

Production of Betalains by Suspension Cultures of *Basella rubra* L.

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The red-violet betacyanins are evidently suitable as natural color additives for some food systems. Red-violet cell suspension cultures of *Basella rubra* were found to accumulate the betacyanins. The media containing 2, 4-D and kinetin effectively produced red pigments. In particular, in the case of leaf, the medium containing 1 μ M of 2, 4-D effectively produced red pigments. Betaxanthins was produced better than betacyanins in the case of leaf.

Introduction

Betalains, especially betacyanins, have received much attention by chemists and biologists^{1), 2)} and even by the food industry. The red-violet betacyanins are evidently suitable as natural color additives for some food systems^{3), 4)}. Although the total chemical synthesis has been achieved⁵⁾, isolation of betacyanins from natural sources would still be the more economical technique for production of these pigments.

In this report we describe our investigation of the improvement in the formation and yields of betacyanins in suspension cultures of *Basella rubra*.

Materials and Methods

Plant material

Cell cultures of *Basella rubra* L. were initiated from leaves and stems of sterile grown seedlings on a Murashige and Skoog medium (MS medium)⁶⁾ agar containing 3 μ M of 2, 4-D and 3 μ M of kinetin. Leaves (5mm X 5mm) and stems (20mm X 5mm) were cut into pieces or blocks with a surgical knife and placed in MS medium

supplemented with various concentration (0, 0.01, 0.1, 1, 10, 100 μ M) of 2, 4-D, NAA, IAA, BA, kinetin and zeatin. The culture were incubated in dark at 25°C.

Results and Discussion

Effects of hormones on red pigments production

Table 1 shows the effects of hormones on red pigments production using leaf tissue. The media containing 2, 4-D and kinetin effectively produced red pigments. In particular, the better results were obtained with the medium containing 1 to 10 μ M of 2, 4-D and 1 to 10 μ M of kinetin.

Effects of tissues on red pigment production

Table 2 shows the effects of tissues on red pigments production with the medium containing 2, 4-D and kinetin. In particular, in the case of leaf, the medium containing 1 μ M of 2, 4-D effectively produced red pigments (Fig.1(a)). In the case of stem, the medium containing 1 μ M of 2, 4-D except that without kinetin effectively produced red pigments (Fig.1(b)).

Table 1. Effects of hormones on red pigments production

| Red pigments production (%) | | | | | | | | | |
|-----------------------------|-----|-----|---------------------------|-----|-----|--------------------------|-----|-----|---|
| BA (μM) | | | Kinetin (μM) | | | Zeatin (μM) | | | |
| 1 | 10 | 100 | 1 | 10 | 100 | 1 | 10 | 100 | |
| 2, 4-D (μM) | | | | | | | | | |
| 1 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 0 |
| 10 | 100 | 100 | — | 100 | 100 | — | 100 | 50 | — |
| 100 | 0 | — | 0 | 0 | — | 0 | 50 | — | 0 |
| NAA (μM) | | | | | | | | | |
| 1 | 0 | 0 | 50 | 0 | 50 | 0 | 100 | 0 | 0 |
| 10 | 50 | 50 | — | 100 | 50 | — | 50 | 100 | — |
| 100 | 100 | — | 100 | 0 | — | 100 | 100 | — | 0 |
| IAA (μM) | | | | | | | | | |
| 1 | 0 | 50 | 0 | 0 | 0 | 0 | 50 | 100 | 0 |
| 10 | 0 | 0 | — | 0 | 0 | — | 100 | 100 | — |
| 100 | 0 | — | 0 | 0 | — | 100 | 0 | — | 0 |

Red pigments production was observed after 14 days culture.

