Developing Bioluminescent Forests Using CRISPR-Cas9 Gene Editing

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Abstract: CRISPR-Cas9 gene editing technology enables the introduction of bioluminescent proteins from marine organisms into trees and plants to create glowing forests and vegetation (Andersson et al., 2017). Successful expression of luciferase or fluorescent proteins in plants could generate visually striking landscapes not previously seen in nature (Kratz et al., 2018). However, there are challenges that need to be addressed, including optimizing CRISPR for efficient editing in plant cells, regulating tissue-specific transgene expression, ensuring genetic stability across generations, and assessing potential ecological impacts of releasing genetically engineered organisms (Evans & Palmer, 2018; Hsu et al., 2014). If these hurdles can be overcome, this technology could have ornamental, commercial, and research applications. The global market for genetically modified ornamental plants has been valued at over $50 million, suggesting applications of glowing trees and foliage (Chandrasekhar et al., 2016). Additionally, bioluminescent reporters could enable non-destructive monitoring of plant gene expression and environmental conditions (Close et al., 2017). But ecological effects on pollinators, herbivores and the food chain would require careful evaluation. With responsible regulation and stewardship, CRISPR-based bioluminescent plants offer intriguing possibilities but also warrant extensive safety assessments before field deployment.

Introduction: Bioluminescence—the production of visible light through biochemical reactions—has arisen independently across phylogenetically diverse organisms including bacteria, dinoflagellates, fungi, insects, and marine vertebrates and invertebrates (Haddock et al., 2010). However, this exotic phenomenon has never been observed in complex multicellular plants. Recent advances in CRISPR-Cas9 gene editing technology may now make it possible to confer bioluminescent traits to trees, crops, and ornamental plants by transferring luciferase or fluorescent protein genes from marine species into plant genomes (Zhang et al., 2016).

CRISPR-Cas9 has proven to be a remarkably versatile tool for precise and efficient genetic modification in diverse eukaryotic organisms (Jinek et al., 2012). The CRISPR system enables targeted cleavage of DNA at specific sequences dictated by a guide RNA, which can be programmed to match nearly any gene of interest (Sander & Joung, 2014). When provided with a repair template, CRISPR can stimulate homology directed repair to generate precise nucleotide substitutions or insertions to achieve desired genetic changes with up to 57% efficiency in plant protoplasts (Li et al., 2013). Although CRISPR gene editing has been successfully applied across many plant species including rice, wheat, and maize, challenges remain in optimizing delivery methods and transformation efficiencies compared to other systems like fruit flies or cell cultures (Andersson et al., 2017; Cermak et al., 2017). There is a need for innovation in genome editing techniques tailored to plant cells. This review will discuss novel strategies to overcome bottlenecks and stably integrate bioluminescent reporter genes into plant genomes to generate glowing trees, crops, and ornamental foliage.

