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**Note**

# Blue light enhances the accumulation of eicosapentaenoic acid in a liverwort, *Marchantia polymorpha* L.

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**Abstract** Liverwort, *Marchantia polymorpha* L. synthesizes various polyunsaturated fatty acids such as arachidonic acid (AA) and eicosapentaenoic acid (EPA), neither of which is produced by higher plants. Here, we report the effects of light quality and intensity on the accumulation of AA and EPA in liverwort. In the range of the light examined, the relative content of EPA to total fatty acid was highest under blue light although that of AA did not vary. Illumination with blue light for a short period efficiently improved the accumulation of EPA without great deficit of growth. At a higher intensity of white light, the relative content of EPA increased. The optimum light intensity for AA and EPA accumulation was found to be 80 photon flux density $\mu$mol $m^{-2}$ $s^{-1}$.

**Key words:** Arachidonic acid, blue light, eicosapentaenoic acid, liverwort, polyunsaturated fatty acid.

Polyunsaturated fatty acids (PUFAs), including linoleic acid (LA), $\alpha$-linolenic acid (ALA), arachidonic acid (AA) and eicosapentaenoic acid (EPA), are essential fatty acids for human health because human beings are not able to desaturate oleic acid to LA. Among the PUFAs, AA and EPA are known to act not only as components of membrane lipids but also as precursors of eicosanoids such as prostaglandins, leukotriens, and thromboxanes (Granström 1984; Smith 1989; Wang and DuBois 2010). Moreover, AA and EPA have recently attracted attention as dietary supplements due to their effects on human health. The main source of PUFAs such as AA and EPA, which vegetable plants do not produce, is fish oil. However, it is predicted that fish resources will decrease, creating the need to establish a sustainable source of these PUFAs. Plants have the potential to act as ecological and economical factories for the production of valuable PUFAs. Bryophytes and alga synthesize C20 and C22 fatty acids, including AA and EPA, although higher plants produce mostly up to C18 fatty acids such as LA and ALA (Gill and Valivety 1997; Grimsley et al. 1981; Nichols and Appleby 1969; Shinmen et al. 1991; Zhukova and Aizdaicher 1995). In addition, it has recently been reported that transgenic higher plants are able to produce AA and EPA (Kajikawa et al. 2008; Napier 2007; Qi et al. 2004; Wu et al. 2005).

Bryophytes contain relatively high levels of AA and EPA (Gellerman et al. 1975; Shinmen et al. 1991). Approximately 10% of the total fatty acids contained in the thallus and cultured cells of liverwort, *Marchantia polymorpha*, are AA and EPA (Kajikawa et al. 2008, Shinmen et al. 1991). Chiou et al. (2001) examined the optimization of production of PUFAs using cultured liverwort cells and reported that PUFA production was strongly associated with cell growth. This suggests that the accumulation of AA and EPA was promoted by the optimization of growth conditions, including light, temperature and nutrition.

Light is one of the most important environmental factors that affect plant growth. Several studies indicate that light conditions modify the composition of PUFAs. In soybean cell cultures, the relative contents of C18:2 and C18:3 fatty acids decreased in response to darkness (Collados et al. 2006). In unicellular algae, illumination may induce the desaturation of fatty acids (Klyachko-Gurvich et al. 1999). However, little is known about the effects of light quality and intensity on PUFA accumulation in plants.

In this study, we investigated the effects of light quality and intensity on AA and EPA accumulation in liverwort thallus. To develop an economical plant factory

Abbreviations: AA, arachidonic acid; ALA, $\alpha$-linolenic acid; EPA, eicosapentaenoic acid; FW, fresh weight; LA, linoleic acid; LED, light-emitting diode; PUFA, polyunsaturated fatty acid

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as a new source of these valuable PUFAs, it is important to achieve increased productivity. Also, the optimization of light conditions is needed to enhance the accumulation of AA and EPA in liverwort thallus, as was reported in the study of cultured liverwort cells (Chiou et al. 2001). Here, we report the effect of blue light on EPA accumulation in liverwort thallus and discuss efficient light conditions for PUFA accumulation.

