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OPTOGENETICS 2.0 IN PLANT SYSTEMS: EVOLUTION, APPLICATIONS, AND CHALLENGES FOR PRECISION CROP IMPROVEMENT

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ABSTRACT
Optogenetics has revolutionized biological research by providing precise spatial and temporal control over the cellular processes through the use of light-sensitive proteins. Although initially it was developed for neuroscience applications, this technology is now rapidly advancing plant biology. However, plants pose unique challenges for optogenetic approaches due to their reliance on light for photosynthesis and the presence of natural photoreceptors that could interfere with synthetic systems. Despite these challenges, significant progress has been made in adapting optogenetic tools for plant research. Researchers have utilized naturally occurring light-sensitive proteins such as phytochromes and cryptochromes to design synthetic switches and transcriptional regulators. These systems enable precise regulation of gene expression and signaling pathways in response to specific light wavelengths. Additionally, advances in synthetic biology have facilitated the development of optogenetic tools that are orthogonal to plant endogenous photoreceptors, minimizing potential interference and enhancing the specificity of the system. Such novel optogenetic tools include PULSE (Plant Usable Light-switch Elements) system, BLINK1 (Blue Light-induced K^+ Channel) system, Highlighter system (based on cyanobacterial CcaS-CcaR pathway) and GtACR1 (based on anion channelrhodopsins). These approaches allow precise and high-resolution control of gene expression in plants in response to light. It functions effectively in a typical light environment by utilizing naturally occurring chromophores. This breakthrough offers a powerful tool for advancing plant biology and holds great potential for improving crop traits and agricultural practices.

KEYWORDS: Optogenetics, Photoreceptors, PULSE System, BLINK1 System, Highlighter, GtACR.

INTRODUCTION

Optogenetics is the use of light to manipulate biological processes at high quantitative and spatiotemporal resolutions in a reversible way with minimal negative consequences. It controls and analyzes biological processes using light-responsive proteins. It was initially developed to investigate neural circuits. Combining optics, genetics, and bioengineering, optogenetics uses light (opto) to activate light-sensitive proteins that are heterologously expressed in order to control cellular activity. To gain accurate insights into biological processes, particular non-invasive manipulations of cellular functioning are desired. The most popular techniques for studying biological processes are genetic experiments that manipulate gene activity or chemical treatments that affect cellular functioning. However, in areas like studying the selectivity or penetration rate of the applied chemicals, pleiotropic effects of genetic change, or temporal gaps between genetic manipulation and analysis, both techniques have limited spatiotemporal precision and specificity. As a result, it may become challenging to assess the effects on the processes under study. By utilizing genetically encoded light-sensitive proteins found in a variety of organisms and minimally invasive optical methods that allow for a high spatiotemporal resolution, optogenetics can overcome these challenges. This enables the direct and controlled modification of biological activities at the subcellular level as well as in tissues and cell types. By fusing proteins of interest (POI) to light-sensitive dimerizing proteins, optogenetics can regulate the activity of POIs or direct them to a particular subcellular region. This review focuses on the evolution, different systems of optogenetics, their applications and challenges relevant to plant science.

In 1979, Richard Fork was the first to utilize light to stimulate mouse neurons. Rafael Yuste later showed how to activate neurons in mice with intact tissue using a laser light. However, the first genetically targeted technique to

control genetically sensitive neurons was created in 2002 by Boris Zemelman (Zemelman et al., 2002). Later on, Karl Deisseroth provided the first example of a channelrhodopsin-based single-component optogenetic system in 2005 (Boyden et al., 2005). Starting with light-regulated gene expression systems (Müller et al., 2014; Ochoa-Fernandez et al., 2016; Ochoa-Fernandez et al., 2020; Chatelle et al., 2018) and a synthetic light-gated potassium channel (Cosentino et al., 2015; Papanatsiou et al., 2019) various optogenetic techniques have been used in plant science till this time. The method utilizes naturally occurring or artificially created proteins that can undergo conformational changes by absorbing light energy. The first article shows the characterization of channelrhodopsin-1 (ChR1) from Chlamydomonas reinhardtii as a light-gated proton channel. In the following year, it was reported that rapid light-induced depolarizing currents can be conducted by microbial opsins, which mediate chemotaxis in Chlamydomonas reinhardtii that is expressed in human embryonic kidney cells (Nash et al., 2011). Since the beginnings of optogenetics, there has been tremendous progress in the types and qualities of opsins, as well as the genetic techniques that are used to target particular cell types. The use of the optogenetic toolbox allows for precise control over which cells should be activated or inhibited. This is genetically specified by particular promoters and location through targeted light stimulation that is not possible with electrical stimulation or systemically applied ASMs (Zemelman et al., 2002).To manipulate and analyze cellular receptors (Armbruster et al., 2024), signaling cascades (Kramer et al., 2021), gene expression (Ohlendorf et al., 2022), or cell state (Shkarina & Broz, 2024) a wide variety of light-dependent switches responsive to the entire visible light spectrum as well as to UV and near-infrared (NIR) light were created by fusing genetically encoded light-sensitive protein switches to the effector proteins.

