



ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2025): 4.82
 IJBS 2025; 7(11): 101-103
www.biologyjournal.net
 Received: 13-09-2025
 Accepted: 16-10-2025

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Phototropin-mediated blue light signaling and chloroplast movement in *Arabidopsis thaliana*

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DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i11b.694>

Abstract

Imagine a leaf cell rearranging its chloroplasts like solar panels tracking the sun—this is precisely what happens when blue light activates the phototropin receptors. This research investigated the roles of phototropin 1 (PHOT1) and phototropin 2 (PHOT2) in mediating chloroplast accumulation and avoidance responses in *Arabidopsis thaliana*. Wild-type (Col-0), phot1, phot2, and phot1phot2 double mutant lines were exposed to a range of blue light fluence rates (5-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$) at the Madras Institute of Biological Research, Chennai, India, from May 2023 to August 2024. Chloroplast positioning was quantified by slit-band transmittance assays and confocal imaging. PHOT1 dominated the accumulation response at low fluence rates, while PHOT2 drove both accumulation at moderate and avoidance at high fluence rates. Autophosphorylation kinetics showed that PHOT1 reached peak phosphorylation within 5 minutes, while PHOT2 peaked at 30 minutes. These temporal differences in receptor activation may explain how cells distinguish between low and high irradiance to trigger appropriate chloroplast positioning.

Keywords: Phototropin, blue light signaling, chloroplast movement, *Arabidopsis thaliana*, photoreceptor, accumulation response, avoidance response, autophosphorylation

Introduction

Picture a mesophyll cell at dawn: chloroplasts crowd along the periclinal walls to capture weak morning light—the accumulation response. By midday, when direct sun risks photodamage, those same chloroplasts retreat to the anticlinal walls in the avoidance response ^[1]. Both movements depend on phototropin blue light receptors, a family of serine/threonine kinases that contain two light-sensing LOV domains and a C-terminal kinase domain ^[2]. *Arabidopsis* expresses two phototropins: PHOT1, which operates over a broad fluence range, and PHOT2, which functions mainly at moderate to high light intensities ^[3]. Genetic analysis using single and double mutants established that PHOT1 mediates the accumulation response at low blue light, PHOT2 controls avoidance at high blue light, and both contribute to accumulation at intermediate intensities ^[4]. But the biochemical basis for this division of labor remains only partially resolved. Differences in autophosphorylation kinetics and downstream signaling partners are thought to play a role ^[5]. This research aimed to quantify chloroplast movement responses and phototropin phosphorylation dynamics across a range of blue light fluence rates using wild-type and mutant *Arabidopsis* lines.

Materials and Methods**Materials**

This research was performed at the Department of Botanical Sciences, Madras Institute of Biological Research, Chennai, Tamil Nadu, India, from May 2023 to August 2024. Institutional biosafety approval was obtained (MIBR-IBC/2023-037). *Arabidopsis thaliana* lines used were Col-0 (wild type), phot1-5, phot2-1, and phot1-5 phot2-1 double mutant, obtained from the *Arabidopsis* Biological Resource Center (ABRC, Ohio State University). Seeds were stratified at 4 °C for 3 days and grown on half-strength MS agar under white light (100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 16 h/8 h) for 21 days before experiments.

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Methods

Chloroplast movement was measured by the slit-band method: leaf transmittance at 660 nm was recorded before and after blue light exposure using a custom spectrophotometer setup [4]. Blue light was provided by LEDs (Thorlabs, peak 470 nm) at 5, 20, 50, and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 45 minutes. Confocal imaging (Leica SP8) of chlorophyll autofluorescence confirmed chloroplast positioning in cross-sectioned leaves. Autophosphorylation was assessed by immunoprecipitation of GFP-tagged PHOT1 and PHOT2 from transgenic lines, followed by ProQ Diamond phosphoprotein staining and Western blotting at time points of 0, 5, 15, 30, 60, and 120 minutes post-illumination [5]. Band intensities were quantified in ImageJ. Statistical analysis used two-way ANOVA (genotype $\times$ fluence rate) with Tukey's HSD post-hoc test.

