

The influence of light spectrum on morphogenesis of orchid germs *in vitro*

L V Khotskova¹, G Ya Stepanyuk¹, M S Yamburov¹, T P Astafurova¹ and S B Turanov²

¹National Research Tomsk State University, Lenin Avenue 36, Tomsk, 634050, Russia

²National Research Tomsk Polytechnic University, Lenin Avenue 30, Tomsk, 634050, Russia

E-mail: tyrsb@yandex.ru

Abstract. The aim of the study is to reveal growth and developments specificities of orchid (*Cymbidium hybridum* F1) germinant *in vitro* cultured early in the ontogenesis in relation to various light spectra. Chromatic and achromatic luminous tubes (Philips, TLD 18 W) were used as lighting sources. 4 light patterns were tested: control group – colorless (white) light WL; group 1 – WL with addition of red WL+RL, group 2 – WL and blue WL+BL, group 3 – WL and green WL+GL. The authors showed that during *C. hybridum* F1cultivation in type breeding ground, morphometric growth indexes of root and leaf and wet weight of germs raised with the increase of red light part in PAR (photosynthetically active radiation) flux, though stem length was twice bigger under the mix of colorless and green light, and also blue light addition to achromatic emission aided the reduction of germ parameters, in contrast with control group. Growth characteristics of plants, cultivated under photoculture conditions, can be adjusted by light with varied spectral content.

1. Introduction

In photoculture conditions growth and development of plants depends heavily on spectral and energetic characteristics of artificial light sources, which lack optimum spectral range ratio and require continual improvements [1]. Orchid family is economically valuable and leads in production sector, widely used as crop and pot culture [2-3]. However, numerous representatives of the orchid family are described as plants with slow development process; they come into reproductive period just by 7-10 years, what inhibits reproduction and cultivation with the use of traditional methods.

The goal of the research is to reveal growth and developments specificities of orchid germinant *in vitro* early in the ontogenesis in relation to various light spectra.

2. Equipment and Methods

The *in vitro* cultured tissue of subtropical orchid *Cymbidium Showgirl* × *Cymbidium Lilian Stewart* (*C. hybridum* F1; figure 1) was chosen as test object. Samples were provided by Siberian Botanic Garden of National Research Tomsk State University with help of reproduction [4].





Figure 1. Flowering *Cymbidium Showgirl* × *Cymbidium Lilian Stewart* plant in collector's fund of Siberian Botanic Garden of National Research Tomsk State University (photo by Khotskova L V 2018).

Family reproduction of orchids *in vitro* was carried out by generally recognized procedure with the use of Murashige and Skoog medium [5]. Within 15 months of cultivation *in vitro* cultured *C. hybridum* F1 germs had obtained differentiated shoots with 4-5 leaves layers and 2-3 roots, sufficient for their planting *ex vitro* in non-sterile conditions. At this growth stage of germinant morphometric parameters were measured: the length of stem, root and leaf, assimilating area on a per intact plant basis and wet germs weight. 3 containers with germs, 10 of which were then selected for analysis, were used per light settings. Statistical analysis of orchid germinant morphometric parameters was performed using MS Excel 2007 programme pack. For plants, grown under PAR (photosynthetically active radiation) light, statistical significance of differences was evaluated with use of Student's t-test ($p \leq 0.05$) and then contrasted with control group (achromatic light). Data in the pictures is arithmetical average \pm average error.

