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EFFECT OF LIGHT WAVELENGTH ON IN VITRO ORGANOGENESIS OF A *CATTLEYA* HYBRID

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The effect of light wavelength on multiplication, tissue growth and pigment content was studied in *Cattleya intermedia* × *C. aurantiaca* microcutting cultures. The initial explants were shoots regenerated from protocorm-like bodies. Modified MS medium containing 5.0 mg·l^{-1} BA, 0.2 mg·l^{-1} zeatin and 1.0 mg·l^{-1} NAA, solidified with Difco agar, was used for adventitious regeneration of shoots and aerial roots. The rate of organ initiation depended on the wavelength of the monochromatic light applied. Red and blue treatments were effective in triggering photomorphogenesis in the evaluated material. The propagation coefficient reached 11.7 under red light, 10.6 under blue, 8.3 under white and 6.2 in darkness. Total chlorophyll and carotenoid content were highest in cultures illuminated with white light, gradually decreasing from the blue to the red and the far red treatments. Blue light treatment improved the efficiency of micropropagation and benefitted initiation of rhizogenesis and aerial root elongation, and the resulting plants were true to type.

Key words: *Cattleya*, monochromatic light, adventitious buds, rhizogenesis, chlorophylls, carotenoids.

INTRODUCTION

European greenhouses are filled with many epiphytic orchid species and hybrids produced over many years of growing and hybridizing. Orchids are propagated both from seeds and vegetatively, but the process is slow. Multiplication of micropropagated material is faster than by any conventional method of propagation, and in vitro culture techniques currently are standard practice for orchid culture (Chen and Chang, 2004; Chen et al., 2004; Tuong and Tanaka, 2004). Plant morphogenesis is influenced by many environmental factors such as nutrients and the quality, duration and intensity of light, temperature regime, type of carbon source, gas composition in the culture vessel (especially CO_2 concentration), and the number of air exchanges during culture (Kirdmanee et al., 1992; Hahn and Paek, 2001; Li et al., 2002). There are many reports on the effects of nutrition and growth regulators used for in vitro cultivation of a variety of orchids, but much fewer focusing on the effect of irradiation on bud initiation and subsequent growth (Ichihashi, 1990; Adelberg et all, 1997; Tanaka et al., 1998; Islam et al, 2000). Modifying the duration and intensity of particular wavelengths has been shown to affect morphogenesis in different ways (Vince-Pure, 1994).

Here we report research on the effects of light quality on micropropagation of a valuable interspecific *Cattleya* hybrid. We also estimated chlorophyll and carotenoid content in this material propagated under different light regimes. The long-term purpose is to obtain high quality *Cattleya intermedia* × *C. aurantiaca* plantlets for further multiplication and transfer to soil.

MATERIALS AND METHODS

The plant material used was a *Cattleya* Lindl. hybrid obtained by crossing *Cattleya intermedia* and *C. aurantiaca*, originating from the Orchid Collection of the Botanical Garden of Jagiellonian University. Explants were obtained from mericloned cultures, cloned pathogen-free and subcultured at 8-week intervals. They were maintained in tissue culture for about a year. Shoots ~5 mm high with three or four leaves, regenerated from a mass of protocorm-like bodies, were excised and placed on 50 ml

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Abbreviations: BA – 6-benzylaminopurine; NAA – 1- naphthaleneacetic acid; MS – Murashige and Skoog medium; PLBs – protocorm-like bodies.

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TABLE 1. Effect of light wavelength on the number and length of adventitious shoots and aerial roots, and on the weight of regenerated *Cattleya* plantlets after 90 days of exposure for 16 hours a day

Light treatment	Propagation coefficient	Shoot length [mm]	Fresh weight [mg]	Dry matter [mg]	Number of roots per shoot	Root length [mm]
white	8.39ab*	10.25b	298.56ab	19.21ab	2.63b	0.40c
blue	10.64bc	10.10b	345.68ab	26.55c	4.14c	0.41c
red	11.70c	12.97c	393.13c	21.67bc	2.12ab	0.23b
far red	6.17a	7.02a	242.65a	20.56ab	1.50ab	0.03a
no light	6.21a	6.87a	236.96a	14.53a	1.00a	0.02a

*Means followed by the same letter in each column do not significantly differ at p = 0.05.

