Scientific Research Publishing

American Journal of Plant Sciences, 2016, 7, 2412-2428
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Phototropism in the Marine Red Macroalga *Pyropia yezoensis*

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How to cite this paper: Takahashi, M. and Mikami, K. (2016) Phototropism in the Marine Red Macroalga *Pyropia yezoensis*. *American Journal of Plant Sciences*, **7**, 2412-2428.
http://dx.doi.org/10.4236/ajps.2016.717211

Received: October 5, 2016
Accepted: December 5, 2016
Published: December 8, 2016

Abstract

Phototropism is a response to the direction of light that guides growth orientation and determines the shape of plants to optimize photosynthetic activity. The phototropic response is present not only in terrestrial plants but also in water-living algae. However, knowledge about phototropism in Bangiophycean seaweeds is limited. Here, we examined the phototropic response of the red alga *Pyropia yezoensis* to elucidate the regulatory mechanism of phototropism in Bangiophyceae. When leafy gametophytes and filamentous sporophytes of *P. yezoensis* were cultured under directional light, phototropism was observed in the gametophytes. Conchosporangia on the sporophytes also exhibited phototropism. Phototropism was positive in the majority of gametophytes and conchosporangia but in some cases was negative. In addition, a strong phototropic response occurred under white light, whereas blue and red light elicited minor and no responses, respectively. This observation is in contrast with the phototropic response in terrestrial plants and several algae, in which blue light is responsible for positive phototropism. Surprisingly, the genome of *P. yezoensis* has no homologues of the photoreceptors for blue and red light, revealing differences in the regulation of phototropism between terrestrial plants and *P. yezoensis*. Studies on the phototropism in *P. yezoensis* could shed light on the evolutional divergence of phototropic responses in plants.

Keywords

Phototropism, Gametophyte, Sporophyte, Conchosporangia, *Pyropia yezoensis*

1. Introduction

Phototropism is defined as the response of plants to directional light that directs growth orientation to optimize photosynthetic activity and energy production [1] [2]. Since the

discovery of the pivotal role of auxin in phototropism [3] [4], the Cholodny-Went hypothesis has been generally accepted. This hypothesis posits the lateral movement of auxin from the illuminated to the shaded side where it promotes cell elongation and curvature of the coleoptile towards the light.

The study of phototropism was extensively advanced using a genetic approach and the dicotyledon *Arabidopsis thaliana* [5] [6] [7]. The identification of phototropin, a plasma membrane-associated blue light receptor consisting of an N-terminal light-sensing domain with two light, oxygen or voltage (LOV) domains and a C-terminal serine/threonine kinase domain, was another major finding that furthered our understanding of the process of phototropism [8] [9] [10] [11]. To date, photoperception and activation of phototropins have been extensively analyzed, and several phototropin-signaling components such as nonphototropic hypocotyl 3 (NPH3) and phytochrome kinase substrate 1 (PKS1) have been identified [12] [13]. Moreover, red light receptor phytochromes, and a class of blue light receptors, the cryptochromes, are involved in fine-tuning phototropin activity by repression of the negative regulators ATP binding cassette B19 (ABCB19), which is an auxin efflux carrier, and root phototropism 2 (RPT2) which participates in blue light-induced phototropism [14] [15]. Thus, all classes of photoreceptors known in *A. thaliana* play a role in the early phase of phototropism.

The formation of the auxin gradient has also been extensively studied. Initially, three families of auxin transporters were studied: auxin resistant 1 (AUX1), the ABC transporters (specifically ABCB19), and the PIN-FORMED (PIN) family [16]. The asymmetric distribution of auxin, results from polar transport through the activity of these transporters [15] [17]. Subsequently, studies of the auxin signal transduction pathway identified the auxin receptors transport inhibitor response 1 (TIR1, also called auxin signaling F-box, AFB) and auxin binding protein 1 (ABP1), the negative regulators auxin/indole-3-acetic acid (Aux/IAA) proteins, and the auxin response factors (ARFs) [18] [19]. Cell-to-cell movement of auxin mediated by auxin transporters establishes an auxin gradient, and the auxin that accumulates in the shaded side activates a signal transduction cascade that leads to expression of genes that stimulate cell elongation at the shaded side specifically [5] [6] [7]. Thus, the molecular mechanisms that regulate phototropism are now mostly elucidated in *A. thaliana*.

The polarity in zygotes of brown algae is determined by the direction of light; the illuminated and shaded sides develop into vegetative and rhizoid cells, respectively [20]. Although evidence for photopolarization is limited to brown algal zygotes, these findings suggest the ability of seaweeds to recognize the direction of light. Indeed, phototropic responses have been found in water-living seaweeds: according to the excellent review by Rico and Guiry [21] for example, negative phototropism of rhizoids and positive phototropism of the thallus were observed [22]-[27]. Subsequently, phototropism responses to directional blue light were found in many species belonging to the Chlorophyceae, Phaeophyceae and Rhodophyceae [21]. Since 1996, no studies on seaweed phototropism have been reported except for the identification of a blue light receptor,

aureochrome, in the photosynthetic stramenopile algae [28] [29]. Thus, detailed information on the process and regulatory mechanism of phototropism in seaweeds is limited.

