Plant Pathology Seminar Series

Bioluminescent fungi, a source of genes to monitor plant stresses and changes in the environment

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Bioluminescence is a natural phenomenon of light emission by a living organism resulting from oxidation of luciferin catalyzed by the enzyme luciferase (Dubois 1887). This process serves as a powerful biological tool for scientists to study gene expression in plants and animals. A wide diversity of living organisms is bioluminescent, including some fungi (Shimomura 2006). For many of these organisms, the ability to emit light is a defining feature of their biology (Labella et al. 2017; Verdes and Gruber 2017; Wainwright and Longo 2017). For example, bioluminescence in many organisms serves purposes such as attracting mates and pollinators, scaring predators, and recruiting other creatures to spread spores (Kotlobay et al. 2018; Shimomura 2006; Verdes and Gruber 2017).

Oliveira and Stevani (2009) confirmed that the fungal bioluminescent reaction involved reduction of luciferin by NADPH and a luciferase. Their findings supported earlier studies by Airth and McElroy (1959) who found that the addition of reduced pyridine nucleotide and NADPH resulted in sustained light emission using the standard luciferin-luciferase test developed by Dubois (1887). Additionally, Kamzolkina et al. (1984;1983) and Kuwabara and Wassink (1966) purified and crystallized luciferin from the fungus *Omphalia flavida*, which was active in bioluminescence when exposed to the enzyme prepared according to the procedure described by Airth and McElroy (1959). Decades after, Kotlobay et al. (2018) showed that the fungal luciferase is encoded by the *luz* gene and three other key enzymes that form a complete biosynthetic cycle of the fungal luciferin from caffeic acid. These enzymes include hispidin-3-hydroxylase, hispidin synthase, and caffeyl pyruvate hydrolase encoded by *h3h*, *hisps,* and *cph*, respectively. The genes were found in the fungi *Neonothopanus nambi*, *Neonothopanus gardneri*, *Mycena citricolor*, and *Panellus stipticus* (Kotlobay et al. 2018). The *luz* gene was identified by amplifying cDNA and cloning the gene into the GAP-pPic9K vector using BamHI/NotI restriction sites. The GAP-pPic9K vector was constructed from the plasmid vector pPic9K by replacing the inducible alcohol oxidase promoter (AOX1) and alpha-factor signal sequences with the glyceraldehyde-3-phosphate dehydrogenase (GAP) promoter from the yeast *Pichia pastoris*. Identification of the *luz* gene resulted in discovery of the gene cluster with *h3h* and *hisps*. To test whether *hisps* produced the luciferin precursor in a heterologous system, reconstruction of the bioluminescent pathway was performed by co-integration of *luz*, *h3h*, and *hisps* from *N. nambi*, and 4'-phosphopantetheinyl transferase (*npgA*) from *Aspergillus nidulans*, into the genome of *P. pastoris* strain GS115 by electroporation. The light produced by *P. pastoris* was visible to the naked eye. This study showed that the fungal bioluminescent pathway can be produced and used in an inexpensive manner since no specialized imaging equipment is needed to visualize the bioluminescence (Kotlobay et al. 2018).

Khakhar et al. (2020) and Mitiouchkina et al. (2020) introduced the genes identified by Kotlobay et al. (2018) into the *Agrobacterium tumefaciens* plasmid as a vector to induce autoluminescence in tobacco and other plant species. They demonstrated how the fungal bioluminescence pathway can be used to develop reporters to monitor plant gene activity in live plant tissues over time, examine details of the response to plant hormones, and build reporters that emit light without the need to add chemicals. This system has many potential applications, including helping plants attract insects to pollinate flowers, and building plant biosensors that emit light in response to environmental signals (Khakhar et al. 2020; Kotlobay et al. 2018). The discovery of these genes involved in converting caffeic acid to luciferin, coupled with light emission, presents a new way of generating and recycling luciferin, eliminating the need for exogenous addition of luciferin, which is expensive and toxic. This system also avoids the need for bacterial bioluminescence genes in eukaryotic systems, as the former are cumbersome technically and fail to produce enough light for visualization without a microscope (Khakhar et al. 2020; Krichevsky et al. 2010; Mitiouchkina et al. 2020).

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Zoom Link: https://wsu.zoom.us/j/93395333254?pwd=OVlwWk8xcnNadVluVjFYUW5hWWx1dz09
Meeting ID: 933 9533 3254
Passcode: 305936
Call in number: 1 253 215 8782

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