solidified modified MS medium in a 250 ml Erlenmeyer flask. The proliferation medium was composed of MS salts and vitamins, supplied with 2 $mg \cdot l^{-1}$ adenine sulphate, 5.0 $mg \cdot l^{-1}$ benzylaminopurine, 0.2 $mg \cdot l^{-1}$ zeatin, 1.0 $mg \cdot l^{-1}$ naphthaleneacetic acid and 3% (w/v) sucrose, solidified with 0.8% (w/v) agar (Bacto Agar, Difco). The pH was adjusted to 5.5. Cultured material was transferred every six weeks onto fresh medium of the same composition for three months. The cultures were maintained at 23±2°C with a 16 h photoperiod under light of different spectra: the control, white (390–760 nm, Tungsram F33 40 W lamp); far red (770–800 nm, 100 W incandescent light and filters: standard filter no. 405 orange + standard filter no. 420 deep blue, Compact light B. V. Amsterdam), red (647–770 nm, Philips TLD 36 W) and blue (450–492 nm, Philips TLD 36 W). Radiometric measurements were made in the horizontal plane 35 cm above the cultures. Solar spectral irradiance in the range 300–1100 nm, expressed in $\mu mol\ m^{-2}s^{-1}$, was measured (with wavelength drive intervals of 2 nm) with an LI–1800 spectroradiometer (LI-COR U.S.A.). The measurements and data integration in quantum units ($\mu mol\ m^{-2}s^{-1}$) in the 300–1000 nm range and photosynthetically active radiation (PAR 400–700 nm) were graphically interpreted using PC–188 ver. 3.01 (LI-COR) by Bach and Malik (1999). The photosynthetically active radiation was 60 $\mu mol\ m^{-2}s^{-1}$ except for red and far red light (20 $\mu mol\ m^{-2}s^{-1}$). Additional control material was maintained under continuous darkness throughout the whole course of the experiment, with the rest of the incubation conditions as given earlier. Each experimental combination was done twice in 25 replicates. A single replicate was a flask with 25 explants. The biometric data recorded after three months of culture for the whole experimental design were as follows: number of regenerated adventitious shoots and aerial roots, shoot length and root length. From each treatment, ten samples consisting of several shoots were collected at random from different flasks at the end of the three-month culture period, and fresh and dry matter were measured. For dry weight determination the material was dried at 105°C for 30 min and then kept at 60°C to constant weight. Additionally, chlorophyll *a* and *b* as well as carotenoid content were determined by light absorption spectroscopy in acetone extracts of 200 mg plant samples, according the method described by Bach and Świderski (2000). Absorbance values at 662, 645 and 470 nm were recorded with a Jasco V–530 spectrophotometer. For light microscopy, material fixed in 3% glutaraldehyde followed by post-fixation in 2% osmium tetroxide was sectioned transversely. Sections 1 µm thick were cut from randomly selected Epon 812 blocks with a Tesla 490 A ultramicrotome. The results were subjected to ANOVA using STATISTICA 6.1. Duncan's multiple range t-test was used; significance was assumed at p = 0.05.

RESULTS

The presented experiments yielded information on the differential morphogenetic response of *Cattleya* micropropagated under different monochromatic light regimes. Adventitious bud formation was enhanced by red and blue light, and after three months of culture the propagation coefficients of material exposed to those light wavelengths were significantly higher: 11.7 under red and 10.6 under blue light, only ~8 under white light, and ~6 in continuous darkness (Tab. 1). Elongation of initiated shoots was most pronounced under red light, but elongation of aerial roots in regenerated plantlets was greatest under blue light. Blue light was also most effective in initiation of rhizogenesis (Fig. 1). The weight of cultures was depended upon the number of regenerated organs. The highest fresh weight was for cultures under red light, and dry weight was highest for cultures under blue light. The propagation coefficient and biomass weight under far red treatment were comparable to the results under constant darkness (Tab. 1). Light wavelength strongly affected the anatomy of regenerated vegetative organs. Cross sections of leaves initiated in vitro showed that blue light favored proper anatomical