Pyropia yezoensis belongs to the family Bangiophyceae and is a model species for red seaweeds [30]; the nuclear, plastid and mitochondrial genomes of this seaweed have been sequenced [31] [32] [33]. We have investigated the light-dependent release of asexual spores of *P. yezoensis* and the establishment of polarity in these spores [30] [34] [35] [36]. However, it remains unclear whether the establishment of polarity in asexual spores depends on the direction and color of light. Phototropism can serve as a useful model to analyze the formation of the polarized axis that determines growth direction. Currently, in contrast to the Florideophyceae family, in which phototropism has been extensively studied [21] [37], there are only two reports on phototropism in the Bangiophyceae family, in *P. yezoensis* and *P. tenera* [38] and *Porphyra umbilicalis* [37]. Migita and Kim [38] documented the phototropic response in sporophytes and conchosporangia of *P. yezoensis*.

In this study, we examine the phototropic responses of gametophytes, sporophytes and conchosporangia of *P. yezoensis*. We extend the previous observations by studying the light color requirement and locating the photo-perception and bending sites through the dissection of the processes that meditate phototropic curvature. Our results help to clarify the regulatory mechanisms of phototropism and photopolarization in Bangiophycean seaweeds. In addition, to identify a potential mechanism that underlies the phototropic response, a large-scale survey of red algal EST information was performed to confirm the presence of homologs of factors that are involved in the regulation of phototropism in other eukaryotes.

2. Materials and Methods

2.1. Seaweed Culture

Leafy gametophytes and filamentous sporophytes of *P. yezoensis* strain U-51, which were obtained from the Marine Resources Research Center of Aichi Fisheries Research Institute, were cultured separately in 500 mL Provasoli-Enriched Seawater (PES) medium [39] with slight modification by replacing the Tris [Tris (Hydroxymethyl) aminomethane] buffer to HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], at 15˚C under 60 μmol photons m^{-2} s^{-1} white light with a photocycle of 10 h light and 14 h dark. The medium was refreshed weekly until the appearance of monosporangia and carposporangia in the gametophytes and conchosporangia on the sporophytes.

To induce the release of monospores and carpospores, thalli were transferred to a 90 × 20 mm Petri dish containing PES medium, and incubated for 5 - 10 min on ice under 60 μmol photons m^{-2} s^{-1}. After removal of the thalli from the dish, monospores and carpospores released into the medium naturally sedimented on a 20 × 20 mm cover glass in a 35 mm dish containing PES medium, and subsequently cultured for further examination. Conchosporangia were prepared from sporophytes through extensive chopping with a blade before transfer to a 20 × 20 mm cover glass in 35 mm dish con-

taining PES medium.

2.2. Directional Light Irradiation

For unilateral light exposure, a culturing box (H 6.5 cm × W 10.5 cm × D 9.0 cm) with only one opened side was used. The box was wrapped with black paper to minimize reflected and scattered light. The Petri dishes in the box were incubated at 15˚C under 60 μmol photons m^{-2} s^{-1} (10L:14D) from a 40-W white fluorescent tube (FL40SS EX-N/37-H, Toshiba, Tokyo, Japan), and light-emitting blue (470 nm, MIL-B18A, Panasonic, Osaka, Japan) or red (660 nm, MIL-R18A) diodes for duration indicated in the text. The direction of individuals was recorded for 100 randomly chosen samples that were cultured for an appropriate duration (see Results section). Subsequently, the direction of light was changed by 90 degrees in clockwise direction and the direction of samples was recorded after 5 d culture under the new light direction. Each experiment was performed three times. In these experiments, germlings from monospores or carpospores and conchosporangia were observed using an inverted light microscope (CKX-41, Olympus, Tokyo, Japan) with a camera (DP26, Olympus). Statistical analysis was performed using *t*-test and was carried out between vertical (Top + Bottom) and horizontal (Left + Right) ratio of each experimental group. Furthermore, it was also carried out vertical (Top + Bottom) or horizontal (Left + Right) to each other before and after the change of light irradiation direction. Differences were reported as significant when $P < 0.05$.

2.3. Homology Searches for Genes Encoding Putative Photoreceptors in Red Seaweeds

Genes from *Arabidopsis thaliana* encoding photoreceptors such as phototropin 1 (accession no, AEE78073), phototropin 2 (accession no, AED97004), cryptochrome 1 (accession no, AEE 82696), cryptochrome 2 (accession no, AEE-27693), cryptochrome DASH (cryptochrome 3; accession no, AED93369), phytochrome A (accession no, AEE28462), phytochrome B (accession no, AEC06808), phytochrome C (accession no, AED94021), were used as queries for web-based homology BLAST searches for *P. yezoensis* ESTs (http://est.kazusa.or.jp/en/plant/porphyra/EST/) and ESTs of *P. purpurea* and *P. umbilicalis* (NoriBLAST: http://dbdata.rutgers.edu/nori/). Amino acid sequences of aureochrome 1 of the brown seaweed *Ectocarpus siliculosus* (accession no, CBJ-25875) and PHY3 of the fern *Adiantum capillus-veneris* (superchrome; accession no, BAA36192) as well as those of protein domains like LOV, cryptochrome_C, phytochrome region and bZIP_AUREO-like were also used as queries.

