Medicinally Important Secondary Metabolites and Growth of *Hypericum perforatum* L. Plants as Affected by Light Quality and Intensity

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*Hypericum perforatum* L. (St. John’s wort) is a traditional medicinal plant that is mainly used for the treatment of neurological disorders and depression. The present study was undertaken to investigate the effects of different light quality, including blue, white, and red light, in combination with two light intensities, 250 and 500 μmol m⁻² s⁻¹ photosynthetic photon flux (PPF) on the concentrations of medicinally important secondary metabolites in the leaf tissues and growth of *H. perforatum* plants. The results revealed that growth was greatest in those plants grown under white and red light with 500 μmol m⁻² s⁻¹ PPF. The hypericin and pseudohypericin contents of the plants grown under red light with 250 μmol m⁻² s⁻¹ PPF were 1.9 and 1.9 times higher, respectively, than those grown under red light with 500 μmol m⁻² s⁻¹ PPF. The differences in contents between blue and white light treatment were not significant for plants grown with 250 μmol m⁻² s⁻¹ PPF. Our result revealed that the contents of medicinally important secondary metabolites in the tissues of *H. perforatum* plants can be altered by controlling the light environment.

Keywords: artificial light, blue light, controlled environment, hyperforin, hypericin

INTRODUCTION

*Hypericum perforatum* L. (St. John’s wort) is a traditional medicinal plant that is mainly used for the treatment of neurological disorders and depression (Alan and Miller, 1998). Moreover, recent studies have identified *H. perforatum* as a good source of anticancer compounds (Shenpp et al., 2002). The major medicinal components of *H. perforatum* are hypericin, pseudohypericin, and hyperforin (Barnes et al., 2001). In 1998, the sales of *H. perforatum* exceeded $570 million worldwide (Gruenwald, 1999).

In our previous studies, we have demonstrated that the concentrations of hypericin and pseudohypericin, and the growth of *H. perforatum* plants grown in controlled environments with artificial light were higher and greater, respectively, than plants grown in a greenhouse or in the field (Zobayed and Saxena, 2004; Mosaleeyanon et al., 2005). We also reported that environmental factors, such as CO₂ concentration, light irradiance, and air temperature, affect the concentrations of medicinal components (Mosaleeyanon et al., 2005; Zobayed et al., 2005; Couceiro et al., 2005), and light quality affect the growth (Nishimura et al., 2006) of *H. perforatum*. However, so far there

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is no report on the effects of light quality on the concentrations of the medicinal components and growth of *H. perforatum* plants grown in controlled environments.

The effect of light on higher plants is mediated through two main physiological processes. Firstly, via photosynthesis, light provides the energy source required by plants. Secondly, light is a signal, received by photoreceptors, that functions to regulate growth, differentiation, and metabolism (Goto, 2003). Furthermore, light quality is known to have strong effects on the secondary metabolites concentrations of many plant species (Afreen et al., 2005; Jenkins et al., 1995). Therefore, we hypothesized that different light quality will alter the content of medicinal components of *H. perforatum* plants. The objective of the current study was to investigate the effects of light quality and intensity on the concentrations of the major medicinal components and the growth of *H. perforatum* plants under controlled environmental conditions.

**MATERIALS AND METHODS**

*Plant material and growing conditions*

Seeds of *H. perforatum* plants were sown in 72-cell plug seedling trays (Takii Seed Co., Ltd., Kyoto, Japan) filled with a commercial soil mixture (Yanmar Agricultural Equipment Co., Ltd., Osaka, Japan). Fourteen days after sowing, seedlings were transplanted to individual pots (bore diameter, 6.0 cm; capacity, 120 mL), filled with the commercial soil mixture. The seedlings (fresh weight, 101.1±30.6 mg; dry weight, 9.7±2.7 mg; and number of unfolded leaves, 6–8), grown for 21 d after sowing, were used as the plant material. The seedlings were transplanted in a controlled-environment room with air temperatures of 28 ± 1/25 ± 1°C (light/dark period) and a 16 h d⁻¹ light period provided by cool-white fluorescent lamps (FHF32-EX-N-H, Matsushita Electric Industrial Co., Ltd., Osaka, Japan). The CO₂ concentration was 1,600±100 μmol mol⁻¹ and the photosynthetic photon flux (PPF) measured at the soil surface was 300 μmol m⁻² s⁻¹.

