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Effect of methyl jasmonate and *p*-coumaric acid on anthocyanin composition in a sweet potato cell suspension culture

Nadia Plata, Izabela Konczak-Islam*, Shalini Jayram, Kylie McClelland, Tracey Woolford, Philip Franks

Food Science Australia, CRC for Bioproducts, Riverside Life Sciences Centre, 16 Julius Avenue, Riverside Corporate Park, North Ryde, NSW 2113, Australia

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Abstract

Methyl jasmonate and *p*-coumaric acid added individually to the basal Murashige and Skoog (MS) medium induced significant changes in the composition of anthocyanin pigments accumulated in a sweet potato PL cell suspension culture. Methyl jasmonate added in concentration of 4.5–44.5 μM promoted biosynthesis of anthocyanins with more complicated molecular structures, among them di-acylated compounds, while the total amount of accumulated pigments remained unchanged. Feeding the precursor *p*-coumaric acid was superior to the effect of methyl jasmonate. The addition of 2 mM *p*-coumaric acid resulted in a complete conversion of non-acylated anthocyanins cyanidin 3-sophoroside-5-glucoside (YGM-0a) and peonidin 3-sophoroside-5-glucoside (YGM-0b) into their mono- and di-acylated derivatives: YGM-0f′, -7a and -7e. These changes were concomitant with an approximately 2-fold increase of the total amount of accumulated pigments. Possible pathways of acylation and methylation in this system are discussed. It is suggested that acylation of YGM-0a and YGM-0b in the PL suspension culture has been limited by the insufficient amount of endogenous *p*-coumaric acid.

Keywords: Anthocyanin; *p*-Coumaric acid; Methyl jasmonate; Sweet potato; Natural colorant; Pigment composition

1. Introduction

Anthocyanins—a large group of water-soluble pigments responsible for red-to-purple and red-to-blue colors of fruits and vegetables, commonly used in acidic solutions as a red pigment in soft drinks, jams, confectionery and bakery products, have been identified to possess strong chemopreventive activities such as antimutagenicity, anti-hypertension, antioxidative potential, and reduction of liver injury [1]. Anthocyanin-rich extracts from grape, bilberry and chokeberry applied at a level from 0.1 to 10.0 mg monomeric anthocyanin/100 ml growth medium inhibited the proliferation of the human colon cancer cell line, HT-29, within 24 h of treatment. Maximum inhibition was consistently observed with the chokeberry extract, suggesting that pigment profile may impact biological activity of anthocyanins [2]. Similarly, Yoshimoto et al. [3,4] reported that among purple-fleshed sweet potato anthocyanins cyanidin possesses significantly higher antimutagenic activity than peonidin. In the linoleic acid system under neutral conditions (pH 7.0) cyanidin-based pigments exhibited significantly stronger antioxidative activity than pelargonidin- and peonidin-based pigments [5]. In recognition of the valuable properties of anthocyanins—the unusual combination of bringing an attractive color to the food as well as strongly enhancing the health-beneficial properties, research projects have been undertaken to develop anthocyanin-rich functional foods, such as the “Anthocyanin bioactivities” project of the European Union Framework Programme “Quality of life” [6]. Designing an “anthocyanin-tailored” composition for functional foods which should serve as a carrier of ingredients with special health benefits without reducing their bioavailability, has been undertaken [7].

A high-anthocyanin accumulated PL cell line established from storage root of the purple-fleshed sweet potato (*Ipomoea batatas* L.) cv. Ayamurasaki accumulates predominantly cyanidin-type pigments with identical or very similar molecular structures to anthocyanins accumulated in the Ayamurasaki field-grown storage root [8,9]. While maintained on basal Murashige and Skoog (MS) medium major anthocyanins biosynthesized in the sweet potato PL suspension culture are cyanidin 3-sophoroside-5-glucoside (YGM-0a) and peonidin 3-sophoroside-5-glucoside (YGM-0b)

* Corresponding author. Tel.: +61-2-9490-8563; fax: +61-2-9490-8524.
E-mail address: izabela.konczak-islam@csiro.au (I. Konczak-Islam).

