Chloroplast cold-avoidance response in *Marchantia polymorpha* occurs independently of changes in endogenous phototropin expression

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Abstract:

When exposed to fluctuating light intensity, chloroplasts move towards weak light (accumulation response), and away from strong light (avoidance response). In addition, cold treatment (5°C) induces the avoidance response even under weak light conditions (cold-avoidance response). These three responses are mediated by the phototropin (phot), which is a blue-light photoreceptor and has also been found to act as a thermosensory protein that perceives temperature variation. Our previous report indicated that cold-induced changes in phot biochemical activity initiate the cold-avoidance response. In this study, we further explored the induction mechanism of the cold-avoidance response in the liverwort *Marchantia polymorpha* and examined the relationship between changes in the amount of phot and the induction of the cold-avoidance response. The switch between the accumulation and avoidance responses occurs at a so-called 'transitional' light intensity. Our physiological experiments revealed that a cold-mediated decrease in the transitional light intensity leads to the induction of the cold-avoidance response. While artificial overexpression of phot decreased the transitional light intensity as much as cold treatment did, the amount of endogenous phot remained unchanged by cold treatment in wild-type *M. polymorpha*. Taken together, these findings show that the cold-avoidance response is initiated by a cold-mediated reduction of the transitional light intensity, independent of the amount of endogenous phot. This study provides a clue to understand the mechanism underlying the switch in direction of chloroplast relocation in response to light and temperature.

Keywords:

Accumulation response; Avoidance response; Chloroplast movement; Cold-avoidance response; *Marchantia polymorpha;* Phototropin

1. Introduction

Plants sense ambient light and temperature to adapt to an ever-changing environment. In response to light and temperature changes, the intracellular chloroplast position is precisely controlled. Under weak light conditions (e.g., under canopy), chloroplasts move towards light to increase light capture for photosynthetic efficiency (accumulation response) [1]. Under strong light conditions (e.g., under direct sunlight), chloroplasts move away from light to avoid photodamage (avoidance response) [2]. Chloroplast position is also affected by temperature [3], notably cold. Under warm conditions, weak light induces the accumulation response; however, under cold conditions (5°C) the same light level induces chloroplast avoidance (Fig. 1). This movement, referred to as the cold-positioning (or cold-avoidance) response, has been studied in a number of plant species, including the moss *Funaria hygrometrica* [3], evergreen ferns (e.g., *Adiantum capillus-veneris*) [4], the liverwort *Marchantia polymorpha* [5] and *Arabidopsis thaliana* [6]. In *M. polymorpha*, the cold-avoidance response contributes to the reduction of photoinhibition under cold conditions [6].

Earlier studies showed that the accumulation, avoidance and cold-avoidance responses are all mediated by the blue-light (BL) photoreceptor phototropin (phot) [4,6-9]. Phot has two photosensory light, oxygen, or voltage (LOV) domains (LOV1 and LOV2) at its N-terminal region and a serine/threonine kinase domain at its C-terminal region. Phot perceives BL via its LOV domains and mediates the change of chloroplast position upon photoactivation of the LOV domains and kinase-mediated autophosphorylation [10]. A decrease in ambient temperature prolongs the lifetime of the photoactivated LOV2 domain, resulting in an increase in autophosphorylation [6]. The cold-induced enhancement of the photochemical and biochemical activities of phot (i.e., prolonged lifetime of photoactivated LOV2 and increased autophosphorylation status) is essential for induction of the cold-avoidance response [6], and further study is required to fully understand this induction.

The switch in chloroplast behavior between the accumulation and avoidance responses occurs at their 'transitional' light intensity (Fig. 1A). In the cold-avoidance response, because the chloroplast moves away from even weak BL (wBL) under cold conditions [6], it is hypothesized that the cold-avoidance response is induced by a cold-mediated reduction of the transitional light intensity (Fig. 1B). However, this hypothesis remains to be tested. Artificial overexpression of phot has been suggested to decrease the transitional light intensity in *A. capillus-veneris* and *A. thaliana* [11,12]. In addition, expression levels of endogenous phot are affected by both light [7,8,13-16] and temperature [16] conditions. Based on these findings, we postulate that a variation in the endogenous amount of phot may be involved in the induction of the cold-avoidance response.