Research Gap in Plant Optogenetics:

Over the past three decades, optogenetics has transformed mammalian cell biology and neuroscience, yet its use in plants is still in its infancy. Rhodopsins, phytochromes, phototropins, and cryptochromes are some of the light-responsive proteins that have revolutionized biological research especially in animal systems, by enabling precise spatial and temporal control of cellular processes (Shikata & Denninger, 2022). However, since plants depend on light for photosynthesis, growth, development, and environmental signaling, so implementing these technologies to plants presents certain challenges. As a result, optogenetic techniques often encounter endogenous crosstalk with native photoreceptors, such as phytochromes, cryptochromes, and phototropins, as well as unwanted activation in ambient light and low availability of orthogonal chromophores (Shikata & Denninger, 2022). Furthermore, the use of optogenetic system has been limited to model species, protoplasts, or transient expression systems in controlled laboratories and less evaluation has been done in field conditions or agricultural crops. Although with the recent advancement in optogenetic systems such PULSE, BLINK1, Highlighter, and GtACR1 systems has significantly improved gene regulation in plants, there still exists a lot of unsolved issues about the creation of reliable, multichromatic, and orthogonal optogenetic platforms that avoid crosstalk with the endogenous signaling pathways. In order to transform plant optogenetics from a potent laboratory research tool into a useful technology for precision crop improvement, climate-resilient agriculture, and next-generation smart farming, these issues must be addressed. In this review, we provide a comprehensive overview of the evolution of plant optogenetics, the molecular mechanisms underlying and key optogenetic tools developed for plants. This review also highlights the current innovations and applications in plant biology, current issues and research gaps, and explores future possibilities, such as integrating optogenetics with cutting edge technologies like synthetic biology, CRISPR/Cas genome editing, and artificial intelligence.

Components of an Optogenetic System:

Two essential components of an optogenetic system are photoreceptors and transcriptional regulators. Transcriptional regulators are proteins that attach to DNA and control gene expression, while photoreceptors are light-sensitive proteins that translate light impulses into biochemical signals. Plant photoreceptors are wavelength-dependent natural light sensors (Christie & Zurbriggen, 2021). Phytochromes, cryptochromes, phototropins, ZEITLUPE, and UVR8 are some of the plant receptors that are found in nature. Phytochromes are photoreceptors sense red and far-red light and control germination, flowering, and response to shadow avoidance. Cryptochromes are photoreceptors that detect blue light and regulate circadian rhythms, flowering and photomorphogenesis. Similarly, phototropins are also blue light-sensitive photoreceptors that regulate phototropism and chloroplast mobility. Blue light (BL) activates phototropins which are the plasma membrane-associated kinases. Two phototropins (phot1 and phot2) are found in flowering plants, including Arabidopsis (Christie & Murphy, 2013; Fankhauser & Christie, 2015). At its N terminus, phototropins have two light-, oxygen-, or voltage-sensing domains (LOV1 and LOV2). Although both LOV1 and LOV2 bind flavin mononucleotide (FMN) as a chromophore and serve as blue light sensors, they have different functions in controlling phototropin activity (Hart et al., 2019). The photoreceptors ZEITLUPE and UVR8 are sensitive to blue light and ultraviolet light which regulate growth and development. Additionally, it is also seen that plants detect UVB radiation via UVB-induced monomerization of the photoreceptor protein UV resistance locus 8 (UVR8), which forms homo-dimers in the absence of UVB (Wu et al., 2012; Christie et al., 2012a).

