Prediction of prospective leaf morphology in lettuce based on intracellular chloroplast position

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ABSTRACT

In the accumulation response, chloroplasts move toward weak blue light, staying at positions along the periclinal cell wall. By contrast, in the avoidance response, chloroplasts move away from strong blue light, escaping to positions along the anticlinal cell wall. The accumulation response maximizes light capture and the avoidance response reduces photodamage. The intracellular positioning of chloroplasts is important for optimizing photosynthesis and may have common signals with the regulators that determine leaf morphology, another factor that affects photosynthesis. Here, we propose that intracellular chloroplast position can be used to predict prospective leaf morphology in lettuce (*Lactuca sativa*). To test this, we induced the accumulation or avoidance response in lettuce cells through exposure to the appropriate strength of blue light and observed the growth of the plants. Our results indicated that leaf area increased in response to weak blue light inducing the accumulation response, and leaf thickness increased in response to strong blue light inducing the avoidance response.

Keywords

Accumulation response; Avoidance response, Blue light; Chloroplast position, *Lactuca sativa*, Leaf morphology; LED; Light-emitting diode; Plant factory; Red light.

28 **1. Introduction**

29 Optimizing photosynthesis is important for regulating plant size and improving
30 seed yield. For optimization of photosynthesis, the intracellular positioning of
31 chloroplasts changes in response to environmental conditions such as light and
32 temperature (Fujii and Kodama, 2018; Wada, 2013). For example, under strong light in
33 warm conditions, chloroplasts localize along the anticlinal cell wall by moving away
34 from the light, in a process termed the avoidance response (Wada, 2013). Similarly,
35 under weak light in cold conditions, chloroplasts localize along the anticlinal cell wall,
36 in a process termed the cold-avoidance response (Kodama et al., 2008; Ogasawara et al.,
37 2013). The avoidance and cold-avoidance responses may reduce photodamage caused
38 by chloroplast photoinhibition under warm and cold conditions, respectively (Fujii et al.,
39 2017; Kasahara et al., 2002). In addition, under weak light in warm conditions,
40 chloroplasts localize along the periclinal cell wall by moving towards the light, in a
41 process termed the accumulation response. Physiologically, the accumulation response
42 is believed to maximize photosynthesis, a theory that was recently confirmed
43 experimentally through comparisons between various *Arabidopsis thaliana* mutants that
44 show unusual chloroplast positioning (Gotoh et al., 2018). However, the effects of these
45 responses in regard to the growth of wild-type plants from economically important
46 species, such as vegetable crops, remained to be determined.

47 The chloroplast avoidance and accumulation responses are mediated by the
48 blue-light (BL) photoreceptor phototropin (Jarillo et al., 2001; Kagawa et al., 2001;
49 Sakai et al., 2001). Recently, we reported that phototropin is a thermosensory protein
50 that mediates the cold-avoidance response (Fujii et al., 2017). Therefore, in many plant
51 species, chloroplast positioning is altered by changes in BL intensity and in response to
52 changes in temperature. Under artificial conditions such as those in a plant growth
53 facility (or plant factory), chloroplast positioning should be influenced only by BL
54 intensity, because temperature is normally kept constant. In this study, to investigate the

effect of chloroplast positioning in plants under such conditions, such as vegetables grown in plant factories, we analyzed the effects on lettuce (*Lactuca sativa*) grown at a constant warm temperature and under strong and weak BL, which induce the avoidance and the accumulation responses, respectively.

2. Materials and methods

2.1. Plant materials and growth conditions

Lettuce (*Lactuca sativa*) seeds (No. 03503, Tohoku Seed Co. Ltd., Tochigi, Japan) were used. The seeds were planted in soil and cultivated under the tested light conditions. For growth test, the seedlings were grown under blue light-emitting diodes (LEDs) and red LEDs (ISL-150x150-H4RB, CCS Inc., Kyoto, Japan) lights in an incubator (IJ101, Yamato Scientific Co., Ltd., Tokyo, Japan) at 20°C for 21 days.