The goals of this study were two-fold: first, to determine if a cold-mediated reduction of the transitional light intensity initiates the cold-avoidance response, and second, to determine if changes in phot amounts play a role in the induction of the cold-avoidance response. *M. polymorpha* was chosen for this study because it has a single copy of the gene for phot (Mpphot) that can mediate the accumulation, avoidance, and cold-avoidance responses [6,17].

2. Materials and Methods

2.1. Plasmid Construction

To construct a plasmid to generate the complementation line of Mpphot-Citrine (35S::Mpphot-Citrine/Mp*phot*^{KO}), we used the Gateway cloning system (Invitrogen) and the binary vector pMpGWB306 [18], which includes *Citrine* for fusion at the 3′-end of the target gene under the control of the cauliflower mosaic virus (CaMV) 35S promoter. The LR reaction (Invitrogen) was performed with a mixture of pMpGWB306 and the donor plasmid pDONR207-MpPHOT [19], and the resulting plasmid pMpGWB306-

91 MpPHOT was used for transformation of *M. polymorpha*.

92

93 *2.2. Plant Materials and Growth Conditions*

94 Thalli of *M. polymorpha* were asexually cultured on half-strength B5 medium
95 under 75 μmol photons m^{-2} s^{-1} continuous white light (FL40SW, NEC Corporation). Light
96 intensity was measured using a LI-250A light meter (LI-COR Biosiences). The Mp*PHOT*
97 knockout line (Mp$phot^{KO}$) was generated, and kindly provided, by Komatsu et al. [17]. In
98 this study, transformation of *M. polymorpha* was performed by the G-AgarTrap method
99 [20,21], which is an *Agrobacterium*-mediated transformation method used for
100 gemmalings. To generate the transformants, we used the binary vector pMpGWB106-
101 MpPHOT [19] for Mpphot-Citrine overexpression lines (35S::Mpphot-Citrine/WT), and
102 pMpGWB306-MpPHOT for the complementation line of Mpphot (35S::Mpphot-
103 Citrine/Mp$phot^{KO}$). The Citrine-expressing line (35S::Citrine/WT) was generated in our
104 previous study [20]. G2 gemmalings were used throughout.

105

106 *2.3. Observation of Chloroplast Positioning*

107 One-day-old gemmalings were incubated in temperature-controlled incubators
108 (IJ100, Yamato Scientific Co., LTD) and light conditions were adjusted using blue light-
109 emitting diodes (LEDs; OptoSupply Limited) and a LI-250A light meter. Chlorophyll
110 fluorescence images were captured using a fluorescence microscope MZ16F (Leica
111 Microsystems) equipped with a DP73 digital camera (Olympus) and quantification of
112 chloroplast positioning was performed using the P/A ratio method with ImageJ software,
113 as previously described [5]. Briefly, fluorescence intensity was measured at 30 points
114 (0.625 μm each, equivalent to 1 pixel) along the anticlinal cell walls (A) and 30 points
115 (39.1 μm^2 each, equivalent to 10 × 10 pixels) along the periclinal cell walls (P). This
116 procedure was repeated five times, and then the P/A ratio was calculated as a mean of the
117 five P/A values. The standard deviation was calculated using Excel software (Microsoft).

118

119 *2.4. Immunoblot Analysis*

120 Immunoblot analysis was performed as previously described [6]. Briefly, four-day-old
121 gemmalings were collected in liquid nitrogen and kept at -80°C until use. The frozen
122 plants were homogenized in SDS sample buffer (2% SDS, 10% glycerol, 0.01%
123 bromophenol blue, 5% mercaptoethanol, and 0.25 M Tris-HCl pH6.8) using a mortar and
124 pestle, and then boiled at 95°C for 5 min. The samples were centrifuged at 14,000 g for
125 10 min, and the supernatants used. Total protein amount was quantified by Bradford-XL
126 assay (APRO Science). Fifteen µg of protein was subjected to SDS-PAGE with 8% gels
127 and transferred to a polyvinylidene difluoride (PVDF) membrane. MpPHOT proteins
128 were detected with rabbit antibody raised against the custom-made synthetic peptide
129 (C+VDERAPPSKGSAKE) (Eurofins Genomics) and horseradish peroxidase-conjugated
130 anti-rabbit IgG (Thermo Scientific). Both antibodies were used at dilution of 1:2000. For
131 histone detection, anti-Histone H3 antibody (Abcam) at dilution of 1:5000 and
132 horseradish peroxide-conjugated anti-rabbit IgG (Thermo Scientific) at dilution of 1:2000
133 were used. Chemiluminescent signal was detected using ECL-Select (GE Healthcare) and
134 Light Capture (ATTO). For image of Rubisco large subunit (RBCL), fifteen µg protein
135 was subjected to SDS-PAGE with 8% gels and stained with conventional Coomassie blue
136 method.