3. Results

3.1. Phototropism in Filamentous Sporophytes

When filamentous sporophytes were exposed to directional light during culture, no phototropic response was observed (**Figure 1(a)** and **Figure 1(b)**), even though Migita and Kim [38] reported positive phototropism under similar conditions. Because

changing the direction of light and exposure to blue or red light did not cause phototropic growth (data not shown), we concluded that *P. yezoensis* sporophytes lack the capacity for phototropic response.

3.2. Phototropism in Conchosporangia

As shown in **Figure 2(a)**, conchosporangia showed a clear phototropic response after seven days of culture under directional white light from the top, which is consistent with the data from Migita and Kim [38]. However, it is notable that both positive and negative phototropic curvature was observed, and not all conchosporangia showed a phototropic response. Indeed, 65.6% and 22.5% of the examined conchosporangia showed positive and negative phototropism, respectively, and the remaining 11.9% did not respond (**Table 1** and **Figure 3(a)**). In addition, when exposed to blue light from the top, positive and negative responses were observed in 39.6% and 27.3% of conchosporangia respectively, whereas exposure to unilateral red light did not cause a phototropic response (**Table 1** and **Figure 3(a)**). Thus, *P. yezoensis* conchoporangia respond to the direction of light. Since white light remained more effective than blue light alone (**Table 1** and **Figure 3(a)**), another color of light, besides blue and red, might be involved in phototropism of conchosporangia.

The phototropic response of conchosporangia was supported by examination of the effects of changing the direction of white light by 90 degrees clockwise. Conchosporangia exposed to light from the left for 5 d after 7 d of light from above responded both positively and negatively (**Figure 2(b)** and **Figure 2(c)**). The percentage of conchosporangia showing growth responsive to the original direction of light was reduced from 65.6% to 15.2%, whereas the percentage responding to the new light direction increased to 51.0% and 26.2% for positive and negative curvatures (**Table 1** and **Figure 3(b)**). Thus, most conchosporangia respond to the direction of white light (positive 51.0%, negative 26.2%, total 77.2%). In addition, because only the tip cell responded to the light (**Figure 2(d)-(h)**), it appears that conchosporangia perceive the light direction at the tip cell and elongate by tip growth, which is shown

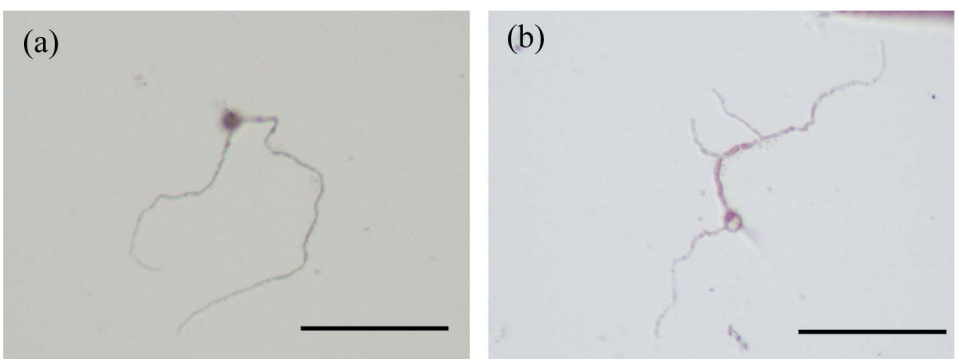


Figure 1. Absence of a phototropic response in filamentous sporophytes of *Pyropia yezoensis*. (a) (b) Sporophytes after 7 d culture under directional white light from above. Scale bars = 100 μm.