Emergence of the Optogenetics:

There are two eras in optogenetics, optogenetics 1.0 which is the first generation of optogenetics and the other one is optogenetics 2.0 which is the second generation of optogenetics. The early 2000s saw the beginning of optogenetics 1.0 which is based on opsins, such as bacteriorhodopsins, halorhodopsins, and channelrhodopsins (ChRs). It aids in comprehending the dynamics of neural activity that control cognition, perception and behavior in both health and illness. However, on the other hand, optogenetics 2.0 involves photoreceptors and includes plants, animals, fungi, and microbes. They combine a photosensory module with an effector module to create synthetic photoswitches. It is used for optical regulation of enzyme activity, kinase and receptor activity, subcellular localization of proteins and organelles, protein stability, and control of gene expression. In order to control a variety of cellular processes, optogenetics 2.0 uses the engineering of microbial and plant photoreceptors to translate information in the form of photons into a molecular function mediated by a change in protein conformation or enzymatic activity (Dagliyan & Hahn, 2019; Kolar et al., 2018**).** This field has advanced rapidly since the introduction of a red-light gene-expression switch in yeast cells in 2002 (Shimizu-Sato et al., 2002**)** and the heterologous expression of photoactivated adenylyl cyclases (PACs) in oocytes and mammalian cells for the production of the second messenger cAMP in 2007 (Schröder-Lang et al., 2007). The field of optogenetics has made rapid advancement with numerous applications in animal, yeast, and microbial systems, and the methods have begun to take root in plants.

Optogenetic tools engineered for plant gene expression control:

a) PHYB/PIF Heterodimerization: It is the interaction between phytochrome B (PHYB) and phytochrome-interacting factors (PIFs), which are basic helix–loop–helix (bHLH) proteins. Phytochromes can photoconvert between red and far-red absorbing forms called Pr and Pfr, which can be used in optogenetics to switch between active (Pr) and inactive (Pfr) states, respectively, by binding a linear tetrapyrole chromophore (phytochromobilin), which is made from heme **(**Rockwell & Lagarias, 2010**).** Numerous photo-reversible dimerization techniques have been developed based on this interaction between PHYB and PIFs. Xie et al. (2011), used Phytochromes to regulate SA and JA signaling pathways for controlled resistance to Magnaporthe grisea in rice. The use of PHY-based technologies in non-plant systems is restricted due to the lack of chromophore or the need to synthesize it in vivo through genetic manipulation. The first red light-inducible gene expression system was developed in yeast (Shimizu-Sato et al., 2002**).**

b) CRY2/CIB Heterodimerization: It is the interaction of cryptochrome 2 (CRY2) and cryptochrome-interacting bHLH 1 (CIB) proteins. In mammalian cells, it functions as an optogenetic dimerizer to regulate transcription, protein translocation, and Cre recombinase-mediated DNA recombination under blue light **(**Kennedy et al., 2010). Furthermore, it was also stated that CRYs binds flavin adenine nucleotide (FAD) as a chromophore and dimerizes after being photoactivated to commence signaling (Wang et al., 2017**;** Ponnu & Hoecker, 2022).

c) LOV Caging/Uncaging: It is a light-mediated activation of caged molecules that regulates protein activity. Blue light causes conformational changes in LOV domains, which can affect a fused protein activity by unveiling domains for its interaction or substrate binding domains or by altering its stability or localization by releasing signal for destruction. Light-dependent receptor autophosphorylation is largely regulated by LOV2, while LOV1 is believed to regulate the function of LOV2. LOV domains are versatile photosensory modules that can control a wide variety of biological outputs in bacteria, fungi, and plants (Glantz et al., 2016**)**. Depending on the LOV domain involved, light-induced adduct production can be reversed in the dark and thermally decays back to the ground state in tens to thousands of seconds **(**Christie et al., 2012b**).** This vast range in adduct decay kinetics has created new opportunities to control the activity of LOV-based photoreceptors. The LOV2 photosensory module has amino acid substitutions that can either increase or decrease adduct decay (Hart et al., 2019**)**. Additionally, it is also demonstrated that slow-cycling variations in Arabidopsis showed improved responsiveness that translated into greater plant growth under light-limiting conditions, and also showed that these variants can change phototropin sensitivity in planta.