137

138

139 **3. Results**

140 *3.1. Chloroplasts avoid weak blue light (wBL) under cold conditions*

141 Using temperature-regulated microscopy with a microbeam irradiation system,
142 we previously reported that under cold conditions (5°C), chloroplasts moved away from
143 wBL that induced the accumulation response at 22°C [6]. However, in the study,
144 physiological evidence of wBL avoidance under cold conditions was limited to

145 microbeam analysis [6]. Here, we further analyzed whether chloroplasts move away from
146 wBL under cold conditions.

147 In *M. polymorpha*, both cold and BL were required to elicit the cold-avoidance
148 response [6]. Building on the result, to explore whether the cold-avoidance response is
149 due to avoidance from wBL under cold conditions, we tested the effects of changing light
150 direction. First, to induce the cold-avoidance response, wild-type (WT) cells were
151 incubated under 25 μmol photons m^{-2} s^{-1} of wBL (BL25) with vertical irradiation at 5°C
152 (Fig. 2A). In WT cells, chloroplasts were positioned along the anticlinal cell walls as
153 cold-avoidance response (Fig. 2B). When the light direction was changed from vertical
154 to horizontal (Fig. 2A), the chloroplasts along the anticlinal cell walls moved to the
155 periclinal cell walls (Fig. 2B), indicating chloroplast avoidance from BL25. This result is
156 consistent with the microbeam analysis in our previous study [6].

157

158 *3.2. Cold treatment decreases the transitional light intensity between the accumulation*
159 *and avoidance responses*

160 We hypothesized that the cold-avoidance response is induced by a cold-mediated
161 decrease of the transitional light intensity (Fig. 1A, B). If this is the case, the accumulation
162 response would be induced under much weaker BL in cold conditions. To investigate
163 whether the accumulation response is induced at 5°C, WT cells were incubated under
164 different intensities of wBL (5, 10, 15 and 25 μmol photons m^{-2} s^{-1}; BL5, BL10, BL15
165 and BL25, respectively) (Fig. 2C, D). Chloroplast position was quantified by a P/A ratio
166 method, developed in earlier studies [4,5]. The P/A ratio is an arbitrary unit calculated by
167 dividing the chlorophyll fluorescence intensity along the periclinal cell wall by that along
168 the anticlinal cell wall, with a higher value indicating periclinal positioning of
169 chloroplasts and vice versa. In WT cells, the cold-avoidance response was observed at
170 5°C under BL10, BL15 and BL25 (Fig. 2C), and at these higher BL intensity levels, we
171 observed a decrease in P/A ratios (Fig. 2D). However, the cold-avoidance response was

172 not observed at 5°C under BL5 (Fig. 2C) and the P/A ratio was unchanged during the
173 incubation of these samples (Fig. 2D). These results suggest that BL5 induces the
174 accumulation response at 5°C. However, because similar chloroplast positioning was
175 observed under dark conditions at 5°C [6], it is also possible that the cells were unable to
176 respond to the extremely weak intensity of BL5. To clarify whether the accumulation
177 response is induced under BL5 or not, an additional test was carried out. Following the
178 induction of the cold-avoidance response at 5°C for 24 h under BL25, the light intensity
179 was changed to BL5 or dark conditions (Fig. 2E). After incubation for 24 h, BL5
180 conditions induced the accumulation response at 5°C, whereas under dark conditions
181 there was no change in chloroplast position (Fig. 2F). These results indicate that the
182 accumulation response is induced under BL5 at 5°C. Taken together, these results indicate
183 that the transitional light intensity is modulated by the temperature change (Fig. 1A, B).
184