As light sources for the experiments chromatic and achromatic luminescent tubes (Philips, TLD 18 W) were chosen. This lamp type is widely applied among commercial flower production and have spectrum, which covers almost whole range of PAR. Nevertheless, it is known that each PAR region (red, green or blue) separately is inapplicable for plants cultivating in protected ground conditions [1]. During the experiments 4 light patterns for seed sprouting and further cultivation were tested: control group – colorless (white) light WL; group 1 – WL with addition of red WL+RL, group 2 – WL and blue WL+BL, group 3 – WL and green WL+GL. Lighting variants were separated by lightproof screens. Light intensity of luminescent tubes was aligned to protocorm level in all experiments and remained at 3000 lux. Cultures were maintained at the temperature $23 \pm 2^\circ\text{C}$ and atmosphere relative humidity 65%, 16-hour photoperiod. To evaluate and analyze irradiation facilities, PPFs of all colour combinations at plants surface were calculated with consideration of glass container (figure 2). The measurements were conducted by applying spectrophotometer TKA-FAR (Russia, Saint-Petersburg) at the level of plants in nutriculture medium.

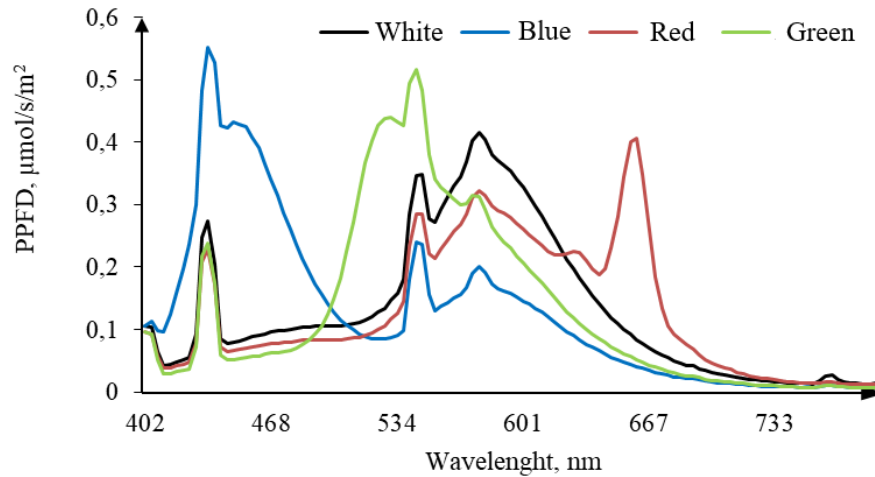


Figure 2. Photosynthetic photon flux density (PPFD, $\mu\text{mol}/\text{cm}^2$) of luminescent tubes (Philips, TLD 18 W) at the surface of *C. hybridum* plants *in vitro* cultured: control group – achromatic (white) light WL, group – WL and red light WL+RL, group 2 – WL and blue light WL+BL, group 3 – WL and green light WL+GL.

Figure 2 depicts that PAR wavelengths ratio redistributed on different sections of the plant under the flux superimposition from varied light sources. In addition, the width of peaks, peak value and spectrum ratio (BL, GL, RL) changes as well. Under mixed white and red light flux additional far-red spectrum peak appears.

3. Results and Discussion

It has been known, that light intensity and spectral structure affect growth and development of plants cultured *in vitro* [6-10]. In this research we detected, colorless light, combined with chromatic light, affects differently on growth and development of cultivated in typical medium orchids. Throughout continuous adaptation to varied spectral content, the combinations of white light + green or red light increased stem length of sample plants (70% and 13% higher than control group relatively), while white light + blue light lead to the degradation of stem length (figure 3). With the addition of blue light to colorless light root length of germs slightly differed from the control group, whereas in contrast the it raised by 85% and 193% over control with green and red light supplements relatively (figure 3). Total leaves area and wet weight of the germinant multiplied as well under long-wavelength spectral region (figure 4). Maximum gain of morphometric parameters occurred under WL+RL, compared with the control group.