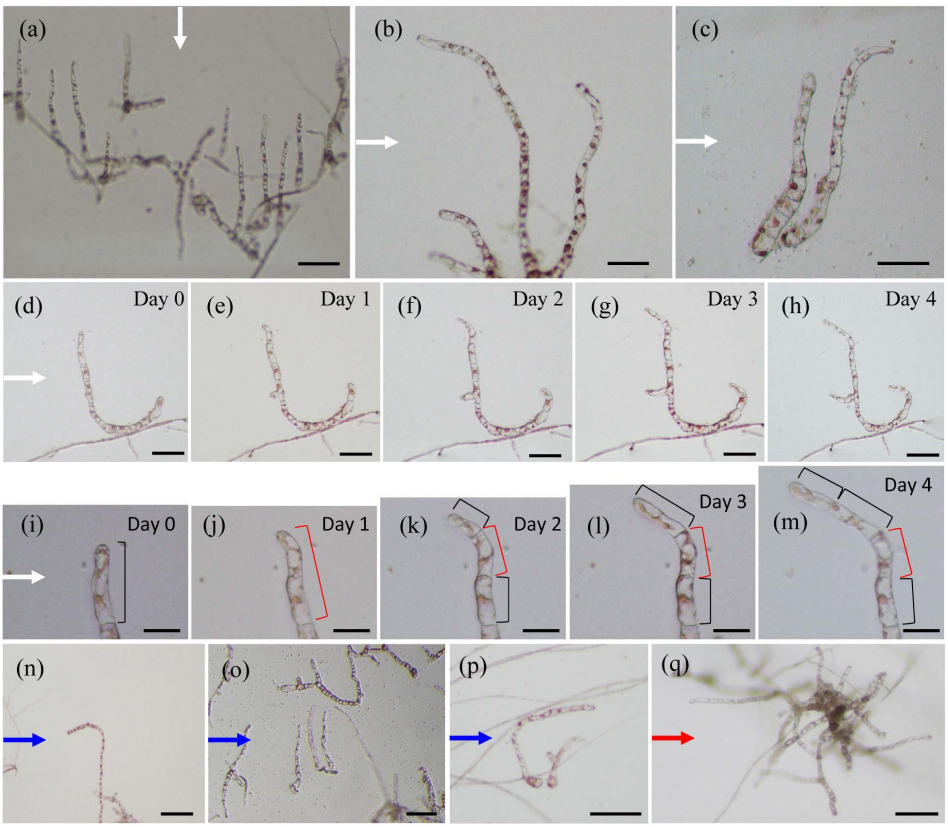
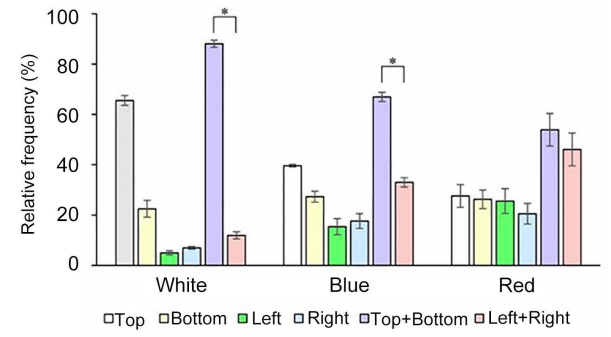


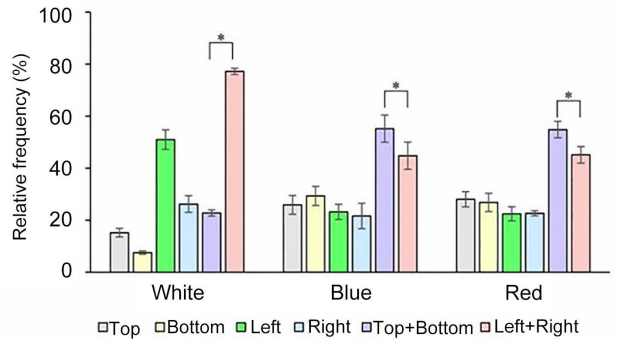
Figure 2. Phototropic response of *Pyropia yezoensis* conchosporangia. (a) Conchosporangia unilaterally illuminated with white light from above for 7 days. (b) (c) Positive (b) and negative (c) phototropic responses to unilateral light from the left for 5 d after 7 d illumination from above; (d)-(h) Temporal view of response to directional light from the left. The phototropic response and cell division in the tip cell were sequentially observed. (d) to (h) represent images taken at day 0, 1, 2, 3 and 4, respectively. New branch formation was also observed in a light-direction dependent manner; (i)-(m) Enlarged sequential images of the tip cell during the phototropic response. Individual cells are indicated by black brackets, with the exception of cells responsible for bending, which are indicated by red brackets; (n)-(p) Effects of directional blue light exposure from the left for 5 d after 7 d illumination from above. Positive (n) and negative (p) curvatures and no response (o) were observed. (q) Lack of phototropic response under red light exposure as for blue light in (n)-(p). The arrows indicate the color and direction of the light. Scale bars = 100 μm (a, n-p), 50 μm (b-m).

clearly in the enlarged photos in **Figure 2(i)-(m)**. Moreover, the formation and tip growth of branches also depended on the light direction (**Figure 2(e)-(h)**). These findings demonstrate the ability of the tip cell of conchosporangia to perceive and respond to the light direction to promote directional tip growth.

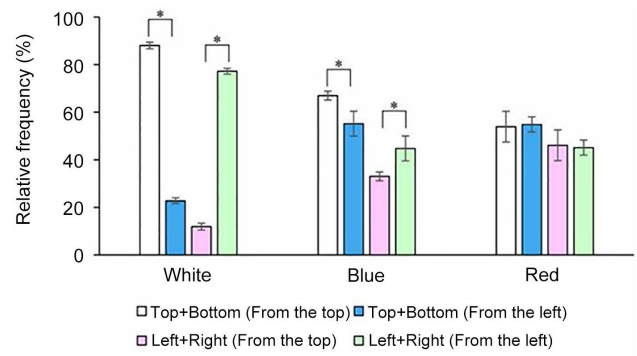
In contrast to the effect of white light, a subsequent exposure to blue light did not result in a clear response in conchosporangia (**Table 1** and **Figure 3(b)**). Some conchosporangia showed phototropism (**Figure 2(n)** and **Figure 2(p)**), although others did not (**Figure 2(o)**), and changing the light direction reduced the percentage growing in original positive growth direction from 39.6% to 25.9% and in-



(a)



(b)



(c)