185 *3.3. Artificial overexpression of Mp*phot *in* M. polymorpha *induces the avoidance*
186 *response under wBL without cold treatment*

187 We next considered the possibility that changes in endogenous phot expression
188 may play a role in the change in transitional light intensity, as previous studies using *A.*
189 *capillus-veneris* and *A. thaliana* suggest that a reduction in the transitional light intensity
190 can be the result of artificial overexpression of phot [11,12]. In *A. capillus-veneris* at 25°C,
191 transient overexpression of the Ac*PHOT2-GFP* gene induced the avoidance response
192 under wBL [11]. Similarly, the avoidance response was observed under wBL in transgenic
193 *A. thaliana* overexpressing the At*PHOT2-GFP* gene at 23°C [12]. Consistent with these
194 previous studies, we found that artificial overexpression of Mpphot fused to the yellow
195 fluorescent protein Citrine (Mpphot-Citrine) in WT cells (35S::Mpphot-Citrine/WT)
196 induced the avoidance response under BL25 at 22°C (without cold treatment), and degree
197 of the avoidance response was dependent on Mpphot-Citrine expression levels (Fig. 3A,
198 B and C). The accumulation response at 22°C and cold-avoidance response at 5°C could

199 be induced in the overexpression line under BL5 (Fig. 3D, E), indicating that the
200 transitional intensity is reduced by overexpression of Mpphot-Citrine. Therefore, it is
201 conceivable that increase of the endogenous amount of Mpphot might play a role in
202 induction of the cold-avoidance response.
203
204 *3.4. Alteration of endogenous Mp*phot *is not related to the induction of the cold-*
205 *avoidance response*
206 To examine the role of Mpphot expression in the induction of the cold-
207 avoidance response, the endogenous amount of Mpphot was determined using
208 immunoblot analysis. In the 4-day-old gemmalings under BL25 at 22°C, the endogenous
209 amount of Mpphot slightly increased at 6 and 24 h (Fig. 4A). On the other hand, under
210 BL25 at 5°C, the endogenous amount of Mpphot was not changed at 1, 3 and 6 h, and
211 slightly increased at 24 h (Fig. 4A). Given that the cold-avoidance response under BL25
212 at 5°C was observed at 6 h (Fig. 2D), the Mpphot amount appears to be unrelated to the
213 induction of the cold-avoidance response. To confirm this result, we examined the
214 complementation lines of Mpphot-Citrine under the control of the CaMV35S promoter
215 with the genetic background of Mp*phot*^{KO} (35S::Mpphot-Citrine/Mp*phot*^{KO}), as
216 CaMV35S promoter activity is reported to be suppressed by cold treatment in tobacco
217 plants [22]. In transgenic *M. polymorpha* expressing Citrine under the control of the
218 CaMV35S promoter (35S::Citrine/WT), we also confirmed that the promoter activity is
219 slightly suppressed by 5°C treatment in *M. polymorpha* (Fig. 4B). In the complementation
220 lines (35S::Mpphot-Citrine/Mp*phot*^{KO}), the cold-avoidance response was clearly
221 observed (Fig. 4C) indicating that the induction of the cold-avoidance response does not
222 require for increase of Mpphot amount. Given that cold treatment increased the
223 autophosphorylation status of phot [6,23], we conclude that an increase of phot
224 autophosphorylation status decreases the transitional light intensity without alteration of
225 the endogenous amount of Mpphot.

226

227 **4. Discussion**

228 *4.1. The transitional light intensity between the accumulation and avoidance responses*
229 *can be changed*