2.2. Observation of chloroplast positioning

For chloroplast observations, the seedlings were grown for 18 days under white light from fluorescent tubes in a growth cabinet (LH-240SP, Nippon Medical & Chemical Instruments Co., Ltd., Osaka, Japan) and then transferred to weak or strong BL (5 or 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively). To make it possible to clearly observe the mesophyll cells, a detached lettuce leaf was deaerated and permeated with water. Mesophyll cells were observed by light microscopy (BX60; Olympus, Tokyo, Japan) under a 100 $\times$ oil-immersion objective (UPlanApo 100 $\times$ /1.35 oil). The images were captured using a digital camera (DP72; Olympus) with cellSens software (Olympus).

2.3. *Measurements of leaf area, thickness, and biomass*

To measure leaf area, lettuce leaves were sandwiched in a clear plastic folder to flatten them, and images were captured using a scanner (imageRUNNER ADVANCE C5045, Canon Inc., Tokyo, Japan). From the images, leaf area was measured by ImageJ (Schneider et al., 2012), and the means and standard deviations were calculated. To measure leaf thickness, a digital caliper (100, MonotaRO Co., Ltd., Hyogo, Japan) was used, and the means and standard deviations were calculated. To measure biomass, the harvested aboveground biomass of the lettuce was measured as the fresh weight, and then oven-dried at 105°C for measurement of the dry weight. The means and standard deviations of the weights were calculated.

3. **Results and discussion**

To optically control chloroplast positions in lettuce cells, we used 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ as weak BL (BL5) and 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ as strong BL (BL50) and compared lettuce growth under the two conditions. When we irradiated lettuce cells with BL5 for 3 h, chloroplasts localized along the cell periphery at the top side of mesophyll cells and accumulated along the periclinal cell wall at the bottom side of the cell (Fig. 1A). This BL5-induced chloroplast positioning in lettuce cells is similar to that induced by weak light in plant species that grow under strong sunlight (Higa and Wada, 2016; Ishishita et al., 2016). When we irradiated lettuce cells with BL50 for 3 h, chloroplasts escaped from the periclinal position (Fig. 1B). In this study, BL5 and BL50 are referred to as the accumulation and the avoidance conditions, respectively.

For longer-term culture of lettuce plants, we added red light, 125 or 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (RL125 or RL250, respectively), to BL5 and BL50 to provide sufficient light energy for photosynthesis. Lettuce seedlings were incubated under the indicated BL and RL

109 conditions at 20°C for 3 weeks. When combined with either RL125 or RL250, the BL5
 110 seemed to enhance lettuce growth compared with BL50 (Fig. 2). To assess this
 111 quantitatively, we measured leaf area, thickness, and biomass. In combination with
 112 either RL125 or RL250, BL5 resulted in significantly larger leaf area compared with
 113 BL50 (Fig. 3A and B). However, the leaf thickness was significantly greater under
 114 BL50 than under BL5 (Fig. 3C and D). The results suggest that BL5 enhances leaf area
 115 and BL50 enhances leaf thickness. We then measured the fresh and dry weights of the
 116 aboveground biomass and found that fresh weight under BL5, in combination with
 117 either RL125 or RL250, was significantly higher than that under BL50 (Fig. 4A and B).
 118 Unexpectedly, however, dry weight under BL5 was comparable to that under BL50 (Fig.
 119 4C and D), indicating that the biomass production was the same.

120 In a previous study using *A. thaliana*, leaf area and biomass (fresh and dry
 121 weights) increased in conditions that induced the accumulation response (Gotoh et al.,
 122 2018). In the present study with lettuce, however, we found that the dry weight, and
 123 hence the biomass, was comparable under the accumulation (BL5) and avoidance
 124 (BL50) conditions (Fig. 4A–D), which is partially inconsistent with that report (Gotoh
 125 et al., 2018). It may be relevant that in our experiments we compared two physiological
 126 conditions (BL5 and BL50), but Gotoh et al. compared genetically distinct groups
 127 (wild-type plants and plants with mutations related to chloroplast positioning) (Gotoh et
 128 al., 2018). Further study will be needed to determine whether and how this difference in
 129 experimental design might have caused the inconsistency.