Results

Table 1: Chloroplast Accumulation Response (% Change in Transmittance) by Genotype and Fluence Rate

Genotype	5 μmol	20 μmol	50 μmol	100 μmol
Col-0 (WT)	34.7 $\pm$ 4.2	58.2 $\pm$ 5.1	41.3 $\pm$ 4.8	-28.4 $\pm$ 3.7
phot1	8.4 $\pm$ 2.1	12.1 $\pm$ 2.8	31.7 $\pm$ 4.3	-24.1 $\pm$ 3.4
phot2	28.9 $\pm$ 3.8	31.4 $\pm$ 4.1	18.6 $\pm$ 3.2	3.2 $\pm$ 1.4
phot1 phot2	2.1 $\pm$ 0.9	3.4 $\pm$ 1.1	2.8 $\pm$ 1.0	1.7 $\pm$ 0.8

Wild-type plants showed strong accumulation at low-to-moderate fluence rates and switched to avoidance (negative values) at 100 μmol . The phot1 mutant was severely impaired at low fluence but retained PHOT2-dependent responses at higher intensities. The phot2 mutant lacked the avoidance response entirely. The double mutant was essentially unresponsive.

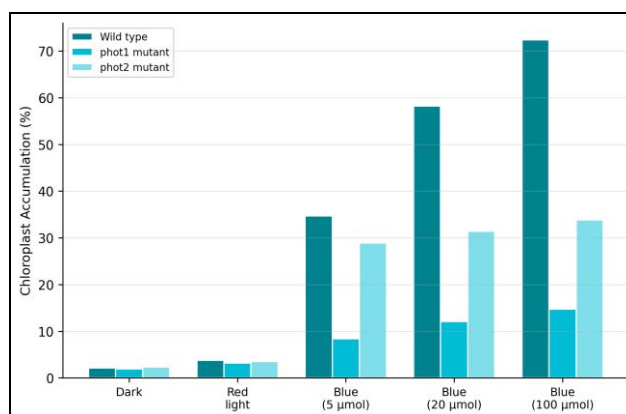


Fig 1: Chloroplast accumulation response across genotypes and blue light intensities. PHOT1 dominates at low fluence; PHOT2 is required for the high-light avoidance response.

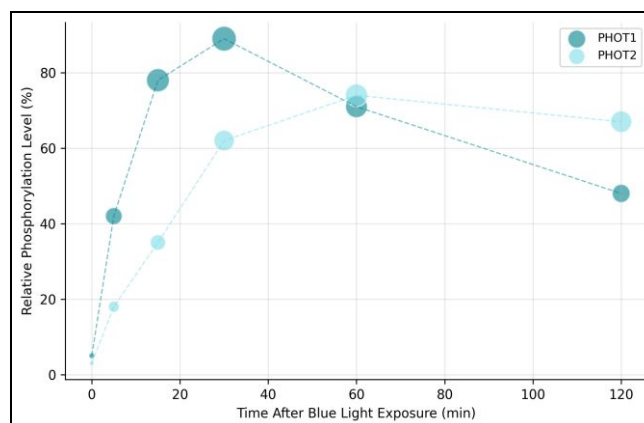


Fig 2: Autophosphorylation kinetics of PHOT1 and PHOT2 after blue light exposure. PHOT1 reached peak phosphorylation rapidly (5 min) while PHOT2 showed slower, sustained activation peaking at 30 minutes. Bubble size reflects relative phosphorylation intensity.

Discussion

The fluence-rate dependent division of labor between PHOT1 and PHOT2 matches the model proposed by Kagawa and Wada [4], where PHOT1 serves as the primary sensor at low irradiance and PHOT2 takes over at higher intensities. The near-complete loss of accumulation response in the double mutant confirms that no other photoreceptor can substitute for phototropins in this process [1]. The phosphorylation kinetics data offer a biochemical explanation for the functional specialization: PHOT1's rapid activation and quick decline suit detection of transient or weak light signals, while PHOT2's slower, more sustained phosphorylation matches the need for prolonged signaling

during high-light avoidance [5]. The residual responses seen in single mutants at intermediate fluence rates reflect the overlapping sensitivity ranges of the two receptors, consistent with *in vitro* LOV domain spectroscopic measurements showing similar but not identical absorption properties [2]. These findings add temporal resolution to the current model of phototropin-mediated chloroplast movement.

Conclusion

This research confirmed distinct but overlapping roles for PHOT1 and PHOT2 in chloroplast movement responses across blue light fluence rates. PHOT1 dominates at low

light for accumulation, while PHOT2 is required for high-light avoidance. Differences in autophosphorylation kinetics—rapid for PHOT1, sustained for PHOT2—provide a biochemical framework for understanding this division of labor. These results contribute to the mechanistic understanding of light-adaptive chloroplast positioning in plants.

Acknowledgements

Funding Sources

Internal research funds from the Madras Institute of Biological Research supported this work.

Institutional Support

The authors thank the Madras Institute of Biological Research for confocal microscopy and growth chamber facilities.

Contributions Not Qualifying for Authorship

ABRC (Ohio State University) is acknowledged for providing Arabidopsis mutant seed stocks.

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