Photo-responses of the fruit body formation in two ectomycorrhizal fungi Alnicola lactariolens and Hebeloma vinosophyllum

Quyen Bao-Thuy Ho1,2,*, Akira Suzuki1,3 and Thao Phuong Nguyen4

1Graduate School of Horticulture, Chiba University, 648 Matsudo, Matsudo City, Chiba Prefecture 271-8510, Japan, 2Department of Microbiology, Faculty of Biology, University of Science, Vietnam National University in Ho Chi Minh City, 227 Nguyen Van Cu, District 5, Ho Chi Minh City, Vietnam, 3Faculty of Education, Chiba University, 1-33 Yayoi-cho, Inage Ward, Chiba City, Chiba Prefecture 263-8522, Japan, 4Southern Institute of Ecology, Vietnam Academy of Science and Technology, 1 Mac Dinh Chi, District 1, Ho Chi Minh City, Vietnam


Effect of light on fruit body formation was examined in two ectomycorrhizal fungi Alnicola lactariolens and Hebeloma vinosophyllum in vitro without host plants. Fruit body initiation of A. lactariolens and H. vinosophyllum was accelerated by light irradiation. Both fungi required light for their fruit body maturation. In both fungi, stipe length became shorter whereas stipe diameter became larger according to the increment of light intensity from 1.3 μmol m⁻²s⁻¹ to 42.2 μmol m⁻²s⁻¹. Pileus diameter of A. lactariolens tended to be smaller at higher light intensity while that of H. vinosophyllum was not definitely changed by light intensity. Dry weight of fruit bodies per culture in A. lactariolens decreased with the increment of light intensity and showed the highest value by 1.3 μmol m⁻²s⁻¹ light irradiation. However, dry weight of fruit bodies per culture in H. vinosophyllum was constant irrespective of light intensity.

Key words: Agaricomycetes, Biomass, Light intensity, Pileus diameter, Stipe length

Introduction

The environmental factors affecting the fruit body formation in fungi have been studied for last decades (Moore, 1998). Among the environmental factors, light is one of principal factors for the fruit body formation of many agaricomycetous mushrooms (Suzuki, 1979, 2012). Light plays an essential role in different developmental processes of fruit body formation in Agaricomycetes. Fruit body initiation of some agaricomycetous mushrooms, such as Schizophyllum commune (Perkins and Goldon, 1969), Pleurotus

* Corresponding author: Quyen Bao-Thuy Ho; e-mail: thquyen21@yahoo.com
ostreatus (Eger et al., 1976) and Coprinellus congregatus (syn.: Coprinus congregatus) (Durand and Furuya, 1985) are induced by a brief irradiation of light. Some agaricomycetous mushrooms, such as Agaricus arvensis (Couvy, 1974) and Lentinus tigrinus (syn.: Panus tigrinus) (Bobbitt and Crang, 1974) require light for normal primodium development. In Lentinula edodes (syn.: Lentinus edodes), light is especially required for basidium and basidiospore formation (Komatsu, 1963). An agaricomycetous mushroom Polyporus arcularius (syn.: Favolus arcularius) requires light not only for the primodium formation (Kitamoto et al., 1968) but also for the pileus initiation (Horikoshi et al., 1974). In Flammulina velutipes, light is the critical factor in the morphological changes that take place during fruit body development (Sakamoto, 2004). In Coprinopsis cinerea (syn.: Coprinus macrorhizus), light triggers nuclear fusion, but inhibits progress in meiosis, in other words meiosis never proceeds without the darkness, during basidiospore formation (Kamada, et al., 1978). Most researches in photo-responses in fruit body formation in agaricomycetous mushrooms have been done by saprobic fungi (Suzuki, 1979, 2012). Because of the difficulty in the fruiting of ectomycorrhizal fungi in pure culture without host plant, a small number of researches have been done about their photo-morphogenesis. Chalciporus rubinellus (syn.: Boletus rubinellus) requires light irradiation for fruit body initiation (McLaughlin, 1970). Laccaria laccata requires light irradiation not only for fruit body initiation but also for fruit body development (Davis and Jong, 1976). Light stimulates the primordium formation of Hebeloma vinosophyllum, but not remarkably affects the progress in fruit body development (Suzuki, 1979). In contrast, Hebeloma radicosum does not require light for primordium formation but for differentiation and maturation of primordium (Kaneko and Sagara, 2002). H. vinosophyllum (Deng and Suzuki, 2008) and Alnicola lactariolens (unpublished data) have high fruiting abilities in pure culture.