*Experimental apparatus, treatments, and growing conditions*

*Growth chamber and light sources*

The experiment was conducted in growth chambers (incubator MIR-553, Sanyo Electric Biomedical Co., Ltd., Osaka, Japan). Three types of fluorescent lamp, blue (FPL55EB, Matsushita Electric Works Ltd.), white (FPL55EX-L, Iwasaki Electric Co., Ltd., Tokyo, Japan), and red (FPL55ER, Matsushita Electric Works Ltd.), were used as light sources. Three-millimeter-thick sharp-cut glass filters (42 cm x 42 cm) were fitted 5 cm below the fluorescent lamps. Commercial standard filters, L-39 (flint glass, half-value: 390 nm, Kenko Co., Ltd., Tokyo, Japan) and L-52 (amber glass, half-value: 520 nm, Kenko Co., Ltd.), were used for the blue and white, and the red fluorescent lamps, respectively.

*Treatments and growing conditions*

The experiment was designed to evaluate the effects of three different light qualities (blue, white, and red light) and two levels of PPF (250 and 500 μmol m⁻² s⁻¹).

The spectral characteristics of each fluorescent lamp with a sharp-cut glass filter are listed in Table 1. There was a difference in the red: far-red (R/FR) ratio for the three light sources; however, the difference in the phytochrome photorequilibrium (Pfr/P) value, which is an indicator of phytochrome response to R/FR, was relatively small (approximately 0.1). The common environmental conditions were a 27/24°C air temperature (light/dark period), a 16 h d⁻¹ light period, and a 1,000 μmol mol⁻¹ CO₂ concentration. The CO₂ concentration was controlled using an infra-red CO₂ gas analyzer (ZFP9, Fuji Electric Systems Co., Ltd., Tokyo, Japan), which was installed in the growth chamber. The planting density was 278 m⁻². In order to prevent the collapse of the seedlings, the plants were provided with supports of commercial wire and thread. As the seedlings grew, the distance between the lamps and the growing points of the plants was adjusted to maintain a constant PPF at the canopy level. The positions of the plant materials within the treatments were
Table 1 Spectral characteristics of each fluorescent lamp with sharp-cut glass filters.

<table>
<thead>
<tr>
<th>Photon flux (μmol m⁻² s⁻¹)</th>
<th>Blue</th>
<th>White</th>
<th>Red</th>
</tr>
</thead>
<tbody>
<tr>
<td>300–400 nm (UV)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>400–500 nm (B)</td>
<td>81.5</td>
<td>11.9</td>
<td>0.3</td>
</tr>
<tr>
<td>500–600 nm (G)</td>
<td>13.4</td>
<td>37.3</td>
<td>15.4</td>
</tr>
<tr>
<td>600–700 nm (R)</td>
<td>2.3</td>
<td>43.0</td>
<td>74.3</td>
</tr>
<tr>
<td>700–800 nm (FR)</td>
<td>2.5</td>
<td>7.5</td>
<td>9.9</td>
</tr>
<tr>
<td>B/R (400–500 nm/600–700 nm)</td>
<td>35.0</td>
<td>0.28</td>
<td>0.005</td>
</tr>
<tr>
<td>R/FR (600–700 nm/700–800 nm)</td>
<td>0.94</td>
<td>5.70</td>
<td>7.54</td>
</tr>
<tr>
<td>Phytochrome photoequilibrium (Pfr/P)*</td>
<td>0.67</td>
<td>0.79</td>
<td>0.80</td>
</tr>
</tbody>
</table>

* The value of phytochrome photoequilibrium was calculated using the equation proposed by Hanyu et al. (1996).

rerearranged to minimize the variation of PPF. Subirrigation with a nutrient solution (Otsuka hydroponic composition (1/4 strength) adjusted to EC 1.2 dS/m and pH 6.0, Otsuka Chemical Co., Ltd., Osaka, Japan: 4.2 mmol l⁻¹ NO₃⁻, 1.3 mmol l⁻¹ H₂PO₄⁻, 1.0 mmol l⁻¹ Ca²⁺, 0.38 mmol l⁻¹ Mg²⁺, 2.2 mmol l⁻¹ K⁺, 0.4 mmol l⁻¹ NH₄⁺) was applied once a day after germination. The experimental period was 14 d. On day 14, the fresh and dry weights of the leaves, stems, and roots were measured.

