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Note

Effects of wavelength of LED-light on *in vitro* asymbiotic germination and seedling growth of *Bletilla ochracea* Schltr. (Orchidaceae)

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Abstract The effects of different light wavelengths on *in vitro* asymbiotic germination of mature seeds and seedling growth of *Bletilla ochracea* Schltr. were examined using five wavelengths from light emitting diodes with peak wavelengths of 470 nm (blue), 525 nm (green), 590 nm (orange), 625 nm (red), and from white light emitting diodes at 40 μmol m^{-2} s^{-1}. Gellan gum solidified New Dogashima medium without plant growth regulators was used as germination medium. Cultures were maintained at 25°C under 24 h continuous lighting or darkness. The frequencies of seed germination three weeks after sowing reached more than 60% in all light conditions examined including continuous darkness. The highest frequencies of seed germination of 74% were achieved using lighting both with green and orange light emitting diodes, though strong inhibition of seed germination by the specific wavelength was not recognized. Seedling growth of this species was greatly inhibited by darkness. The most effective wavelength of light for rhizoid formation was revealed to be in the range of 590 nm (orange light) and 625 nm (red light), and almost no rhizoid was formed in the darkness. After 3 months of culture, leave width was expanded under white and blue light emitting diodes and was narrower under green, orange and red light. Seedlings grown under white and blue light emitting diodes resulted in thicker pseudobulbs.

Key words: LED, light wavelength, orchid, seed germination.

Light is an important environmental factor affecting plant development. Although the effect of light on seed germination of orchids is not generalized (Arditti and Ernst 1984), seed germination of several terrestrial orchid species was inhibited by light (Godo et al. 2010; Harvais 1973; Stewart and Kane 2006; Stoutamire, 1974). Thus, dark conditions have been commonly used for both asymbiotic and symbiotic seed germination of terrestrial orchids (Kitsaki et al. 2004; Lee et al. 2007; Miyoshi and Mii 1998; Rasmussen 1990; Yamazaki and Miyoshi 2006). Lighting also has effects on the growth of orchids, especially in the formation of root system. *Oncidium* seedlings formed both shoots and roots in the dark (Yates and Curtis 1949). On the contrary, Yates and Curtis (1949) described asymbiotic seedlings of *Cattleya* and *Cymbidium* formed shoots but not roots when cultured in the dark. Ichihashi (1982; 1990) also reported that germination and seedling growth of *Bletilla striata*, which is closely related species to *B. ochracea*, formed no root in the dark. The influence of light on seed germination and plant growth has been investigated intensively. However, few studies described the effects of different wavelengths of light on orchid seed germination (Fukai et al. 1997; McKinley and Camper 1997). In the present study, the effects of light wavelength on asymbiotic germination of mature seeds and seedling growth of *B. ochracea,* which is a grassland plant species, were evaluated.

New Dogashima medium (NDM; Tokuhara and Mii 1993) supplemented with 20 g l^{-1} sucrose and no plant growth regulators was used as germination medium. The medium were solidified with 2 g l^{-1} gellan gum (Phytagel; Sigma Chemical Co., St. Louis, USA). The pH of the medium was adjusted to 5.4 and the medium was sterilized by autoclaving at 121°C. Seven milliliters of media was dispensed into each well of a six-well plate (Falcon Multiwell; Becton Dickinson Labware, Franklin Lake, NJ, USA). Mature seeds of *B. ochracea* stored at 5°C for 14 months were surface sterilized with a solution of NaOCl (1% available chlorine) that contained 10 g l^{-1} surfactant (polyoxyethlene sorbitan monolaurate) for 10 min and then washed five times with sterilized distilled

