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BRIEF COMMUNICATION

Induction of betalain pigmentation in hairy roots of red beet under different radiation sources

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Abstract

The effect of different radiation sources – blue (B), red (R), R plus B (RB), B plus far red (BFr), R plus far red (RFr) – was tested on the growth of hairy roots and betalain accumulation in *Beta vulgaris* L. (red beet). Light emitting diodes were used as radiation sources. The growth of hairy roots under different radiation treatments depended on radiation quality. Highest biomass accumulation was under the BFr treatment. BFr treatment efficiently induced betalain pigmentation in hairy roots. Total sugar and sucrose contents of hairy roots were also greater in this treatment. Thus, the betalain pigmentation in the cultured hairy roots can be influenced by radiation quality and BFr is most suitable for accumulation of betalains.

Additional key words: *Beta vulgaris*, betacyanin, betaxanthin, biomass, bioreactor, light emitting diodes, radiation quality.

Beta vulgaris L. (red beet) contains betalains, which are water-soluble nitrogenous pigments, predominantly the yellow betaxanthins and the red violet betacyanins (Bohm and Rink 1988). These pigments are commercially important as natural dyes in food and pharmaceutical industry (Leathers *et al.* 1992). There are efforts to produce betalains by *in vitro* cell suspension culture (Leathers *et al.* 1992). Recently hairy roots have been induced in *B. vulgaris* cultured in small scale or large-scale cultures for the production of betalains (Hamill *et al.* 1986, Kino-Oka *et al.* 1992, Mukundan *et al.* 1998a,b). In plant cell cultures, various conditions (chemical and physical) influence the production of secondary metabolites (Buitelaar and Tramper 1992). Among physical factors, radiation is especially important for accumulation of metabolites. Irradiation enhances anthocyanin production in *Catharanthus roseus* (Knobloch *et al.* 1982), *Vitis* hybrid (Yamakawa *et al.* 1983), *Perilla frutescents* (Zhong *et al.* 1991), *Populus* hybrid (Matsumoto *et al.* 1973), and *Fragaria ananassa* (Sato *et al.* 1996). However, we have no information about betalain pigmentation responses to specific radiation sources. Therefore, we explored whether betalain biosynthesis in hairy root culture can be induced under different radiation stimuli.

The hairy roots of red beet (*Beta vulgaris* L. cv. Detroit dark red) were used throughout the experiments. The hairy roots were induced by leaf disc method with *Agrobacterium rhizogenes* strain A4 described in Taya *et al.* (1992). The hairy roots were maintained by the subcultures in the dark at 25 °C every 10 d in half strength Murashige and Skoog (1962; MS) medium (pH 5.8) containing 30 g dm^{-3} sucrose and no phytohormone.

Hairy roots (5 g dm^{-3}) were cultured in Balloon Type Bubble Bioreactors (airlift type of bioreactor, volume 1000 cm^3 capacity) containing 500 cm^3 of half strength MS medium with 30 g dm^{-3} sucrose. The volume of input

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Abbreviations: B - blue radiation; BFr - blue plus far red radiation; LED - light emitting diode; MS medium - Murashige and Skoog medium; PFD - photon flux density; R - red radiation; RB - red plus blue radiation; RFr - red plus far red radiation.
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air was adjusted to 6.0 vvs (air volume/culture volume/s). The cultures were maintained in growth chambers wherein air temperature was 25 °C and relative humidity was 70 %. The hairy roots were grown for 15 d under the different radiation sources of photon flux density (PFD) of 50 µmol m^{-2} s^{-1}. There were six different radiation treatments: fluorescent (FL, used as control), monochromatic red (R, peak emission of 660 nm), monochromatic blue (B, peak emission of 470 nm), R plus B (RB, 1:1 photon flux density, PFD), B plus Fr (BFr, 1:1 PFD), and R plus Fr (RFr, 1:1 PFD); light emitting diodes (LED; *GF-320S* system *Good Feeling Co.*, Seoul, Korea). The spectral energy distribution of R, B, RB, BFr, RFr and FL is shown in Fig. 1. The spectral distribution of relative energy of B, R, and Fr regions was determined with a spectroradiometer (*LI-1800*, *LI-COR*, Lincoln, USA). In another set of experiments effect of different proportions of B and Fr were tested on the growth of betalain synthesis. On day 15, all the hairy roots in the treatments were sampled for measurements of fresh mass, dry mass and betalain and sugar contents.

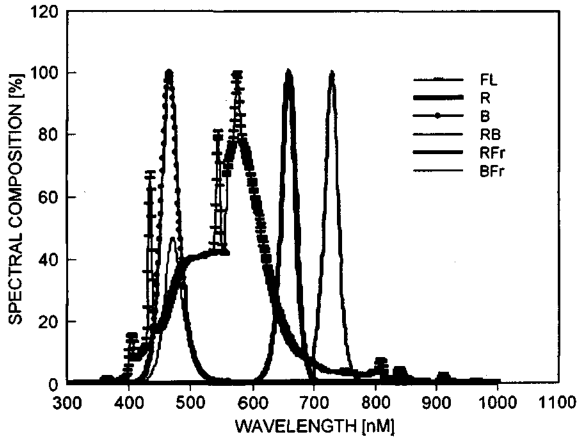

Fig. 1. Spectral composition of radiation sources used for culturing hairy roots. For symbols see Table 1.

Pigments from random samples of hairy roots were analysed spectrophotometrically (*Uvikon 930*, *Kontron Instruments Co.*, Zurich, Switzerland) by the method of Nilsson (1970). Sucrose content was measured by dinitrosalicylic acid method and total sugar content was analysed by phenol sulfuric acid method (Chaplin 1986).