**Extraction and determination of hypericin, pseudohypericin, and hyperforin concentrations**

The leaves from the main stem tips (approximately 0.15 g fresh weight samples) were collected on day 14 and stored at −85°C until used for the analysis of hypericin, pseudohypericin, and hyperforin, as described previously (Mosaleeyanon et al., 2005). The concentrations of the medicinal components in the sampled leaves were expressed as mg g⁻¹ leaf DW, and the total medicinal content of each seedling was calculated by multiplying the concentrations of the medicinal components in the sampled leaves by the total leaf dry weight per plant.

**Statistical analysis**

Twenty-five seedlings were grown per treatment. Ten of these seedlings were used for the measurement of medicinal component concentrations and 10 used for dry weight determinations. The experiment was conducted twice. The data were quantified in terms of the means and standard errors obtained from replicate experiments and subjected to an analysis of variance (ANOVA). The means were compared using the Tukey-Kramer test at a 5% level of significance.

**RESULTS**

The plants grown under high PPF and red light conditions were vigorous with many fully expanded leaves (Fig. 1). The dry weights of *H. perforatum* plants at PPF 500 μmol m⁻² s⁻¹ were significantly greater than those at PPF 250 μmol m⁻² s⁻¹ (Fig. 2). The dry weights of plants grown under red light at PPF 250 μmol m⁻² s⁻¹ (R250) were greater than those grown under blue and white light at PPF 250 μmol m⁻² s⁻¹ (B250 and W250, respectively). The dry weights of plants grown under red light at PPF 500 μmol m⁻² s⁻¹ (R500) were greater than those grown under blue light at PPF 500 μmol m⁻² s⁻¹ (B500), but similar to or less than those of plants grown under white light at PPF 500 μmol m⁻² s⁻¹ (W500). The dry weights at PPF 500 μmol m⁻² s⁻¹ for each light quality were 1.5–2.6 times greater than the respective weights at PPF 250 μmol m⁻² s⁻¹.

The concentrations of medicinal components at PPF 250 μmol m⁻² s⁻¹ were significantly higher than those at PPF 500 μmol m⁻² s⁻¹ when the light quality was same. The hypericin, pseudohypericin, and hyperforin concentrations at PPF 250 μmol m⁻² s⁻¹ were 3.1–3.4, 2.6–3.3, and 1.6–1.9 times higher, respectively, than those at PPF 500 μmol m⁻² s⁻¹.

The concentrations of the medicinal components (hypericin, pseudohypericin, and hyperforin)
of *H. perforatum* plants 35 d after sowing were virtually the same under different light quality treatments with similar light intensity (Fig. 3).

The hypericin, pseudohypericin, and hyperforin contents of plants grown in R250 were the highest among the treatments (Fig. 4). The hypericin and pseudohypericin contents of the plants grown in R250 were 1.9 and 1.9 times higher, respectively, than in those plants grown in R500. The hyperforin contents in plants grown in R250 and R500 were the same. The difference between the contents in plants grown in B250 and W250 was non-significant; however, these contents were generally lower than those in R250 plants.
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Fig. 3 Hypericin, pseudohypericin, and hyperforin concentrations of *H. perforatum* plants grown under different light quality and intensity for 14 d (35 d after sowing). Different letters indicate significant differences between the treatments at $P<0.05$, determined by the Tukey-Kramer test. Error bars represent standard error.

DISCUSSION

The present experiment was undertaken in order to gain an understanding of the influence of light quality and intensity on the concentrations of three major medicinal components in leaves and on the growth of *H. perforatum* plants. The dry weights of the plants were greater under red light than under blue light; however, the concentrations of medicinal components in the leaf tissues were not significantly different among the light quality treatments under similar PPF. Moreover, the hypericin, pseudohypericin, and hyperforin concentrations in plants grown at PPF 250 $\mu$mol m$^{-2}$s$^{-1}$ were significantly higher than those in plants grown at PPF 500 $\mu$mol m$^{-2}$s$^{-1}$. This result differs from the previous studies (Brisken and Gavienowski, 2001; Mosaleeyanon et al., 2005) on the effect of light intensity on the concentrations of the medicinal components of *H. perforatum*. This difference may stem from the fact that the growth stage of the plants sampled in the current study (35 d after sowing) was earlier than that reported previously.