d) UVR8/COP1 Heterodimerization: It is the interaction between ultraviolet-B receptor (UVR8) with CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) protein. It was discovered in Arabidopsis and is triggered by dimer-to-monomer switching after absorbing UV-B radiation through a tryptophan cluster (Trp233, Trp285, and Trp337) within the protein **(**Christie et al., 2012a**;** Yang et al., 2015). The COP 1 protein was used in plant system to promote photomorphogenesis growth (Sassi at al., 2012; Senapati et al., 2019; Podolec & Ulm, 2018). To regulate photomorphogenic growth in plants, the monomeric form of the UVR8 interacts with the COP1 or a number of other proteins, including transcription factors (Favory et al., 2009**).** In contrast to other photoreceptors, the UVR8 protein can sense light without the need for an external chromophore (Christie et al., 2012a**;** Wu et al., 2012**).** Therefore, UVR8-based optogenetic methods do not require synthetic chromophores and can be used even in non-plant systems **(**Ouyang et al., 2021**;** Rizzini et al., 2011). An inherent characteristic of UVR8 is UV-B-induced monomerization, which is mostly unaffected by the light-regulated organization of other protein complexes. Due to this, UVR8 is a quick and reliable on-switch for UV-B optogenetic regulation even in the presence of multichromatic light. Monomeric UVR8 reverts back to the homodimeric state with the removal of UV-B light, thus allowing for light switchable control (Heilmann et al., 2013**).**

e) **Channelrhodopsin-based:** The most popular optogenetic tool in the neurosciences is the channelrhodopsin-2 (ChR2), which is an algal retinal-binding protein. Rhodopsins (opsin + covalently bound retinal = rhodopsin) can functionally express themselves when retinal is present in plants. Rhodopsins have roles as enzymes, ion pumps, or light-gated ion channels. It absorbs visible light and becomes photo-responsive when the chromophore retinal (vitamin A aldehyde) is covalently bound to the protein (opsin). ChR2 regulates cellular signaling, membrane potential, membrane depolarization, and the influx of $Ca2^{+}$ and Na^{+} **(**Christie & Zurbriggen, 2021**).** Wavelengths of different plant photoreceptors are shown in Fig. 1. **(**Christie & Zurbriggen, 2021**)**

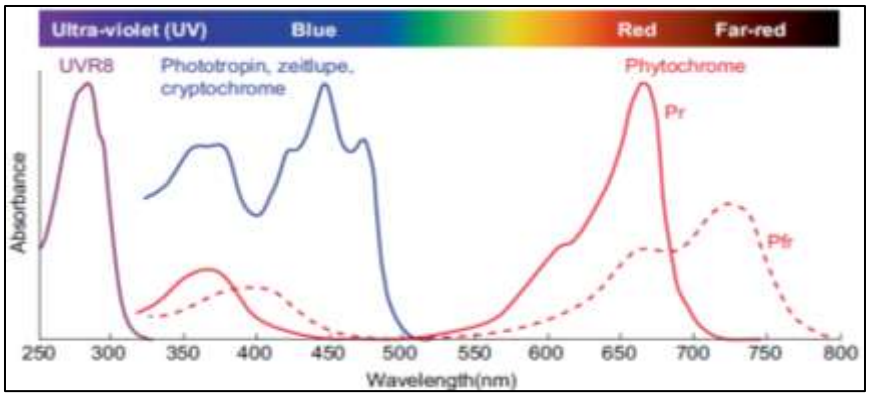

Fig. 1: Plant photoreceptors with their wavelengths

Recent optogenetic approaches for minimizing endogenous crosstalk in plants:
Plants rely on light for growth, development, photosynthesis, and other functions which complicates the use of the optogenetic system in plants. Since many optogenetic technologies rely on plant light-responsive proteins including PHYB, CRY2, PHOT, ZTL (LOV domains), and UVR8, they may interfere with endogenous light signaling pathways present in the plant system. These optogenetic methods are limited to transitory expression systems because the EXPRESSION is induced by the addition of cofactors or by red light, which is necessary for plant growth. Novel strategies have been developed using modern optogenetic technologies such as RNA interference, antisense RNA, CRISPR/Cas genome editing, and synthetic biology to address the situation without crosstalk with the endogenous light signaling system of plants. Such approaches are described below**:**

