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ENGINEERING A NEAR-INFRARED LIGHT-INDUCIBLE GENE EXPRESSION SYSTEM FOR PLANTS USING BPHP1-QPAS1 AND LOV DOMAINS *

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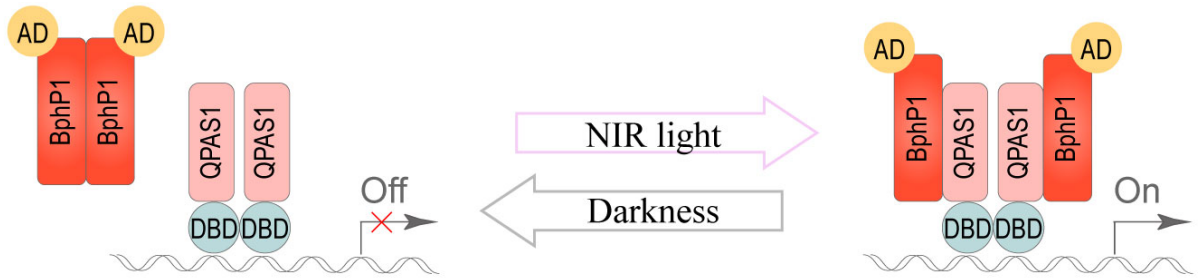
Abstract

The application of optogenetics in plants is limited by their requirement for white-light cultivation, which causes nonspecific activation of light-sensitive proteins [1]. Our proposed solution combines the existing bacterial BphP1-QPAS1 system with additional light-sensitive components and signal peptides to overcome these limitations.

The BphP1-QPAS1 system operates through interaction between the bacterial phytochrome BphP1 and its truncated partner QPAS1 [2]. Heterodimer formation under near-infrared (NIR) light (780 nm) brings together DNA-binding (DBD) and activation (AD) domains, triggering target gene expression (see figure). Although first validated in mammalian cells, the BphP1-QPAS1 system is particularly suitable for plants as its activation wavelength avoids interference with endogenous photoreceptor pathways [3].



A schematic representation of the BphP1-QPAS1 activation

To prevent undesired white-light-induced activity, we fused QPAS1 to the blue-light-sensitive (460–480 nm) LOV domain protein *As*LOV2 [4, 5] and incorporated either nuclear export signals or the RRRG degron.

All constructs were evaluated in *Nicotiana benthamiana* leaves via agroinfiltration, with pEGFP reporter expression assessed by fluorescence microscopy. The desired optogenetic profile, strong pEGFP induction under NIR light with no detectable activation under white light or darkness, was achieved with the *As*LOV2-RRRG degron variant. Functionally, white-light exposure triggered unfolding of the *As*LOV2 C-terminal Jα helix, exposing the fused RRRG degron and subsequently targeting the chimeric protein for degradation. In contrast, NIR illumination maintained the degron in its buried conformation, permitting unimpeded system activation and robust pEGFP expression. This demonstrates that light-inducible degradation is more effective than nuclear export for preventing leakage. While currently validated in *N. benthamiana*, our BphP1-QPAS1/*As*LOV2-RRRG degron system holds promise for both agricultural and fundamental applications. In crop engineering, this light-controlled platform could enable chemical-free regulation of stress resistance or yield-related traits through targeted gene expression. For plant research, integrating optogenetics with CRISPR/Cas9 may allow precise spatiotemporal control of gene editing, facilitating functional studies in specific tissues or developmental stages. Further optimization could expand this technology to staple crops and field conditions, supporting sustainable agriculture with reduced environmental impact.

References

1. Braguy J., Zurbriggen M. D. Synthetic strategies for plant signalling studies: molecular toolbox and orthogonal platforms // The Plant Journal. 2016. Vol. 87, Iss. 1. P. 118–138.

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2. Redchuk T.A. et al. Near-infrared optogenetic pair for protein regulation and spectral multiplexing // Nature Chemical Biology. 2017. Vol. 13, Iss. 6. P. 633–639.

3. Johnson M.P. Correction: Photosynthesis // Essays in Biochemistry. 2017. Vol. 61, Iss. 4. P. 429.

4. Kaya S.G., Hovan A., Fraaije M.W. Engineering of LOV-domains for their use as protein tags // Archives of Biochemistry and Biophysics. 2025. Vol. 763. P. 110228.

5. Kim C. et al. Structural dynamics of protein-protein association involved in the light-induced transition of Avena sativa LOV2 protein // Nature Communications. 2024. Vol. 15, Iss. 1. P. 6991.