Refinements to light sources used to analyze the chloroplast cold-avoidance response over the past century

Yuta Fujii and Yutaka Kodama
Center for Bioscience Research and Education, Utsunomiya University, Tochigi, Japan

ABSTRACT
Chloroplasts alter their subcellular positions in response to ambient light and temperature conditions. This well-characterized light-induced response, which was first described nearly 100 years ago, is regulated by the blue-light photoreceptor, phototropin. By contrast, the molecular mechanism of low temperature-induced chloroplast relocation (i.e., the cold-avoidance response) was unexplored until its discovery in the fern Adiantum capillus-veneris in 2008. Because this response is also regulated by phototropin, it was thought to occur in a blue light-dependent manner. However, until recently, the blue light dependency of this response could not be examined due to the lack of a stable light source under cold conditions. We recently refined the light source to precisely control light intensity under cold conditions. Using this light source, we observed the blue light dependency of the cold-avoidance response in the liverwort Marchantia polymorpha and the phototropin-2-mediated cold-avoidance response in the flowering plant Arabidopsis thaliana. Thus, this mechanism is evolutionarily conserved among land plants.

Light sources used to induce the cold-avoidance response
Senn 1908—Chloroplast relocation movement was investigated in the field or laboratory using sunlight as the light source.1,2 Because no appropriate light source that emits stable light intensity was available at that time, it was challenging to control experimental light conditions.

Kodama et al. 2008—We employed white light fluorescent tubes (FL10W, Matsushita Electric Industrial Co. Ltd.) and fluorescent
Figure 1. Schematic illustration of chloroplast relocation movements. Under warm conditions (e.g., 20–25°C), weak light induces the accumulation response, in which chloroplasts localize along the periclinal cell walls (left), whereas strong light induces the avoidance response, in which chloroplasts localize along the anticlinal cell walls (middle). Under cold conditions (e.g., 5°C), weak light induces the cold-avoidance response, in which chloroplasts localize along the periclinal cell walls (right).

Blue-light dependency of the cold-avoidance response

Because Dr. Senn did not analyze the BL dependency of the cold-avoidance response, we will only describe our two previous studies.5,6 In our previous study using A. capillus-veneris, we analyzed the BL dependency of the cold-avoidance response by performing time-lapse observations under a microscope with a white fluorescent bulb.5 The microscope was placed in a cold room at 4°C, and we successfully observed the white light-induced cold-avoidance response by microscopy. However, unlike the observations under white light, the cold-avoidance response was only partially induced under BL; some chloroplasts moved back to the cell surface during the cold-avoidance response. The difference appeared to be caused by the unstable, low levels of BL obtained from white fluorescent light bulbs covered with blue plastic film.

By contrast, the use of an LED light source clearly revealed the BL dependency of the cold-avoidance response in the liverwort Marchantia polymorpha.6 Because 70–140 μmol photons m⁻² s⁻¹ of white LED light could induce the cold-avoidance response in M. polymorpha,6 we passed approximately 350 μmol photons m⁻² s⁻¹ of white light through blue plastic film to obtain a sufficient intensity of BL. As observed under white-light conditions, chloroplasts relocated from the periclinal wall to the anticlinal wall under BL conditions.6 Thus, the BL dependency of the cold-avoidance response was successfully observed due to the precise control of BL intensity using LED light.

The cold-avoidance response in angiosperms

Previous studies by Dr. Senn and our laboratory suggested that angiosperms do not exhibit the cold-avoidance response.1,5 However, in these studies, the light intensity could not be controlled precisely under low temperature conditions. Recently, Łabuz et al. reported that cold treatment increased the light-induced avoidance response in the angiosperm A. thaliana,8 a response we considered to be similar to the cold-avoidance response in A. capillus-veneris and M. polymorpha. We employed LEDs and successfully induced the cold-avoidance response in A. thaliana;6 our temperature-regulated microscope equipped with LEDs6,9 clearly captured the cold-avoidance response in A. thaliana.6

Using this microscopy system, we also found that the cold-avoidance response in A. thaliana is dependent on phototropin2. Note that A. thaliana has two types of phototropin (phot1 and phot2) that play overlapping roles in light-induced chloroplast movement,3,4 phot1 only mediates the accumulation response, whereas phot2 mediates both the accumulation and avoidance responses. In A. thaliana, the cold-avoidance response was observed in the phot1-5 mutant, but not in the phot2-1 mutant,6 suggesting that phot2, but not phot1, mediates the cold-avoidance response in A. thaliana.6 The phot2-mediated cold-avoidance response was also observed in A. capillus-veneris,5 and the phototropin encoded by a single gene in M. polymorpha was reported to be a phot2-type protein.10 Furthermore, the cold-avoidance response via thermosensation of phot2-type phototropin was observed in a liverwort (M. polymorpha), fern (A. capillus-veneris), and angiosperm (A. thaliana),7 suggesting that the underlying molecular mechanism is evolutionarily conserved in many land plants.
Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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