1. PULSE system (Plant Usable Light-switch Elements)
It is an optogenetic method which in the presence of ambient light, allows the reversible control of gene expression in plants. PULSE is a combination of a red-light-inducible switch and a blue-light-regulated repressor. Red light causes gene expression to become active, while both white light and darkness inactivate it. To regulate transcriptional initiation, it combines two distinct photo-switches: Phytochrome B (PHYB) and phytochrome-interacting factors (PIFs), which are red on modules, and the other one is engineered from the LOV-based transcription factor, EL222, which is a blue off module (Nash et al., 2011). EL222 fused to the EAR repressor domain known as SRDX and inhibit transcription activation. Additionally, in the presence of blue light it dimerizes and binds to the target DNA sequence. Thus, the red-on module only activates transcription in monochromatic red light, whereas the blue-off module represses it in white light (Fig. 2). The PULSE system has been used to modulate immunological responses in plant leaves, resulting in Arabidopsis transgenic plants (Ochoa-Fernandez et al., 2020).

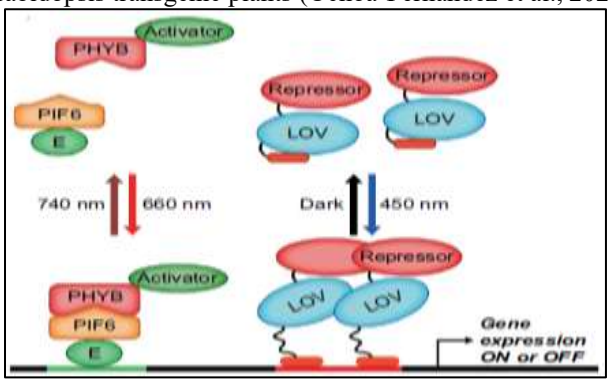

Fig. 2: Schematic representation of the PULSE (Plant Usable Light-Switch Elements) optogenetic system

2. BLINK1 (Blue Light-induced K+ Channel)

In the BLINK1 system, the LOV domain is fused and when it is exposed to blue light, the plasma membrane H^+ ATPase activates and promotes K^+ absorption. BLINK1 can conduct both inward and outward currents based on the gradient of K^+ concentration across the membrane. It closes in the dark and opens in response to blue light. Blue light also promotes stomatal motion, CO_2 assimilation, and water status preservation by increasing K^+ permeability in stomata which results in an overall increase in the biomass (Papanatsiou et al., 2019). Additionally, it was reported that BLINK1 accelerates stomatal closure in the darkness.

3. Highlighter system

It is a light-gated synthetic gene expression system that is designed for use in the plant system. The photo-switchable CcaS–CcaR system of cyanobacteria serves as the basis for this highlighter system. In cyanobacteria, a light-responsive histidine kinase (CcaS) phosphorylates a response regulator (CcaR). Using this principle, in the highlighter system a target gene of interest is expressed when $CcaS_{HL}$ (highlighter-optimised CcaS) phosphorylates $CcaR_{HL}$ (highlighter-optimised CcaR) in response to activating light conditions, which leads to increased binding to its cognate promoter P_{HL}. Blue light suppresses this mechanism, while green light activates it. CcaS functions as a flavin-binding domain. Previously, the CcaS-CcaR system was used to create synthetic optogenetic gene expression systems for prokaryotic hosts, including cyanobacteria (Abe et al., 2014**;** Kobayashi et al., 2019**),** Bacillus subtilis **(**Castillo-Hair et al., 2019**),** and Escherichia coli (Tabor et al., 2011**;** Ariyanti et al., 2021**)**. Furthermore, CcaS may accept the endogenously generated phytochromobilin (PΦB) chromophore, which facilitates photo-switching in plants due to its homology to plant phytochromes (Larsen et al., 2023).

4. GtACR1 anion channelrhodopsins

In controlled-environment agriculture and horticulture crops are typically grown with LED lighting systems that only emit blue and red light (Bantis et al., 2018). However, the addition of green light affects plant growth (Wang et al., 2013**;** Smith et al., 2017), therefore, it is not essential for many crops. In GtACR1 system, it is possible to use green light to activate rhodopsin-based optogenetic tools while growing plants with blue and red light without affecting its growth. GtACR1 (Guillardia theta anion channelrhodopsin-1) is a recently identified family of opsins known as anion channelrhodopsins (ACRs) (Govorunova et al., 2015**).** It was first derived from archaea and has greater negative reversal potential values than ChR2, fast response kinetics, high single channel conductance, and selectivity in narrow wavelength ranges. GtACR1 conducts a flow of anions (such as Cl^{-}) when stimulated by green light, causing an outward current that speeds up repolarization (Govorunova et al., 2016**;** Govorunova et al., 2015**;** Govorunova et al., 2017). GtACR1 is expressed in guard cells in plants and when needed it can be used to close the stomata for particular leaves. A schematic comparison of optogenetic signal cascades in animal and plant systems has been described in Fig. 3**.**