Abbreviations: LED, light emitting diode; MS, Murashige and Skoog; NDM, New Dogashima medium
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water. Approximately 30 seeds were sown on the medium in each well. The plates were sealed with Parafilm™ (American Can Company, Chicago, IL, USA) and incubated at 25±2°C under 24 h continuous light from LEDs with peak wavelengths of 470 nm (blue), 525 nm (green), 590 nm (orange), 625 nm (red), and from white LEDs at 40 μmol m^{-2} s^{-1} or darkness (Figures 1 and 2). The seed germination and rhizoid formation frequencies were scored under a light microscope after 3 weeks of culture. According to Godo et al. (2009; 2010), the germination was considered to have occurred when the embryo size had doubled compared with that

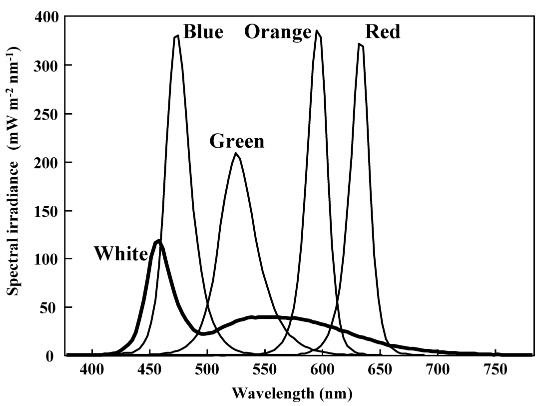

Figure 1. Spectral irradiation of white, blue, green, orange and red light emitting diodes (LEDs) at 40 μmol m^{-2} s^{-1} of PPFD.

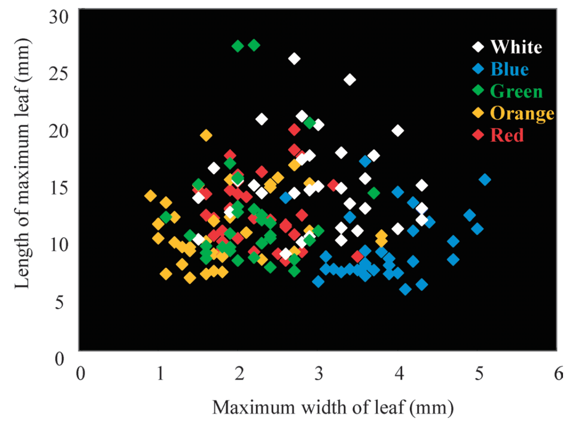

Figure 3. Effects of light wavelength on leaf size of *Bletilla ochracea*. Leaf length and width of 35 plantlets cultured under the each light condition were measured at 3 months after sowing.