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[10]. Reduction of ammonium ion concentration in culture medium was found to enhance accumulation of acylated components, YGM-0f′, -7a and -7e [11]. Highly acylated anthocyanins possess a high thermostability [12], which makes them an important source of anthocyanin-based food colorants and therefore they are of significant interest to the colorant industry.

Development of an efficient tissue culture system for commercial production of anthocyanins requires an integrated approach combining the effects of various enhancement strategies. Elicitation of secondary metabolites in various tissue culture systems with both biotic and abiotic elicitors enhanced the formation of secondary metabolites. Mueller et al. [13] have identified that elicitation with fungal elicitors led to increase of intracellular jasmonate concentration and activities of enzymes responsible in their biosynthesis. Jasmonic acid and its derivatives are considered to be compounds involved in the part of a signal transduction system which induces particular enzymes catalyzing biosynthetic reactions to form defense compounds such as polyphenols or alkaloids [14–16]. Exogenously applied jasmonates have provoked profound effects on accumulation of paclitaxel in *Taxus* cell suspension culture [17], rosmarinic acid in *Lithospermum erythrorhizon* and *Coleus blumei* cultures [18,19], ginsenosides in *Panax ginseng* culture [20] and anthocyanins in strawberry [21] and ohelo cultures [22]. The effects of jasmonates on the composition of accumulated anthocyanins have not been studied.

In respect to sweet potato research Nakatani and Koda [23] demonstrated that jasmonic acid and its related compounds play an important physiological role in the development of storage root. Jasmonic acid present in aqueous and acid ethyl acetate fractions from top organs of sweet potato plant induced tuberization in potato-stem-segment culture. When exogenous jasmonic acid was applied to *in vitro* culture of roots of the sweet potato cv. Beniazuma (cultivar with red skin color) the formation of storage root was promoted. The authors reported that color of roots in treatments with jasmonic acid become red, while roots in control maintained the original white color. This observation may indicate some involvement of jasmonates in the metabolic pathway of anthocyanins in sweet potato.

Another means to enhance the formation of secondary metabolites in plant cell cultures is the feeding of commercially available precursors or metabolic intermediates. Dougall [24] reported that feeding sinapic acid to cultures from wild carrot (*Daucus carota* L.) led to increased accumulation of anthocyanin. Furthermore, wild carrot cell suspension culture was able to metabolize non-natural 3,4-dimethoxy- and 3,4,5-trimethoxy-cinnamic acids and in each case new mono-acylated anthocyanin was formed [25]. The authors concluded that in their system the biosynthesis and accumulation of anthocyanins is not specific for sinapic acid, but other naturally occurring cinnamic acids and a number of “non-natural” cinnamic acids can be incorporated into anthocyanins. Dougall et al. [26] proposed this approach to be used for production of more stable pigments.

In the crude pigment extract of the sweet potato PL cell line Terahara et al. [9] have identified a novel YGM anthocyanin acylated with *p*-coumaric acid. This type of acylation was not found among anthocyanin pigments of field-grown purple-fleshed sweet potato storage roots. Further research indicated that this type of acylation dominates in the biosynthetic pathway of anthocyanins accumulated in the PL cell line (Terahara, personal communication). Therefore it might be suspected that addition of *p*-coumaric acid to the culture medium could contribute towards accumulation of cyanidin-based acylated anthocyanins with increased stability. In this study we have tested the effects of methyl jasmonate and *p*-coumaric acid on the composition of anthocyanin pigments accumulated in the sweet potato PL cell suspension culture.

