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Glowing Plants Can Light Up the Night Sky? A Review

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Abstract

Luminescence, a physical phenomenon that producing cool light in vivo, has been found in bacteria, fungi and anminals but not yet in terrestrial higher plants. Through genetic engineering, it is feasible to introduce luminescence system into living plant cells as biomarkers. Recently, some plants transformed with luminescent systems can glimmer in darkness, which can be observed by our naked eyes and provide a novel lighting resource. In this review, we summarized the development of luminescence in plant cells, followed by exampling the successful cases of glowing plants transformed with diverse luminescent systems. The potential key factors to optimize a glowing plant are also discussed. Our review is useful for the creation of the optimized glowing plants, which can be used not only in scientific research, but also as promising substitutes of artificial light sources in the future.

Glowing Plants Can Light Up the Night Sky? A Review

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Abstract

Luminescence, a physical phenomenon that producing cool light *in vivo,* has been found in bacteria, fungi and anminals but not yet in terrestrial higher plants. Through genetic engineering, it is feasible to introduce luminescence system into living plant cells as biomarkers. Recently, some plants transformed with luminescent systems can glimmer in darkness, which can be observed by our naked eyes and provide a novel

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lighting resource. In this review, we summarized the development of luminescence in plant cells, followed by exampling the successful cases of glowing plants transformed with diverse luminescent systems. The potential key factors to optimize a glowing plant are also discussed. Our review is useful for the creation of the optimized glowing plants, which can be used not only in scientific research, but also as promising substitutes of artificial light sources in the future.

Keywords:

Glowing plant; Luminescence; Genetic engineering; Bioluminescent system; Fluorescent protein

Introduction

Luminescence is a common physical phenomenon to create light, which was discovered among more than 10,000 species, i.e., bacteria, fungi, protists and animals (Desjardin et al., 2008; Haddock et al., 2010; Meighen, 1991). Luminescence is a kind of specific communication approaches for signaling, such as alarming predators (Burford & Robison, 2020), trapping prey (Verdes & Gruber, 2017; Wainwright & Longo, 2017) and attracting mates (Ellis & Oakley, 2016). During the past years, it was found that fluorescent proteins (FPs) and bioluminescent systems are responsible for induction of most native luminescence (Kim & Paulmurugan, 2021). FPs generate photoluminescece that is excited by specific emission without consumption of ATP (Chudakov et al. 2010), while bioluminescent systems exhibit light by oxidation of a specific substrate, luciferin, with co-factors, e.g., ATP and NADH, and catalyzed by the specific enzyme, luciferase (Fleiss & Sarkisyan, 2019).

Despite luminescence was found among many species, native terrestrial plants haven't been reported to produce luminescence yet. Bioluminescent systems and FPs have been initially proven to be powerful toolkits as biomarkers and biosensors in living plant cells. Recently, based on the newly-discovered bioluminescent systems and enhancement of suboptimized luminescence, the glowing plants were created (Reuter et al., 2020). The glowing plants can be used as the illumination for reading and writing (Kwak et al., 2017), though most of them are currently gloomy. As cool light, bioluminescence and fluorescence are softer and more effective compared with traditional artificial light, which makes glowing plants as valuable source for aesthetics and lighting. However, the pratical application of glowing plants was currently not availble due to cytotoxicity of most bioluminescent systems, and lack of understanding of optical characteristics and biosynthetic pathways of bioluminescent systems. Solving those problems are helpful for developing the optimized glowing plants in the future (Kaskova et al. 2016; Strack 2019).

Here, we summarized the literatures of rebuilding bioluminescent systems and FPs to make glowing plants. The key points for design and optimization of glowing plants, and the potential applications of glowing plants are also proposed. These glowing plants might be useful in scientific researching, as well as promising substitutes of artificial light to light up the night sky in the future.

Bioluminescence in plants: beyond as the biomarkers

During past decades, tremendous efforts have been invested for the development of optical non-invasive biomarkers in plant cells. The first bioluminescent system that was introduced into plants is firefly luciferase-luciferin system (Ow et al., 1986). Firefly luciferase can be expressed independently or fused with proteins of interst in plant cells, which provides a useful tool to study protein expression and localization, and protein-protein interactions (PPIs) (Guan et al., 2013; Sanchez et al., 2018). To detect PPIs, firefly luciferase is splited as N- and C-terminal halves and fused with two candidates of interactional proteins (Chen et al. 2008). However, firefly luciferin could not be biosynthesized in plant cells yet, so that it have to be supplied exogenously, which causes low cost-efficency.

To break those bottlenecks, several strategies were established, including using FPs as alternative biomarkers and searching novel luciferase-luciferin bioluminescent systems that can be completely biosynthesized in plants. In 1995, the green fluorescent protein (GFP) gene from *Aequorea victoria* was successfully expressed in*Arabidopsis* , which brings novel revoluion in plant research (Hu & Cheng, 1995). Since then, FPs as tags are widely used in plants to monitor protein localization *in vivo* , recognize specific plant cells and tissues,

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obtain cell-specific transcriptomes and record plant cell fate selection (Ckurshumova et al., 2011). Spectral characteristics of FPs could be altered to reduce autofluorescence background from plant tissues. Various FPs covering most fluorescent ranges (i.e. yellow, blue, cyan, green, and red) have been adapted for plant cells (Blatt and Grefen, 2014). However, there are still problems remained when using FPs, including cellular damage caused by excited laser, signal weakening by plant tissues and migrating from plasmodesmata due to its smaller molecular weight (Brunkard & Zambryski 2017).

