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Note

Effect of medium composition on the production of anthocyanins by hairy root cultures of *Ipomoea batatas*

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Abstract We obtained hairy roots of *Ipomoea batatas* cv. Ayamurasaki by infection of *Agrobacterium rhizogenes* A13. Accumulation of anthocyanins in hairy roots was observed when they were cultured under continuous light irradiation, whereas it was not observed when they were cultured in the dark. The amount of accumulated anthocyanins in hairy roots increased as a result of choosing the medium and increasing the sucrose concentration in the medium. Accumulation of anthocyanins was also observed in the hairy roots cultured in liquid medium in the dark by choosing medium.

Key words: Anthocyanins, hairy root, *Ipomoea batatas*.

Ipomoea batatas cv. Ayamurasaki is a cultivar of sweet potato with purple-flesh which accumulates a large amount of anthocyanins in the tuberous root (Yamakawa et al. 1997). Anthocyanins accumulated in *I. batatas* cv. Ayamurasaki are expected to be used as a natural food colorant because of their antioxidant activity, antimutagenic activity, and attractive color (Odake et al. 1994; Furuta et al. 1995; Suda et al. 1997; Yoshimoto et al. 1999). An anthocyanin-producing cultured cell line was established recently from storage root of *I. batatas* cv. Ayamurasaki (Konczak-Islam et al. 2000), but production of anthocyanins by hairy root cultures has not been reported. As we expected hairy roots and tuberous roots to be similar in the capability of anthocyanin production, we obtained hairy root cultures of *I. batatas* cv. Ayamurasaki in order to produce anthocyanins. The culture condition of hairy roots for the accumulation of anthocyanin was determined in this paper. This is the first report that shows production of anthocyanins by hairy root cultures of *I. batatas* cv. Ayamurasaki.

Expanded leaves of *I. batatas* cv. Ayamurasaki intact plant were sterilized with 10% antiformin for 15 min and rinsed with sterile water for three times, and then they were infected with *Agrobacterium rhizogenes* A13 as previously reported (Otani et al. 1993). Hairy roots were induced about 4 weeks after the infection of *A. rhizogenes* A13. They were cut off from leaf disks and transferred to fresh Linsmaier-Skoog (LS) medium (Linsmaier and Skoog 1965) solidified with 0.32% Gelrite (Wako Pure Chemicals, Japan) containing 250 mg/l of cefotaxime (Wako Pure Chemicals, Japan). Integration of T-DNA was confirmed by amplification of *rol* gene by PCR as reported previously (Kiyokawa et al. 1992, data not shown). The transformed hairy roots obtained were cultured on LS medium at 26°C in the dark, and subcultured every 4 weeks. When they were cultured on LS medium containing 3% sucrose in the dark, no coloration was observed (Figure 1A). However, when they were cultured under continuous light irradiation ($25–35\ \mu E\ m^{-2}\ s^{-1}$), a small amount of anthocyanin was accumulated (Figure 1B). A quarter-strength LS (1/4 LS) medium and PRL-4C medium (Gamborg 1966) containing 3% sucrose were also employed for culturing hairy roots. The most intense coloration was observed in hairy roots cultured on PRL-4C medium (Figure 1B–D). Anthocyanin was extracted from hairy roots cultured on those media for 3 weeks and the color value of extracted pigments was calculated following the method of Konczak-Islam et al. (2000). Hairy roots which had been cultured for 3 weeks were ground to powder with mortar and pestle, and then immersed in 50% acetic acid overnight. The volume of acetic acid solution was adjusted to 20 times equivalent of the sample weight. After centrifugation, supernatants were diluted with McIlvaine's buffer (pH 3.0) to 4 times volume. Absorbance at 530 nm was determined using a spectrophotometer (U-2001; Hitachi, Japan). The color value (CV) for the pigment extract was calculated using following formula: $CV = 0.1 * OD_{530} * 4 * 20/1\ gFW$. Several lines of hairy roots were tested, and the color value was different from line to line even on the same medium. However, the color value was highest for extracts from hairy roots cultured on PRL-4C medium in all lines tested (Figure 2).