2. Materials and methods

2.1. Plant material and culture conditions

The high-anthocyanin accumulating PL cell line established previously from a storage root of the purple-fleshed sweet potato, cv. Ayamurasaki [8] has been used for this study. Suspension cultures were initiated by transferring about 1 g (fresh weight) of callus to 50 ml of liquid medium in 250 ml Erlenmayer flasks. The MS [27] basal medium supplemented with 1.0 mg/l 2,4-D was used as a maintenance medium. The cultures were incubated on a rotary shaker (130 rpm) at 25 °C in the dark. The medium was changed weekly.

The cells from suspension cultures, maintained for at least 3 months, were used for the experiments. Cell aggregates (250 mg) from 7-day-old subcultures were placed in 100 ml Erlenmayer flasks containing 25 ml medium. The samples (five replications) were harvested after 14 days of culture.

2.2. Preparation of MS media with methyl jasmonate and p-coumaric acid

Both chemicals were purchased from Sigma (Sigma Chemical, St. Louis, MO, USA). Methyl jasmonate in concentrations 4.5, 13.4, 22.3 and 44.5 μM and *p*-coumaric acid in concentrations of 0.1, 0.5, 1.0 and 2.0 mM were added to the basal MS medium individually. Before addition *p*-coumaric acid was dissolved in small amount of dimethylsulfoxide. Methyl jasmonate was filter sterilized, while *p*-coumaric acid was co-autoclaved with other media components. The media were supplemented with 3% sucrose and with no growth regulators. The pH was adjusted to 5.8 before autoclaving. A basal MS medium enriched with 3% sucrose was used as a control.

2.3. Determination of growth

Growth was measured by removing the aggregates from the medium, rinsing them with distilled water, separating them from the liquid by vacuum filtration and weighing. The results are presented as fresh weight (g/l).

2.4. Extraction of anthocyanins

Cell aggregates separated from the culture medium by vacuum filtration were ground and steeped in 15% (methyl jasmonate experiment) or 50% (*p*-coumaric acid experiment) acetic acid for 16 h. The volume of acetic acid solution was adjusted to 20 times equivalent of the sample weight. The samples were centrifuged at 12 000 × *g* for 10 min. The supernatants were used for identification of anthocyanins and quality analysis.

2.5. Anthocyanin identification and HPLC analysis

The supernatant diluted 10-fold with McIlvaine's buffer solution [28], pH adjusted to 3.0, was used for the measurement of the optical densities at 520 nm with spectrophotometer UV-1601 (Shimadzu, Japan). Color value (CV) of the pigment extract was calculated using the following formula: $CV = 0.1 \times OD_{520} \times D_1 \times D_2$ (CV/g FW), where OD_{520} is the spectrophotometric reading at 520 nm, and D_1 and D_2 the levels of dilution [8].

HPLC analysis was performed as described previously [8]. The HPLC system consisted of two LC-10AD pumps, SPD-M10A diode array detector, CTO-10AS column oven, DGU-12A degasser, SIL-10AD auto-injector and SCL-10A system controller (Shimadzu, Kyoto, Japan) equipped with Luna (3μ C18(2), 4.6 mm × 100 mm, Phenomenex, USA) column. Analytical HPLC was run at 35 °C and monitored at 520 nm. The following solvents in water with a flow rate of 1 ml min^{-1} were used: A (1.5% phosphoric acid) and B (1.5% phosphoric acid, 20% acetic acid and 25% acetonitrile). The elution profile was a linear gradient elution for B of 25–85% during 40 min in solvent A. The chromatograms were recorded and the relative concentrations of pigments were calculated from the peak areas. Identification of anthocyanins was carried out comparing the peaks with YGM-0a (cyanidin 3-sophoroside-5-glucoside) and YGM-0f′(cyanidin 3-(*E*)-*p*-coumaroyl-sophoroside-5-glucoside) standards isolated from the PL cell line [9] and standard peaks of purple-fleshed sweet potato YGM anthocyanins: YGM-1a (cyanidin 3-(6,6′-caffeoyl-*p*-hydroxybenzoylsophoroside)-5-glucoside), YGM-1b (cyanidin 3-(6,6′-dicaffeoylsophoroside)-5-glucoside), YGM-3 (cyanidin 3-(6,6′-caffeoylferuloylsophoroside)-5-glucoside), YGM-4b (peonidin 3-(6,6′-dicaffeoylsophoroside)-5-glucoside), YGM-5a (peonidin 3-(6,6′-caffeoyl-*p*-hydroxybenzoylsophoroside)-5-glucoside), and YGM-6 (peonidin 3-(6,6′-caffeoylferuloylsophoroside)-5-glucoside) [29].

