits of three cultivars (Williams Jiao, Brazil Jiao, and Mi Jiao) hanging in the same position on the axis of a tree and from the six developmental stages (20, 40, 60, 80, 100, 120 days after fruit set) were harvested between August and November in 2002 from the experimental orchard of Guangxi Agricultural Vocation-Technical College, Nanning, China.

Reagents Pyrocatechol, DL-dopa, gallic acid, pyrogallol, phloroglucinol were obtained from Wako Pure Chemical Co., Osaka. Folin & Ciocalteu's phenol reagent, bovine serum albumin, Tween 80, (+)-catechin, chlorogenic acid and (–)-epicatechin were purchased from Sigma Chemical Co., St. Louis. 3-Hydroxytyramine (tyramine) and L-dopa were obtained from Aldrich Chemical Co., St. Louis. Dopamine was purchased from Nacalai Tesque Co., Kyoto. Glucoamylase was purchased from Nagase Biochemical Co., Osaka. Other reagents were obtained from Dahao Chemical Co., Guangdong, China.

Preparation of pulp PPO Fifty grams of banana pulp from the middle portion of the fruits, three to eight depending on their size, was homogenized with 50 ml of 0.1 M sodium phosphate-0.1 M potassium phosphate buffer (0.1 M PB, pH 7.0) con-

* To whom correspondence should be addressed.
E-mail: fujitas@cc.saga-u.ac.jp

taining 3% Tween 80 and 0.1% glucoamylase, and the homogenate was hydrolyzed by glucoamylase at 37°C for 3 h. The hydrolyzate was filtered through cotton cloth, and the filtrate was centrifuged at 10,300 *g* for 20 min (2°C). The supernatant was used as stock solution of PPO.

Preparation of peel PPO Fifty grams of peel pooled as pulp was homogenized with 0.1 M PB (pH 7.0) containing only 3% Tween 80 and placed at 5°C for 1 h. After filtration of the homogenate through cotton cloth, the filtrate was centrifuged at 10,300 *g* for 20 min (2°C), and the supernatant was used as stock solution of PPO.

Determination of PPO activity and protein For PPO assay, the stock solution was diluted 20 to 40-fold by 0.01 M PB (pH 7.0) and determined for PPO activity by the colorimetric method according to Yang *et al.* (2000, 2001). One unit of the enzyme activity was defined as an increase in absorbance of the mixture at 420 nm (ΔA_{420}) of 0.1 per min and per ml enzyme solution (1 cm light path).

Protein was determined by Hartree method (Hartree, 1972) using bovine serum albumin as standard.

Results and Discussion

Occurrence of PPO in banana fruit The nine cultivars of banana fruits used in this study are commercial bananas in China. In the PPO preparation procedure, the detergent (Tween 80) was added because the banana pulp and peel PPO present mainly as a latent form (Sojo *et al.*, 1998; Fujita *et al.*, 2001). Because the polysaccharide in banana pulp interfered with filtration and centrifugation, the polysaccharide in the pulp homogenate was first hydrolyzed by glucoamylase (Yang *et al.*, 2000).

Table 1 shows pulp PPO activities from nine banana cultivars against various polyphenols. All pulp PPOs tested strongly oxidized for dopamine and tyramine. Oxidation rate of catechol by the enzyme was about one-half that of dopamine. However, the activity towards (+)-catechin, (–)-epicatechin, L-dopa and DL-

dopa was less than one-fourth of that toward dopamine. These PPOs had fairly weak and/or no activity towards such trihydroxybenzenes as pyrogallol, gallic acid, and phloroglucinol. Similar substrate specificities of PPO had been found in other banana cultivars (Palmer, 1963; Galeazzi *et al.*, 1981; Galeazzi & Sgabieri, 1981; Thomas & Janave, 1986; Yang *et al.*, 2000; Yang *et al.*, 2001). These results indicated that all banana pulp PPOs tested had both tyrosinase and *o*-diphenolase activities as reported by Thomas and Janave (1986).