Since anthocyanin production was reported to be

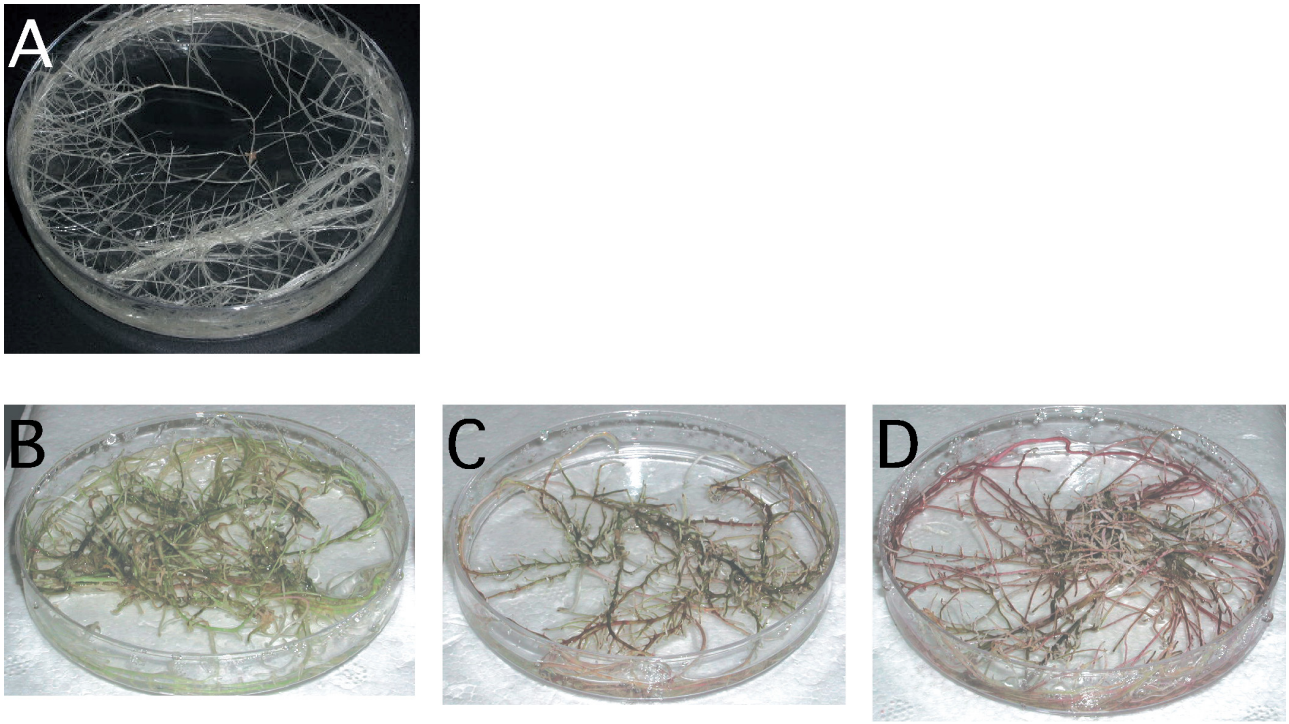

Figure 1. Hairy roots of *Ipomoea batatas* cv. Ayamurasaki cultured for 4 weeks on LS medium in the dark (A), and LS medium (B), 1/4 LS medium (C), and PRL-4C medium (D) under continuous white light, respectively.