Figure 3. Effects of unilateral light on determination of growth direction in *Pyropia yezoensis* conchosporangia. (a) Comparison of the relative frequency of growth direction after 7 d white, blue and red light from above; (b) Comparison of the relative frequency of growth direction after the second exposure of white, blue and red light from the left for 5 d subsequent to the experiments in (a). The directions shown indicate the direction toward which the conchosporangia grew. For these panels, in addition to top, bottom, left and right growth directions, frequencies of top + bottom and left + right are also represented to allow consideration of each instance of phototropic response as the sum of positive and negative curvature; (c) Comparison of frequencies of top + bottom and left + right between irradiation from top and left. Error bars mean ± SD of triplicated experiments and an asterisk indicates a significantly difference at $P < 0.05$.

creased that showing the second positive growth direction from 15.4% to 23.2% (Figure 3(b)). These effects were not observed under exposure to red light (Figure 2(q) and Figure 3(b)). Thus, blue light is involved in the phototropic response of conchosporangia, albeitits phototropic effect is weaker than that caused by white light.

3.3. Phototropism of Monospore Germlings

When monospores were exposed to directional white light immediately after release, germination was not photopolarized (Figure 4(a)). By contrast, germlings that were cultured for 7 d responded to the direction of white light both positively and negatively (Figure 4(b)), although, similar to conchosporangia, not all germlings showed a phototropic response (Figure 4(c)). In this case, positive and negative curvatures were observed in 43.2% and 13.4% of all germlings, respectively, and thus 43.4% germlings did not respond to directional light from the top (Table 1 and Figure 5(a)). Surprisingly, neither red nor blue light resulted in a phototropic response in germlings (Table 1 and Figure 5(a)).

Table 1. Growth direction of conchosporangia and monospore germlings. The headings indicate the direction(s) toward which conchosporangia and monospore germlings grew.

	Top	Bottom	Left	Right	Top + Bottom	Left + Right
Conchosporangia						
7-day culture under unilateral light irradiation from the top						
White	65.6 ± 2.0	22.5 ± 3.4	4.9 ± 0.9	7.0 ± 0.6	88.1 ± 1.4	11.9 ± 1.4
Blue	39.6 ± 0.5	27.3 ± 2.2	15.4 ± 3.2	17.6 ± 3.0	67.0 ± 1.9	33.0 ± 1.9
Red	27.6 ± 4.5	26.3 ± 3.7	25.6 ± 4.9	20.5 ± 4.1	53.9 ± 6.5	46.1 ± 6.5
5-day culture under changing the direction of light irradiation from the top to the left						
White	15.2 ± 1.7	7.6 ± 0.7	51.0 ± 3.8	26.2 ± 3.2	22.8 ± 1.2	77.2 ± 1.2
Blue	25.9 ± 3.6	29.3 ± 3.7	23.2 ± 3.0	21.6 ± 4.9	55.2 ± 5.2	44.8 ± 5.2
Red	28.0 ± 2.9	26.8 ± 3.5	22.5 ± 2.7	22.7 ± 1.0	54.9 ± 3.2	45.1 ± 3.2
Monospore germlings						
7-day culture under unilateral light irradiation from the top						
White	43.2 ± 4.4	13.4 ± 12.0	26.5 ± 5.9	16.9 ± 2.7	56.6 ± 7.6	43.4 ± 7.6
Blue	31.0 ± 5.9	15.5 ± 9.5	21.7 ± 4.2	31.8 ± 8.5	46.5 ± 4.7	53.5 ± 4.7
Red	23.7 ± 3.8	23.0 ± 7.4	22.1 ± 5.3	31.2 ± 1.1	46.7 ± 5.3	53.3 ± 5.3
5-day culture under changing the direction of light irradiation from the top to the left						
White	25.7 ± 4.4	15.9 ± 2.6	30.5 ± 0.9	27.9 ± 1.4	41.6 ± 1.8	58.4 ± 1.8
Blue	18.5 ± 2.6	21.6 ± 2.9	35.6 ± 2.8	24.3 ± 2.9	40.1 ± 5.3	59.9 ± 5.3
Red	23.2 ± 0.8	29.9 ± 3.0	23.5 ± 3.7	23.4 ± 3.1	53.1 ± 3.3	46.9 ± 3.3

mean ± SD (%).

