International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 930-933 www.ijprajournal.com ISSN: 2249-7781

Fungal Bioluminescent System: New Way to Develop Glowing Ornamental Plants

Ganesuni Lakshmi Prasanna* and Laveti Gowthami
*Ph. D Scholar, Department of Plant Pathology and Ph. D Scholar, Department of Floriculture, Dr. YSRHU, College of Horticulture, Anantharajupeta, YSR Kadapa – 516105, Andhra Pradesh

Submitted: 01-02-2022 Accepted: 10-02-2022

ABSTRACT: The recent advances in the application of bioluminescence through incorporated green fluorescent proteins (GFPs) gene expression and also light emission from luciferase requires exogenous addition of a luciferin substrate in ornamental plants existence have lagged in the lower back of the recent development of effective genetic tools. Bioluminescence is a phenomenon of emission of light by organism, has been observed in wide range of bacteria, fungi, insect, marine invertebrates and animals except terrestrial greater flora. Through genetic engineering, it is manageable to introduce bioluminescent genes into living plant cells as biomarkers. the fungal luciferase and other three key enzymes that form the biosynthetic cycle of the fungal luciferin from caffeic acid, a simple and widespread metabolite that were used to express autoluminescence in eukaryotes. This overview is beneficial for the advent of the optimized glowing plants, which can be used not only in scientific research purpose as biomarkers, but also well promising substitutes of artificial lighting sources in the future.

Keywords: Bioluminescence, Luciferase, Gene expression, Genetic engineering, Caffeic acid, and Eukaryotes.

I. INTRODUCTION

Bioluminescence is a physical phenomenon to emit light, which was discovered in wide variety of organisms such as bacteria, fungi, insect, marine invertebrates and animals (Haddock et al, 2010). The mechanism of light emission is broadly conserved by an enzymatic oxidation reaction by a luciferase enzyme turns a luciferin substrate into a high energy intermediate, which decays to produce light in firefly, in this the light is emitted when a flavin pigment, luciferin, is oxidized in the presence of luciferase, an enzyme also produced by this organism, Renilla luciferases and in bacteria, the described pathway in bacterial has limited the application in eukaryotes which were used to build reporters to study gene expression in plants and other eukaryotes system (Shimomura, 2006). The light produced these organisms is usually blue-green in colour. But there is one major constrain that fails to express uniform delivery and penetration of the luciferin substrate, especially in matured plant tissues. However, these reporter genes also require added chemicals to the tissues to emit light. These chemicals lean to be expensive and many of these chemicals may not penetrate uniformly into the plant tissues of targeted, which limits the potential applications of the reporters in scientific studies.

Recently, it has also been discovered that fungi have a bioluminescence pathway that converts a molecule known as caffeic acid into luciferin. The chemical caffeic acid is a common molecule in plants, so it is possible that fungal bioluminescence pathway could be used to build reporters that produce light without any other additional chemicals. These genes that encode the enzymes of the fungal bioluminescence pathway inserted into the tobacco plants (Khakhar et al., 2018). An autoluminescence pathway which is genetically encodable in plants would have considerable potential with the fungal bioluminescent system being a suitable candidate for the development of such technology. Autoluminescent plants genetically engineered with reporters to express bioluminescence have not been widely adopted because of low light output. Because of this, scientist has developed engineered tobacco plants with a fungal bioluminescence system to produce or emit self-sustained bioluminescence that is visible to the naked eye. Here, in this review we described the function and evolution of the key genes responsible for the bioluminescence of the fungus (Neonothopanus nimbi) and it shown that the expression of fungal genes is more sufficient to engineer auto-luminescent eukaryotes mostly plants.