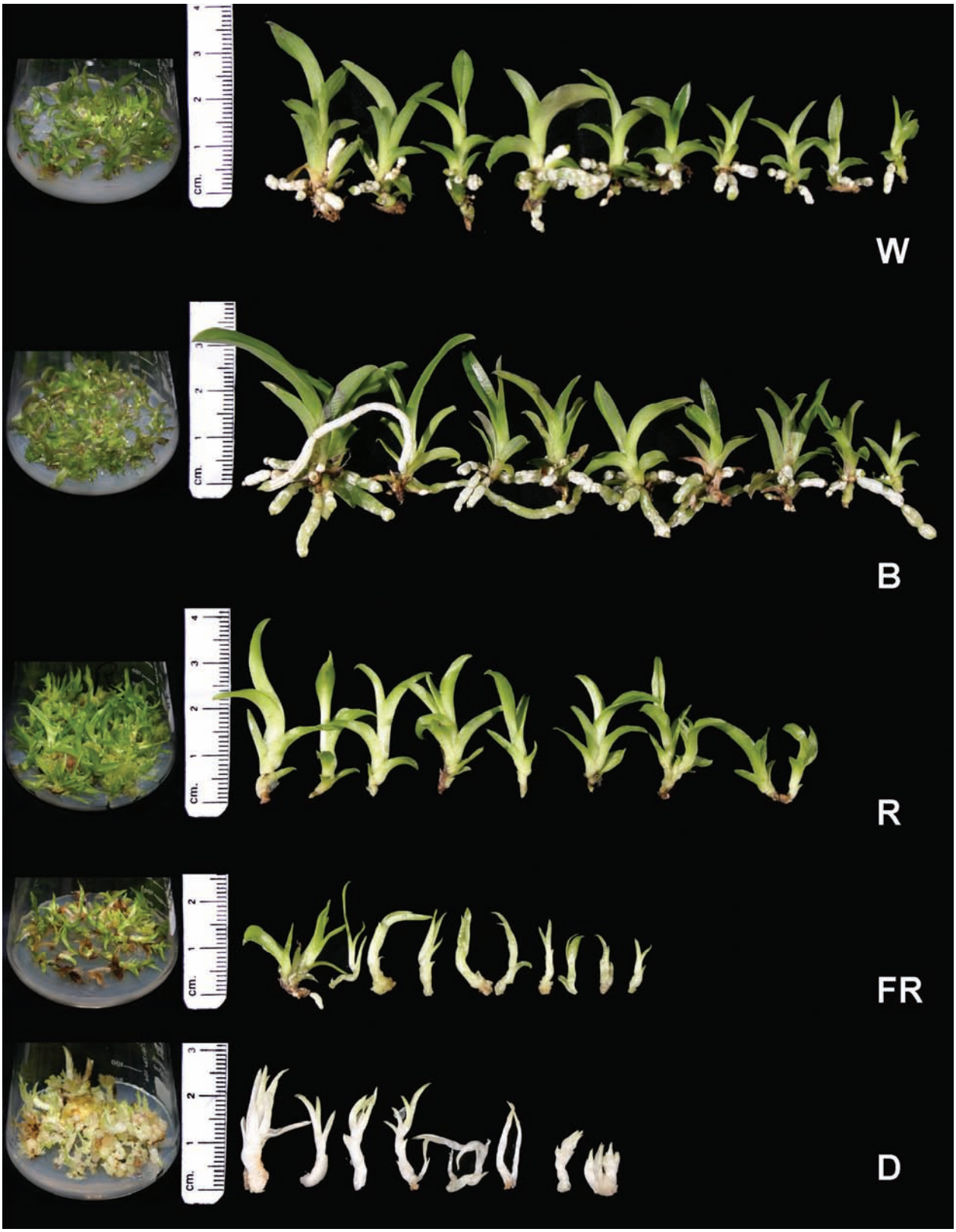

Fig. 1. Effects of white (W), blue (B), red (R), far red (FR) light and continuous darkness (D) on organogenesis in *Cattleya intermedia* × *C. aurantiaca* shoot explants cultured in vitro.