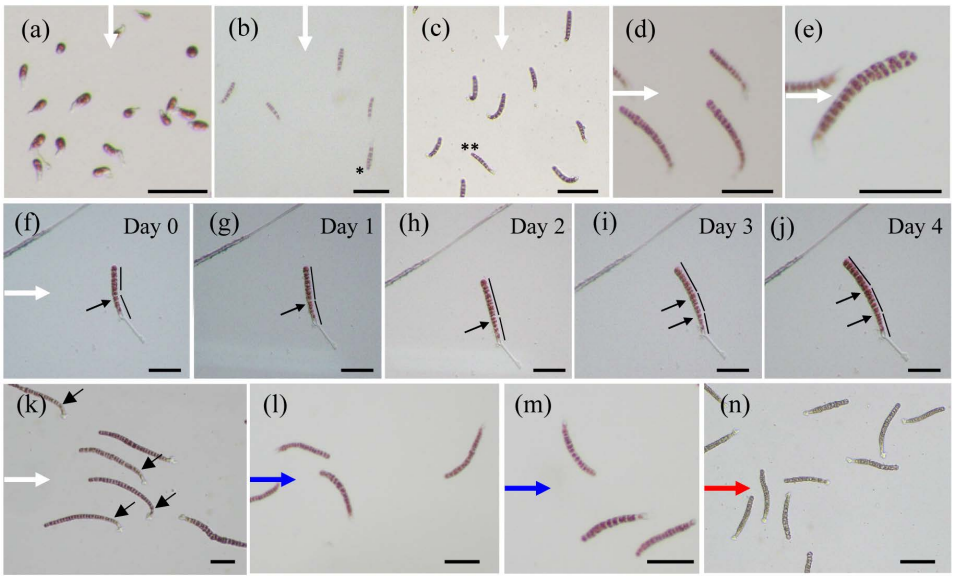
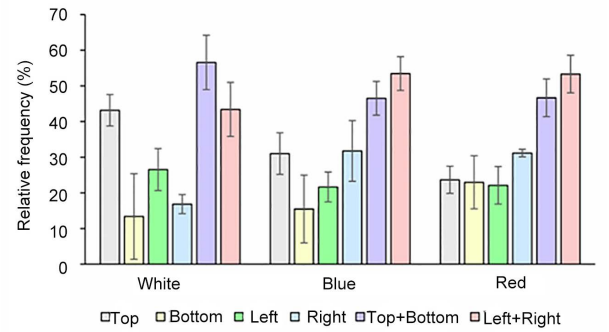


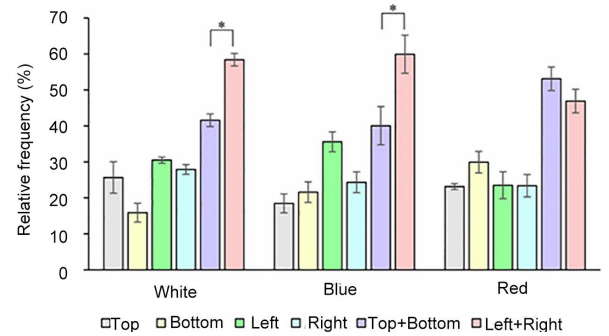
Figure 4. Phototropic response of *Pyropia yezoensis* monospore germlings. (a) Monospores directly after germination under unilateral exposure to with white light from above for 1 d. Photopolarized germination was not observed; (b) (c) Monospore germlings after unilateral exposure to white light from above for 7 d. Germlings that respond positively and negatively (asterisk in (b)) to the direction of white light, and germination without photopolarization (double-asterisk in (c)) were observed; (d) (e) Positive (d) and negative (e) phototropic responses to unilateral light from the left for 5 days after the experiments (b) (c); (f)-(j) Temporal view of the response to the directional light from the left. The phototropic response in germlings was sequentially observed. (f) to (j) represent images taken at day 0, 1, 2, 3 and 4, respectively. Arrows indicate cells that may be responsible for bending and the black lines reveal the direction of each corresponding part of germlings; (k) Final images of phototropic response to unilateral exposure of white light from the left. Black arrows indicate cells that may be responsible for bending; (l) (m) Effects of directional blue light exposure from the left for 5 d after 7 d light exposure from above. Positive and negative curvatures and no response were observed; (n) Lack of phototropic response under red light exposure. Arrows denote the color and direction of irradiated light. Scale bars = 100 μm (a-e, k-n), 50 μm (f)-(j).

Next, we monitored the effect on germlings of changing the light direction to the left after 7 d illumination from above. Monospore germlings illuminated from the left for 5 d responded both positively and negatively (**Figure 4(d)-(e)**). These results demonstrate that the direction of white light determines the growth direction of germlings, although the efficiency was not 100% (**Table 1** and **Figure 5(b)**). **Figure 4(f)-(k)** shows the continuous observation of germlings cultured underexposure of white light from the left. The growth direction of the germlings gradually adjusted toward to the light direction. It appears that the cells close to the holdfast are responsible for the initial bending during the first few days (**Figure 4(f)-(h)**), and that the cells located above these cells are responsible for the additional bending due to illumination for more than 3 d (**Figure 4(i)** and **Figure 4(j)**), which completes the phototropic response as shown in **Figure 4(k)**.

Interestingly, blue light reduced the number of germlings that grew toward the



(a)



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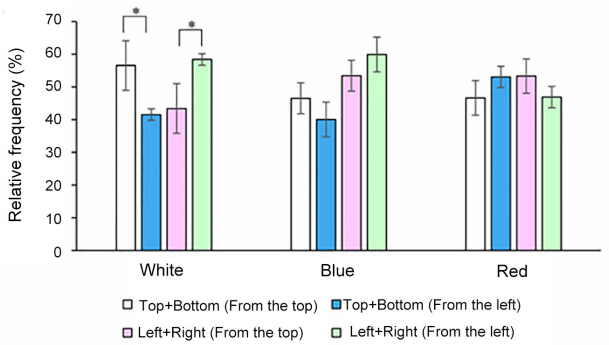

(c)