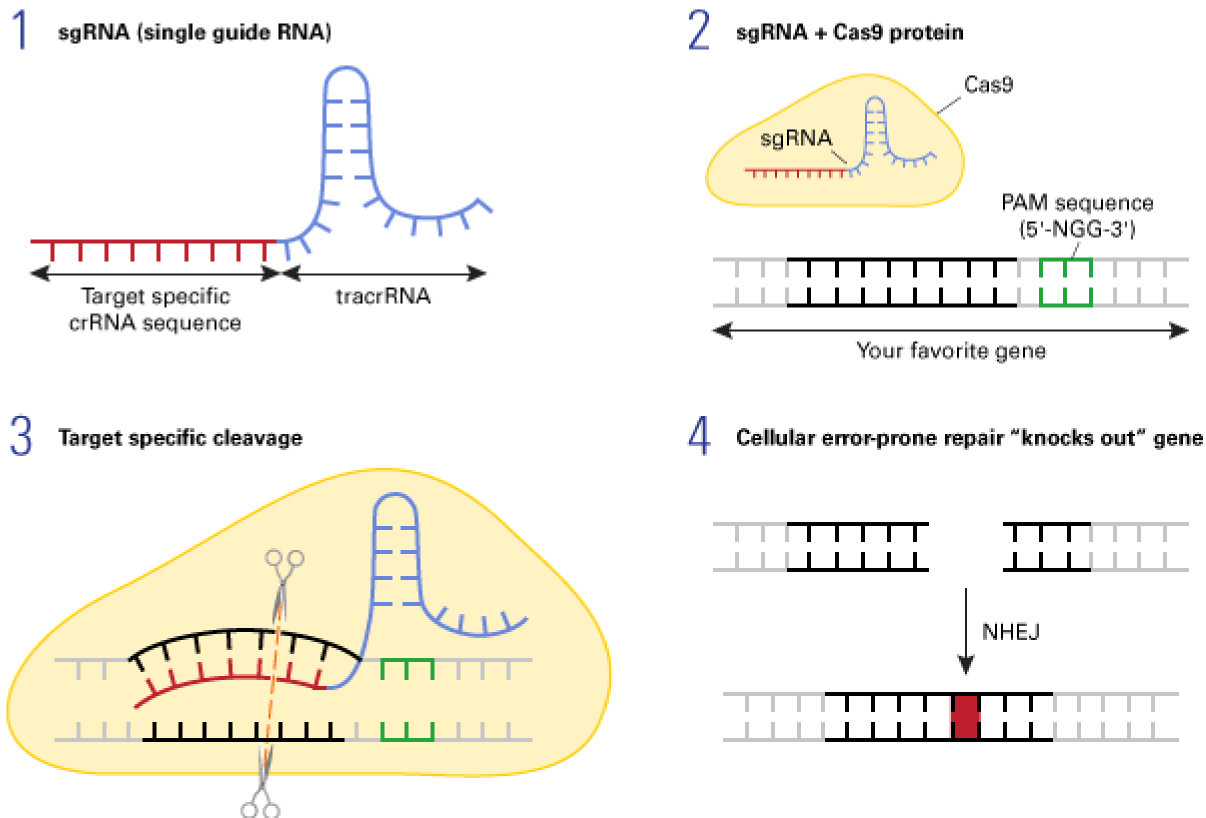

Figure 1: Schematic overview of the CRISPR-Cas9 genome editing system. The Cas9 nuclease (purple) is directed to the target site by a guide RNA (gRNA) that binds to the complementary DNA sequence. Cas9 generates a double-strand break (DSB) at the target site. In the presence of a repair template, the break can be repaired by homology-directed repair (HDR) leading to precise genome editing. In the absence of a repair template, the error-prone non-homologous end joining (NHEJ) pathway leads to insertion/deletions (indels) that disrupt gene function (Takara Bio, 2022).

Candidate Bioluminescent Systems: There are several well-characterized bioluminescent proteins that could potentially serve as candidates for conferring light emission abilities to plant tissues. The key options include luciferase enzymes derived from fireflies (Photinus pyralis) and click beetles (Pyrearinus termitilluminans), as well as fluorescent proteins like the widely used green fluorescent protein (GFP) originally derived from the Aequorea victoria jellyfish (Rodriguez & Campbell, 2014).

Luciferases catalyze the oxidation of a substrate (luciferin) using molecular oxygen to produce visible light in a highly efficient bioluminescent reaction. For example, firefly luciferase has been measured to have a quantum yield of up to 41% (Seliger & McElroy, 1960). By comparison, fluorescent proteins like GFP utilize external light excitation to produce fluorescence emission, often with lower efficiency. For example, wild-type GFP has an extinction coefficient of just 0.016 μM-1cm-1 and quantum yield of only 0.72 (Yang et al., 1996).