In this study, we, therefore, investigated the photo-responses of fruit body formation in A. lactariolens and H. vinosophyllum to different light intensities, as the model organisms for the investigation into the effect of light on the fruit body formation of ectomycorrhizal fungi in vitro.

Materials and methods

Pre-cultivation

Alnicola lactariolens CHU7001 (Chiba University Collection, Japan) and Hebeloma vinosophyllum HCMUS-C2 (Ho et al. 2012) were maintained at 5°C in darkness. They were pre-cultured on the MY agar medium ([malt extract 10 g/L (Difco, Detroit, USA), yeast extract 2 g/L (Difco, Detroit, USA) and agar
15 g/L (Nakalai Tesque, Kyoto, Japan) sterilized at 120°C for 15 minutes] in a petri dish.

Effect of light on the fruit body formation of Alnicola lactariolens and Hebeloma vinosophyllum

Mycelium agar discs (5 mm in diameter) of A. lactariolens and H. vinosophyllum pre-cultured on the MY agar plates were separately cut from the sub-peripheral region of actively growing mycelial colony of each fungal isolate and inoculated separately on the center of the MY agar slants. Ten culture slants were prepared for each treatment.

After inoculation, one set of the cultures were incubated at 25.0 ± 0.5°C in darkness and then exposed continuously to different light intensities (1.3, 3.0, 4.6, 11.4, 13.7, and 42.2 μmol m⁻²s⁻¹, respectively) provided by the white fluorescent lamps (FL10W-B, Hitachi, Tokyo, Japan). Light intensities were varied by changing the distance from the lamps to the surface of culture slants. Another set of the cultures were grown for 10 days in darkness. Thereafter, they were exposed continuously to different light intensities same as the above.

The cultures of A. lactariolens and H. vinosophyllum were also exposed to 1.3 μmol m⁻²s⁻¹ for different light periods (0.25 hour, 0.5 hour, 1 hour, and 12 hours per day, respectively) just after the inoculation.

Observations were made at 1-day interval. The responses of the cultures to light exposure were determined as the time required for the fruit body initiation (defined as fruit body shaft formation) and the time required for the fruit body maturation. The details of nodulus, fruit body shaft, primodium, and mature fruit body were described in Deng and Suzuki (2008). The sizes of the largest mature fruit body were measured with a digital calliper. The stipe and pileus diameters were measured at the largest direction. Stipe diameter was measured at the junction of the pileus and the stipe. The fruit bodies were weighted after being dried at 60°C for 24 hours and put in a desiccator for 12 hours.

The dark control was conducted with 300 culture slants of each species incubated at 25.0 ± 0.5°C continuously in darkness for 30 days. Ten culture slants of each species were observed every day for recording the fruit body shaft formation in order to know the fruit body initiation time.

Statistical analysis

Data were analyzed by one-way ANOVA, and significant differences between treatments were determined by Tukey-Kramer test. All statistical
analyses were performed using Statcel2 software (OMS Publishing Co, Tokorozawa, Saitama, Japan).