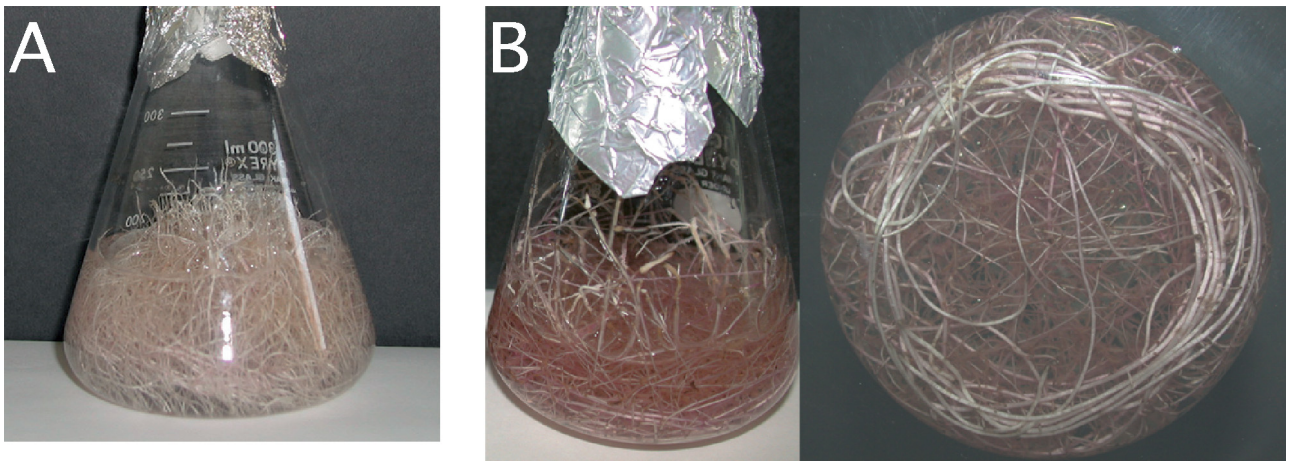

Figure 4. Hairy roots cultured in liquid medium in the dark. (A) Hairy roots cultured in 300-ml Erlenmayer flasks containing 200 ml of LS medium for 4 weeks. (B) Hairy roots cultured in 100-ml Erlenmayer flasks containing 50 ml of PRL-4C medium for 4 weeks.

enhanced by increase of sucrose concentration in the medium (Yamakawa et al. 1983; Sato et al. 1996), the sucrose concentration in PRL-4C medium was modified from 3 to 1 and 5%. In all lines, the most intense coloration was observed in hairy roots cultured on medium containing 5% of sucrose, and extracts from these hairy roots also showed the higest anthocyanin content on solid medium (Figure 3).

Liquid cultures of hairy roots were initiated by transferring about 5 g (fresh weight) of hairy roots to 200 ml of LS liquid medium (2% sucrose) in 300-ml Erlenmayer flasks. The flasks were rotated on a rotary shaker (100 rpm) at 25°C in the dark. For analysis of anthocyanin accumulation, 0.2 g of hairy roots were inoculated to 50 ml of PRL-4C medium containing 6% (w/v) of sucrose in 100 ml Erlenmayer flasks. These flasks were then rotated on rotary shaker (80 rpm) at 25°C under continuous light irradiation or in the dark. When hairy roots were cultured in the dark for 3 weeks, accumulation of anthocyanins in hairy roots was observed 3 weeks after transfer to PRL-4C medium containing 6% sucrose, though accumulation was not observed during hairy roots were cultured in liquid LS medium (Figure 4). Pigments were extracted from the

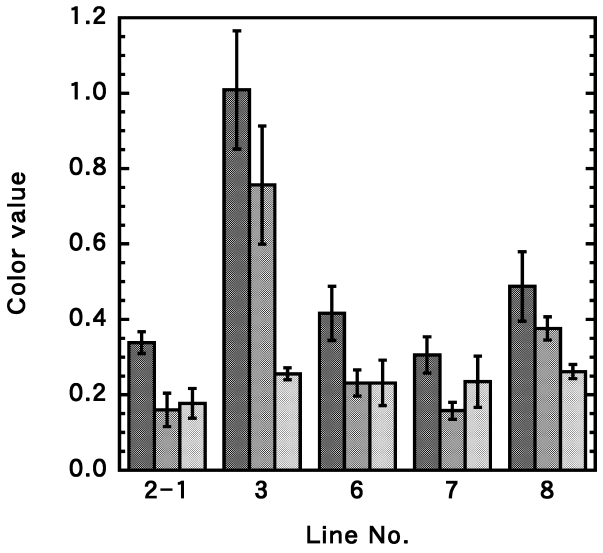

Figure 2. The color value of pigments extracted from hairy roots cultured on solidified medium for 3 weeks. From darkest bars to brightest bars, each group of bars shows the color value of pigments extracted from hairy roots cultured on PRL-4C medium, 1/4 LS medium, and LS medium, respectively. Several lines of hairy roots were tested, and the highest color value was observed in hairy roots cultured on PRL-4C medium.