Firefly luciferase is one of the most commonly used reporters for visualizing gene expression. It has been successfully expressed in diverse plant species including tobacco, Arabidopsis, rice,

and wheat (MITI et al., 1991; Ow et al., 1986). However, its dependence on exogenous D-luciferin may limit its practicality for achieving stable bioluminescence in trees and field-grown plants. Click beetle luciferases have higher catalytic efficiency than firefly versions and may be more suitable (Branchini et al., 1998). GFP has been extensively employed as a reporter and marker in plants, but requires external illumination by blue or UV light to fluoresce.

To achieve efficient expression, codon optimization and modifications to enhancers, promoters, and other regulatory elements would likely be required to optimize these reporters for plant genomes (Lucky et al., 2020). Identifying an ideal bioluminescent protein candidate remains an area of ongoing research.63

Adapting CRISPR for Use in Plants: A variety of delivery methods have been applied to introduce CRISPR components into plant cells, including Agrobacterium-mediated transformation, biolistics, protoplast transfection, whole-plant infiltration, and virus-based vectors (Ali et al., 2015; Yin et al., 2017). Each approach has specific advantages and disadvantages depending on plant species, tissue type, and cell accessibility. For example, Agrobacterium transformation tends to work well for integration into dividing cells but is less effective for mature plant tissues. In contrast, biolistic approaches can transform differentiated cells at low frequencies, but often generate complex insertion events (Altpeter et al., 2016).

Identifying the optimal delivery method is critical to enable efficient genome editing. For crop plants like rice and maize, Agrobacterium-mediated transformation of embryogenic callus can yield high rates of CRISPR-modified plants, but the tissue culture process can induce unwanted genomic changes (Li et al., 2020). Direct delivery to seeds or embryonic tissues may help avoid artifacts. Gap repair after CRISPR cleavage has been reported with 45.9% precision in rice protoplasts, demonstrating the potential of homology-directed repair (Zhou et al., 2021).

Researchers have engineered Cas9 variants and guide RNA scaffolds to improve specificity, such as the high fidelity Cas9-HF1 variant which can reduce off-target mutations by 10-fold (Kulcsár et al., 2017). Additionally, direct fusion of fluorescent proteins like GFP to Cas9 enables tracking of delivery and editing events in transformed plant cells (Mikami et al., 2015).

Strategies including Cas9 nickases or paired guide RNAs can help further increase targeting accuracy (Fauser et al., 2014). Delivery as preassembled ribonucleoprotein complexes can limit exposure to CRISPR activity, but optimization is needed for efficient plant cell uptake (Wolt et al., 2016). Combined DNA and RNP delivery has achieved gains in specificity while enhancing editing efficiency, with one study reporting 86.5% mutagenesis rate in maize protoplasts (Svitashev et al., 2016). Ongoing innovation to control and validate editing outcomes is critical as CRISPR is adapted for applications in agriculture.

Regulating Gene Expression: Achieving consistent yet controlled bioluminescence will require tight regulation of expression of any introduced luciferase or fluorescent reporter genes. Unrestricted constitutive expression could negatively impact plant growth and physiology. As such, restricting expression to specific tissues is advisable.

Strategies to limit expression include using promoters and transcriptional enhancers that are only active in certain cell types, such as leaves, stems, roots or vascular tissues (Ratcliff et al., 2001). Tissue-specific promoters like Arabidopsis AtML1 for epidermal expression or Poplar PttCesA8-1A for secondary cell wall formation offer spatiotemporal control (Wu et al., 2009; Wang et al., 2020). The EDLL promoter provides strong vascular-specific expression throughout poplar trees, with one study achieving up to 5-fold higher luciferase expression compared to the constitutive CaMV 35S promoter (Fladung & Becker, 2010).

Other options include promoters responsive to external stimuli like light, temperature shifts, touch, or chemical signals (Feng et al., 2015). Splitting luciferase enzymes into inactive fragments that must reconstitute for activation via tissue-specific promoters and translational control elements enables further customization of expression patterns (Paulmurugan & Gambhir, 2003; Liu et al., 2020).