230 Light-induced chloroplast movement is widely found in photosynthetic
231 organisms from algae to land plants [3]. The transitional light intensity between the
232 accumulation and avoidance responses differs among various plant species [24].
233 Furthermore, the transitional light intensity can be reduced in response to low temperature,
234 inducing the cold-avoidance response [4-6]. In this study, we analyzed light fluence rate
235 dependency of the cold-avoidance response. Under cold conditions, chloroplasts moved
236 in a light fluence rate-dependent manner (Fig. 2C, D), as occurs during the avoidance
237 response at a standard growth temperature [25]. This result supports the conclusion that
238 there is a cold-induced reduction of the transitional light intensity (Fig. 1A, B). Because
239 the cold-avoidance response has been found to moderate photodamage in *M. polymorpha*
240 [6], plasticity of the transitional light intensity is vital for plants exposed to cold stress. In
241 *A. thaliana*, the transitional light intensity is controlled by two types of phot: Atphot1 and
242 Atphot2. Atphot1 specifically regulates the accumulation response, while Atphot2
243 regulates both the accumulation and avoidance responses [7-9]. In this context, the
244 coordination mechanism of the transitional light intensity in *A. thaliana* appears to be
245 more complex than that in *M. polymorpha*. Further analysis of the transitional light
246 intensity in various plant species and genetic mutants may lead to better understanding of
247 the transition mechanism of chloroplast movements.

248

249 *4.2. Photoactivated phototropin amount changes the transitional light intensity*

250 Intracellular phot proteins are more frequently activated under strong light than
251 weak light conditions. Weak light irradiation at lower temperatures, prolongs the lifetime
252 of photoactivated phot [6], and, like the effect of strong light irradiation at a standard

253 growth temperature, results in the increase of photoactivated phot population. Therefore,
254 we concluded that the intracellular amount of photoactivated phot determines the
255 transition from the accumulation response to the avoidance response. Furthermore,
256 considering the effect of phot overexpression on the transitional light intensity (Fig. 3),
257 the transition mechanism maybe based on the absolute amount of photoactivated phot in
258 the cell rather than the ratio of active/inactive phot. How an increase in photoactivated
259 phot proteins triggers the switch from the accumulation to avoidance responses remains
260 unknown. Because phot autophosphorylation is also increased under strong-light/warm
261 or weak-light/cold conditions [6,10,23], we speculate that in order to induce the
262 avoidance response, an increase of photoactivated phot amount may change the phot
263 phosphorylation status, possibly via intermolecular phosphorylation activity [26,27].
264 Notably, the recruitment of phot to microdomains at the plasma membrane is involved in
265 phot autophosphorylation [28]. Further autophosphorylation analysis regarding the
266 spatial density of photoactivated phot may clarify the switching mechanism between the
267 accumulation and avoidance responses.
268
269 *4.3. Alteration of phot expression does not occur for induction of the cold-avoidance*
270 *response in* M. polymorpha
271 The cold-avoidance response is initiated by the temperature-dependent
272 biochemistry of the phot LOV2 domain [6]. However, in *A. thaliana*, alteration of the
273 transcript and protein levels of Atphot1 and Atphot2 in response to light [7,8,13-16] and
274 temperature [16] was reported. Notably, Łabuz et al. reported that lower temperature
275 decreases Atphot1 expression, and increases Atphot2 expression [16]. In this study, we
276 showed that the cold-avoidance response can be induced regardless of change in the
277 endogenous Mpphot amount (Fig. 4A) and even without its own promoter (Fig. 4B),
278 indicating that transcriptional regulation of Mpphot is not required for the cold-avoidance
279 response in *M. polymorpha*. Mpphot is a phot2-type protein, which regulates both the

accumulation and avoidance responses [17]. Consistent with Mpphot, Atphot2 expression in *A. thaliana* was reported to be unchanged at a temperature range from 4°C to 23°C [16]. Interestingly, cold treatment had a more significant impact on the decrease of Atphot1 expression than the increase of Atphot2 expression [16], implying that the expression of Atphot1 might be more sensitive to cold than that of Atphot2. The gene duplication of phot [29] and functional differentiation between phot1 and phot2 may have enabled land plants to have different transitional light intensities and chloroplast movements, allowing species to expand their range of habitat.

Author contributions

YK conceived and supervised the study; YF, YO and ST performed experiments; YF and YK wrote the manuscript.

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Disclosures

The authors declare no conflicts of interest.

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395 *Figure Legends*

396

397 **Fig. 1. Schematic diagram showing the transitional light intensity of the**
398 **accumulation and avoidance responses.** Solid vertical lines indicate points of the
399 transitional light intensity under warm (A; 22°C) and cold (B; 5°C) conditions.