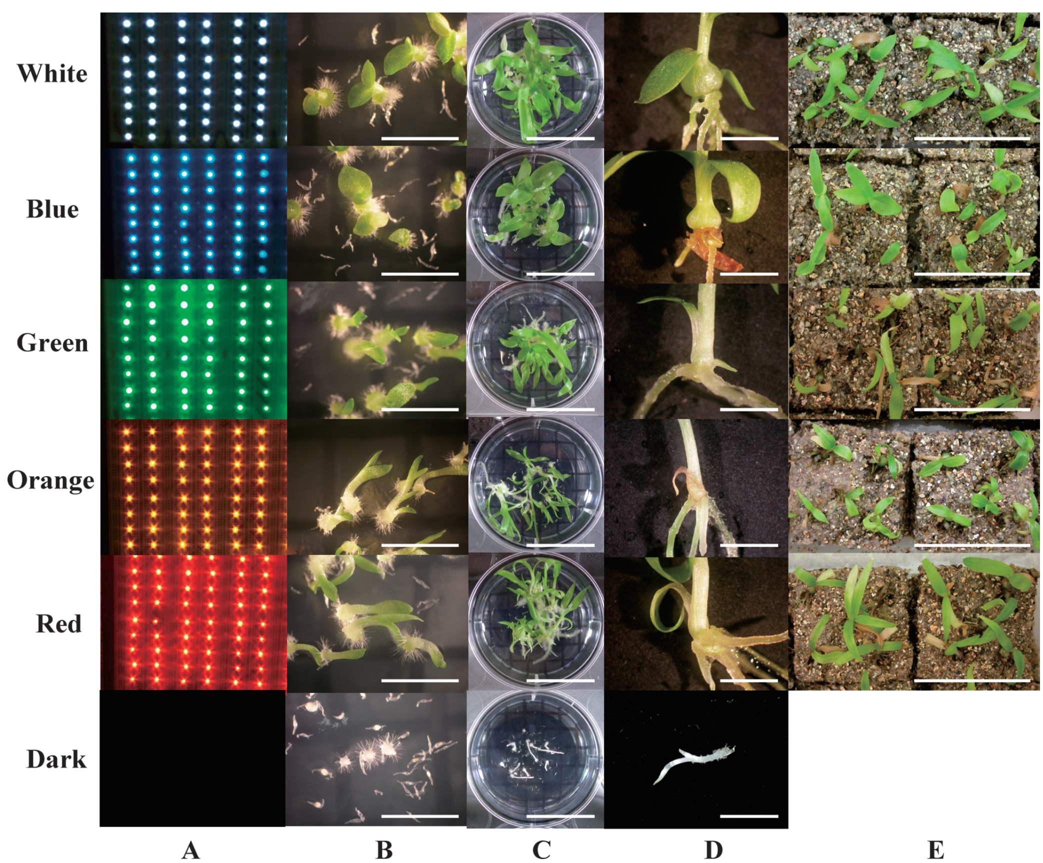

Figure 2. Effects of wavelength of light on germination of mature seed and seedling growth of *Bletilla ochracea.* Mature seeds were sown on New Dogashima medium supplemented with 20 g l^{-1} sucrose and no plant growth regulators and incubated at 25±2°C under 24 h continuous light from light emitting diodes (LEDs) of white, blue (470 nm), green (525 nm), orange (590 nm), and red (625 nm) at 40 μmol m^{-2} s^{-1} or darkness. (A) Lighting of LEDs. (B) Seed germination after 6 weeks of sowing. Bar=5 mm. (C) Seedlings after 3 months of sowing. Bar=2 cm. (D) Morphology of pseudobulb after 3 months of sowing. Bar=5 mm. (E) Plantlets after 3 months of transplanting to Florialite 552 for acclimatization (=after 6 months of sowing). Bar=5 cm.

Table 1. Effects of different wavelengths of LED-light on symbiotic seed germination, rhizoid formation and pseudobulb growth of *Bletilla ochracea* Schltr

LED	Peak wavelength (nm)	Frequency of seed germination (%)[a]	Frequency of rhizoid formation (%)[a]	Pseudobulb width (mm)[b]
White	(460, 560)*	64.5 ± 9.8ab	35.8 ± 11.4c	2.8 ± 0.6a
Blue	470	63.1 ± 3.8ab	16.5 ± 3.5d	2.4 ± 0.6b
Green	525	74.0 ± 9.0a	51.6 ± 15.5bc	2.0 ± 0.4c
Orange	590	74.2 ± 2.8a	71.7 ± 6.6a	1.4 ± 0.5d
Red	625	71.9 ± 7.6ab	67.4 ± 3.1ab	1.6 ± 0.3d
Dark	—	61.8 ± 6.6b	0.9 ± 0.8d	—[c]

[a] Data were scored 3 weeks after seed sowing. [b] Data were scored 3 months after seed sowing. Mean value ± SD are given for six replications. Values followed by same letters in each column are not significantly different at $p<0.05$. [c] Not measured. * sharp peak at 460 nm with a broad peak at 560 nm

immediately after sowing. The frequencies of seed germination and rhizoid formation were taken as the percentage of germinated seeds and rhizoid forming protocorms relative to the total number of seeds inoculated in one well. Mean values were obtained from six replicate wells for each treatment. Data were analyzed using ANOVA and correlation analysis using software (Statview; Abacus Concepts, Inc., Berkeley, CA, USA). Fisher's PLSD was used to compare means. Three months after sowing, the leaf length, leaf width and pseudobulb width of plantlets were measured. Subsequently these plantlets were transplanted to Florialite 552 (Nisshinbo Holdings Inc., Tokyo) and kept moist in a container at 25±2°C under white light (40 μmol m^{-2} s^{-1}) with a 16 h photoperiod for acclimatization.