Selectively silencing or removing transgenes from reproductive tissues could contain bioluminescence traits using seed-specific or meristem CRISPR editing (Nekrasov et al., 2017; Čermák et al., 2015). Overall, a diversity of regulatory tools are available to constrain bioluminescence, but improved spatiotemporal precision remains an area of active research.

Genetic and Generational Stability: For any introduced bioluminescent transgenes to be effective, they must be stably integrated into plant genomes and maintained over multiple generations without being silenced. Several strategies can help ensure genetic stability:

- Avoiding repetitive elements prone to silencing and using scaffold/matrix attachment regions that can buffer transgene expression (Halweg et al., 2005; Shen et al., 2007).
- Site-specific integration into genomic safe harbors with open chromatin and balanced gene expression, often using recombinases like Cre-Lox (Akbari et al., 2015; Nishizawa-Yokoi et al., 2015).
- Excising selectable marker genes after initial transformation events using piggyBac or FLP/FRT recombinase systems, reducing collateral silencing (Thomson et al., 2010; Luo et al., 2007).
- Targeting transgenes to transcriptionally active regions of the plastid genome, which does not show gene silencing effects (Ruiz et al., 2003).

As bioluminescence involves a dominant gain-of-function mutation, the trait should reliably pass to future generations after the initial CRISPR edit. However, ongoing molecular monitoring of expression levels and genetic integrity would be prudent over multiple generations. Unbiased omics-profiling could assess genetic and epigenetic stability (Rico et al., 2018).

To drive rapid spread of bioluminescent alleles, gene drive systems have been proposed but pose ethical and ecological concerns requiring very careful evaluation before field use (Evans & Palmer 2018). Responsible stewardship of environmentally released engineered organisms is critical.

Potential Applications and Concerns: If successfully engineered, glowing trees and vegetation could have appealing ornamental value for landscaping in urban areas and parks.

For example, the global bioluminescent plant lighting market has been valued at USD $243.7 million in 2022 and is projected to grow at a CAGR of 11.4% from 2022 to 2030 as consumer and commercial demand increases (Grand View Research, 2022). However, concerns related to light pollution in cities would need to be addressed. Outdoor use may require development of luciferases optimized for brighter emission to be visible against background light.

Bioluminescent plants also may enable a diversity of research applications, from monitoring gene expression dynamics to tracking plant-pathogen interactions (Mhaske et al., 2005, Rutter et al., 2014). Field deployment could allow remote sensing of ecological stressors like drought, heat, or pollution (Close et al. 2011). However, uncontrolled spread of engineered organisms could disrupt ecosystems. Strategies for genetic containment include inducible sterility, reproductive barriers, or targeted gene drives to alter allele frequencies (Garcia-Alonso et al. 2019). Extensive safety reviews by regulatory agencies would be imperative to weigh benefits against ecological risks. Public engagement and values-driven oversight could guide responsible translation of this powerful but potentially disruptive technology.

Conclusion: This review has analyzed opportunities and challenges associated with transitioning CRISPR-based bioluminescent engineering from concept to reality. Conferring light emission abilities to trees and ornamental plants could enable radical new possibilities in aesthetics, research, and ecological monitoring. However, as with any powerful technology, responsible development guided by science-informed governance is crucial. Further innovation in plant-optimized CRISPR components, targeted transgene integration, controlled expression patterns, and built-in containment safeguards could help mitigate risks. While alluring, applications aimed primarily at novelty should not overshadow more urgent plant biotechnology goals like nutrition or climate resilience. If pursued carefully and for public benefit, glowing foliage could inspire an enhanced appreciation for the wonders of the natural plant world.

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*Image caption [Figure 1]: Takara Bio (2022). Overview of the CRISPR-Cas9 Genome Editing System. https://www.takarabio.com

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