3. Results and discussion

3.1. Effect of methyl jasmonate

The addition of methyl jasmonate to the basal MS medium induced changes in the composition of crude pigment extract of the PL suspension culture (Table 1). These changes were independent of dose. In the presence of methyl jasmonate the relative concentration of YGM-0a, previously identified as cyanidin 3-sophoroside-5-glucoside, a non-acylated anthocyanin [9], showed a significant decrease of 37%. This decrease was directly proportional to an increase of relative concentration of a non-identified cell line specific anthocyanin YGM-7a. Methyl jasmonate also induced accumulation of YGM-7e, which was not detected among anthocyanins accumulated in suspension culture grown on basal MS medium. The peaks YGM-7a and -7e elute on the reversed phase HPLC column with longer retention times than YGM-0a peak and therefore they have a more complicated molecular structures. The peak YGM-7e has recently been identified as peonidin 3-(6,6′-di-*p*-coumaroylsophoroside)-5-glucoside (Terahara, personal communication). In the presence of methyl jasmonate, a significant increase of the relative concentrations of YGM-3 and -3′ have also been recorded. YGM-3 has been identified as cyanidin 3-(6,6′-caffeoylferuloylsophoroside)-5-glucoside [29]. Therefore the addition of methyl jasmonate to the culture medium in our system enhanced the accumulation of highly acylated anthocyanins. It was reported previously that in the PL suspension culture the decrease of NH_4^+ concentration below 7.5 mM in MS basal medium significantly decreased accumulation of YGM-0a and increased accumulation of YGM-0f′ (cyanidin 3-(*E*)-*p*-coumaroyl-sophoroside-5-glucoside), a mono-acylated anthocyanin [11]. Simultaneous increase of the YGM-0g′, -3, -3′, -7a and -7e anthocyanins have also been reported. In the present experiment we have detected changes in the relative concentrations of non-acylated YGM-0a and di-acylated YGM-3, -3′, -7a and -7e, while the level of mono-acylated YGM-0f′ remained constant. Our future research interest will be directed towards understanding the mechanism of these changes.

The addition of methyl jasmonate into the medium did not have any effect, neither on cell growth nor on total amount of accumulated pigment (Fig. 1). In contrast in a strawberry cell suspension culture the presence of 3 mg/l of methyl jasmonate in the medium added at day 3 of the growth period increased total anthocyanin accumulation by 30%, while the accumulation of biomass was slightly inhibited [21]. In ohelo cell suspension culture the effect of methyl jasmonate on total accumulation of anthocyanin strongly depended on the applied concentration. In the presence of 0.05–0.5 μM jasmonic acid anthocyanin accumulation increased however it began to decline at 5–50 μM, and by 500.0 μM it was less than the control without elicitor. Methyl jasmonate did not effect biomass accumulation [22].

Table 1
Relative concentrations of YGM anthocyanin pigments accumulated in the PL sweet potato suspension culture grown for 14 days in MS medium supplemented with different levels of methyl jasmonate