Table 2. Effects of tissues on red pigments production

| Red pigments production (%) | | | | | | | | | |
|-----------------------------|------|-----|-----|-----|---------------------------|------|-----|-----|-----|
| Leaf | | | | | Stem | | | | |
| Kinetin (μM) | | | | | Kinetin (μM) | | | | |
| 0 | 0.01 | 0.1 | 1 | 10 | 0 | 0.01 | 0.1 | 1 | 10 |
| 2, 4-D (μM) | | | | | | | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.1 | 50 | 50 | 50 | 50 | 50 | 0 | 0 | 50 | 50 |
| 1 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 100 |
| 10 | 0 | 100 | 100 | 100 | 0 | 50 | 50 | 50 | 100 |

Red pigments production was observed after 13 days culture.

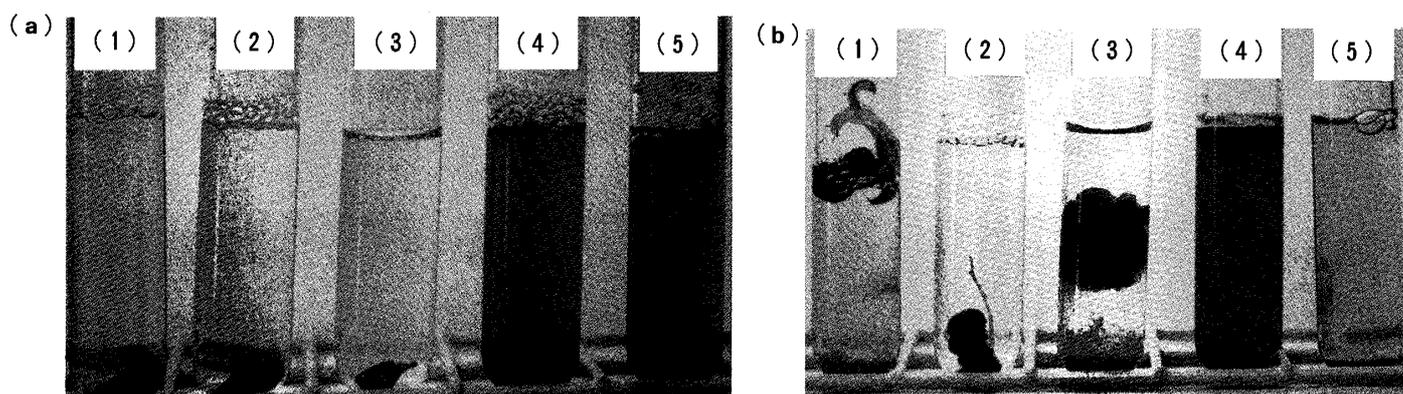


Fig.1 Effects of leaf(a) and stem(b) tissues on red pigment production.

Concentration of 2, 4-D and kinetin were (1), 1 and 0; (2), 0.01 and 0; (3), 0.1 and 0; (4), 1 and 1; (5), 10 and 1 μM , respectively.

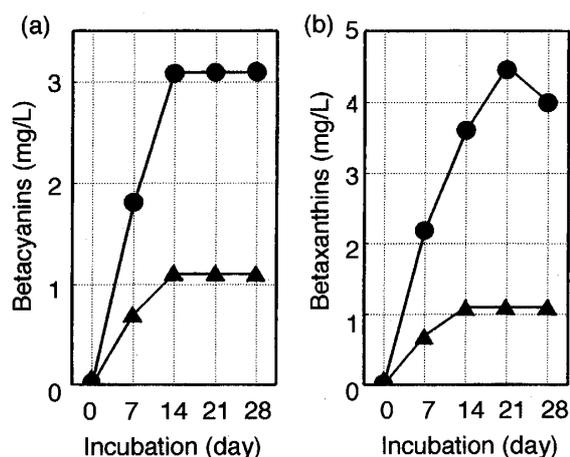


Fig.2 The time course of production of betacyanins (a) and betaxanthins (b) of *B. rubra* cell cultures.

●, leaf; ▲, stem.

Betaxanthins and betacyanins of *B. rubra* cell cultures

Figure 2 shows the time course of production of red pigments. The better results were obtained using leaf rather than stem. In the case of leaf, betaxanthins was produced better than betacyanins. On the other hand, in the case of stem, the production of betacyanins was almostly same as that of betaxanthins.

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