Results and discussions

Responses of the fruit body formation in Alnicola lactariolens to different light intensities

Fruit body initiation and fruit body maturation tended to delay according to the increment of light intensity in the cultures exposed to light just after the inoculation (Table 1). Time required for fruit body initiation was nearly constant in the cultures exposed to light after 10 days of dark cultivation. Fruit body maturation also tended to delay according to the increment of light intensity in the cultures exposed to light after 10 days of dark cultivation but this tendency was not confirmed in the cultures exposed to light just after the inoculation (Table 1). It took about 5 – 6 days for fruit body initiation after starting of light exposure irrespective of light intensity in both starting time of light exposure (Table 1). This indicates that minimum dark period to attain full photo-sensitivity seems to be 9 – 11 days.

Table 1. Responses of fruiting in Alnicola lactariolens to different light intensities

<table>
<thead>
<tr>
<th>Light exposure starting after</th>
<th>Light intensity (μmol m⁻² s⁻¹)</th>
<th>Days required for</th>
<th>Number of fruit bodies per culture</th>
<th>Total dry weight of fruit bodies (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fruit body initiation</td>
<td>Fruit body maturation</td>
<td></td>
</tr>
<tr>
<td>Inoculation</td>
<td>1.3</td>
<td>12.8 ± 0.3a</td>
<td>30.4 ± 0.2a</td>
<td>1.2 ± 0.1a</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>13.2 ± 0.2ae</td>
<td>30.8 ± 0.2ae</td>
<td>1.6 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>4.6</td>
<td>14.0 ± 0.3bc</td>
<td>31.3 ± 0.4ac</td>
<td>1.4 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>11.4</td>
<td>14.1 ± 0.3be</td>
<td>31.5 ± 0.2bc</td>
<td>1.3 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>13.7</td>
<td>14.9 ± 0.3ab</td>
<td>32.5 ± 0.2d</td>
<td>1.1 ± 0.1a</td>
</tr>
<tr>
<td></td>
<td>42.2</td>
<td>16.0 ± 0.3cd</td>
<td>34.8 ± 0.1e</td>
<td>1.1 ± 0.1a</td>
</tr>
<tr>
<td>10 days of dark cultivation</td>
<td>1.3</td>
<td>14.5 ± 0.2a</td>
<td>30.3 ± 0.2a</td>
<td>1.3 ± 0.1a</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>14.7 ± 0.2a</td>
<td>30.9 ± 0.3ab</td>
<td>1.8 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>4.6</td>
<td>15.2 ± 0.4a</td>
<td>31.4 ± 0.2b</td>
<td>1.6 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>11.4</td>
<td>15.1 ± 0.1a</td>
<td>32.2 ± 0.2bc</td>
<td>1.9 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>13.7</td>
<td>15.9 ± 0.3b</td>
<td>32.7 ± 0.2c</td>
<td>1.4 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>42.2</td>
<td>15.3 ± 0.2ab</td>
<td>35.4 ± 0.2d</td>
<td>1.4 ± 0.2a</td>
</tr>
</tbody>
</table>

Days required for fruiting was determined by a fruit body appeared at the earliest. Numbers of fruit bodies were determined at the time of maturation of the fruit body at the earliest. Mean ± SE (n=10). For each light exposure experiment starting at each incubation time, different letters in the same rows are significantly different at P < 0.05 according to Tukey-Kramer test.
Fig. 1. Effect of light intensity on the morphology of mature fruit bodies in *Alnicola lactarioiens*. Sizes of pileus and stipes in the graph are shown by the fruit body having the longest stipe. A, C, E: Light exposure starting just after inoculation. B, D, F: Light exposure starting after 10 days of dark cultivation. Vertical bars indicate the standard error (n = 10). Different letters on the shoulder of vertical bars indicate significantly different at P < 0.05 according to Tukey-Kramer test.

Dry weights of fruit bodies decreased when the light intensity increased in both starting time of the exposure. Maximum dry biomasses were 17.4 ± 0.4 mg and 15.8 ± 0.8 mg at 1.3 μmol m⁻² s⁻¹ in the cultures exposed to light just after the inoculation and in those exposed to light after 10 days of dark cultivation, respectively (Table 1).