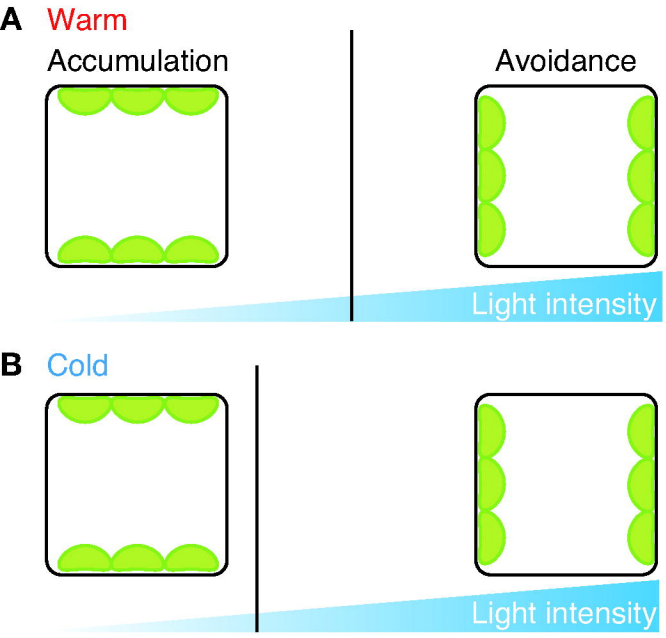
400

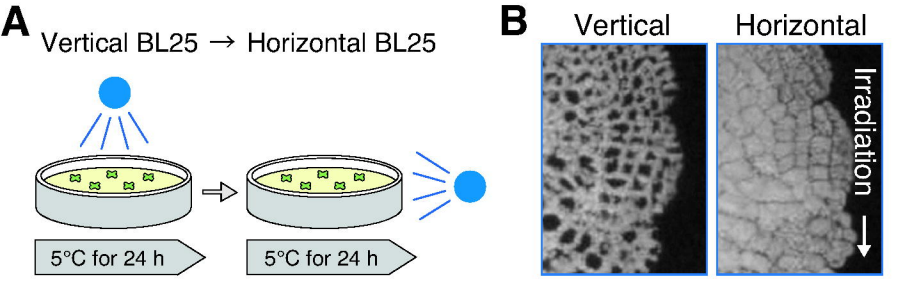
401 **Fig. 2. Cold-induced reduction of the transitional light intensity**. (A) Diagram of the
402 experimental conditions used to induce the cold-avoidance response with vertical or
403 horizontal irradiation of blue-light. After incubation under 25 μmol photons m^{-2} s^{-1} of
404 blue-light (BL25) with vertical irradiation at 5°C for 24 h, the WT gemmalings were
405 irradiated by horizontal BL25. (B) Chlorophyll fluorescence images after irradiation of
406 vertical (left) or horizontal (right) BL as shown in (A). The white arrow indicates the light
407 direction. (C) Chloroplast positioning in WT gemmalings incubated at 5°C for 12 h with
408 5, 10, 15, and 25 μmol photons m^{-2} s^{-1} of blue-light (BL5, BL10, BL15, and BL25,
409 respectively). (D) The P/A ratios, calculated from chlorophyll fluorescence images after
410 incubation for 0, 2, 6, 8, 12, and 24 h. Error bars indicate standard deviations. The
411 representative chlorophyll fluorescence images of WT gemmalings incubated for 12 h are
412 shown in (C). (E) Diagram of experimental conditions used to confirm cold-induced
413 reduction of the transitional intensity. After pre-incubation under BL25 at 5°C for 24 h,
414 WT gemmalings were transferred to BL5 or dark and incubated at 5°C for 24 h. (F)
415 Representative chlorophyll fluorescence images of WT gemmalings after pre-incubation
416 (BL25) and incubation under BL5 (from BL25 to BL5) or dark (from BL25 to Dark) as
417 shown in (E).