Herms and Mattson (1992) reported that the allocation of photosynthate to secondary metabolism is relatively low because, in resource-enriched environments, photosynthate is preferentially allocated to growth processes especially during the early stages of plant growth (vegetative stage). In the current study, *H. perforatum* plants grown at PPF 500 $\mu$mol m$^{-2}$s$^{-1}$ for 35 d (harvested prior to the flower bud initiation) exhibited a substantially higher allocation of photosynthate for growth compared to those grown at PPF 250 $\mu$mol m$^{-2}$s$^{-1}$. Therefore, at PPF 500 $\mu$mol m$^{-2}$s$^{-1}$, plant
growth was promoted and the concentrations of secondary metabolites, such as medicinal components, were low.

The dry weights of plants grown in R250 were greater than those grown in B250 and W250, whereas those grown in R500 were greater than those in B500, but similar to those in W500. Thus, under both PPF conditions, the dry weights of plants grown in the red light treatments were greater than those of plants grown in the blue light treatments. The differences in the dry weights of the *H. perforatum* plants among the different light quality treatments were not due to the spectral characteristic or the photosynthetic ability of the leaves, but due to the amount of light actually absorbed by the leaves. In other words, the number of leaves of plants grown under red light was greater than that of plants grown under blue light (Fig. 1; Nishimura et al., 2006). In this study, the responses of *H. perforatum* plants to light quality differed from the responses to light intensity; not only the rate of each wavelength per PPF, but also the amount of photon flux of each wavelength may be related to dry weights. At PPF 250 μmol m⁻²s⁻¹, the dry weights of plants grown in R250 were greater than those in W250; this is probably because the amount of red light in R250 is higher than that in W250. However, at PPF 500 μmol m⁻²s⁻¹, the dry weights in R500 were almost the same as those in W500, even though the amount of red light in R500 was higher than that in W500. There are two possible reasons that might account for this observation. Firstly, the absorption of red light was saturated in R500 and W500, and the absorption in W500 was similar to that in R500. Secondly, the absorption of red light was saturated in R500 and not saturated in W500, but the B/R ratio in W500 was relatively high (0.28); thus, growth was promoted. There are many reports

**Fig. 4** Hypericin, pseudohypericin, and hyperforin contents of *H. perforatum* plants grown under different light quality and intensity for 14 d (35 d after sowing). Different letters indicate significant differences between the treatments at *P* < 0.05, determined by the Tukey-Kramer test. Error bars represent standard error.
indicating that the growth rate of plants was highest under a B/R ratio of 0.1–0.2 (Goto, 2004).

The contents of three major medicinal compounds in the plants grown in R250 were the highest among the treatments; the hypericin, pseudohypericin, and hyperforin concentrations in R250 were almost the same as those in B250 and W250, and were 6.3–8.1, 4.0–5.5, and 3.7–5.9 times higher, respectively, than those at PPF 500 μmol m⁻²s⁻¹. The leaf dry weights in R250 were 1.4 times higher, than those in both B250 and W250, and 0.5–0.9 times higher than in blue and white light at PPF 500 μmol m⁻²s⁻¹. In a recent study, Afreen et al. (2005) also indicated that red light and UV-B radiation have effects on the glycyrrhizin content of Glycyrrhiza plants grown in controlled environments. In the current study, the optimal light environment for the production of the medicinal components of H. perforatum plants was red light with 250 μmol m⁻²s⁻¹ PPF.

In conclusion, our results suggest that it is possible to increase the production of specific medicinal components by controlling the light environment. In fact, the content of hypericin per plant in the current study (under red light) was about 1.7 times than that of the field grown plants reported by Mosaleeyan et al. (2005). It should be mentioned that the planting density in the current study was about 23.2 times higher than the field grown plants reported by Mosaleeyan et al. (2005). Therefore, control of the light environment can be an important technique for producing medicinal plants with high medicinal contents under controlled environmental conditions.

REFERENCES