One to two fruit bodies per culture slant were formed irrespective of exposure to in both starting time under different light intensities, and there was no statistically significant difference among the cultures (Table 1).
In darkness, a few fruit body shafts were formed after 20 days of cultivation, but ceased to develop. These indicate that light is not essential factor for fruit body induction of *A. lactariole*ns but accelerates the fruit body initiation and necessary to fruit body development.

![Fig. 2. Effect of light intensities on fruit body formation in *Alnicola lactariole*ns. Light exposure just after inoculation. (A-F) to different light intensities (1.3, 3.0, 4.6, 11.4, 13.7, and 42.2 μmol m⁻²s⁻¹, respectively) with light exposure starting after inoculation. Bar A-F: 18 mm.](image)

Stipe became shorter according to the increment of light intensity irrespective of the starting time of light exposure (Figs. 1, 2). Maximum stipe lengths were 57.9 ± 2.5 mm and 76.5 ± 2.3 mm at 1.3 μmol m⁻²s⁻¹ in the cultures exposed to light just after the inoculation and those exposed to light after 10 days of dark cultivation, respectively.

The stipe diameter tended to be larger at higher light intensity and the saturation intensities were around 13.7 μmol m⁻²s⁻¹ and around 4.6 μmol m⁻²s⁻¹ in the cultures exposed to light just after the inoculation and in those exposed light after 10 days of dark cultivation, respectively (Fig. 1). The pileus diameter tended to be smaller at higher light intensity in both starting time of light exposure. Maximum values were 7.2 ± 0.4 mm and 6.1 ± 0.5 mm at 1.3 μmol m⁻²s⁻¹ in the cultures exposed to light just after the inoculation and in those exposed to light after 10 days of dark cultivation, respectively.

These results indicated that the total dry weight of fruit bodies, fruiting time, and morphology of fruit bodies were affected by light intensity in *A. lactariole*ns.
**Responses of the fruit body formation in Hebeloma vinosophyllum to different light intensities**

Changes of time required for fruit body initiation and fruit body maturation according to light intensity were not observed irrespective of starting time of light exposure (Table 2). After 10 days of dark cultivation, mycelia in the cultures covered the whole slant surface and the part of the test tube inner surface. It took about 4 days for fruit body initiation after starting of the light exposure irrespective of light intensity in both starting time of light exposure (Table 2). This indicates that minimum dark period to attain full photo-sensitivity seems to be 7 – 8 days.

**Table 2.** Responses of *Hebeloma vinosophyllum* fruiting to different light intensities

<table>
<thead>
<tr>
<th>Light exposure starting after</th>
<th>Light intensity (μmol m(^{-2})s(^{-1}))</th>
<th>Days required for</th>
<th>Number of fruit bodies per slant</th>
<th>Total dry weight of fruit bodies (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation</td>
<td>1.3</td>
<td>11.2 ± 0.8ab</td>
<td>20.7 ± 1.0a</td>
<td>1.6 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>11.6 ± 0.7b</td>
<td>20.6 ± 0.6a</td>
<td>1.9 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>4.6</td>
<td>9.6 ± 0.2ab</td>
<td>20.7 ± 0.4a</td>
<td>2.1 ± 0.4a</td>
</tr>
<tr>
<td></td>
<td>11.4</td>
<td>9.3 ± 0.3a</td>
<td>17.3 ± 0.6b</td>
<td>1.8 ± 0.3a</td>
</tr>
<tr>
<td></td>
<td>13.7</td>
<td>10.6 ± 0.3ab</td>
<td>20.9 ± 0.8a</td>
<td>2.0 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>42.2</td>
<td>10.9 ± 0.4ab</td>
<td>20.1 ± 0.6ab</td>
<td>1.6 ± 0.2a</td>
</tr>
<tr>
<td>10 days of dark cultivation</td>
<td>1.3</td>
<td>14.1 ± 0.2a</td>
<td>21.1 ± 0.2a</td>
<td>1.3 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>13.7 ± 0.3a</td>
<td>20.7 ± 0.3a</td>
<td>1.4 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>4.6</td>
<td>13.4 ± 0.2a</td>
<td>20.6 ± 0.2a</td>
<td>1.7 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>11.4</td>
<td>13.4 ± 0.2a</td>
<td>20.6 ± 0.2a</td>
<td>1.3 ± 0.1a</td>
</tr>
<tr>
<td></td>
<td>13.7</td>
<td>13.7 ± 0.2a</td>
<td>20.8 ± 0.2a</td>
<td>1.7 ± 0.1a</td>
</tr>
<tr>
<td></td>
<td>42.2</td>
<td>13.8 ± 0.2a</td>
<td>20.9 ± 0.3a</td>
<td>1.2 ± 0.1a</td>
</tr>
</tbody>
</table>