Different activities of the pulp PPOs toward dopamine were found among the banana cultivars: they were in between 5.11 and 25.72 units/mg protein. Relatively high PPO activity (18.41 to 25.72 units/mg protein) was detected in such AAA and AA genome groups as Brazil Jiao (AAA), Williams Jiao (AAA), Nalong Jiao (AAA), Red Dacca Jiao (AAA), Gong Jiao (AA), and Mi Jiao (AA). These values were about three times higher than the PPO activities of the AAB and ABB groups (5.11 to 7.83 units/mg protein), of Ji Jiao (AAB), Fen Jiao (ABB) and Da Jiao (ABB). Similar results were observed using catechol as substrate. These results suggested that the activity of pulp PPO of the AAA and AA genome groups was generally higher than that of the ABB and AAB groups.

Table 2 shows peel PPO activities of the nine banana cultivars. These peel PPO activities toward phenolic compounds were almost the same as those of pulp PPOs. All peel PPOs tested strongly oxidized dopamine and tyramine. The oxidation rate of catechol by the enzyme was 50–90% of that of dopamine. This is a little different from that of the banana pulp PPO noted above. However, the activity towards (+)-catechin, (–)-epicatechin, L-dopa and DL-dopa was less than one-fourth of that toward dopamine. And the PPOs had fairly weak and/or no activity towards such trihydroxybenzenes as pyrogallol, gallic acid, and phloroglucinol. These results also indicated that banana peel PPOs had both tyrosinase and *o*-diphenolase activities, and that dopamine was a main substrate for the enzymes. Banana peel

Table 1. Pulp PPO activities from nine banana cultivars.

Banana cultivars	PPO activity (units/mg protein)					
	Tyramine	Dopamine	Catechol	Catechin	Epicatechin	Chlorogenic acid
Williams Jiao (<i>M.cavendishii</i> L.; AAA)	23.22±1.21	23.51±1.35	12.47±1.34	6.60±0.34	6.23±0.39	1.21±0.23
Brazil Jiao (<i>M. cavendishii</i> L.; AAA)	25.46±1.49	25.72±1.48	15.26±1.04	6.07±0.43	6.79±0.44	2.86±0.37
Nalong Jiao (<i>M. cavendishii</i> L.; AAA)	24.70±1.51	24.09±1.71	15.91±0.72	5.77±0.27	6.57±0.39	3.28±0.19
Red Dacca Jiao (<i>M. sapientum</i> L.; AAA)	16.63±1.09	18.41±1.08	8.36±0.44	4.27±0.26	1.59±0.27	4.35±0.31
Mi Jiao (<i>M. acuminata</i> Colla; AA)	21.51±1.36	21.59±1.69	13.02±0.81	2.43±0.30	3.67±0.43	3.21±0.28
Gong Jiao (<i>M. acuminata</i> Colla; AA)	22.68±1.41	22.82±1.35	13.21±0.73	7.34±0.31	6.01±0.34	3.85±0.33
Ji Jiao (<i>M. paradisiaca</i> L.; AAB)	4.24±0.48	5.11±0.52	2.15±0.23	0.76±0.08	0.36±0.04	0.32±0.03
Fen Jiao (<i>M. paradisiaca</i> L.; ABB)	6.42±0.55	7.73±0.55	3.61±0.30	1.34±0.24	2.00±0.31	0.63±0.02
Da Jiao (<i>M. sapientum</i> L.; ABB)	7.82±0.58	7.83±0.54	4.58±0.30	2.30±0.31	1.79±0.28	0.59±0.02

Banana cultivars	PPO activity (units/mg protein)				
	L-Dopa	DL-Dopa	Gallic acid	Pyrogallol	Phloroglucinol
Williams Jiao (<i>M.cavendishii</i> L.; AAA)	5.21±0.39	5.21±0.28	0	0	0
Brazil Jiao (<i>M. cavendishii</i> L.; AAA)	7.59±0.33	6.89±0.29	0	0.39±0.04	0
Nalong Jiao (<i>M. cavendishii</i> L.; AAA)	6.68±0.44	5.59±0.29	0	1.16±0.20	0
Red Dacca Jiao (<i>M. sapientum</i> L.; AAA)	3.01±0.33	3.68±0.23	0	0.68±0.04	0.57±0.04
Mi Jiao (<i>M. acuminata</i> Colla; AA)	2.71±0.25	3.06±0.30	0	1.13±0.18	0.35±0.04
Gong Jiao (<i>M. acuminata</i> Colla; AA)	5.45±0.46	4.64±0.32	0	1.67±0.18	0.33±0.03
Ji Jiao (<i>M. paradisiaca</i> L.; AAB)	0.98±0.08	0.72±0.05	0	0.42±0.04	0
Fen Jiao (<i>M. paradisiaca</i> L.; ABB)	1.07±0.15	1.62±0.19	0	0	0
Da Jiao (<i>M. sapientum</i> L.; ABB)	1.40±0.13	2.11±0.39	0	0	0