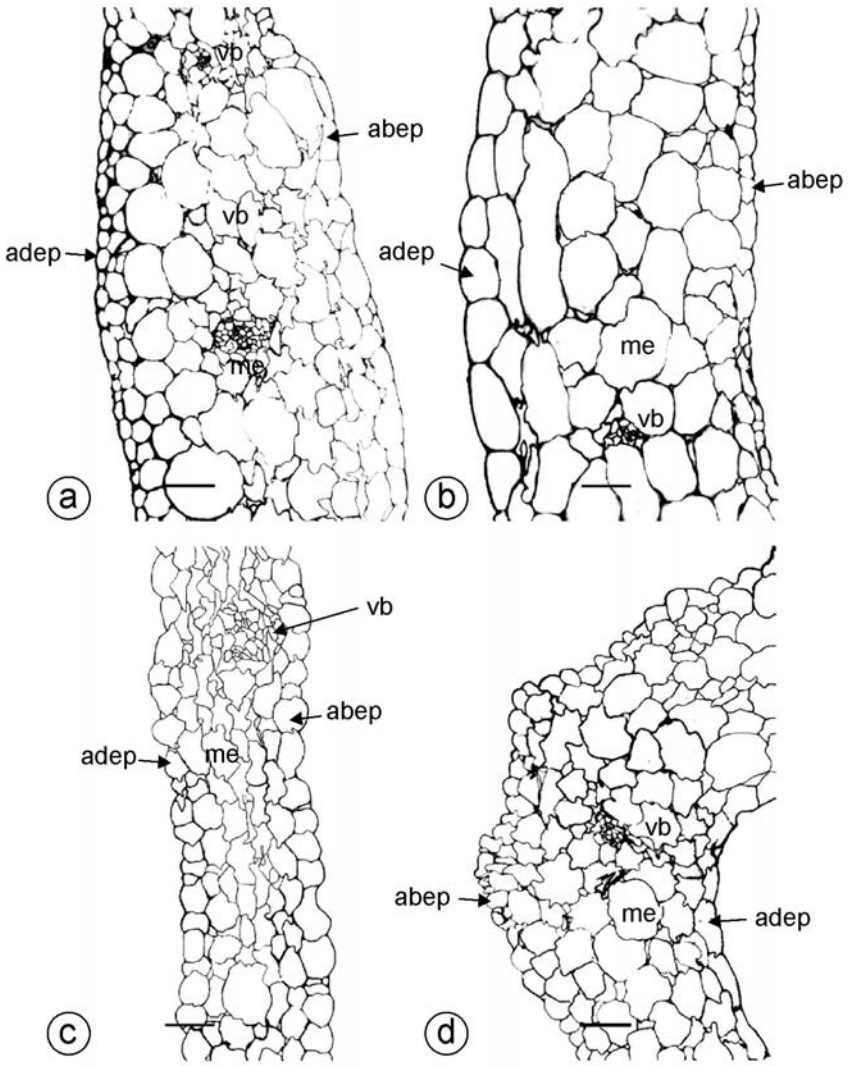

Fig. 2. Transversal section of middle part of leaf blades regenerated under white (**a**), blue (**b**), and red (**c**) light, and continuous darkness (**d**). abep – abaxial epidermis; adep – adaxial epidermis; vb – vascular bundle; me – mesophyll. Bar = 74 μm.

development, whereas red light caused collapse of part of the mesophyll cells, and reduction of leaf blades (Fig. 2). The concentration of pigments in *Cattleya* leaves also depended upon the wavelength of the applied light. Data on pigment content per fresh weight of plants are shown in Figure 3. Both total chlorophylls and total carotenoids were highest in control plants illuminated with white light, ~50 mg/100 g and ~10 mg/100 g, respectively, and decreasing gradually from the blue to the red and the far red treatments. The lowest values were in plants grown in darkness. The same correlations were found for chlorophyll *a* and *b* treated separately (Fig. 4). Photosynthetic pigment levels were lower under illumination with monochromatic light than under white light, but in terms of the analyzed plant material's morphological and anatomical features, irradiation with blue monochromatic light was most favorable for *Cattleya intermedia* × *C. aurantiaca* cultures. Total chlorophyll *a* and *b* for the blue treatment was ~42 mg/100 g fresh weight, not much lower than under white light; it was apparently sufficient for the photosynthetic activity of mixotrophic culture, and at the same time beneficial to photomorphogenesis.