Another alternative strategy that developing novel bioluminescent systems was emerging. After elucidation of the luciferase-luciferin pairs original from bacteria and fungi, two distinguished optical reporters have been developed from these systems and now accessible in plant science. These systems have been described in their entirety and could be biosynthesized in plant cells. However, due to cytotoxicity and low photon yield of bacterial bioluminescent reactions in eukaryotes (Kotlobay et al., 2018), since autoluminescent system was firstly reported in *Nicotiana tabacum* , only few bacterial luciferase-luciferin pairs were reported in other transgenic plants (Cui et al., 2014; Krichevsky et al., 2010). Fungal bioluminescent system is an unique genetically encodable eukaryote luciferase-luciferin system, which was introduced into plant cells recently (Khakhar et al., 2020; Mitiouchkina et al., 2020). Less cytotoxicity and high luminescent intensity make fungal bioluminescent system as one of the most promising optical molecular tools (Reuter et al., 2020).

Nowdays, protein-directed revolution and synthetic biology boosted the development for novel bioluminescent systems. Luciferase engineering could be used to optimize the natural bioluminescent systems, such as increasing bioluminescent intensity and stability. Rational designing novel bioluminescent systems provides two representative cases, Nano Luciferase (NanoLuc) (England et al., 2016) and Nano-lantern (Doerr, 2013). NanoLuc is a small subunit (19 kDa) from shrimp *Oplophorous gracilirotris* luciferase (Hall et al., 2012). Split NanoLuc was developed to screen PPIs, which avoids imposing steric hindrance caused by huge firefly luciferase halves (Wang et al., 2020). Nano-lantern is an artificially designed luminescent protein fused with enhanced*Renilla reniformis* luciferase and yellow FP Venus. ATP production from chloroplast could be observed by Nano-lantern (Saito et al., 2012). Today, fluorescence and bioluminescence is no longer a new tool for molecular and celluar biology, but becoming a novel light sources, with which glowing plants have been obtained.

Ornamental glowing plants with FPs or nano-lanterns

Ornamental glowing plants would be available when the fluorescence or bioluminescence was bright enough. The reported bioluminescent plants are summaried and shown in **Table 1** . The gene expression casstte of FPs are easily to be integrated into genome and FPs can accumulated enormously in plant cells, so it is feasible to generate fluorescent plants. For example, CpYGFP, a yellowish-green FP from marine plankton*Chiridius poppei* , can be excited at 509 nm laser line and emission was collected at 517 nm which is a red-shifted visible light (Masuda et al. 2006). Commercial plants were transformed with*CpYGFP* gene, and green fluorescence was observed from the flowers when emission filter were used (Sasaki et al. 2014; Kishi-Kaboshi et al. 2017). Additionally, eYGFP and eYGFPuv, two derivatives of CpYGFP, were expressed in the flowers of another flowering plant, *Petunia hybrida* . Green fluorescence was macroscopically observed from the flowers with naked eyes, when they were illuminated with visible and ultraviolet LED, respectively. Among the various colors of flowers, white flowers contain less petal pigments, indicating that less exciation light and fluorescence would be absorbed. As a result, white flowers were chosen in these trials (Chin et al., 2018). Many FPs maintain fluorescent stability under acidic conditions, thus keeping high activity in the acidic intracellular environment of higher plants that pH approximately 4.5-7.2. To enhance fluorescence, nano-lanterns were introduced into plant cells. Recently, green enhanced nano-lantern gene was transferred into *A. thaliana* , which could be lightened under the excitation of blue light. The luminescence became remarkably brighter when the suitable substrate furimazine was added (Furuhata et al., 2020). These results indicate it is possible to obtain commercial ornamental fluorescent flowers. Characteristics of FPs provides the fluorescent plants a specific proposed implementations, such as dried fluorescent flowers and fluorescent plant specimens. It is reported that embedding in fine grained silica gels, the desiccated fluorescent flowers transferred with *CpYGFP* gene remained glowing for more than a year, because some FPs are capable to

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generate fluorescence without water (Sasaki et al., 2014). However, fluorescent plants utilized as alternative light sources for street or reading lamp in completely darkness is currently difficult, and these application might be satisfied by glowing plants with luciferase-luciferin bioluminescent systems.

Glowing plants with bioluminescent systems

Up to now, three bioluminescent systems that origin from firefly, bacteria and fungi have been reconstructed in plants. The firefly luciferase-luciferin system is a kind of D-luciferin-dependent systems, which is one of the most intensively studied bioluminescent systems (Fleiss & Sarkisyan, 2019). In the presence of Mg^{2+}, oxygen and ATPs, reduced firefly luciferin can be catalyzed by firefly luciferase, and generate yellow-green luminescence. To prolong glowing, CoA could be used to deoxidize the oxidized luciferin (Gosset et al., 2020). Ow. et al. (1986) introduced *Photinus pyralis* luciferase gene into *N. tabacum* and *Daucus carota* for the first time. Firefly luciferase was expressed during growing process of the transgenic plant and accumulated in various plant organs, e.g., roots, stems and leaves. When watering the tobacco with solution of firefly luciferin and ATP, the substrate was transmitted to every tissues through the vasculature and react with luciferase, leading to lightening of the whole transgenic tobacco. Due to the technical difficulty to synthesize firefly luciferin in plant cells, the firefly luciferins can only be exogenously supplied. In 2017, a novel nano-technique named pressurized bath infusion of nanoparticles (PBIN) was utilized to introduce the whole firefly bioluminescent system. In PBIN, luciferin and coenzyme A (CoA) were packaged into two different nano capsules, and luciferase was linked onto the surface of silica-PEG nanocarriers. These nanoparticles could reduce luciferin cytotoxicity and diminish chemical denaturation of luciferase to some extent, causing a long light-emitting duration of up to 8 hours. To transmit the whole firefly bioluminescent system, the plant was immersed in solution containing the nanoparticles in a closed pressured aqueous chamber, in which pressurization delievered the nanoparticles into mesophyll cells through stomata. Once these nanoparticle gathered in mescophyll cells, it glowed, and produced the brightest glowing plants to date. The PBIN-based light-emitting plants were also the only glowing plants which could be modulate “on” and “off” states when CoA- and dehydroluciferin-nanoparticles were provided, respectively, thus providing viability as alternative light sources (Kwak et al., 2017).