Each value is expressed as the mean±SE of three trials.

contained a large amount of dopamine (Riggin *et al.*, 1976; Tono *et al.*, 1999), and dopamine was strongly oxidized by peel PPO (Yang *et al.*, 2001). These results indicated that the browning of banana peel was caused by enzymatic oxidation of dopamine.

Different activities of the peel PPOs toward dopamine were also found among the nine banana cultivars, being between 6.75 and 26.86 units/mg protein. High activity (18.97 to 26.86 units/mg protein) was observed in such AAA and AA genome groups as Brazil Jiao (AAA), Williams Jiao (AAA), Nalong Jiao (AAA), Red Dacca Jiao (AAA), Gong Jiao (AA), and Mi Jiao (AA). Contrarily, PPO activities in the AAB and ABB groups Ji Jiao (AAB), Fen Jiao (ABB) and Da Jiao (ABB) were less than one-half those of the AAA and AA groups (6.75 to 11.97 units/mg protein). These results indicated that the peel PPO activity in AAA and AA genome group bananas was higher than that in the AAB and ABB groups.

In addition, it was also observed that the browning rate of pulp and peel of AAA and AA genome group bananas were higher

than those of the AAB and ABB groups.

The above results suggested that pulp and peel PPOs had two functions of tyrosinase and *o*-diphenolase, and specific activity toward dopamine and tyramine of pulp and peel PPO in the AAA and AA groups was higher than those of the ABB and AAB groups. These results also indicated that banana PPO could be a dopamine oxidase. These substrate specificities of banana pulp and peel PPO differ from those of Japanese pear (Tono *et al.*, 1986), eggplant (Fujita & Tono, 1988), lettuce (Fujita *et al.*, 1991), and apple (Murata *et al.*, 1992), which strongly oxidize (–)-epicatechin and/or chlorogenic acid.

Change of PPO activity in banana during fruit development

As described above, AAA and AA genome group bananas have strong PPO activity. In addition, Williams Jiao (AAA) and Brazil Jiao (AAA) are the most predominant cultivars of big-fruit banana, and Mi Jiao (AA) is one of the favorite cultivars of small-fruit banana. Therefore, these three cultivars were selected and used in this experiment. Figure 1 shows the changes in aver-

Table 2. Peel PPO activities from nine banana cultivars.

Banana cultivars	PPO activity (units/mg protein)					
	Tyramine	Dopamine	Catechol	Catechin	Epicatechin	Chlorogenic acid
Williams Jiao (<i>M. cavendishii</i> L.; AAA)	20.55±1.56	20.73±1.58	11.52±0.82	3.56±0.27	2.78±0.22	0.98±0.18
Brazil Jiao (<i>M. cavendishii</i> L.; AAA)	22.26±1.60	22.02±1.59	15.86±0.64	8.58±0.56	3.31±0.23	2.45±0.25
Nalong Jiao (<i>M. cavendishii</i> L.; AAA)	21.96±1.30	21.79±1.38	15.37±0.78	8.35±0.28	2.31±0.31	2.48±0.25
Red Dacca Jiao (<i>M. sapientum</i> L.; AAA)	18.88±1.43	19.56±1.66	11.23±0.47	3.51±0.20	2.98±0.26	3.86±0.34
Mi Jiao (<i>M. acuminata</i> Colla; AA)	18.61±1.45	18.97±1.32	12.48±0.78	2.86±0.17	2.78±0.32	1.21±0.10
Gong Jiao (<i>M. acuminata</i> Colla; AA)	26.82±1.78	26.86±1.76	19.58±0.83	5.73±0.28	5.95±0.46	3.72±0.30
Ji Jiao (<i>M. paradisiaca</i> L.; AAB)	10.18±0.44	10.41±0.89	9.30±0.64	1.42±0.24	1.60±0.14	0.42±0.04
Fen Jiao (<i>M. paradisiaca</i> L.; ABB)	11.25±0.89	11.97±0.96	7.17±0.41	1.24±0.13	3.85±0.42	1.90±0.22
Da Jiao (<i>M. sapientum</i> L.; ABB)	5.69±0.78	6.75±0.72	3.61±0.32	2.06±0.29	2.48±0.22	1.12±0.12