Peak	Methyl Jasmonate (μM)				
	0	4.5	13.4	22.3	44.5
YGM-0a	32.3 ± 1.5[a]	27.6 ± 4.2	27.8 ± 2.7	27.8 ± 2.1	25.4 ± 2.8
YGM-0b	11.6 ± 2.5	10.1 ± 0.6	10.4 ± 2.3	10.4 ± 1.4	10.1 ± 2.4
YGM-0c	2.2 ± 0.1	2.1 ± 0.2	2.0 ± 0.2	1.9 ± 0.1	1.8 ± 0.2
YGM-0d	4.9 ± 1.3	3.9 ± 0.2	4.3 ± 0.6	4.4 ± 0.8	4.9 ± 0.9
YGM-0f	0.9 ± 0.1	1 ± 0.8	0.8 ± 0.1	1.1 ± 0.1	0.9 ± 0.1
YGM-0f′	12.9 ± 0.9	12.7 ± 0.5	12.7 ± 0.8	11.3 ± 0.5	12.3 ± 0.6
YGM-0g	11.3 ± 0.5	10.1 ± 0.5	10.7 ± 1.1	10.6 ± 0.8	11.1 ± 1.1
YGM-0g′	3.0 ± 0.9	3.8 ± 0.6	3.4 ± 0.5	3.4 ± 0.7	3.9 ± 0.9
YGM-0i	4.1 ± 0.9	4.1 ± 0.4	4.0 ± 0.9	5.0 ± 0.7	5.0 ± 1.4
YGM-2	2.8 ± 0.8	3.2 ± 0.3	3.4 ± 0.7	3.7 ± 1.2	2.7 ± 0.6
YGM-3	3.3 ± 0.5	4.2 ± 0.4	4.4 ± 0.3	4.2 ± 0.8	5.1 ± 0.8
YGM-3′	2.8 ± 0.6	4.7 ± 0.5	4.6 ± 0.6	4.8 ± 0.8	5.3 ± 0.9
YGM-5b	0.9 ± 0.6	2.2 ± 0.2	1.5 ± 0.9	1.6 ± 0.1	1.6 ± 0.9
YGM-6	2.2 ± 0.9	3.4 ± 0.2	3.3 ± 0.7	2.9 ± 0.9	3.5 ± 0.3
YGM-7a	1.4 ± 2.4	7.5 ± 0.4	6.2 ± 0.9	5.0 ± 1	8.2 ± 0.9
YGM-7e	ND[b]	2.4 ± 0.3	1.9 ± 0.2	1.9 ± 0.8	2.5 ± 0.3

[a] Standard deviation of five independent values.
[b] Peak not detected.

In the sweet potato PL suspension culture we did not obtain any increase in total anthocyanin accumulation with the addition of methyl jasmonate. However, our system is different to the cited strawberry and ohelo systems. The PL sweet potato culture accumulates anthocyanins in the dark, but both strawberry and ohelo suspension cultures for anthocyanin accumulation required light illumination. Synergistic effect of jasmonic acid and light on total anthocyanin accumulation in grape suspension culture has been reported [30]. It is possible for the same effect to occur among methyl jasmonate and light. On the other hand both strawberry and ohelo suspension cultures consist of mottled aggregates. For example, in strawberry suspension culture the amount of pigmented cells at day 5 was higher in the medium with 3 mg/l methyl jasmonate in comparison to the control: 52.7 and 4.6%, respectively [21]. Previously it was reported that treatment of roots of the sweet potato cv. Beniazuma with jasmonic acid not only promoted the formation of storage root but also induced red pigmentation of the skin, while control roots maintained their original white color [23]. Therefore it can be assumed that the presence of jasmonates might be beneficial to trigger the expression of anthocyanin biosynthetic genes in the non-pigmented cells, which results in increase of total pigment accumulation. In the high-anthocyanin accumulated sweet potato PL cell line the aggregates are uniformly pigmented black-purple. Thus, it can be suspected that a similar effect on the total anthocyanin accumulation in our system would not be observed.