The second bioluminescent system is discovered in three Gram-negative motile rods luminous bacteria genera, that is *Vibrio* ,*Photobacterium* , and *Xenorhabdus* . The *lux* operons (*luxCDABEG*) are highly conserved through all luminous bacterial, which encode the whole bacterial bioluminescent system (Close et al., 2009). Gene *luxA* and *luxB* encode α and β subunits of bacterial luciferase, respectively, and *luxCDEG* encodes biosynthesis pathway of tetradecanal. Krichevsky et al. (2010) transferred the whole *Photobacterium leiognathi lux* operon into tobacco chloroplast and obtained the first auto-luminescent plant. Although only very dim green luminescence was observed, this auto-emission needs neither exogenous luciferin nor optical excitation. However, the cytotoxicity of the bacterial luciferin, long-chain fatty aldehyde, is ineluctable to the eukaryotic cells. It is reported that a low concentration of bacterial luciferin *n-decyl* aldehyde can cause toxity to many model eukaryotes, including mammalian and higher plant cells (Hollis et al., 2001), which hindered application of bacterial bioluminescent system as biomarkers or illuminants.

Recently, the third bioluminescent system originated from fungi was used to generate auto-stronge-glowing plants.The biosynthetic pathway of fungal luciferin is named caffeic acid cycle (Kotlobay et al., 2018). A gene cluster encoded four key enzymes catalyze the caffeic acid cycle in*Neonothopanus gardneri* , including fungal luciferase (Luz), hispidin-3-hydroxylase (H3H), hispidin synthase (Hisps), and caffeylpyruvate hydrolase (CPH). Hisps is post-translationally activated by another enzyme, NPGA (4’-phosphopantetheinyl transferase). Precursor of fungal luciferin is hispidin, which can be convert to fungal luciferin, termed 3-hydroxyhispidin. Then the luciferin is oxidized by Luz to yield photons and oxy-luciferin, named caffelpyruvic acid, followed by hydrolyzing the oxy-luciferin to pyruvic acid and caffic acid. Caffic acid can be recycled to hispidin by Hips (Purtov et al. 2015; Oba et al. 2017). Fungal caffeic acid cycle shares three intermediary metabolites with three major biosynthetic pathways of vascular plants, including shikimate, lignins and flavonoid anthocyanins condensed tannins. These common molecules make a bridge connecting the gap between fungal caffeic acid cycle and the native biosynthetic pathway in plant cells. As a result, it is reasonable

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to design a sophisticated fungal luciferin synthetic pathway reconstituted into tobacco metabolic routines (Mitiouchkina et al., 2019). Mitiouchkina et al. (2020) created a luminescent *N. tabacum* through synthetic biology, which stably displayed green luminescence. Gene of *Luz* , *H3H* ,*Hisps* , and *CPH* were transferred in the tobacco. The auto-luminescent tobacco was brighter than other reported auto-light-emitting plants. Coninsistantly, Khakhar et al. (2020) described the transiently transformation and expression of fungal bioluminescent system in both model and commercial plant species.*NPGA* gene was also transferred besides the four gene mentioned above. Stronge luminescence was generated from cotyledons, leaves, and petals of these genetically modified plants. The fungal bioluminescent system produces brighter light and shows no cytotoxicity and growth inhibition to plant cells, compared with that employed bacterial luminescent system (Kotlobay et al., 2018). However, drawbacks are also existed on the plant-expressed fungal bioluminescent system. For example, low pH and temperature in plant cells reduced photon yield (Reuter et al., 2020).

Synthetic routine of luciferin: footstone of auto-glowing plants

Here we define "plantern" (a chimera of "plant" and "lantern" in both semantics and biology) as transgenic plant that is capable to produce visible light need neither external illumination nor exogenous substances, and the bioluminescence is bright enough to be observed with naked eyes. Feasibility of designing "planterns" as streetlights have been evaluated according to photosynthetic efficiency and luminous energy output. To produce as much light as tranditional streetlights, glowing plants have to output more than 1000 lumens by the transformed firefly bioluminescent system. It was estimated that a fast-grow tree glowing with the firefly luciferase-luciferin pairs only have to divert approximately 0.3 % of the stored energy to generate bright-enough bioluminesence during the nighttime (Reeve et al., 2014). In addition to producing light, planterns are also leveraged for whole-plant imaging and studying spatiotemporal expression of genes during the entire life-cycle, for limitations on delivering exogenous luciferin to the whole plant and tissue would no longer be a bottleneck (Reuter et al., 2020).

Rebuilding luciferin biosynthetic routine is the priority of generating "planterns", which could be achieved by introducing all genes associated with luciferin biosynthesis (Krichevsky et al., 2010) or integrating luciferin biosynthetic routine into native plant metabolic pathway (Khakhar et al., 2020; Mitiouchkina et al., 2019). In the latter situation, the complicated biosynthesis pathway could be simplified. The unclarified intermediate steps would even be skipped if products of the unknown enzymatic reaction have been found in plant metabolome. Once the luciferin biosynthetic pathway in plant cells is reconstrcuted, common plant genetic engineering technique would be used to make the transgenic glowing plants (**Figure 1**).