For each treatment there were three replicates and experiments were repeated twice. Values were analysed by using statistical package of *SAS* system (version *6.21*, *SAS Institute Inc.*, Cary, USA). Statistical significance between mean values was assessed by Duncan's multiple range test.

Bioreactor culture is widely adopted to enhance metabolic productivity in plant cell or hairy root cultures. The hairy root cultures of *B. vulgaris* grown in bioreactors showed a dependence of biomass formation (fresh and dry mass) on applied radiation quality (Table 1). Biomass of hairy roots was comparatively less in the cultures grown under R, B, RB, and RFr irradiations than under FL. However, the biomass of hairy roots was comparatively greater in BFr cultures than in the FL cultures.

Table 1. Fresh and dry mass and contents of total sugars and sucrose in hairy roots of *Beta vulgaris* grown in airlift bioreactor under different radiation treatments for 15 d (FL - fluorescent, R - red, B - blue, RB - red + blue, BFr - blue + far red, RFr - red + far red). Mean separation within the columns by Duncan's multiple range test at 5 % level.

Radiation treatment	Fresh mass [g dm^{-3}]	Dry mass [g dm^{-3}]	Total sugars [mg g^{-1}(d.m.)]	Sucrose [mg g^{-1}(d.m.)]
FL	50.8 a	5.6 a	24.2 b	4.2 b
R	38.3 b	3.2 c	14.5 c	2.0 c
B	46.2 ab	4.8 ab	29.9 a	8.5 a
RB	47.0 ab	5.6 a	28.6 a	4.2 b
BFr	52.3 a	5.8 a	31.1a	5.5 b
RFr	39.9 b	4.1 bc	13.2 c	2.2 c

In comparison to hairy roots grown under FL, R and RFr irradiations drastically reduced the betacyanin and betaxanthin contents (Fig. 2). In contrast, BFr irradiation enhanced the accumulation of both betacyanin and bataxanthin. The betacyanin and betaxanthin contents of hairy roots grown under B and RB were similar to those of hairy roots grown under FL.

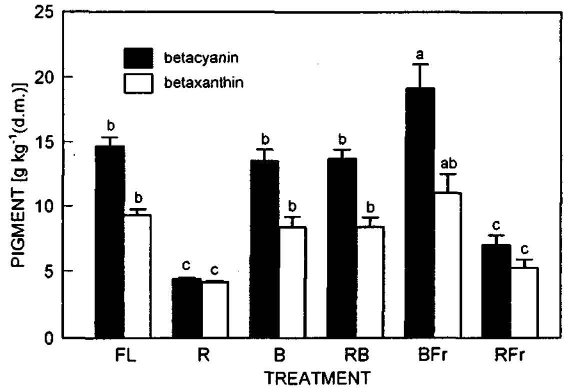

Fig. 2. Betacyanin and betaxanthin contents of hairy roots of *Beta vulgaris* grown in airlift bioreactor under different radiation treatments for 15 d. For symbols see Table 1.

Plant responses to red, blue, and UV radiation evoke different expression via signal perception and transduction pathways (Kendrick and Kronenberg 1993). Most of *in vitro* studies show some effects of radiation on the accumulation of betalain in cultured cells (Bianco-Colomos and Huges 1990, Bohm *et al.* 1991) although details of responses have not yet been clarified. Blue radiation was more effective than red radiation in inducing betalains in seedling of *Amaranthus* (Obrenovic 1985) and callus cultures of *Portulaca* (Kishima *et al.*

1995). In our study also B alone has induced greater betalain synthesis than R.

In the present experiments the BFr irradiation induced pigment accumulation comparable to all other treatments. Hence this pigmentation requires both B and Fr spectrum and phytochrome may be stimulated by Fr to produce low amount of pigment. Furthermore, the large betalain accumulation in hairy roots following BFr irradiation probably represents co-action of transduction via blue and red photoreceptors (Mohr 1993).

Changes in radiation quality were accompanied by unspecific increase in total sugar and sucrose contents (Table 1). They were lower in the hairy roots grown under R and RFr treatments than under FL. By contrast, B enhanced sugar synthesis. The presence of larger amounts of total sugars and sucrose might be responsible for active growth of hairy roots as well as accumulation of betalains.

For further characterisation of hairy roots they were grown under different ratios of B and Fr (Table 2). Among the four treatments studied the 1:1 (PFD ratio of B and Fr) was best suited for accumulation of betalains.

Table 2. Betalain content of hairy roots of *Beta vulgaris* grown in airlift bioreactor under blue plus far red (BFr) radiation treatments for 15 d. Mean separation within the columns by Duncan's multiple range test at 5 % level.

B + Fr ratio	Betacyanin [mg g^{-1}(d.m.)]	Betaxanthin [mg g^{-1}(d.m.)]
1:0	13.6 b	8.2 b
1:0.5	19.5 a	11.1 a
1:1	20.8 a	11.3 a
1:1.5	11.0 b	7.4 b

Photomorphogenesis in higher plants is under the control of at least three different photo-transduction system involving phytochrome, blue radiation, and UV photoreceptors (Kendrick and Kronenberg 1993). Thus, betalain pigmentation in *Beta vulgaris* hairy roots could be activated through BFr during bioreactor cultivation. However, additional studies are necessary to establish firmly the involvement of phytochrome and/or B absorbing photoreceptors in photoresponses of hairy roots.

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