130 When we compared the fresh weights of plants grown under the two BL conditions,
 131 the weight for the plants grown under BL5 was much higher than that for the plants
 132 grown under BL50 (Fig. 4A and B). Given that the dry weights under the two
 133 conditions were comparable (Fig. 4C and D), this implied that lettuce grown under BL5
 134 had a higher water content. Because higher BL intensity increases the degree of
 135 stomatal opening (Kinoshita et al., 2001), we suspected that the BL50 condition might

136 result in more open stomata, resulting in greater transpiration from stomata and thus
137 lower water content.

138 Although the leaf morphology was very different between the accumulation (BL5)
139 and avoidance (BL50) conditions, the dry weights were comparable (Fig. 4C and D).
140 Plant photosynthetic production under the two conditions thus seems to be same,
141 suggesting that some form of compensatory growth occurs in lettuces under BL5 as
142 compared to the BL50 conditions (namely, homeostasis). This compensatory growth
143 may be based on varying strategies for leaf light capture during lettuce growth. In the
144 BL5 accumulation conditions, because chloroplasts localize along the periclinal cell
145 wall of the uppermost palisade cells, light penetration into deeper cells within the leaf
146 could be expected to be inhibited (Gotoh et al., 2018). To efficiently capture light so as
147 to maximize photosynthesis during growth, leaf area might be increased, or possibly
148 leaf thickness might be decreased. By contrast, in the BL50 avoidance conditions,
149 chloroplasts localize along the anticlinal cell wall of the uppermost cells. Because the
150 light can penetrate into cells deeper in the leaf (Gotoh et al., 2018), leaf thickness might
151 be increased to efficiently capture light, or leaf area might be decreased. Lettuce may
152 thus have a regulatory mechanism to compensate for variations in chloroplast
153 positioning.

154 Based on our observations, we propose that chloroplast position responds to a
155 signal that also controls leaf morphology of lettuce. The relationship could also be
156 direct—that is, the accumulation and the avoidance responses could serve as signals to
157 enhance leaf area and leaf thickness, respectively. Based on these observations, we can
158 predict prospective leaf morphology of lettuce by observing chloroplast position and we
159 can modulate leaf morphology by altering light conditions. Given that chloroplast
160 position can be changed by BL in many plant species, and that leaf morphology is
161 important for many vegetable food crops, this control strategy may be employed for
162 many economically important plants in artificial growth conditions.

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219 *Figure legends*

220

221 **Fig. 1.** Optical control of chloroplast positioning in lettuce cells.

222 (A, B) Intracellular chloroplast position in the accumulation response and the avoidance
223 response in lettuce under BL5 (A) and BL50 (B), respectively.

224

225 **Fig. 2.** Lettuces cultivated under accumulation (BL5) and avoidance (BL50) conditions.

226 (A, B) Photographs of lettuces cultivated under BL5 (A) or BL50 (B) in combination
227 with RL125. (C, D) Photographs of lettuces cultivated under BL5 (C) or BL50 (D) in
228 combination with RL250. White bars indicate 10 cm.

229

230 **Fig. 3.** Leaf area and leaf thickness of lettuces cultivated under accumulation (BL5) and
231 avoidance (BL50) conditions.

232 (A, B) Comparison of leaf area in the BL5 and BL50 conditions in combination with
233 RL125 (A) or RL250 (B). Bars indicates standard deviations. (C, D) Comparison of leaf
234 thickness between the BL5 and BL50 conditions in combination with RL125 (C) or
235 RL250 (D). In all panels, asterisks indicate statically significant differences between
236 conditions (Student's *t* test, $P < 0.01$).

237

238 **Fig. 4.** Fresh and dry weights of lettuces cultivated under the accumulation (BL5) and
239 avoidance (BL50) conditions.

240 (A, B) Comparison of fresh weights of leaves between the BL5 and BL50 conditions in
241 combination with RL125 (A) or RL250 (B). Asterisks indicate statically significant
242 differences (Student's *t*-test, $P < 0.01$). (C, D) Comparison of dry weight between the
243 BL5 and BL50 conditions in combination with RL125 (C) and RL250 (D). Student's *t*
244 test indicated no significant difference between the two samples ($P = 0.86$ in C and $P =$
245 0.63 in D).

Fig. 1

Fig. 2

Fig. 3

Fig. 4