Besides those well-known bioluminescent systems that have been reconstructed in plant metabolome, new bioluminescent systems were also discovered recently. More alternatives of intracellular illuminants would be available once unveiling the mysteries of newly-found bioluminescent systems (Kaskova et al., 2016). Some of these systems might be suitable for plant cells or whose synthetic pathways could be reconstituted in plant metabolic routine. Recently, a novel yellow bioluminescent system from annelid worms in the genus *Tomopteris* , kinds of pelagic polychaetes, was separated and characterized (Glagoleva et al., 2020). The worm luciferin turned out to be aloeemodin, which had been also discovered in plants, such as *aloe* and *senna*(Heidemann et al., 1996). *Arachnocampa luminosa* , a New Zealand luminescent glowworm, can generate blue-green light. The glowworm luciferase shares 31% sequence identity with firefly luciferase, but catalyzing an entirely new luciferin whose biosynthetic pathway begins from xanthurenic acid and tyrosine (Watkins et al., 2018). Dinoflagellate, notorious protist responsible for causing the surface of the ocean to sparkle at night, produce bluish-green gimmer under mechanical disturbance (Valiadi & Iglesias-Rodriguez, 2013). The dinoflagellate luciferin is believed as derivative of chlorophyll$a$ because of their similar molecular structures (Topalov & Kishi, 2001). Genes associated with reaction converting chlorophyll$a$ into dinoflagellate luciferin could be introduced into parts of chloroplasts when synthetic routine of dinoflagellate luciferin is clarified.

Optimization of "planterns"

One of the problems is low luminescent intensity, which hinders the population of planterns. Though some

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glowing plants generated a bright emission of 1.44×10^{12} photons/s (Kwak et al. 2017), many other auto-glowing plants produce a much dimmer luminescence of 1.3×10^{6} photons/s (Krichevsky et al., 2010) and 1.6×10^{9} photons/s (Mitiouchkina et al., 2020), which is only bright enough to see in entire darkness with naked eyes. Enhancement of whole-plant glowing intensity could be considered as follow. Bioluminescent intensity is determined by both characteristics and accumulation of luciferase-luciferin pairs. Plant intracellular conditions also interfere bioluminescent intensity. Once the luminescence is produced, transparency window (TW) effects transmission efficiency (Strack, 2019).TW can be measured and sketched by spectrophotometer (Mitiouchkina et al., 2020). Because the TW is effected by pigments, plant species, apparatus and growth status, the bioluminescent spectrum should be altered according to TW.

As a result, the improvement of glowing intensity could be achieved from two aspects, i.e., the optimization of bioluminescent systems and the transgenic strategies. By engineering of luciferase, promotes feasibility to enhance bioluminescent intensity, spectral characteristics, pH and thermal stability by random mutagenesis, site-specific mutagenesis and *in scilicon* design (Branchini et al., 2019; Fujii et al., 2007; Modestova & Ugarova, 2016). Protein fusion or spilt could also alter bioluminescent characteristics. A chimeric firefly luciferase of North American firefly (*Photinus pyralis*) and Japanese firefly (*Luciola italica*) luciferase exhibited 1.4-fold enhanced bioluminescence quantum yield compared with the wide-type firefly luciferases (Branchini et al., 2014). Nano-lantern and NanoLuc are shown to improve luminescent intensity significantly, though exogenously supplied luciferin is necessary. Similar nano-lantern or NanoLuc might be developed based on the other bioluminescent systems whose luciferin could be biosynthesized, e.g., fungal bioluminescent system. Recently, as structural basis for the spectral difference was gradually clarified, bioluminescent color could also be changed by designing luciferin. It was reported that firefly luciferins with elongated π-system, such as phenyl or phenol, exhibit an obvious red-shift of emission spectrum (Yao et al., 2020). It was reported that firefly luciferin analog with less conjugated π-system present blue-shift in absorption and fluorescence (Pirrung et al., 2019). The designed luminescent pairs provide more selections, and these luciferin analogs might be able to be biosynthesized in plant cells. Given that the potentially cytotoxicity and growth inhibition to higher plants caused by bioluminescent systems, protecting groups were introduced into luciferin is reported possible, which can decrease cytotoxicity and enhance the accumulation (Yuan et al., 2017).

To optimize transgenic methodologies, substanial accumulation of luciferase-luciferin pairs can be achieved via common genetic engineering technique, such as codon optimization, inserting translational enhancer and several tandemly ranging the gene cluster (Sasaki et al., 2014). Engineering subcellular organelles (e.g. chloroplast and vacuole) into specified "scintillon" is another promising strategy. Increasing the accumulation of the whole luciferin pathway can be achieved through the development of chloroplast transformation technique (Kwak et al., 2019; Jin & Daniell, 2015). Bacterial bioluminescent system has been stably expressed in chloroplast (Krichevsky et al., 2010). Maternal inheritance of the chloroplast genome prevents genes from escaping into the environment through pollen grains (Yu et al., 2020), which may helps popularize planterns as alternative light sources in future (Callaway, 2013). Meanwhile, vacuoles could be employed to accumulate FPs or luciferase-luciferin pairs. Proteins associatd to bioluminescence can be fused with sequence specific vacuolar sorting signals (Viegas et al., 2017). The potential strategy to enhance the suboptimized glowing plants would be helpful for designing better glowing plants in future.

Perspective

Glowing plants, especially "planterns", showed promising prespections in detecting microbiome changes in built environment, and also can be use as natural illumination (Reuter et al., 2020; Stewart et al., 2018). The goal of generating "planterns" will promote the development of plant biotechnology and benefit synthetic biology in return. Creating "planterns" will beone of the research hotspots because many pioneering designs leave several suboptimization to be enhanced. Commerical popularization of "planterns" will probably achieve another success, given the fact that several genetic modified fluorescent zebrafish species are still existing in worldwide aquarium despite controversy (Knight 2003; Stewart 2006). We believe that it will be possible to read or write under the luminescence of desk "planterns", drive beneath the light of street

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"planterns", and enjoy the mysterious atmosphere provided by garden "planterns" in the future.

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Conflict of Interest

There is no conflict of interest.

References

Blatt, M. R., & Grefen, C. (2014). *Arabidopsis Protocols* . Totowa, NJ: Humana Press. pp. 487–507.