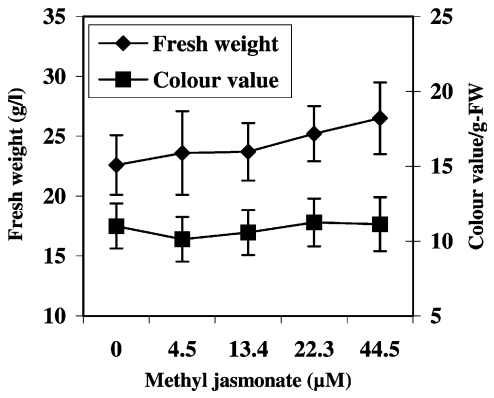


Fig. 1. Total anthocyanins and biomass accumulation in the sweet potato PL suspension culture grown for 14 days in MS medium modified with different levels of methyl jasmonate. Vertical bars represent standard deviation of five replications.

3.2. Effect of p-coumaric acid

Feeding the PL suspension culture with commercially available *p*-coumaric acid induced significant changes in the composition of crude pigment extract (Table 2). In the control treatment non-acylated pigments YGM-0a and YGM-0b (peonidin 3-sophoroside-5-glucoside [10]), dominated and constituted over 60% of the total anthocyanins as calculated according to the peak area. With an increase of *p*-coumaric acid level relative concentrations of these anthocyanins dramatically decreased and instead YGM-0f′, -g′, -3′, -7a and -7e were accumulated (Fig. 2).

The relative concentrations of YGM-0a and YGM-0f′ accumulated in the control treatment and in the medium modified with 2 mM of *p*-coumaric acid were examined. A decrease of 98% of the relative concentration of YGM-0a and an increase of 111% of YGM-0f′ were recorded. No changes occurred in the relative concentrations of YGM-0f′ between the treatments with 1.0 and 2.0 mM

Table 2
Relative concentrations of YGM anthocyanin pigments accumulated in the PL sweet potato suspension culture grown for 14 days in MS medium supplemented with different levels of *p*-coumaric acid

Peak	*p*-Coumaric acid (mM)				
	0	0.1	0.5	1.0	2.0
YGM-0a	49.4 ± 1.4[a]	50.7 ± 2.7	42.8 ± 1.8	33.2 ± 3.2	1.0 ± 0.2
YGM-0b	15.3 ± 1.4	15.3 ± 0.5	12.4 ± 2.3	7.7 ± 0.8	0.5 ± 0.1
YGM-0c	1.6 ± 0.1	1.6 ± 0.1	2.1 ± 0.1	3.5 ± 0.2	0.7 ± 0.1
YGM-0d	3.8 ± 0.3	3.2 ± 0.3	2.9 ± 0.3	2.8 ± 0.2	1.8 ± 0.3
YGM-0f	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
YGM-0f′	10.6 ± 0.9	10.8 ± 1.5	18.3 ± 2.2	22.7 ± 1.5	22.4 ± 1.3
YGM-0g	8.6 ± 0.7	8.3 ± 0.7	8.7 ± 1.2	7.1 ± 0.8	2.6 ± 0.3
YGM-0g′	1.5 ± 0.1	2.1 ± 0.4	5.3 ± 0.9	6.8 ± 0.7	10.1 ± 0.9
YGM-0i	3.1 ± 0.3	3.0 ± 0.2	2.9 ± 0.4	2.3 ± 0.1	2.2 ± 0.4
YGM-2	2.1 ± 0.1	1.8 ± 0.4	ND[b]	ND	ND
YGM-3	1.0 ± 0.2	0.8 ± 0.1	0.6 ± 0.07	0.7 ± 0.1	0.4 ± 0.1
YGM-3′	0.9 ± 0.1	0.8 ± 0.2	1.4 ± 0.1	1.9 ± 0.3	3.2 ± 0.1
YGM-5b	0.4 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	1.6 ± 0.1	1.9 ± 0.1
YGM-6	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.01	0.8 ± 0.1	1.8 ± 0.1
YGM-7a	0.3 ± 0.01	0.2 ± 0.1	1.3 ± 0.2	5.5 ± 0.8	34.7 ± 2.1
YGM-7e	ND	ND	0.5 ± 0.1	2.1 ± 0.2	14.7 ± 0.9

[a] Standard deviation of five independent values.
[b] Peak not detected.