Figure 5. Effects of unilateral light on determination of growth direction in monospore germlings of *Pyropia yezoensis*. (a) Comparison of the relative frequency of growth direction after 7 d exposure to white, blue and red light from above; (b) Comparison of the relative frequency of growth direction after a second exposure to white, blue or red light from the left for 5 d subsequent to the experiments in (a). The directions shown indicate the direction toward which the monospore germlings grew. For these panels, in addition to top, bottom, left and right growth directions, frequencies of top + bottom and left + right are also represented to allow consideration of each instance of phototropic response as the sum of positive and negative curvature; (c) Comparison of frequencies of top + bottom and left + right between irradiation from top and left. Error bars mean ± SD of triplicated experiments and an asterisk indicates a significantly difference at $P < 0.05$.

original direction from 31.0% to 18.5% and increased growth along the new direction from 21.7% to 35.6% (Table 1, Figure 4(l), Figure 4(m) and Figure 5(b)), whereas red light had no effect (Table 1, Figure 4(n) and Figure 5(b)). Thus, gametophytes of *P. yezoensis* respond to the direction of white light, and blue light might be involved in this phototropic response.

3.4. Sequence Searches for Photoreceptor Homologues

Using the amino acid sequences of phototropins, cryptochromes, phytochromes, superchrome and aureochrome as queries, a BLAST search was performed against the EST databases for *P. yezoensis*, *P. umbilicalis* and *P. purpurea*. Surprisingly, the results indicated that these red seaweeds might lack homologs of any of the photoreceptors that have been identified in green plants and brown seaweeds. These findings are consistent with previous reports [29] [40] [41] [42].

4. Discussion

In the present study, we observed phototropism in gametophytes and conchosporangia, but not in sporophytes, of the marine red seaweed *P. yezoensis*. The phototropic response was observed in the tip cells of conchosporangia and in the cells close to the holdfast of the gametophytes (Figure 2 and Figure 4). There are, however, two unique features of phototropism in *P. yeziensis*. The first is that, although positive phototropism occurred in most cases, negative phototropism was also observed in both gametophytes and conchosporangia (Table 1, Table 2, Figure 3 and Figure 5). The second is that phototropic curvature was not observed in all examined gametophytes and conchosporangia (Table 1, Table 2, Figure 3 and Figure 5), indicating differences in the sensitivity to the light direction between individual organisms. It remains unclear whether these unique phototropic features have a significant biological function in the

Table 2. Comparison of frequencies of growth direction of conchosporangia and monospore germlings between irradiation from top and left. The headings indicate the directions toward which conchosporangia and monospore germlings grew.

	Top + Bottom		Left + Right	
Irradiation	From the top	From the left	From the top	From the left
		Conchosporangia		
White	88.1 ± 1.4	22.8 ± 1.2	11.9 ± 1.4	77.2 ± 1.2
Blue	67.0 ± 1.9	55.2 ± 5.2	33.0 ± 1.9	44.8 ± 5.2
Red	53.9 ± 6.5	54.9 ± 3.2	46.1 ± 6.5	45.1 ± 3.2
		Monospore germlings		
White	56.6 ± 7.6	41.6 ± 1.8	43.4 ± 7.6	58.4 ± 1.8
Blue	46.5 ± 4.7	40.1 ± 5.3	53.5 ± 4.7	59.9 ± 5.3
Red	46.7 ± 5.3	53.1 ± 3.3	53.3 ± 5.3	46.9 ± 3.3

mean ± SD (%).

growth of *P. yezoensis*. Interestingly, our results in **Figure 1** are inconsistent with those of Migita and Kim [38], who reported positive phototropism in sporophytes. The lack of phototropic response in sporophytes in our study might be due to the fact that the response to the light direction could prevent boring of sporophytes into shells. Moreover, although white light effectively induced phototropic curvature, conchosporangia and gametophytes showed a weak receptivity to blue light (**Table 1**, **Table 2** and **Figures 2-5**), which is inconsistent with the general consensus that blue light plays a central role in phototropism in seaweeds [21]. Taken together, our results therefore indicate the presence of novel mechanisms that regulate phototropism in *P. yezoensis*.

The uniqueness of this phototropic machinery in *P. yezoensis* is supported by the results of the homology searches for photoreceptor genes. The blue light-receptor phototropin, which recognizes and absorbs blue light through LOV domains, plays a central role in the phototropic response in terrestrial plants [8] [10] [11]. However, the genomes of *P. yezoensis* have no homologue of this LOV domain-containing photoreceptor. In addition, a large scale-EST survey did not identify homologues of other photoreceptors present in terrestrial plants and brown seaweeds. Because white and blue light, but not red light, promote phototropic bending in gametophytes and conchosporangia, unknown photoreceptors might be present in *P. yezoensis* and may function as regulators whose activity is not sufficient to mediate phototropism in all examined individuals. These findings also indicate differences in regulatory mechanisms of phototropism between terrestrial plants and *P. yezoensis*.