Branchini, B. R., Fontaine, D. M, Southworth, T. L., Huta, B. P., Racela, A, Patel, K. D., & Gulick, A. M. (2019). Mutagenesis and Structural Studies Reveal the Basis for the Activity and Stability Properties That Distinguish the *Photinus* Luciferases*scintillans* and *pyralis* . *Biochemistry* ,*58* (42), 4293–4303. *doi:* 10.1021/acs.biochem.9b00719

Branchini, B. R., Southworth, T. L., Fontaine, D. M., Davis, A. L., Behney, C. E., & Murtiashaw, M. H. (2014). A *Photinus pyralis*and *Luciola italica* Chimeric Firefly Luciferase Produces Enhanced Bioluminescence. *Biochemistry* , *53* (40), 6287–6289.*doi:* 10.1021/bi501202u

Brunkard, J. O., & Zambryski, P. C. (2017). Plasmodesmata enable multicellularity: new insights into their evolution, biogenesis, and functions in development and immunity. *Current Opinion in Plant Biology* , *35* , 76–83. *doi:* 10.1016/j.pbi.2016.11.007

Burford, B. P., & Robison, B. H. (2020). Bioluminescent backlighting illuminates the complex visual signals of a social squid in the deep sea. *Proceedings of the National Academy of Sciences* ,*117* (15), 8524–8531. *doi:* 10.1073/pnas.1920875117

Callaway, E. (2013). Glowing plants spark debate. *Nature* ,*498* (7452), 15–16. *doi:* 10.1038/498015a

Chen, H., Zou, Y., Shang, Y., Lin, H., Wang, Y., Cai, R., Tang, X., & Zhou, J. (2008). Firefly Luciferase Complementation Imaging Assay for Protein-Protein Interactions in Plants. *Plant Physiology* , *146* (2), 368–376. *doi:* 10.1104/pp.107.111740

Chin, D. P., Shiratori, I., Shimizu, A., Kato, K., Mii, M., & Waga, I. (2018). Generation of brilliant green fluorescent petunia plants by using a new and potent fluorescent protein transgene. *Scientific Reports* , *8* (1). *doi:* 10.1038/s41598-018-34837-2

Chudakov, D. M., Matz, M. V., Lukyanov, S., & Lukyanov, K. A. (2010). Fluorescent Proteins and Their Applications in Imaging Living Cells and Tissues. *Physiological Reviews* , *90* (3), 1103–1163.*doi:* 10.1152/physrev.00038.2009

Ckurshumova, W., Caragea, A. E., Goldstein, R. S., & Berleth, T. (2011). Glow in the Dark: Fluorescent Proteins as Cell and Tissue-Specific Markers in Plants. *Molecular Plant* , *4* (5), 794–804. *doi:* 10.1093/mp/ssr059

Close, D. M., Ripp, S., & Sayler, G. S. (2009). Reporter Proteins in Whole-Cell Optical Bioreporter Detection Systems, Biosensor Integrations, and Biosensing Applications. *Sensors* , *9* (11), 9147–9174. *doi:* 10.3390/s91109147

Cui, B., Zhang, L., Song, Y., Wei, J., Li, C., Wang, T., Wang, Y., Zhao, T., & Shen, X. (2014). Engineering an Enhanced, Thermostable, Monomeric Bacterial Luciferase Gene As a Reporter in Plant Protoplasts. Ed. Hector Candela. *PLoS ONE* , *9* (10), e107885.*doi:* 10.1371/journal.pone.0107885

Desjardin, D. E., Capelari, M., & Stevani, C. (2007). Bioluminescent*Mycena* species from São Paulo, Brazil. *Mycologia* , 99(2), 317–331. *doi:* 10.1080/15572536.2007.11832592

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Doerr, A. (2013). Nano-lantern lights the way. *Nature Methods* ,*10* (2), 104–104. *doi:* 10.1038/nmeth.2356

Ellis, E. A., & Oakley, T. H. (2016). High Rates of Species Accumulation in Animals with Bioluminescent Courtship Displays.*Current Biology* , *26* (24), 1916–1921.*doi:* 10.1016/j.cub.2016.05.043

England, C. G., Ehlerding, E. B., & Cai, W. (2016). NanoLuc: A Small Luciferase Is Brightening Up the Field of Bioluminescence.*Bioconjugate Chem* , *27* (5), 1175–1187.*doi:* 10.1021/acs.bioconjchem.6b00112

Fleiss, A., & Sarkisyan, K.S. (2019). A brief review of bioluminescent systems (2019). *Current Genetics* , *65* (4), 877–882.*doi:* 10.1007/s00294-019-00951-5

Fujii, H., Noda, K., Asami, Y., Kuroda, A., Sakata, M., & Tokida, A. (2007). Increase in bioluminescence intensity of firefly luciferase using genetic modification. *Analytical Biochemistry* ,*366* (2), 131–136. *doi:* 10.1016/j.ab.2007.04.018

Furuhata, Y., Sakai, A., Murakami, T., Nagasaki, A., & Kato, Y. (2020). Bioluminescent imaging of Arabidopsis thaliana using an enhanced Nano-lantern luminescence reporter system. *PLOS ONE* ,*15* (1), e0227477. *doi:* 10.1371/journal.pone.0227477

Glagoleva, A. Y., Shoeva, O. Y., & Khlestkina, E. K. (2020). Melanin Pigment in Plants: Current Knowledge and Future Perspectives.*Frontiers in Plant Science* , *11* ,*doi:* 10.3389/fpls.2020.00770

Gosset, P., Taupier, G., Crégut, O., Brazard, J., Mély, Y., Dorkenoo, K., Léonard, J., & Didier, P. (2020). Excited-State Proton Transfer in Oxyluciferin and Its Analogues. *The Journal of Physical Chemistry Letters* , *11* (9), 3653–3659.*doi:* 10.1021/acs.jpclett.0c00839

Guan, Q., Wu, J., Zhang, Y., Jiang, C., Liu, R., Chai, C., & Zhu, J. (2013). A DEAD Box RNA Helicase Is Critical for Pre-mRNA Splicing, Cold-Responsive Gene Regulation, and Cold Tolerance in*Arabidopsis* . *The Plant Cell* , *25* (1), 342–356.*doi:* 10.1105/tpc.112.108340

