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

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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 phototropin2-mediated cold-avoidance response in the flowering plant *Arabidopsis thaliana*. Thus, this mechanism is evolutionarily conserved among land plants.

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Chloroplasts alter their subcellular localization in response to environmental stimuli such as light and temperature. This response, known as chloroplast relocation movement, was thoroughly characterized by Gustav Senn in 1908.[1,2] He performed detailed experiments using handmade equipment and identified various types of chloroplast relocation movement in algae, and in aquatic and terrestrial plants.[1] Many other researchers have focused on light-induced chloroplast movement; chloroplasts move toward weak light (accumulation response), but away from strong light (avoidance response) (Fig. 1). In the early 21st century, phototropin was identified as a blue-light (BL) photoreceptor that regulates light-induced responses.[3,4] In many species, ranging from algae to flowering plants, light-induced responses occur in a BL-dependent manner and are mediated by phototropin.

In 1908, Gustav Senn also reported that low temperature treatment alters the subcellular distribution of chloroplasts in the moss *Funaria hygrometrica*,[1] but he did not observe any changes in angiosperms (flowering plants) such as *Oxalis acetosella*. In 2008, we observed the same response (named the cold-positioning response or cold-avoidance response) in the fern *Adiantum capillus-veneris*; chloroplasts accumulated along the periclinal cell wall under weak white light at 25°C, whereas they relocated to the anticlinal cell wall after a temperature shift to 4°C,[5] Fig. 1). Similar to Senn's observations,[1] we did not observe the cold-avoidance response in the angiosperm *Arabidopsis thaliana*.[5] In addition to these consistent observations, we discovered that the degree of the cold-avoidance response in *A. capillus-veneris* is enhanced by higher levels of weak white light. Interestingly, like the strong light avoidance response, the cold-avoidance response is also mediated by phototropin.[5] Thus, we speculated that the cold-avoidance response is regulated in a BL-dependent manner. However, because the cold-avoidance response in *A. capillus-veneris* was partially induced under BL conditions, it was unclear whether the BL dependency of the cold-avoidance response is indeed dependent on BL.[5] In our recent study, we refined the light source used for irradiation and successfully identified the BL dependency of the cold-avoidance response in the liverwort *Marchantia polymorpha*.[6] Furthermore, the use of a refined light source allowed us to observe the cold-avoidance response in the angiosperm *A. thaliana*.[6] Here, we describe the refinements made to the light source over the past 100 years to analyze the cold-avoidance response and to examine the evolutionary conservation of the cold-avoidance response in many 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

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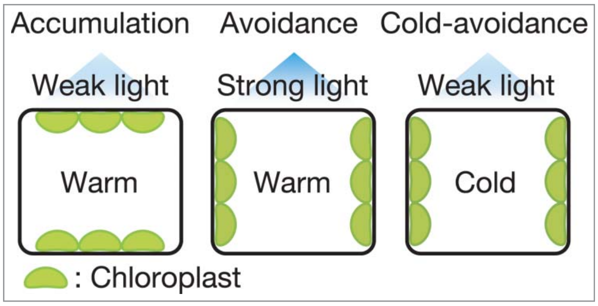

Figure 1. Schematic illustration of chloroplast relocation movements. Under warm conditions (e.g., 20–25°C), weak light induces the accumulation response, in which chloroplasts localize along the periclinal cell walls (left), whereas strong light induces the avoidance response, in which chloroplasts 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).

bulbs (EFA15EN/12-R, Toshiba Lighting & Technology) in our experiments.[5] The maximum intensity of the fluorescent light was approximately 80 μmol photons m^{-2} s^{-1} at 25°C. However, the intensity of the fluorescent light might be unstable under cold conditions due to fluctuations in gas pressure inside the tube. To isolate BL from white light, we used a blue plastic film (No. 72, Tokyo Butai Showmei, Tokyo), but the BL intensity was reduced by approx. 38% in the blue region (peak at 450 nm) as it passed through the plastic film (Fig. 2). Thus, we were unable to precisely control the light intensity used to induce the cold-avoidance response.

Fujii et al. 2017—To produce a stable light source at an intensity strong enough to induce the cold-avoidance response, we employed light-emitting diodes (LEDs) (OptoSupply, Hong Kong) and a direct-current stabilized power supply (AD-8723D, A&D Company).[6,7] The use of stable light from white LEDs enabled us to accurately analyze the cold-avoidance response.[6,7] To produce BL, we isolated BL from white LED light by passing the light through blue plastic film (No. 72, Tokyo Butai Showmei, Tokyo)[6]; alternatively, we used blue LEDs (OptoSupply, Hong Kong).[6]

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 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[5]. 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.[5] 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.

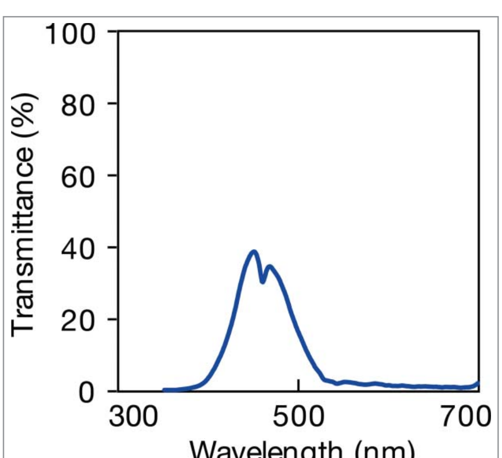

Figure 2. Transmittance of the blue plastic film used to produce the blue light source (No. 72, Tokyo Butai Showmei, Tokyo). The spectrum (350–700 nm) was obtained using NanoVue (GE Healthcare).

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 LEDs[6,9] 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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