To investigate the effect of light on AA and EPA contents, we used the female E line of *M. polymorpha* as described by Okada et al. (2000) and grew gemmae which arise from single initial cells in gemma cups on M51C solid medium (Takenaka et al. 2000). Since the liverwort thalli grown from gemmae showed nearly constant levels of AA and EPA relative to total fatty acid during 5 weeks (data not shown), we used young thalli, regardless of developmental stage.

To examine the effect of light quality on AA and EPA content, liverwort gemmae were grown for 5 weeks under continuous illumination with blue (470 nm), green (525 nm), and red (660 nm) LEDs (light-emitting diodes) with 30 photon flux density $\mu$mol m$^{-2}$ s$^{-1}$. The blue light (HLMP-BB11) was provided by Hewlett Packard (CA, USA). The green light (TOL-55hUGdCUu-ETE) was provided by TAIWAN OASIS (Taipei, Taiwan). The red light (SDL-5N3KR) was provided by SANDER (Taipei, Taiwan). Then, we analyzed fatty acid compositions using approximately 0.2 g of each liverwort thallus. Fatty acid methyl esters were prepared and analyzed by gas chromatogram as described previously (Hamada et al. 2006) using GC-2010 (Shimazu, Kyoto, Japan) and column Omegawax 250, 30 m×0.25 mm (SIGMA-ALDRICH, MO, USA). After 5 weeks, growth was markedly repressed by blue light and slightly by green light compared to red light (Table 1). Especially, under blue light, thalli became pale green (data not shown). Nevertheless, the total fatty acid content per 1g fresh weight (FW) of liverwort did not differ significantly among the three light qualities (Table 1). The levels of AA were nearly equal under all light qualities (Figure 1). On the other hand, EPA concentration under blue light was highest and that under red light was lowest. The former was 1.8-fold higher than the latter. These results suggested that blue light enhanced EPA accumulation. However, we cannot rule out a possibility that red or green light repressed EPA accumulation. To dissolve this problem, further analysis will be required. Under blue light, the relative content of EPA was certainly higher than those under red and green lights, although the growth rate was significantly reduced by long-period illumination with blue light (Table 1). To asses blue light illumination effect for EPA accumulation, we tested short-period illumination. The gemmae were initially grown for 3–4 weeks under white light (FLR40SW/M, NEC, Tokyo Japan; Supplementary Figure 1A) with 50 photon flux density $\mu$mol m$^{-2}$ s$^{-1}$. Then, young thalli at approximately 200 mg were transplanted onto fresh M51C solid medium and grown under blue or white light LED (MSR/7000, Opto Research, Tokyo, Japan; Supplementary Figure 1B) with 50 photon flux density $\mu$mol m$^{-2}$ s$^{-1}$ for 1 week. Compared to white light illumination as a control, the growth rate and total fatty acid content were slightly decreased after 7 days under blue light (Table 2). The relative concentration of EPA was gradually increased by blue light illumination and became 1.5-fold higher than that under white light after 7 days (Figure 2). In contrast, the levels of AA varied little during 7 days under both white and blue lights as was the case with long-period illumination. These findings suggested a positive effect of short-period illumination with blue light on EPA accumulation without a

Table 1. Effect of light quality on growth and PUFA productivity.*

| | Fresh weight (mg plant$^{-1}$) | Total fatty acid ($\mu$g g FW$^{-1}$) |
|---|---|---|
| Blue | 295 ± 82 | 1452 ± 279 |
| Green | 370 ± 146 | 1552 ± 186 |
| Red | 489 ± 169 | 1560 ± 148 |

* The gemmae were grown under three kinds of light with different wavelengths, blue (470 nm), green (525 nm) and red (660 nm). Data are the average ± SD of three independent measurements.