Haddock, S. H. D., Moline, M. A., & Case, J. F. (2010). Bioluminescence in the Sea. *Annual Review of Marine Science* , *2* (1), 443–493. *doi:* 10.1146/annurev-marine-120308-081028

Hall, M. P., Unch, J., Binkowski, B. F., Valley, M. P., Butler, B. L., Wood, M. G., Otto, P., Zimmerman, K., Vidugiris, G., Machleidt, T., Robers, M. B., Benink, H. A., Eggers, C. T., Slater, M. R., Meisenheimer, P. L., Klaubert, D. H., Fan, F., Encell, L. P., & Wood, K. V. (2012). Engineered Luciferase Reporter from a Deep Sea Shrimp Utilizing a Novel Imidazopyrazinone Substrate. *ACS Chemical Biology* , *7* (11), 1848–1857. *doi:* 10.1021/cb3002478

Heidemann, A., Völkner, W., & Mengs, U. (1996). Genotoxicity of aloeemodin in vitro and in vivo. *Mutation Research/Genetic Toxicology* , *367* (3), 123–133.*doi:* 10.1016/0165-1218(95)00084-4

Hollis, R. P., Lagido, C., Pettitt, J., Porter, A. J. R., Killham, K., Paton, G. I., Glover, L. A. (2001). Toxicity of the bacterial luciferase substrate, *n* -decyl aldehyde, to *Saccharomyces cerevisiae*and *Caenorhabditis elegans* . *FEBS Letters* , *506* (2), 140–142. *doi:* 10.1016/S0014-5793(01)02905-2

Jin, S., & Daniell, H. (2015). The Engineered Chloroplast Genome Just Got Smarter. *Trends in Plant Science* , *20* (10), 622–640.*doi:* 10.1016/j.tplants.2015.07.004

Kaskova, Z. M., Tsarkova, A. S., & Yampolsky, I. V. (2016). 1001 lights: luciferins, luciferases, their mechanisms of action and applications in chemical analysis, biology and medicine. *Chemical Society Reviews* , *45* (21), 6048–6077.*doi:* 10.1039/C6CS00296J

Kim, S., & Paulmurugan, R. (2021). Bioluminescent Imaging Systems for Assay Developments. *Analytical Sciences* , *37* (2), 233–247.*doi:* 10.2116/analsci.20R003

Khakhar, A., Starker, C. G., Chamness, J. C., Lee, N., Stokke, S., Wang, C., Swanson, R., Rizvi, F., Imaizumi, T., & Voytas, D.F. (2020). Building customizable auto-luminescent luciferase-based reporters in plants. *eLife* , 9. *doi:* 10.7554/eLife.52786

Posted on Authorea 14 Apr 2021 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.161840162.24514207/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Knight, J. (2003). GloFish casts light on murky policing of transgenic animals. *Nature* , *26* . *doi:* 10.1038/426372b

Kotlobay, A. A., Sarkisyan, K. S., Mokrushina, Y. A., Marcet-Houben, M., Serebrovskaya, E. O., Markina, N. M., Somermeyer, L. G., Gorokhovatsky, A. Y., Vvedensky, A., Purtov, K. V., Petushkov, V. N., Rodionova, N. S., Chepurnyh, T. V., Fakhranurova, L. I., Guglya, E. B., Ziganshin, R., Tsarkova, A. S., Kaskova, Z. M., Shender, V., Abakumov, M., Abakumova, T. O., Povolotskaya, I. S., Eroshkin, F. M., Zaraisky, A. G., Mishin, A. S., Dolgov, S. V., Mitiouchkina, T. Y., Kopantzev, E. P., Waldenmaier, H. E., Oliveira, A. G., Oba, Y., Barsova, E., Bogdanova, E. A., Gabaldón, T., Stevani, C. V., Lukyanov, S., Smirnov, I. V., Gitelson, J. I., Kondrashov, F. A., & Yampolsky, I. V. (2018). Genetically encodable bioluminescent system from fungi. *Proceedings of the National Academy of Sciences* , *115* (50), 12728–12732.*doi:* 10.1073/pnas.1803615115

Krichevsky, A., Meyers, B., Vainstein, A., Maliga, P., & Citovsky, V. (2010). Autoluminescent Plants. Ed. Vladimir N. Uversky. *PLoS ONE* , *5* (11), e15461. *doi:* 10.1371/journal.pone.0015461

Kwak, S., Giraldo, J. P., Wong, M. H., Koman, V. B., Lew, T. T. S., Ell, J., Weidman, M. C., Sinclair, R. M., Landry, M. P., Tisdale, W. A., & Strano, M. S. (2017). A Nanobionic Light-Emitting Plant. *Nano Letters* , *17* (12), 7951–7961. *doi:* 10.1021/acs.nanolett.7b04369

Kwak, S., Lew, T. T. S., Sweeney, C. J., Koman, V. B., Wong, M. H., Bohmert-Tatarev, K., Snell, K. D., Seo, J. S., Chua, N., & Strano, M. S. (2019). Chloroplast-selective gene delivery and expression in planta using chitosan-complexed single-walled carbon nanotube carriers.*Nature Nanotechnology* , *14* , 447–455.*doi:* 10.1038/s41565-019-0375-4

Marin Viegas, V. S., Ocampo, C. G., & Petruccelli, S. (2017). Vacuolar deposition of recombinant proteins in plant vegetative organs as a strategy to increase yields. *Bioengineered* , *8* (3), 203–211. *doi:* 10.1080/21655979.2016.1222994

Masuda, H., Takenaka, Y., Yamaguchi, A., Nishikawa, S., & Mizuno, H. (2006). A novel yellowish-green fluorescent protein from the marine copepod, Chiridius poppei, and its use as a reporter protein in HeLa cells. *Gene* , *372* , 18–25.*doi:* 10.1016/j.gene.2005.11.031