DISCUSSION

Orchids grown in vitro are generally not photosynthetically autonomous and frequently depend on a source of organic carbon such as sucrose (Chen

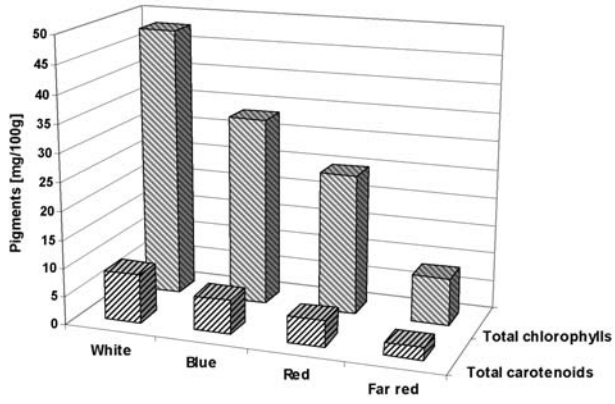

Fig. 3. Chlorophyll and carotenoid content in shoots of hybrid *Cattleya* cultured under monochromatic light regimes.

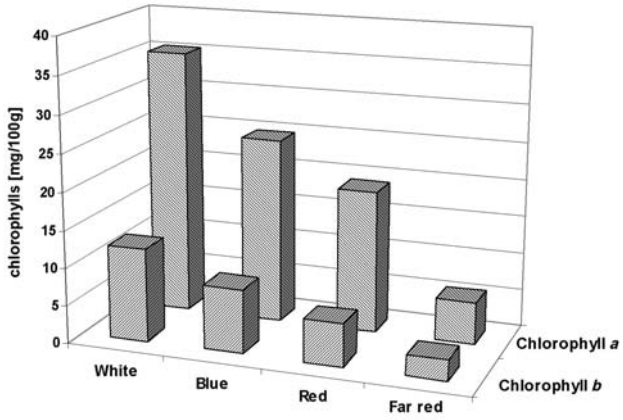

Fig. 4. Chlorophyll *a* and *b* content in *Cattleya intermedia* × *C. aurantiaca* shoots regenerated adventitiously in vitro under monochromatic light regimes. Values for plants grown in darkness were treated as blanks and were subtracted.

and Chang, 2004; Chen et al., 2004; Tuong and Tanaka, 2004), though in recent years it has been shown that CO_2 enrichment and the use of high photosynthetic photon flux can speed the growth rates of both C_3 and CAM orchids in tissue culture (Hahn and Paek, 2001; Li et al., 2002). Light, as a main environmental trigger, plays a central role in regulating plant development. Here we studied light wavelength as a factor in morphogenesis and growth in mixotrophic cultures of *Cattleya intermedia* × *C. aurantiaca* as an experimental model. We demonstrated that it can alter organogenesis in this slow-growing material, and can alter the morphology and anatomy of orchid plantlets obtained adventitiously in vitro. Applying superbright red and blue light-emitting diodes (LED) to *Cymbidium* cultures, Tanaka et al. (1998) found that the fresh and dry weight of plantlets was significantly higher when grown under red and red plus blue LEDs, and that leaves were longest under red. This is consistent with our results with incandescent light. However, attention should be paid to light intensity, another important variable. Culturing *Phaius* and *Vanda* in vitro, Soontornchainaksaeng et al. (2001) observed that light intensity significantly affected dry weight and plantlet height, leaf number, shape and area. In our study the differences in fresh and dry weight of biomass obtained under blue and red light may have been due in part to differences in light intensity.

In *Betula*, as in our *Cattleya* hybrid, light quality was shown to affect not only proliferation rate and shoot elongation but also the anatomy of cultured plantlets (Sæbø et al. 1995). Red light significantly enhanced adventitious bud formation and stem elongation in *Petunia* and *Gerbera*, while blue increased the number of adventitious buds and the fresh weight of obtained shoots of *Freesia*, *Hyacinthus* and *Cyclamen* (Gabryszewska and Rudnicki, 1995; Bach and Malik, 1999; Bach et al., 2000; Bach and Świderski, 2000; Witomska and Koszewska, 2002).

Blue light treatment improved the efficiency of the in vitro propagation system used. The morphology and anatomy of the obtained plantlets was also positively affected, and the resulting plants were true to type. The plantlets obtained under red were not acceptable, because the total chlorophyll and carotenoid content of leaves was much reduced and degenerative changes occurred in the mesophyll. We conclude that light spectra can be used in *Cattleya* organ culture to control morphogenesis and the growth of tissues in vitro. This is important, because techniques successfully adopted for mass propagation of orchids are now widely used not only for clonal propagation but also during genetic manipulation aimed at plant improvement (Tanaka et al., 2005).

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