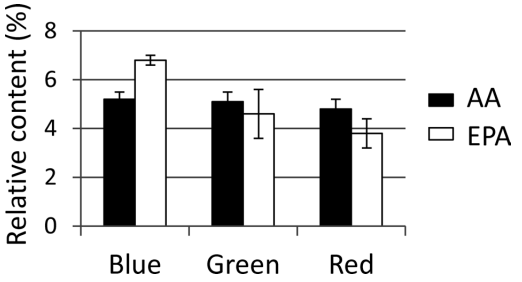


Figure 1. Effect of light quality on the relative contents of AA and EPA. The gemmae were grown under three kinds of light with different wavelengths, blue (470 nm), green (525 nm) and red (660 nm). Closed and open bars indicate the levels of AA and EPA, respectively. Data are the average ± SD of three independent measurements.

Table 2. Effect of short-period illumination with blue light on growth and PUFA productivity.*

| | Fresh weight (mg plant$^{-1}$) | Total fatty acid ($\mu$g g FW$^{-1}$) |
|---|---|---|
| White light | | |
| 1 day | 237 ± 15 | 1239 ± 65 |
| 4 days | 292 ± 3 | 1532 ± 63 |
| 7 days | 543 ± 14 | 1341 ± 102 |
| Blue light | | |
| 1 day | 221 ± 20 | 1245 ± 98 |
| 4 days | 278 ± 45 | 1162 ± 49 |
| 7 days | 454 ± 55 | 1168 ± 48 |

* The gemmae were initially grown under white light for 3–4 weeks, then under white or blue light for 1 week. Data are the average ± SD of three independent measurements.

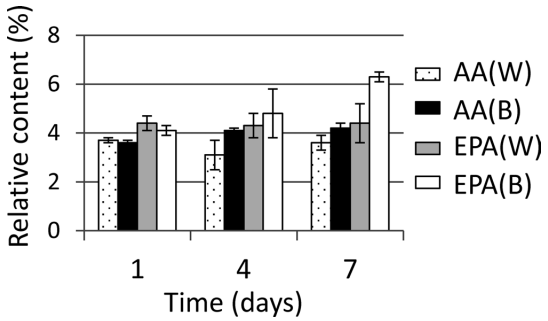


Figure 2. Effect of short-period illumination with blue light on the relative contents of AA and EPA. The gemmae were initially grown under white light for 3–4 weeks, then under white or blue light for 1 week. Dotted and closed bars indicate concentrations of AA under white light (W) and blue light (B), respectively. Grey and open bars indicate the concentrations of EPA under white light (W) and blue light (B), respectively. Data are the average ± SD of three independent measurements.

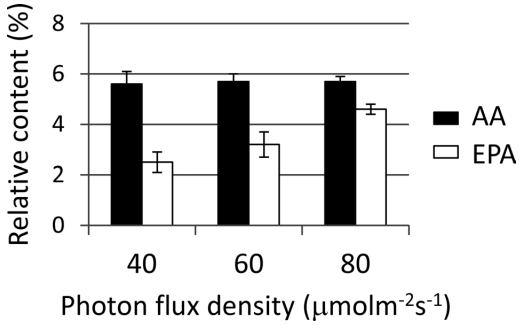


Figure 3. Effect of light intensity on the relative contents of AA and EPA. The gemmae were grown under various light intensities of white light for 5 weeks. Closed and open bars indicate the relative content of AA and EPA to total fatty acid, respectively. Data are the average ± SD of three independent measurements.

Table 3. Effect of light intensity on growth and PUFA productivity.*

| Photon flux density ($\mu$ molm$^{-2}$ s$^{-1}$) | Fresh weight (mg plant$^{-1}$) | Total fatty acid ($\mu$g g FW$^{-1}$) |
|---|---|---|
| 40 | 696 ± 146 | 1995 ± 107 |
| 60 | 1075 ± 126 | 2002 ± 167 |
| 80 | 1088 ± 480 | 2247 ± 93 |

* The gemmae were grown under various light intensities of white light for 5 weeks. Data are the average ± SD of three independent measurements.

significant deficit of growth.