Days required for fruiting was determined by a fruit body appeared at the earliest. Numbers of fruit bodies were determined at the time of maturation of the fruit body at the earliest. Mean ± SE (n=10). For each light exposure experiment starting at each incubation time, different letters in the same rows indicates significantly different at P < 0.05 according to Tukey-Kramer test.

Dry biomasses of fruit bodies and numbers of fruit bodies per culture were not influenced by light intensity irrespective of starting time of the light exposure (Table 2).

Stipe became shorter according to the increment of light intensity irrespective of the starting time of light exposure. The stipe diameter in *H. vinosophyllum* became larger at around 42.2 μmol m\(^{-2}\)s\(^{-1}\) in the cultures exposed to light just after the inoculation and those exposed to light after 10 days of
dark cultivation. The effective light intensity for increment of stipe diameter in *H. vinosophyllum* was higher than in *A. lactariolens* (Figs. 1, 3). The influence of light intensity upon pileus diameter of *H. vinosophyllum* was not definitely shown in our experiment (Fig. 3).

**Fig. 3.** Effect of light intensity on the morphology of mature fruit bodies in *Hebeloma vinosophyllum*. Sizes of pileus and stipes in the graph are shown by the fruit body having the longest stipe, A, C, E: Light exposure starting just after inoculation. B, D, F: Light exposure starting after 10 days of dark cultivation. Vertical bar indicates the standard error (n = 10). Different letters on the shoulder of vertical bars indicates significantly different at P < 0.05 according to Tukey-Kramer test.

In darkness, fruit body shafts of *H. vinosophyllum* were formed after 18 days of cultivation, grew to primodium after 20 days and developed pileus after 24 days. However, the fruit bodies were immature, i.e. no basidiospore discharge.
Light intensity in the range of 1.3 – 42.2 μmol m^{-2}s^{-1} was enough to accelerate the fruit body initiation of both ectomycorrhizal fungi *A. lactariolens* and *H. vinosophyllum*. We, therefore, chose the minimum light intensity at 1.3 μmol m^{-2}s^{-1} in the following experiment.

**Fig. 4.** Effect of light intensities on fruit body formation in *Hebeloma vinosophyllum*. Light exposure just after inoculation. (A-F) to different light intensities (1.3, 3.0, 4.6, 11.4, 13.7, and 42.2 μmol m^{-2}s^{-1}, respectively) with light exposure starting after inoculation. Bar A-F: 18 mm.

**Responses of the fruit body formation in Alnicola lactariolens and Hebeloma vinosophyllum to different light periods**

Fruit body initiation and fruit body maturation in *A. lactariolens* and *H. vinosophyllum* tended to be accelerated according to the increment of light exposure period per day. This tendency was more distinct in the latter species (Tables 1 – 3). Saturation period of light exposure for fruit body initiation and fruit body maturation in *A. lactariolens* was between 0.25 hour/day and 0.5 hour/day. Saturation period of light exposure for fruit body initiation and fruit body maturation in *H. vinosophyllum* was between 1 hour/day and 12 hours/day. In other words, both ectomycorrhizal fungi do not required continous light irradiation for their full photo-response to fruiting.