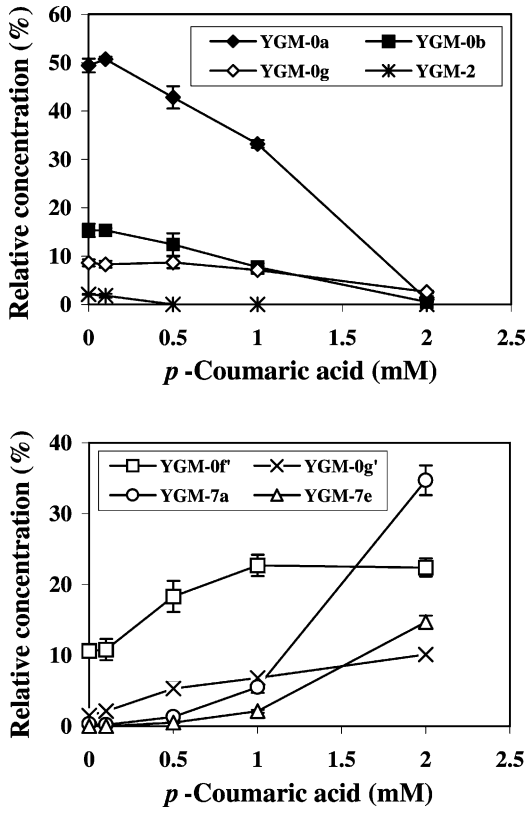
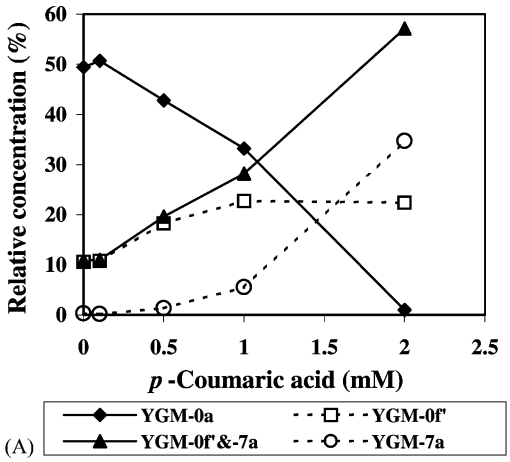
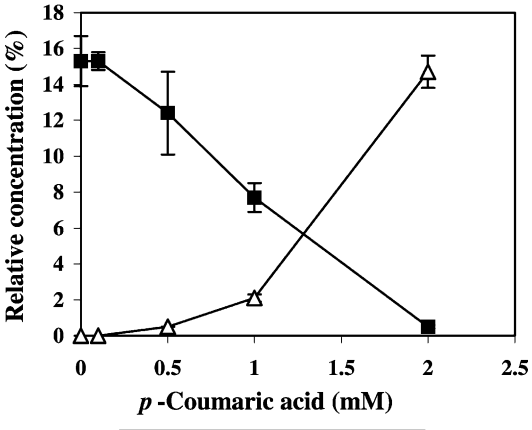


Fig. 2. Effect of different levels of *p*-coumaric acid on relative concentrations of major YGM anthocyanins accumulated in the PL sweet potato suspension culture after 14 days in MS medium. Verticals bars represent standard deviation of five independent replications.