Meighen, E. A. (1991). Molecular biology of bacterial bioluminescence.*Microbiological Reviews* , *55* (1), 123–142.*doi:* 10.1128/MMBR.55.1.123-142.1991

Mitiouchkina, T., Mishin, A. S., Somermeyer, L. G., Markina, N. M., Chepurnyh, T. V., Guglya, E. B., Karataeva, T. A., Palkina, K. A., Shakhova, E. S., Fakhranurova, L. I., Chekova, S. V., Tsarkova, A. S., Golubev, Y. V., Negrebetsky, V. V., Dolgushin, S. A., Shalaev, P. V., Shlykov, D., Melnik, O. A., Shipunova, V. O., Deyev, S. M., Bubyrev, A. I., Pushin, A. S., Choob, V. V., Dolgov, S. V., Kondrashov, F. A., Yampolsky, I. V., & Sarkisyan, K. S. (2020). Plants with genetically encoded autoluminescence. *Nature Biotechnology .doi:* 10.1038/s41587-020-0500-9

Modestova, Y., & Ugarova, N. N. (2016). Color-shifting mutations in the C-domain of L. mingrelica firefly luciferase provide new information about the domain alternation mechanism. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* , *1864* (12), 1818–1826.*doi:* 10.1016/j.bbapap.2016.09.007

Oba, Y., Suzuki, Y., Martins, G. N. R., Carvalho, R. P., Pereira, T. A., Waldenmaier, H. E., Kanie, S., Naito, M., Oliveira, A. G., Dörr, F. A., Pinto, E., Yampolsky, I. V., & Stevani, C. V. (2017). Identification of hispidin as a bioluminescent active compound and its recycling biosynthesis in the luminous fungal fruiting body. *Photochemical & Photobiological Sciences* , *16* (9), 1435–1440.*doi:* 10.1039/C7PP00216E

Ow, D. W., DE Wet, J. R., Helinski, D. R., Howell, S. H., Wood, K. V., & Deluca, M. (1986). Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. *Science* ,*234* (4778), 856–859. *doi:* 10.1126/science.234.4778.856

Pirrung, M. C., Carlson, A. D., De Howitt, N., & Liao, J. (2019). Synthesis and bioluminescence of thioluciferin. *Bioorganic & Medicinal Chemistry Letters* , *29* (19).*doi:* 10.1016/j.bmcl.2019.07.050

Posted on Authorea 14 Apr 2021 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.161840162.24514207/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Purtov, K. V., Petushkov, V. N., Baranov, M. S., Mineev, K. S., Rodionova, N. S., Kaskova, Z. M., Tsarkova, A. S., Petunin, A. I., Bondar, V. S., Rodicheva, E. K., Medvedeva, S. E., Oba, Y., Oba, Y., Arseniev, A. S., Lukyanov, S., Gitelson, J. I., & Yampolsky, I. V. (2015). The Chemical Basis of Fungal Bioluminescence. *Angewandte Chemie International Edition* , *54* (28), 8124–8128.*doi:* 10.1002/anie.201501779

Reeve, B., Sanderson, T., Ellis, T., & Freemont, P. (2014).*Bioluminescence: Fundamentals and Applications in Biotechnology - Volume 2* . Berlin, Heidelberg: Springer Berlin Heidelberg. pp. 3–30.

Reuter, D. N., Stewart, C. N., & Lenaghan, S. C. (2020). Lighting the Way: Advances in Engineering Autoluminescent Plants. *Trends in Plant Science* , *25* (12), 1176–1179.*doi:* 10.1016/j.tplants.2020.08.004

Saito, K., Chang, Y., Horikawa, K., Hatsugai, N., Higuchi, Y., Hashida, M., Yoshida, Y., Matsuda, T., Arai, Y., & Nagai, T. (2012). Luminescent proteins for high-speed single-cell and whole-body imaging. *Nature Communications* , *3* (1). *doi:* 10.1038/ncomms2248

Sanchez, E., Artuso, E., Lombardi, C., Visentin, I., Lace, B., Saeed, W., Lolli, M. L., Kobauri, P., Ali, Z., Spyrakis, F., Cubas, P., Cardinale, F., & Prandi, C. (2018). Structure–activity relationships of strigolactones via a novel, quantitative in planta bioassay.*Journal of Experimental Botany* , *69* (9), 2333–2343.*doi:* 10.1093/jxb/ery092

Sasaki, K., Kato, K., Mishima, H., Furuichi, M., Waga, I., Takane, K., Yamaguchi, H., & Ohtsubo, N. (2014). Generation of fluorescent flowers exhibiting strong fluorescence by combination of fluorescent protein from marine plankton and recent genetic tools in *Torenia fournieri* Lind. *Plant Biotechnology* , *31* (4), 309–318.*doi:* 10.5511/plantbiotechnology.14.0907a

Stewart, C. N. (2006). Go with the glow: fluorescent proteins to light transgenic organisms. *Trends in Biotechnology* , *24* (4), 155–162. *doi:* 10.1016/j.tibtech.2006.02.002

Stewart, C. N., Abudayyeh, R. K., & Stewart, S. G. (2018). Houseplants as home health monitors. *Science* , *361* (6399), 229–230.*doi:* 10.1126/science.aau2560

Strack, R. (2019). Building up bioluminescence. *Nature Methods* ,*16* (1), 20–20. *doi:* 10.1038/s41592-018-0274-x

Topalov, G., & Kishi, Y. (2001). Chlorophyll Catabolism Leading to the Skeleton of Dinoflagellate and Krill Luciferins: Hypothesis and Model Studies. *Angewandte Chemie International Edition* , *40* (20), 3892–3894.*doi:* 10.1002/1521-3773(20011015)40:20<3892::AID-ANIE3892>3.0.CO;2-H