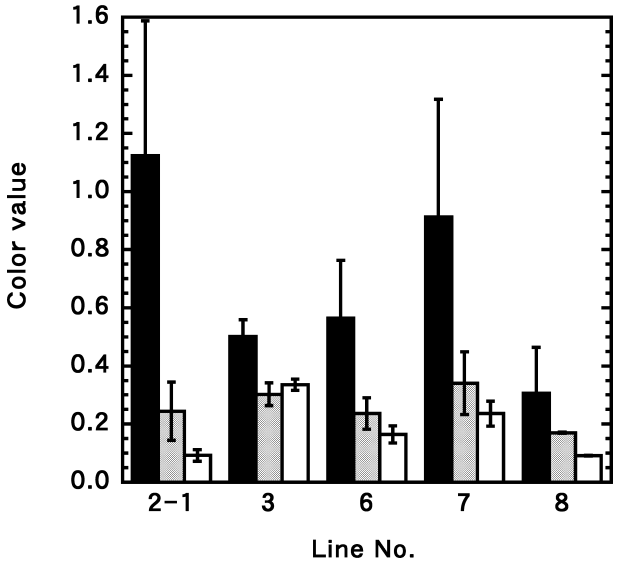

Figure 3. The color value of pigments extracted from hairy roots cultured on PRL-4C medium containing various amount of sucrose. Black, gray, and white bars show the color value of pigment extracted from hairy roots cultured on medium containing 5%, 3%, and 1% of sucrose (w/v), respectively. Several lines of hairy roots were tested, and the highest color value was observed in hairy roots cultured on PRL-4C medium containing 5% of sucrose.

hairy roots, and the color value was calculated (Figure 5). The amount of anthocyanins in hairy roots cultured in liquid PRL-4C medium containing 6% sucrose was comparable to that cultured on solid PRL-4C medium containing 3% sucrose. When hairy roots were cultured under the continuous light, some hairy root cultures

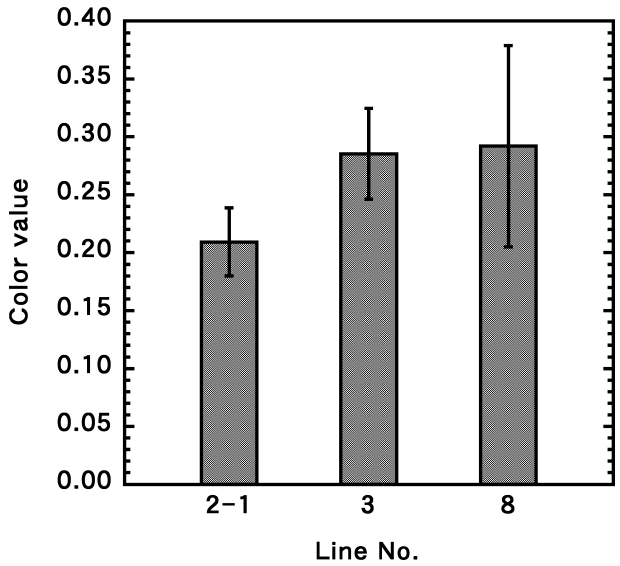

Figure 5. The color value of pigments extracted from hairy roots cultured in liquid PRL-4C medium for 3 weeks.

accumulated higher amount of anthocyanins than those cultured in the dark, and some other cultures turned brown and died. These phenomena would suggest that continuous light irradiation is inhibitory for the growth of hairy roots.

In this paper, hairy root cultures of *I. batatas* on Gelrite-solidified medium produced detectable amounts of anthocyanins only when they were cultured under continuous light irradiation. Previous reports have also shown that production of anthocyanins is induced by light irradiation in many plant species and plant cell cultures (Kakegawa et al. 1987; Zhong et al. 1993; Sato et al. 1996; Zhang et al. 2002). Therefore, it is suggested that light irradiation is a key factor for the production of anthocyanins. Anthocyanins were also produced in hairy roots cultured in PRL-4C liquid medium in the dark, although it was a lower amount than in hairy roots cultured on PRL-4C solid medium under continuous light. This shed light on production of anthocyanins in a large scale culture of hairy roots.

The maximum color value of hairy roots in this report, CV=1.1, was not so high compared with that of storage roots of intact plant, CV=8.9 (Yamakawa et al. 1997). However, the production of anthocyanins might be increased by modifying culture conditions, and hairy roots are thought to be applicable for the industrial production of the anthocyanins of purple-fleshed sweet potatoes.

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