418

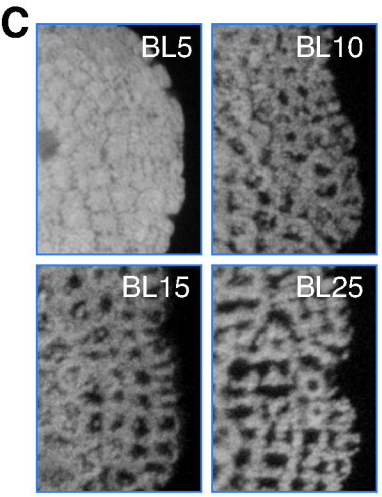
419 **Fig. 3. Overexpression of Mpphot-Citrine decreases the transitional light intensity.**
420 (A) Immunoblot analysis of WT and Mpphot-Citrine overexpression lines (35S::Mpphot-
421 Citrine/WT: OX1, OX2, and OX3) with anti-Mpphot antibody. White and black

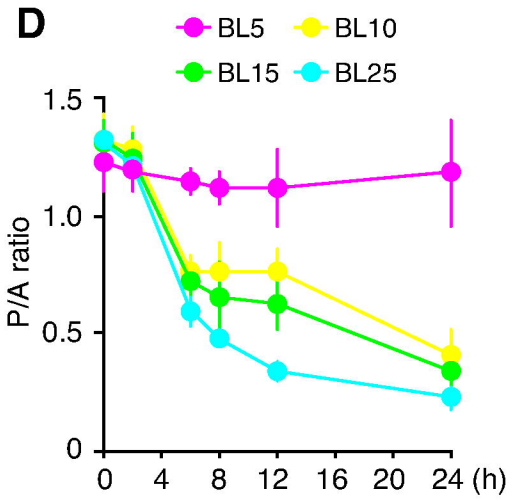
422 arrowheads indicate Mpphot-Citrine and Mpphot, respectively. Rubisco large-subunit
423 (RBCL) was shown as a loading control. (B) Representative images of chlorophyll
424 fluorescence in WT and Mpphot-Citrine overexpression lines. (C) The P/A ratios
425 calculated from the chlorophyll fluorescence images shown in (B) after the incubation
426 under BL25 at 22°C for 12 h. (D) Confirmation of the reduction of transitional light
427 intensity in OX2. In the 1-day-old gemmalings from OX2, the accumulation response
428 was induced under BL5 at 22°C (left panel). The cold-avoidance response was induced
429 under BL5 at 5°C for 24 h (right panel). (E) Time-course of the cold-avoidance response
430 induced under BL5 at 5°C in OX2. The P/A ratios were calculated from the chlorophyll
431 fluorescence images after incubation for 0, 6, 12, and 24 h.
432
433 **Fig. 4. Alteration of Mpphot expression is unrelated to the induction of the cold-**
434 **avoidance response.** (A) Immunoblot analysis of endogenous MpPHOT amount in WT
435 gemmaling, incubated under BL25 at 22°C or 5°C. The black arrowhead and asterisk
436 indicate MpPHOT and non-specific signal, respectively. Histone H3 protein is shown as
437 a loading control. (B) Immunoblot analysis of cold-induced repression of activity of
438 CaMV35S promoter. A transformant expressing Citrine under the control of CaMV35S
439 promoter (35S::Citrine/WT) was used. Coomassie Brilliant Blue (CBB)-stained Rubisco
440 large-subunit (RBCL) was used as a loading control. (C) Chlorophyll fluorescence images
441 showing the cold-avoidance response in complementation lines (35S::Mpphot-
442 Citrine/Mp*phot*^{KO}). After incubation under BL25 at 22°C, the gemmalings were
443 transferred to 5°C under the same light conditions.