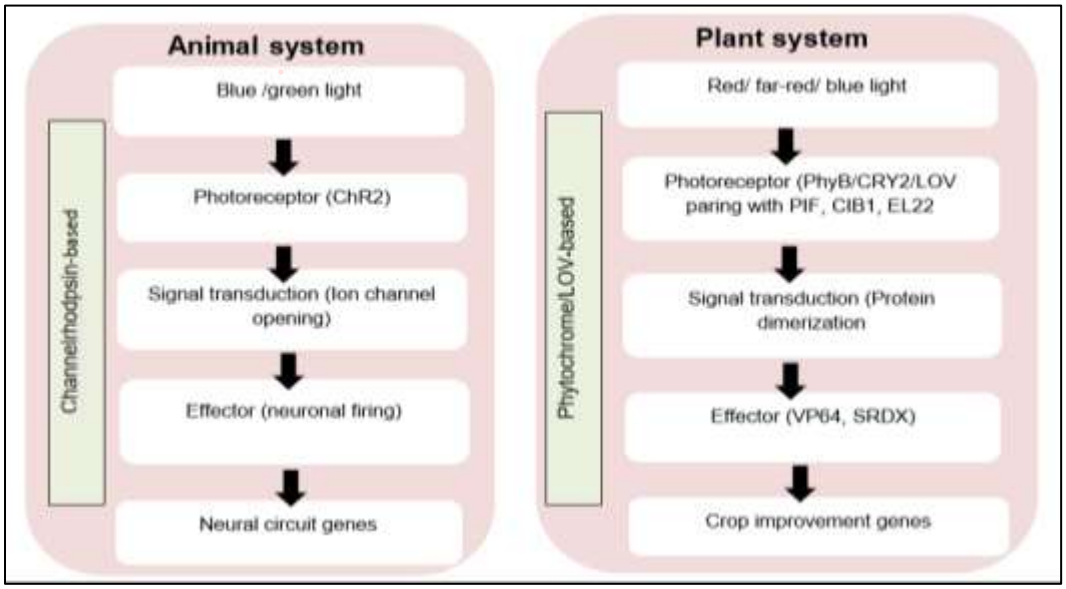

Fig. 3: The core optogenetic signaling cascade for animal and plant system

Applications of optogenetics in crop improvement:

There are several applications of optogenetics in the plant system (Fig. 4). The Optogenetic methods, like GtACR1, that is discussed before, might be helpful in shifting sap flows or stopping harmful microbes from infesting agricultural plants. It is also possible to prevent Ca^{2+} deficit in rapidly growing plant tissues by using green light-induced stomata

closure. Moreover, it was observed that abscisic acid (ABA) induces reduction in transpiration in mature leaves and may prevent tomato fruit blossom end rot (Tonetto et al., 2014). Therefore, GtACR1 may also be employed in the future to prevent $Ca2^{+}$ deficit in phloem-fed tissues by reducing the transpiration of mature leaves. Furthermore, GtACR1 may be employed to stop pathogenic microbes from infecting hosts through open stomata. Many emerging optogenetic technologies have been use till date for biotic (Nie et al., 2023; Wang et al., 2022a; Wu & Yang, 2010; Xiang et al., 2022; Xie et al., 2011; Yang et al., 2015; Yuan et al., 2023) and abiotic stress resistance (Nie et al., 2023; Sharma et al., 2014; Wang et al., 2020; Wang et al., 2022b; Wang et al., 2022c; Yang et al., 2018a; Yang et al., 2021b; Kanojia et al., 2023). Optogenetic tools are also applied for photomorphogenesis, growth and for signaling pathways in plants (Oh et al., 2007; Xu et al, 2023a; Yan et al., 2021; Yang et al., 2021a; Yang et al., 2018b; Zeng et al., 2023; Zhang et al., 2021; Zhao et al., 2022a; Zhao et al., 2023; Zhao et al., 2022b; Zheng et al., 2013; Zhong et al., 2021). List of various optogenetic tools applied in the plants are described in Table 1.