In conclusion, *A. lactariolens* and *H. vinosophyllum* do not require light for fruit body initiation but need light for their maturation. This type of photo-response to fruiting was also reported in an ectomycorrhizal fungus *H. radicosum* (Kaneko and Sagara, 2002) but different from another
ectomycorrhizal fungus *Laccaria laccata* which requires light irradiation both for fruit body initiation and development (Davis and Jong, 1976).

In conclusion, light more drastically affected the morphology of fruit bodies, especially stipe length than fruiting time, number of fruit bodies, and biomass of fruit bodies in both ectomycorrhizal fungi. *H. vinosophyllum* is less sensitive than *A. lactariolens* in photo-morphogenesis.

Table 3. Days required for fruit body formation of *Alnicola lactariolens* and *Hebeloma vinosophyllum* to different light exposure periods per day

<table>
<thead>
<tr>
<th>Light exposure period (hour)</th>
<th>Days required for fruit body initiation in <em>Alnicola lactariolens</em> (Days)</th>
<th>Days required for fruit body initiation in <em>Hebeloma vinosophyllum</em> (Days)</th>
<th>Days required for fruit body maturation in <em>Alnicola lactariolens</em> (Days)</th>
<th>Days required for fruit body maturation in <em>Hebeloma vinosophyllum</em> (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>14.4 ± 0.2a</td>
<td>18.6 ± 0.2a</td>
<td>33.2 ± 0.4a</td>
<td>27.3 ± 0.4a</td>
</tr>
<tr>
<td>0.5</td>
<td>13.6 ± 0.3ab</td>
<td>15.8 ± 0.2b</td>
<td>32.8 ± 0.4ab</td>
<td>26.7 ± 0.5ab</td>
</tr>
<tr>
<td>1</td>
<td>13.1 ± 0.3b</td>
<td>16.6 ± 0.4b</td>
<td>32.2 ± 0.3ab</td>
<td>25.1 ± 0.4b</td>
</tr>
<tr>
<td>12</td>
<td>13.1 ± 0.3b</td>
<td>11.9 ± 0.3c</td>
<td>31.4 ± 0.2b</td>
<td>21.3 ± 0.2c</td>
</tr>
</tbody>
</table>

All experiments were done at 1.3 μmol m⁻² s⁻¹ light irradiation.

Mean ± SE (n=10). Different letters in the same rows are significantly different at P < 0.05 according to Tukey-Kramer test.

In continuous light exposure period:

- Days required for fruit body initiation in *A. lactariolens* and *H. vinosophyllum* were 12.8 ± 0.3 days and 11.2 ± 0.8 days, respectively (see Tables 1, 2).
- Days required for fruit body maturation in *A. lactariolens* and *H. vinosophyllum* were 30.4 ± 0.2 days and 20.7 ± 1.0 days, respectively (see Tables 1, 2).

Present data revealed that ectomycorrhizal fungi would have different photo-responses to fruiting similar as many those to fruiting in saprobic fungi. Moreover, it is expected that *A. lactariolens* and *H. vinosophyllum* are suitable model organisms for further studies about the effect of light on the fruit body formation in ectomycorrhizal association *in vitro* and the mechanism of the photo-morphogenesis of ectomycorrhiza fungi.

Acknowledgements

This work was financially supported in part by a Grand-in-Aid for the Scientific Research (No.19570082) from the Japan Society for the Promotion of Science (JSPS). We sincerely thank Dr. Naohiko Sagara (Prof. Emeritus, Kyoto University, Japan) and Dr Nguyen Binh Truong (Tay Nguyen Institute of Biology, Vietnam) for providing fungal isolates used in this study.
References


(Received 4 November 2012; accepted 30 November 2012)