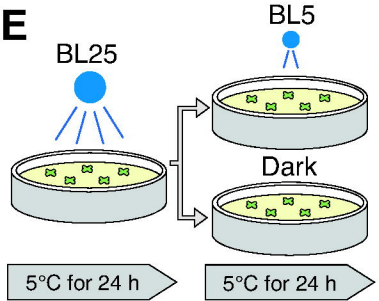

A
Warm
Accumulation
Avoidance
Light intensity
B
Cold
Light intensity

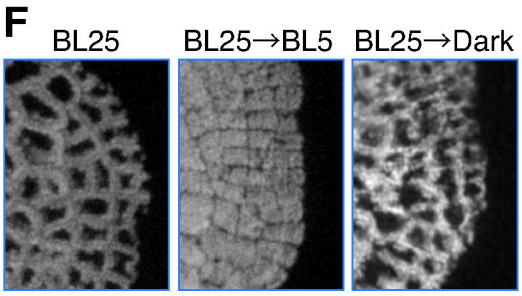

A
Vertical BL25 → Horizontal BL25
5°C for 24 h
5°C for 24 h

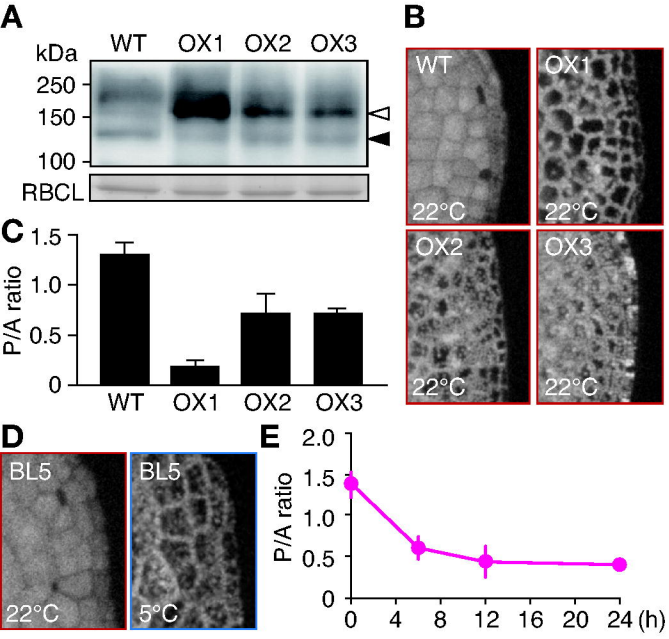
B
Vertical
Horizontal
Irradiation

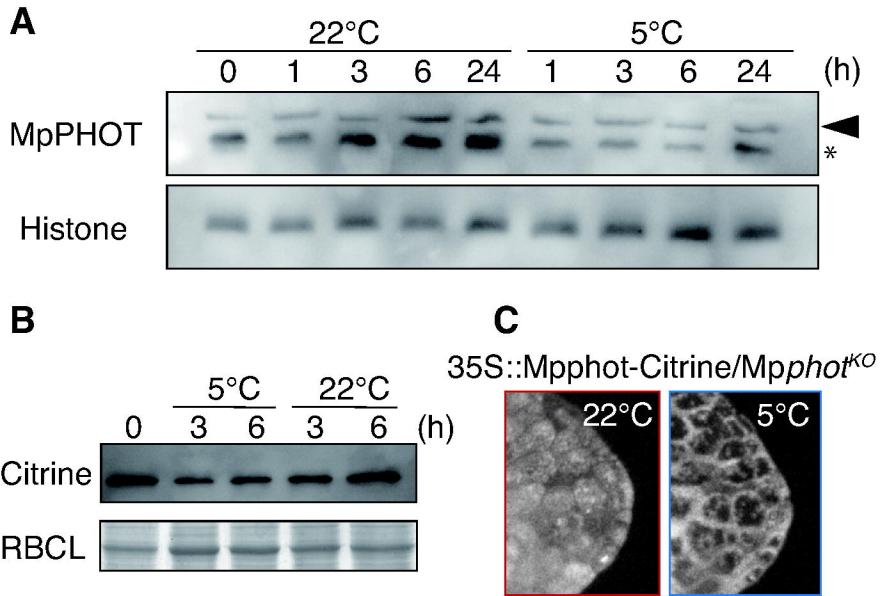

C
BL5
BL10
BL15
BL25


D
BL5
BL10
BL15
BL25
P/A ratio
1.5
1.0
0.5
0
0
4
8
12
16
20
24 (h)


E
BL25
BL5
Dark
5°C for 24 h
5°C for 24 h


F
BL25
BL25→BL5
BL25→Dark


A
kDa
WT
OX1
OX2
OX3
250
150
100
RBCL
B
WT
OX1
OX2
OX3
22°C
C
P/A ratio
1.5
1.0
0.5
0
D
BL5
22°C
5°C
E
2.0
0
4
8
12
16
20
24 (h)


A
22°C
5°C
0 1 3 6 24 1 3 6 24 (h)
MpPHOT
*
Histone
B
5°C 22°C
0 3 6 3 6 (h)
Citrine
RBCL
C
35S::Mpphot-Citrine/Mp*phot*^{KO}
22°C
5°C