To analyze the effect of light intensity on the relative contents of AA and EPA, liverwort gemmae were grown for 5 weeks under continuous illumination by fluorescent white lamp (FL40SS-EX-L/37, Panasonic, Osaka, Japan; Supplementary Figure 1C) with 40, 60, and 80 photon flux density $\mu$mol m$^{-2}$ s$^{-1}$. As a result, the fresh weight of liverwort under 40 $\mu$mol m$^{-2}$ s$^{-1}$ was lower than that under 60 and 80 $\mu$mol m$^{-2}$ s$^{-1}$ (Table 3). Under light intensity above 80 $\mu$mol m$^{-2}$ s$^{-1}$ liverwort growth was significantly less than growth under 80 $\mu$mol m$^{-2}$ s$^{-1}$ (data not shown). This indicated that excess light inhibited liverwort growth and that proper light intensity was important for growth. As expected from the natural habitat of liverwort, light intensity required for liverwort growth was lower than that for higher plants. Total fatty acid content per 1 g FW of liverwort grown under 40 $\mu$mol m$^{-2}$ s$^{-1}$ was slightly lower than that under 80 $\mu$mol m$^{-2}$ s$^{-1}$ but nearly equal to that under 60 $\mu$mol m$^{-2}$ s$^{-1}$ (Table 3). The level of AA was approximately 5.7% of total fatty acid, which level was found to be nearly equal under all light intensities (Figure 3). In contrast, the relative content of EPA to total fatty acid became higher as the light intensity increased. The concentration of EPA under 80 $\mu$mol m$^{-2}$ s$^{-1}$ was 1.8-fold higher than that under 40 $\mu$mol m$^{-2}$ s$^{-1}$. Considering of the growth and content of AA and EPA, optimum light intensity for the AA and EPA accumulation was 80 $\mu$mol m$^{-2}$ s$^{-1}$. Chiou et al. (2001) found that intracellular lipid content of liverwort cultured cells among various light intensities from 3 to 32 $\mu$mol m$^{-2}$ s$^{-1}$ was highest at 20 $\mu$mol m$^{-2}$ s$^{-1}$. This and our study suggested that higher AA and EPA accumulation could be achieved by white light illumination at the proper intensity. However, the concentrations of AA and EPA in cultured cells showed no significant change under different illumination intensities. The constant EPA level in cultured cells may have resulted from a light intensity that was too low to alter the EPA content, or the different regulation of PUFA metabolism between the thallus and cultured cells.

Our present study indicated that the relative content of EPA to total fatty acid was affected, but not that of AA, by the light condition examined. Considering the fact that the same three enzymes, Δ6-desaturase, Δ6-elongase, and Δ5-desaturase are involved in the synthesis of AA from LA and EPA from ALA (Kajikawa et al. 2004), it is suggested that AA and EPA content were dependent on the metabolism of LA and ALA, respectively. Previous studies have reported that light induced the expression of $\omega$-3 desaturase gene which catalyzed desaturation from LA to ALA bringing into ALA increase (Collados et al. 2006). However, whether expression of this gene is regulated by the light quality and intensity is not clear. To our knowledge, there are no reports describing the effect of blue light on PUFA accumulation. Further analysis should contribute to the elucidation of the molecular mechanisms behind the regulation of PUFA metabolism by light quality and intensity.

In this study, we showed that short-period illumination with blue light was effective in achieving a high yield of EPA. Moreover, the growth of liverwort thallus was enhanced by illumination with an appropriate intensity of white light. White light was most efficient for growth and total fatty acid production compared to monochromatic

light (data not shown). For higher productivity of AA and EPA, both plant growth and accumulation of each PUFA are critical factors. Illumination combining white and blue light is an important subject for subsequent investigation. In future, the optimization of other growth conditions such as temperature and nutrition, may further improve productivity of AA and EPA, contributing the development of economical plant factory to produce them.

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