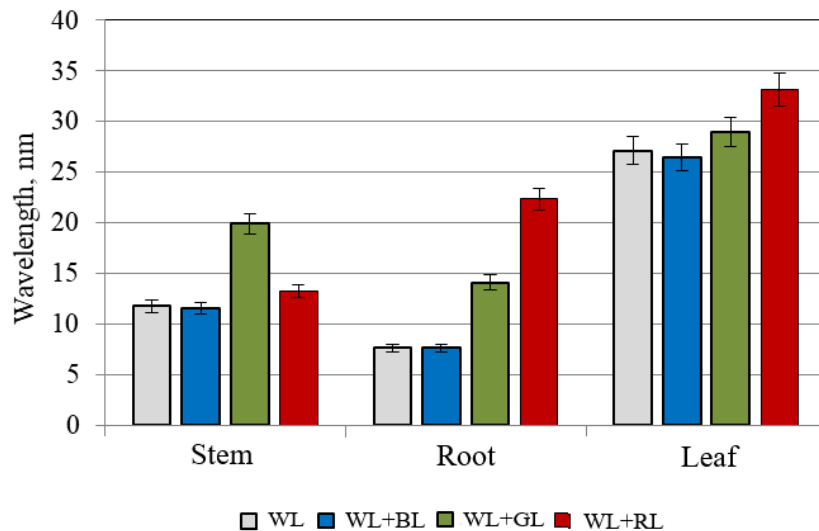


Figure 3. Growth parameters of 15-months *C. hybridum* F1 germs, cultivated *in vitro* in typical medium under varied light spectrum.

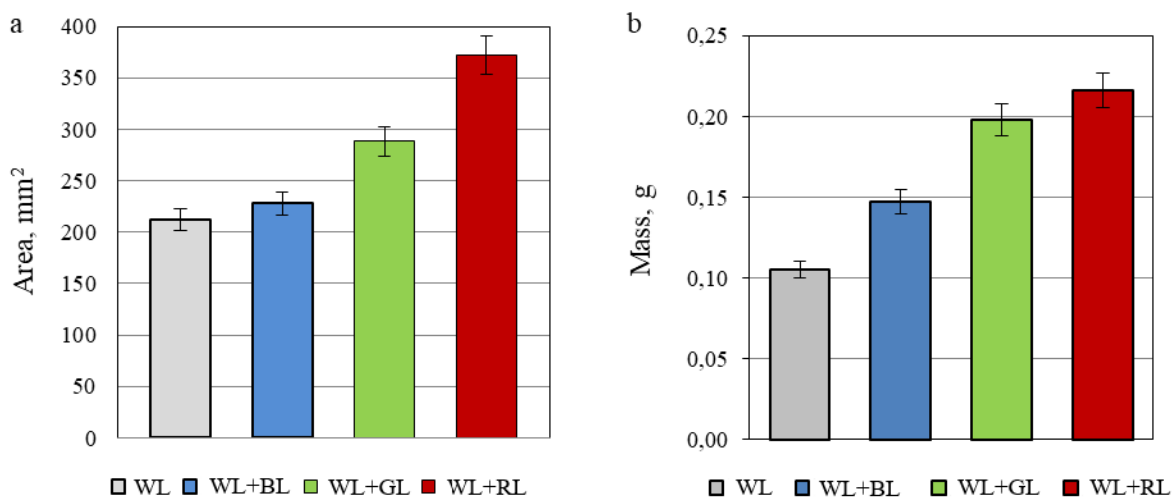


Figure 4. Total area (a) and wet weight (b) of the intact plant leaf (15-months *C. hybridum* F1 germinant, grown *in vitro* in typical medium under varied light spectrum).

Therefore, obtained results illustrate the relation between the development of germs *Cymbidium hybridum* F1 *in vitro* and different light spectrum early in the ontogenesis.

4. Conclusion

Morphometric parameters of germinant root, leaf and wet weight (grown in Murashige and Skoog medium *C. hybridum* F1 *in vitro* cultured) rose with the increase of wavelength of supplementary to achromatic light illuminating source under WL+RL and WL+GL conditions. Moreover, stem was longer under WL+GL combination. Intensified with short-wave spectral region achromatic light diminished stem length of orchid germs. Obtained results of the research ascertain, that long-wave spectral region part intensification in combines light flux enhances linear size and surface area of the

plants, what was previously discovered among different species [11-14]. Under protoculture conditions growth parameters of plants can be adjusted by light emission with variously structured spectrum to modify ornamentality.