p-coumaric acid. According to Terahara et al. [9] YGM-0a is considered to be the common precursor for biosynthesis of cyanidin and peonidin pigments accumulated in the PL cell line and in storage root of purple-fleshed sweet potato. The methylation of cyanidin and/or acylation at the glucopyranoside-hydroxyl group might be repressed in the PL suspension culture grown in the basal MS medium but occurs in the storage root of purple-fleshed sweet potato. Furthermore, according to the same authors, the non-acylated cyanidin YGM-0a is the precursor of the mono-*p*-coumaroylated YGM-0f′, since alkaline hydrolysis of YGM-0f′ produced YGM-0a, and the hydrolysate contained *p*-coumaric acid. The glucopyranosyl group of YGM-0a served as an acyl acceptor group, and acylation probably occurred as the final step in anthocyanin biosynthesis. As mentioned above, with the increase of *p*-coumaric acid concentration from 1 to 2 mM no further increase in the relative concentration of YGM-0f′ was detected, which indicates that no additional acylation of YGM-0a occurred. However, a decrease of the relative concentration of YGM-0a was still recorded in the medium with 2 mM of *p*-coumaric acid (Table 2). The decrease in total relative concentration of YGM-0a and simultaneous increase in the combined relative concentrations of YGM-0f′ and YGM-7a were examined and correlated (Fig. 3A). The result suggests that the biosynthetic pathway of the PL cell line favors acylation of the glucopyranoside hydroxyl group of YGM-0a, and thus produces the mono-*p*-coumaroylated YGM-0f′ in a first reaction. When equilibrium is reached, a second reaction might occur leading to the biosynthesis of YGM-7a, which appeared at a later retention time, thus consuming the remaining non-acylated precursor YGM-0a.



(A)



(B)

Fig. 3. Correlation between relative concentrations of YGM-0a and YGM-0f′&-7a (A) and between YGM-0b and YGM-7e (B) in the PL sweet potato suspension culture after 14 days in MS medium modified with different levels of *p*-coumaric acid. Verticals bars represent standard deviation of five independent replications.

The gradual decrease of the relative concentration of YGM-0b (peonidin 3-sophoroside-5-glucoside) with the increase of *p*-coumaric acid concentration in culture medium highly correlated with gradual increase of the relative concentration of YGM-7e, peonidin 3-(6,6′-di-*p*-coumaroylsophoroside)-5-glucoside (Fig. 3B). The data indicates that YGM-0b is a precursor of the di-*p*-coumaroylated YGM-7e.

A similar approach of feeding hydroxy-cinnamic acid (sinapic acid) to suspension cell culture from wild carrot led to biosynthesis of mono-acylated anthocyanin and increased total anthocyanin accumulation [22]. Feeding of the hydroxy-cinnamic acid to the sweet potato PL suspension culture also resulted in increased accumulation of acylated anthocyanins both mono-acylated and di-acylated. The presence of *p*-coumaric acid also produced a significant increase in the total anthocyanin accumulation expressed as the CV/g FW (Fig. 4). The *p*-coumaric acid did not have any

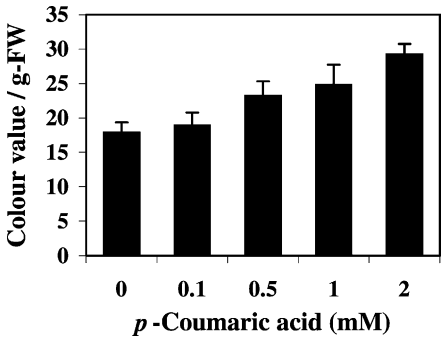


Fig. 4. Accumulation of total anthocyanins in the PL sweet potato suspension culture after 14 days in MS medium modified with different levels of *p*-coumaric acid. Verticals bars represent standard deviation of five independent replications.

significant effect on biomass accumulation (data not presented).

The effect of exogenous supply of *p*-coumaric acid to the PL suspension culture may suggest that endogenous supply of *p*-coumaric acid for the biosynthesis of anthocyanins is limited which leads to the accumulation of non-acylated components, YGM-0a and YGM-0b. However, *p*-coumaric acid limitation may not be the only obstacle which prevents acylation in this system. Previously high concentration of ammonium ion was identified as a factor suppressing acylation of YGM-0a and YGM-0b [11]. Knowledge of the mechanisms of the acylation and/or methylation in the sweet potato PL suspension culture could contribute towards development of an integrated approach that combines the effects of various strategies for enhanced accumulation of the desired anthocyanins.

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