Table 1 Different Applications of Optogenetics in Plants

Sl. No.	Function	Photo-sensor	Tool name	State form	Activation light	Cofactor	Origin	Reference
1	Dimerization	LOV	EL22	Homodimer, DNA-binding	blue	FMN	Erythrobacter litoralis	Ochoa-Fernandez et al., 2020; Nash et al., 2011
2	Dimerization	CarH	-	Homodimer, DNA-binding	Green	AdoB12	Thermus thermophilus	Chatelle et al., 2018
3	Dimerization	PhyB	-	Heterodimer	Red	PΦB	Arabidopsis thaliana	Müller et al., 2014; Ochoa-Fernandez et al., 2020
4	Dimerization	CRY2	-	Heterodimer	Blue	-	Arabidopsis thaliana	Wang et al., 2017
5	Dimerization	OCP2	-	Complemented monomer	Blue-green	Keto-carotenoid	Fremyella thermalis	Piccinini et al., 2022
6	Ionflux	AsLOV2	BLINK1	K^{+} permeable	Blue	FMN	Avena sativa, Chlorella virus	Cosentino et al., 2015; Papanatsiou et al., 2019
7	Ionflux	ChR1 & 2		Cation permeable	Blue to green	All-transretinal	Chlamydomonas reinhardtii	Nagel et al., 2002; Reyer et al., 2020
8	Ionflux	GtACE1		anion permeable	Blue to green	All-transretinal	Guillardia theta	Zhou et al., 2021a
9	Ionflux	GtACE2		anion permeable	Blue	All-transretinal	Guillardia theta	Zhou et al., 2021b
10	Ionflux	ZipACR		anion permeable	Green	All-transretinal	Proteomonas sulcata	Zhou et al., 2021a
11	-	CcaS-CcaR system	Highlighter		Green/red	PΦB	Cyanobacteria	Larsen et al., 2023
12	-	LOV2	LiMETER		Blue		Avena sativa	Li et al., 2025

Challenges of Optogenetics in Plant Science:

Several interconnected issues must be addressed before utilizing optogenetics in the plant system. A major constraint for plant optogenetics is the requirement for precise light quality, intensity, and duration to activate; however, plants are constantly exposed to changing natural light, which makes regulated stimulation challenging in field conditions. Another major challenge of optogenetics is the availability of chromophore, which may be scarce or unevenly distributed in plant tissues, potentially lowering their efficiency. Plant systems have an extremely complex network of photoreceptors, so endogenous light signaling pathways can be disrupted by optogenetic manipulation. Moreover, the introduced light-responsive protein can also accidentally cross-talk with the endogenous signaling system of the plant, thus affecting their growth and development. Another important challenge posed by the optogenetic system in

plants is the efficient expression and appropriate subcellular localization of optogenetic components, as plant cells possess many organelles, rigid cell walls, and compartmentalized signaling. Thermal reversion, where light-activated proteins revert back to their inactive state in the absence of light due to temperature-dependent kinetics, may also hinder the duration and precision of optogenetic control. Finally, in natural environments, several environmental factors such as temperature fluctuations, light intensity, humidity, and stress can greatly impact the efficacy and reproducibility of plant optogenetic systems, thus complicating their practical application.

Future Perspectives: Integrating Optogenetics with cutting-edge technologies:

The integration of plant optogenetics with cutting-edge technologies like artificial intelligence (AI), synthetic biology, and CRISPR/Cas genome editing, will revolutionize the future of optogenetics which will enable precise and sustainable crop improvement methods (Nihongaki et al., 2015; Zetsche et al., 2015; Jumper et al., 2021, Zeng et al., 2024; Liu et al., 2025; Beyer et al., 2024; Fu et al., 2026). Optogenetics offers an ideal platform for the dynamic regulation of plant traits in contrast to traditional genetic engineering, by providing reversible and spatiotemporal control of gene expression. A new technique known as Opto-CRISPR has been developed by combining optogenetic systems with CRISPR/Cas technologies (Huang et al., 2025), which uses light to precisely control genome editing or programmable gene activation (CRISPRa) and repression (CRISPRi). Opto-CRISPR systems have so far primarily develbeen oped for mammalian cells (Yang et al., 2025; Liu et al., 2026), but similar strategies could also be applied to plants to achieve a controlled regulation of genes that control drought tolerance, disease resistance, flowering time, nutrient use efficiency, and yield (Zhou et al., 2026). By restricting genome editing to particular tissue, developmental phase, or environmental condition, such light-inducible genome editing platforms would reduce off-target effects and prevent continuous transgenic expression (Nihongaki et al., 2015; Devarajan, 2023). Through the use of AI-assisted protein engineering and computational design, the next-generation optogenetic system will be further be accelerated (Banerjee & Mitra, 2020; Fu et al., 2026). Machine learning and sophisticated structural prediction tools like RoseTTAFold and AlphaFold predicted microbial opsin structures can be further utilized to predict the protein structures, protein–chromophore interaction optimization, and novel photoreceptors with better spectral features, stability, and limited endogenous crosstalk (Jumper et al., 2021; Watson et al., 2023; Baek et al., 2021; Zhu et al., 2025; Wietek et al., 2024). AI-driven protein design approaches accelerated the discovery of improved microbial opsins and the de novo design of light-sensitive proteins for enhanced tissue penetration, thus indicating the potential of AI for engineering advanced optogenetic tools. These technologies have been primarily utilized in animal systems; however, they offer a potent framework with improved orthogonality, sensitivity, and compatibility with crop species for creating a next-generation plant optogenetic system. Additionally, important regulatory genes and signaling pathways can be identify by the use of AI-driven genomic, transcriptomic, proteomic, and phenotypic data analysis, which are appropriate for optogenetic manipulation, thus enhancing the accuracy and effectiveness of crop engineering (van Dijk et al., 2021). Moreover, crop performance can be monitored continuously, and specific light wavelengths can be provided by utilizing controlled-environment agriculture and smart greenhouses with sensors, computer vision, and programmable LED lighting and to activate optogenetic circuits only when necessary (van Dijk et al., 2021; Singh et al., 2021). The productivity of a crop can be increased by the use of such advanced optogenetic systems by dynamically controlling physiological processes like stomatal movement, stress-responsive pathways, nutrient uptake, photosynthesis, and growth, while using less water, fertilizer, and energy. Although many promising developments have been made in relation to optogenetics, there are several issues that still need to be addressed before adopting this in agriculture. Future studies should focus primarily on creating orthogonal photoreceptors that perform well in natural sunlight, increasing chromophore availability and tissue-specificity and multichromatic regulation, and validating optogenetic platforms in economically significant crop species under practical field conditions. Collectively, there is potential to transform plant biotechnology from static genome modification to dynamic, programmable control of plant biology by combining optogenetics, CRISPR/Cas genome editing, AI-assisted protein engineering, and precision agriculture thus creating new opportunities.

CONCLUSION

Optogenetics uses light-responsive proteins to control and dissect biological functions. With the discovery of channelrhodopsins, the optogenetic system has been boosted and different types of photoreceptors with diverse functions have been used to design light-controllable proteins. A large part of these existing tools can be directly used for plant research. Two of the most important optogenetic system which is recently developed and widely used for plant optogenetics are the PULSE and BLINK1 systems. The red light–activated, blue light–repressed optogenetic tool PULSE is useful to induce the expression of different optogenetic tools at a certain development or physiological stage or environmental condition. Recently, an advanced optogenetic system known as the highlighter system has also been developed using the CcaS-CcaR system from cyanobacteria. Highlighter provides new opportunities for optogenetic perturbation of biological processes with high spatiotemporal resolution in plants. Another important optogenetic tool is the GtACR1 (Guillardia theta anion channelrhodopsin-1), which is a recently identified family of opsins stimulated by green light. This can be used to prevent Ca^{2+} deficit in phloem-fed tissues by reducing the transpiration of mature leaves and can also be employed to stop pathogenic microbes from infecting hosts through

open stomata. Plant optogenetics is still in its infancy and awaits exploration by incorporating additional approaches. Given the high quantitative modulation, spatiotemporal resolution, and the reversible control capabilities, combining different approaches such as those used in PULSE, BLINK1, highlighter, and GtACR1will facilitate the targeted manipulation and study of biological processes in light-grown plants thus provide a compelling basis for future field application for improvement of crop varieties.

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