Banana cultivars	PPO activity (units/mg protein)				
	L-Dopa	DL-Dopa	Gallic acid	Pyrogallol	Phloroglucinol
Williams Jiao (<i>M. cavendishii</i> L.; AAA)	2.97±0.24	1.91±0.20	0	0	0
Brazil Jiao (<i>M. cavendishii</i> L.; AAA)	5.13±0.25	3.85±0.17	0	0	0.85±0.06
Nalong Jiao (<i>M. cavendishii</i> L.; AAA)	6.22±0.34	4.03±0.21	0	0	0.91±0.05
Red Dacca Jiao (<i>M. sapientum</i> L.; AAA)	3.60±0.23	4.12±0.13	0.79±0.03	1.41±0.11	1.58±0.08
Mi Jiao (<i>M. acuminata</i> Colla; AA)	3.15±0.23	3.36±0.14	1.28±0.07	2.67±0.18	0.76±0.03
Gong Jiao (<i>M. acuminata</i> Colla; AA)	5.68±0.32	6.67±0.28	1.05±0.11	2.12±0.13	1.25±0.08
Ji Jiao (<i>M. paradisiaca</i> L.; AAB)	2.49±0.14	2.15±0.15	0	1.59±0.09	1.94±0.20
Fen Jiao (<i>M. paradisiaca</i> L.; ABB)	4.52±0.26	3.25±0.17	0	0	1.51±0.07
Da Jiao (<i>M. sapientum</i> L.; ABB)	2.74±0.17	3.04±0.17	1.25±0.09	1.24±0.12	1.68±0.08

Each value is expressed as the mean±SE of three trials.

Fig. 1. Change of PPO activities during fruit development. PPO activity was determined by the colorimetric method. The reaction mixture consisted of 1.0 ml of 0.02 M aqueous solution of dopamine, 3.9 ml of 0.1 M sodium phosphate-0.1 M potassium phosphate buffer (0.1 M PB, pH 7) and 0.1 ml of the diluted enzyme solution. After incubation at 30°C for 5 min, the absorbance of the mixture at 420 nm (ΔA_{420}) of 0.1 per min and per ml enzyme solution (1 cm light path). Data represent the mean±SE of three trials. □ Fresh weight, ● PPO activity (pulp), ○ PPO activity (peel). Arrow indicates the optimal harvest time.

age weight of the fruits and specific activity of the pulp and peel PPO during fruit development. Average fruit weight of Williams Jiao, Brazil Jiao, and Mi Jiao was 161.1 g, 168.7 g, and 49.8 g, respectively, at 120 days after fruit set. Relatively high specific activity of pulp and peel PPO was found in young fruits of all cultivars used, and the activity markedly decreased as fruit developed. The activity of pulp and peel PPO of Brazil Jiao changed from ca. 75 to 25 units/mg protein and from 40 to 20 units/mg protein, respectively, during fruit development. Similar changes of pulp and peel PPO activity were found in Williams Jiao fruit. On the other hand, the activity of the pulp and peel PPO in Mi Jiao changed from ca. 45 to 20 units/mg protein and from ca. 25 units to 15 units/mg protein, respectively, during the fruit development. Similar changes of PPO during fruit development had been found in Satsuma mandarin (Tono *et al.*, 1989) and Japanese pear (Fujita *et al.*, 1990).

The above results indicated that the activity of pulp and peel PPO from AAA and AA genome group bananas toward dopamine and tyramine was higher than those of the ABB and AAB groups. In addition, we reported that only pulp PPO activity of banana markedly increased and then decreased, showing a peak of climacteric rise in respiration, during ethylene ripening treatment (Fujita *et al.*, 2001). Therefore, it is important to select suitable cultivars and ripening stage of banana to prevent the enzymatic browning in processing.

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