The difference in phototropism between vascular plants and conchosporangia of *P. yezoensis* could be related to lateral cell-to-cell interaction. Phototropism in the multicellular architecture in vascular plants requires differential growth between cells in the illuminated and shaded sides [1] [2] [5] [6] [7], whereas phototropism in the single conchosporangia tip cell requires differences in growth rate between the illuminated and shaded sides within a single cell. To date, single cell-based phototropism has been observed in tip-growing protonemal cells of the mosses *Ceratodon purpureus* and *Physcomitrella patens* [43] [44] [45]. Moreover, in *C. purpureus* the red light-receptor phytochrome is involved in phototropism in the tip cells through re-organization of microfilaments (MFs) [46] [47] [48]. Although red light is not required for phototropism in *P. yezoensis* conchosporangia (**Table 1**, **Table 2**, **Figure 2(q)** and **Figure 3**), it is necessary to address whether light-dependent re-organization of MFs is involved in bending of the tip cell of *P. yezoensis* conchosporangia.

We also observed phototropism at an early developmental phase of gametophytes that were composed of a tandem array of cells along the apical-basal axis (**Figure 4**). Although bending requires differential growth between the illuminated and shaded sides, it is still unclear whether sensing the light direction and asymmetrical elongation are mediated by a single cell or spatially separated cells. Moreover, our findings clearly demonstrate that the ability to recognize the light direction is acquired at a multi-cellular stage during early development of gametophytes from monospores (compare **Figure 4(a)-(c)**). Thus, identification of the cell(s) capable of perceiving the light signal

and bending due to asymmetrical growth is important to elucidate the mechanism of phototropism in gametophytes.

Although the asymmetrical distribution of the phytohormone auxin between the illuminated and shaded sides is critical for phototropism in terrestrial plants [1] [2] [5] [6] [7], it is unknown whether auxin acts as a regulator of phototropism in *P. yezoensis*, and whether auxin is asymmetrically distributed in the bending cell in conchosporangia and gametophytes. The gravitropic response was attenuated by inhibitors of auxin transporters in the tip cell of *C. purpureus* protonemata [49]. In addition, endogenous auxin is present in *P. yezoensis* [50]. Thus, it is interesting to examine whether auxin is involved in phototropism in conchosporangia and gametophytes of *P. yezoensis*. There are in fact no homologues of factors involved in auxin biosynthesis, transport and signal transduction in *P. yezoensis* [50], and it is possible that auxin, if involved, meditates phototropism in *P. yezoensis* through an unknown mechanism.

As shown in **Figure 2(n)-(q)** and **Figure 4(l)-(n)**, it was unexpectedly observed that the color of the conchosporangia and monospore germlings changed to green and bright purple under red and blue light, respectively (summarized in **Supplemental Figure 1**). Such light responses are very similar to the complementary chromatic adaptation (CCA) found in chromatically-adapting prokaryotes such as the cyanobacterium *Fremyella diplosiphon* [51] [52] [53]. CCA is a process of adaptation to differences in light color by modification of the pigment composition in the light-harvesting phycobilisomes, in which exposure to green and red light alter the cellular color to red and green, respectively. Because the photosynthetic machinery of *P. yezoensis* includes phycobilisomes, it is likely that CCA is conserved in the phycobilisome-containing marine photosynthetic eukaryotes such as the red seaweeds. Confirmation of this possibility remains to be addressed.

5. Conclusion

Our work demonstrates that leafy gametophytes and conchosporangia of *P. yezoensis* perceive and respond to the direction of light both positively and negatively, although not all individuals respond to light and blue light has a weaker potential to produce phototropic response than white light. In addition, *P. yezoensis* has no homologs of any light receptors found in terrestrial plants. Because these characteristics are specific to this seaweed, this study of phototropism in *P. yezoensis* sheds light on the evolutionary divergence of photomorphogenesis in plants.

Acknowledgements

We are grateful to the Marine Resources Research Center of Aichi Fisheries Research Institute for kindly providing *P. yezoensis* strain U51. This work was supported in part by KAKENHI (15H0453905).

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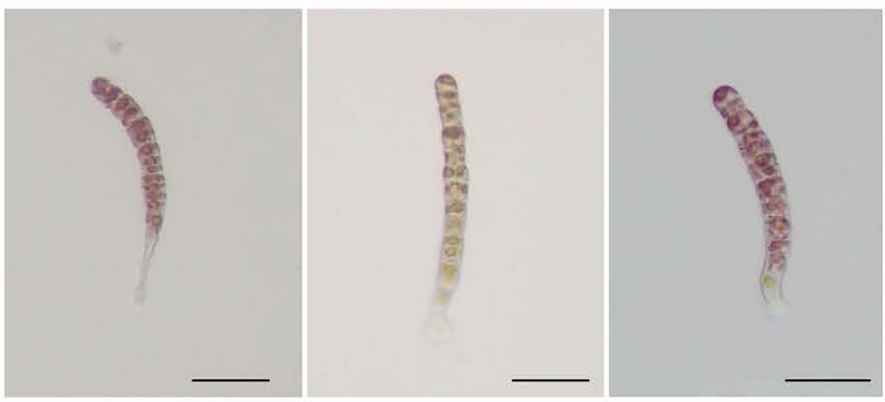
Appendix



Supplemental Figure 1. Comparison of the color of monospore germlings cultured under white (a), red (b) and blue (c) light for 10 d. Scale bars = 50 μm.