Acknowledgement

This research was supported by “Tomsk State University competitiveness improvement program” grant (№ 8.1.29.2018).

The work was supported by the Ministry of Education and Science of the Russian Federation under the State Program in the field of scientific investigations №13.3647.2017/PCH

References

- [1] Bantisa F, Smirnakoub S, Ouzounisc T, Koukounarasa A, Ntagkase N and Radogloub K 2018 Current status and recent achievements in the field of horticulture with the use of light-emitting diodes (LEDs) *Scientia Horticulturae* **235** pp 437–451
- [2] Kolomeitseva G L 2014 *Large-flowered orchids in the collection of Main Botanic Garden named after N V Tsitsin RAS (Cattleya, Cymbidium, Dendrobium, Paphiopedilum, Phalaenopsis)* (Moscow: GEOS) p 296
- [3] De Lakshman, Pathak P, Rao A N and Rajeevan PK 2015 *Commercial orchids* ed Walter de Gruyter (India: GmbH) p 301
- [4] Stepanuk G Ya and Khotskova L V 2010 The results of some tropical and subtropical orchids introduction into Siberian Botanic Garden *The first international Bekkerov readings* (Volgograd: TriAs) pp 199–201
- [5] Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue culture *Physiologia Plantarum* **15** pp 473–497
- [6] Kong S S, Hosakatte N M, Jeong W H, Eun J H and ,Kee Y P 2008 The effect of light quality on the growth and development of in vitro cultured *Doritaenopsis* plants *Acta Physiologiae Plantarum* **30** pp 339–343
- [7] Rodrigues S, Joyce D, Pasqual M, Rodrigues F and de Araujo A G 2010 Etiolation and artificial light in native and hybrid orchids under in vitro cultivation *Ciencia Rural* **40** pp 1941–47
- [8] Hamada K, Shimasaki K, Nishimura Y, Oyama-Egawa H and Yoshida K 2011 Effects of red, blue and yellow fluorescent films on proliferation and organogenesis in *Cymbidium* and *Phalaenopsis* PLB in vitro *Acta Horticulturae* pp 381–84
- [9] Mengxi L, Zhigang X, Yang Y and Yijie F 2011 Effects of different spectral lights on *Oncidium* PLBs induction, proliferation, and plant regeneration *Plant Cell, Tissue and Organ Culture* **106** pp 1–10
- [10] Liu H, Fu Y, Hu D, Yu J and Liu H 2018 Effect of green, yellow and purple radiation on biomass, photosynthesis, morphology and soluble sugar content of leafy lettuce via spectral wavebands “knock out” *Scientia Horticulturae* **236** pp 10–17
- [11] Astafurova T, Lukash V, Goncharov A and Yurchenko V 2010 Phytotron for supplementary LED lighting of plants in greenhouses and at home *Solid-state lighting* **3** p 36–38
- [12] Chung J P, Huang C Y and Dai T E 2010 Spectral effects on embryogenesis and plantlet growth of *Oncidium* ‘Gower Ramsey’ *Scientia Horticulturae* **124** pp 511–16
- [13] Golovatskaya I F, Dorofeev V Yu, Medvedeva Yu V, Nikiforov P E and Karanchuk R A 2013 Optimization of illumination conditions in cultivation process of *Solanum Tuberosum* L. cv. Lugovskoy microcutting in vitro *Tomsk State University Journal of Biology* **24** pp 133–144
- [14] Chen X L, Yang Q C, Song W P, Wang L C, Guo W Z and Xue X Z 2017 Growth and nutritional properties of lettuce affected by different alternating intervals of red and blue LED irradiation *Scientia Horticulturae* **223** pp 44–52