Valiadi, M., & Iglesias-Rodriguez, D. (2013). Understanding Bioluminescence in Dinoflagellates—How Far Have We Come?*Microorganisms* , *1* (1), 3–25.*doi:* 10.3390/microorganisms1010003

Verdes, A., & Gruber, D. F. (2017). Glowing Worms: Biological, Chemical, and Functional Diversity of Bioluminescent Annelids.*Integrative and Comparative Biology* , *57* (1), 18–32.*doi:* 10.1093/icb/icx017

Wainwright, P. C., & Longo, S. J. (2017). Functional Innovations and the Conquest of the Oceans by Acanthomorph Fishes. *Current Biology* , *27* (11), 550–557. *doi:* 10.1016/j.cub.2017.03.044

Wang, F., Zhang, N., Guo, Y., Gong, B., & Li, J. (2020). Split Nano luciferase complementation for probing protein-protein interactions in plant cells. *Journal of Integrative Plant Biology* , *62* (8), 1065–1079. *doi:* 10.1111/jipb.12891

Watkins, O. C., Sharpe, M. L., Perry, N. B., & Krause, K. L. (2018). New Zealand glowworm (Arachnocampa luminosa) bioluminescence is produced by a firefly-like luciferase but an entirely new luciferin.*Scientific Reports* , *8* (1).*doi:* 10.1038/s41598-018-21298-w

Hu, W., & Cheng, C. (1995). Expression of *Aequorea* green fluorescent protein in plant cells. *FEBS Letters* , *369* , 331–334. *doi:* 10.1016/0014-5793(95)00776-6

Posted on Authorea 14 Apr 2021 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.161840162.24514207/v1 — This a preprint and has not been peer reviewed. Data may be preliminary.

Yao, Z., Zhang, B. S., Steinhardt, R. C., Mills, J. H., & Prescher, J. A. (2020). Multicomponent Bioluminescence Imaging with a π-Extended Luciferin. *Journal of the American Chemical Society* ,*142* (33), 14080–14089. *doi:* 10.1021/jacs.0c01064

Yu, Y., Yu, P., Chang, W., Yu, K., & Lin, C. (2020). Plastid Transformation: How Does it Work? Can it Be Applied to Crops? What Can it Offer? *International Journal of Molecular Sciences* ,*21* (14), 48–54. *doi:* 10.3390/ijms21144854

Yuan, M., Ma, X., Jiang, T., Gao, Y., Cui, Y., Zhang, C., Yang, X., Huang, Y., Du, L., Yampolsky, I., & Li, M. (2017). Prolonged bioluminescence imaging in living cells and mice using novel pro-substrates for *Renilla* luciferase. *Organic & Biomolecular Chemistry* , *15* (48), 10238–10244.*doi:* 10.1039/C7OB01656E

Table. 1 . Summary of the reported glowing plants.

Luminescence	Engineered Plants	Luminescent specices	Introduced Genes and/or s
FP-derivative	*Torenia fournieri*	*Chiridius poppei*	*CpYGFP* gene
	Chrysanthemum morifolium	*Chiridius poppei*	*CpYGFP* gene
	Petunia hybrida	*Chiridius poppei*	*eYGFP* gene
	Petunia hybrida	*Chiridius poppei*	*eYGFPuv* gene
Nano-lantern	*Nicotinan benthamiana*	Artificial nano-lantern	*pH2GW7_CT- Nano-lante*
	Arabidopsis thaliana	Artificial nano-lantern	*green enhanced Nano-lante*
Firefly	*Nicotiana tabacum*	*Photinus pyralis*	*firefly luciferase* gene, solu
	Daucus carota	*Photinus pyralis*	firefly luciferase gene, solu
	Spinacia oleracea	unknown	three nanocarriers with fir
	Eruca sativa	unknown	three nanocarriers with fir
	Nasturtium officinale	unknown	three nanocarriers with fir
	Brassica oleracea	unknown	three nanocarriers with fir
Bacterium	*Nicotiana tabacum*	*Photobacterium leiognathid*	*luxCDABEG*
Fungi	*Nicotiana benthamiana*	*Aspergillus nidulans* and *Neonothopanus nambi*	gene of *NPGA*, *H3H*, *Hisp*
	Arabidopsis thaliana	*Aspergillus nidulans* and *Neonothopanus nambi*	gene of *NPGA*, *H3H*, *Hisp*
	Solanum lycopersicum	*Aspergillus nidulans* and *Neonothopanus nambi*	gene of *NPGA*, *H3H*, *Hisp*
	Dahlia pinnata	*Aspergillus nidulans* and *Neonothopanus nambi*	gene of *NPGA*, *H3H*, *Hisp*
	Catharathus roseus	*Aspergillus nidulans* and *Neonothopanus nambi*	gene of *NPGA*, *H3H*, *Hisp*
	Petunia hybrida	*Aspergillus nidulans* and *Neonothopanus nambi*	gene of *NPGA*, *H3H*, *Hisp*
	Rosa rubiginosa	*Aspergillus nidulans* and *Neonothopanus nambi*	gene of *NPGA*, *H3H*, *Hisp*
	Nicotiana tabacum	*Neonothopanus nambi*	gene of *H3H, Hisps, Luz a*

NPGA, 4'-phosphopantetheinyl transferase; H3H, hispidin-3-hydroxylase; Hisps, hispidin synthase; Luz, fungal luciferase; and CPH, caffeylpyruvate hydrolase.

Figure legend:

Figure 1 . Flow chart of creating auto-glowing plants.**(A)** The representative native bioluminescent system is elucidated. **(B)** The optimized biosynthetic pathway of luciferin is then reconstituted into plant metabolomic rountines. **(C)** The associated key genes are amplified, cloned into plasmids, and transformed into the plants. **(D)** Once obtaining glowing plants, luminescent characteristic and effects on these transplants will be tested.

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