Inhibition of seed germination by lighting has been reported for many terrestrial orchids, such as *Calanthe tricarinata* (Godo et al. 2010), *C.* Satsuma (Fukai et al. 1997), *Habenaria macroceratitis* (Stewart and Kane 2006), and *Cypripedium reginae* (Harvais 1973). Dutra et al. (2008) described lighting condition had promotive effects on the seed germination of *Bletia purpurea*, which is closely related to genus *Bletilla*. Our result of *B. ochracea* showed the frequencies of seed germination were in a range of 61.8–74.2% in the wavelengths examined including dark conditions and the highest germination frequency of 74% was obtained using both green and orange LED-light (Table 1). Ichihashi (1990) also described that no statistically significant difference between the dark conditions and under blue, green, yellow or red light conditions on seed germination of *B. striata*. These results suggested that light is not a limiting factor for seed germination in the genus *Bletilla*.

Frequencies of rhizoid formation 3 weeks after sowing were, respectively, 71.7, 67.4, 51.6, 35.8, and 16.5%, under orange, red, green, white, and blue LED-light (Table 1). The most promotive wavelength of light for rhizoid formation was considered to be in the range of 590 nm (orange light) and 625 nm (red light), whereas rhizoid formation was strongly inhibited by the darkness (Table 1). In contrast, Fukai et al. (1997) reported that rhizoid formation of *Calanthe* Satsuma under the dark condition was better than irradiation of either red LED, blue LED, mixed red and blue LED, or fluorescent light.

A tendency of changes in the morphology of seedlings in respect to leaf shape was observed among the wavelength examined. Leaves of seedlings became wide under white or blue LED-light, but the leaf width narrowed and spindly growth under green, orange or red LED-lights were observed (Figure 3). Pseudobulb width was also thicker under white or blue LED-light (Table 1, Figure 2). Seedlings under the darkness were lacking chlorophyll and failed further development (Table 1, Figure 2). Light, irrespective of wavelength was necessary for seedling development of *B. ochracea*. Fukai et al. (1997) reported, however, that the most vigorous protocorm development of *Calanthe* Satsuma was obtained in the dark rather than by the irradiation of red, blue, mixed light of red and blue, and fluorescent light. In its natural habitat, *B. ochracea* grows on open grassy and stony slopes, and most of the members in genus of *Calanthe* are found in shady forest floor. The difference of the seedling growth in response to the light in the two taxa might be the results of adaptation to the environmental conditions of the natural habitats of these orchid taxa.

Inhibition of rooting in darkness for *Bletilla striata* (Ichihashi 1982), *Cattleya* (Yates and Curtis 1949), and *Cymbidium* (Yates and Curtis 1949) were reported previously. In the present study, the root formation after 3 months of sowing was observed under all lighting conditions. However, root formation was hardly observed in the dark (Figure 2).

Orchidaceae is a large family in the plant kingdom with wide range of biodiversity. Thus, the generalization of the effects of light condition on seed germination and seedling growth in this family could not be achieved. Results of this study and descriptions in previous reports indicate that the inhibitory as well as promotive effects of light on the seed germination and the seedling development of the Orchidaceae plants are intrinsic traits for each species. Recently, Godo et al. (2009) described that the effect of light on the seed germination in

Calanthe spp. was influenced by benzyladenine, a plant growth regulator, in the medium. Further studies are necessary for the elucidation of the influence of light, in terms of quality and quantity in each phase of plant development in Orchidaceae which facilitate the establishment of artificial propagation methods and eventually may contribute *ex situ* conservation of endangered species.

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