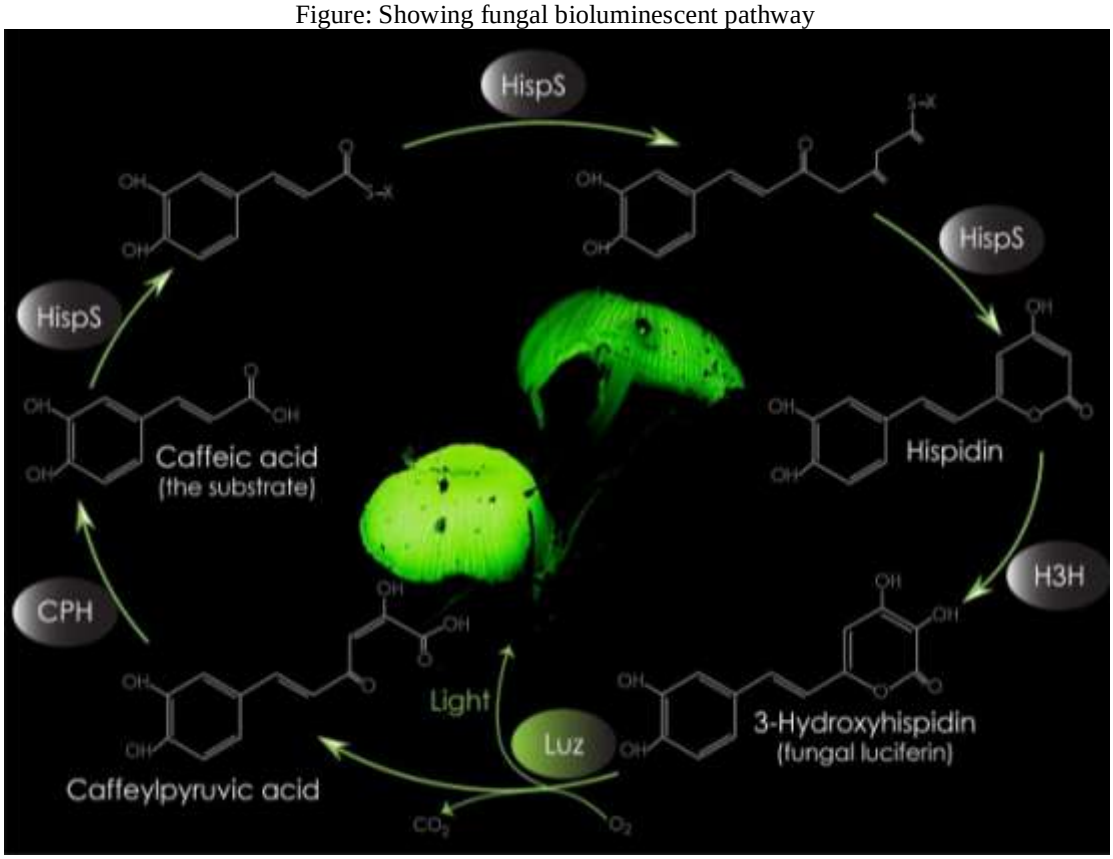
Bioluminescent system used to develop auto-luminescent ornamental plants from fungi

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 930-933 www.ijprajournal.com ISSN: 2249-7781

Ornamental glowing plants could be developed, when the fluorescence or bioluminescence was bright enough to express in plants system. The expression cassettes of these bio-fluorescent proteins are easily to be integrated into the genome, if fluorescent proteins are accumulated enormously in plant cells, then it is feasible to generate fluorescent plants. There are more than100 species of fungi belongs to the order Agaricales emit light by using biochemical reaction. Fungal bioluminescence system utilizes four different components such as the luciferin, which was identified as 3-hydroxyhispidin is a product of molecular oxidation of the simple plant and second one, the fungal metabolite hispidin and the other one undescribed key enzyme, which are NADPH-dependent hydroxylase and finally, an enzyme luciferase.

The bioluminescent fungi make light by using a chemical reaction, which involves luciferin, a luciferase enzyme and molecular oxygen. This chemical reaction, called bioluminescence, is almost similar to how fireflies produce light. To identify and isolate the enzymes which involved in fungal bioluminescent pathway that is luciferase gene, researches has used the genes expressing the Neonothopanus nimbi cDNA library in Pichia pastoris and spraying agar plates with synthetic 3-hydroxyhispidin, and then identified and sequenced a luminescent yeast colony expressing the luciferase gene. The Neonothopanus nambi luciferase, n-Luz, is a 267 amino acids containing protein and has no homolog sequence similarity to conserved protein domains, representing a novel protein family.

Figure: Showing fungal bioluminescent pathway



Genes coding for enzymes that synthesize secondary metabolites are often clustered in fungal genomes (Keller et al., 2005). Researchers hypothesized that clustering may be due to the bioluminescent enzymes cascade; because the cascade is conserved among the fungi which shown bioluminescence and thus looked for genes related to luciferin biosynthesis in the vicinity of the luciferase gene in the Neonothopanus nambi genome. Along with N. nambi, researchers also sequenced genomes and transcriptomes of few bioluminescent fungi like Mycena citricolor,

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 930-933 www.ijprajournal.com ISSN: 2249-7781

Neonothopanus gardneri, and Panellus stipticus and compared these fungi with available genome sequences of bioluminescent and non-bioluminescent fungi and found that the luciferase is a member of a conserved gene cluster, which also includes two other genes viz., h3h and hisps (Grigoriev et al., 2014). The h3h gene has sequence similarity with 3-hydroxybenzoate 6-monooxygenases, enzymes that catalyze oxidation reaction of NADPH-dependent hydroxylase and molecular oxygen and the reaction is identical to that of enzymatic reaction which converts hispidin into luciferin, the h3h gene codes for hispidin-3-hydroxylase (H3H), the enzyme corresponding to the predicted hydroxylase. The cloned gene from Neonothopanus nambi and P. pastoris colonies expressing both nnluz and nnh3h emit light when sprayed with luciferin precursor hispidin. Conservation of this gene cluster suggests that, in contrast to other groups of bioluminescent organisms, bioluminescence evolved in fungi only once, with luz, h3h, and hisps genes which are emerging through gene duplications.

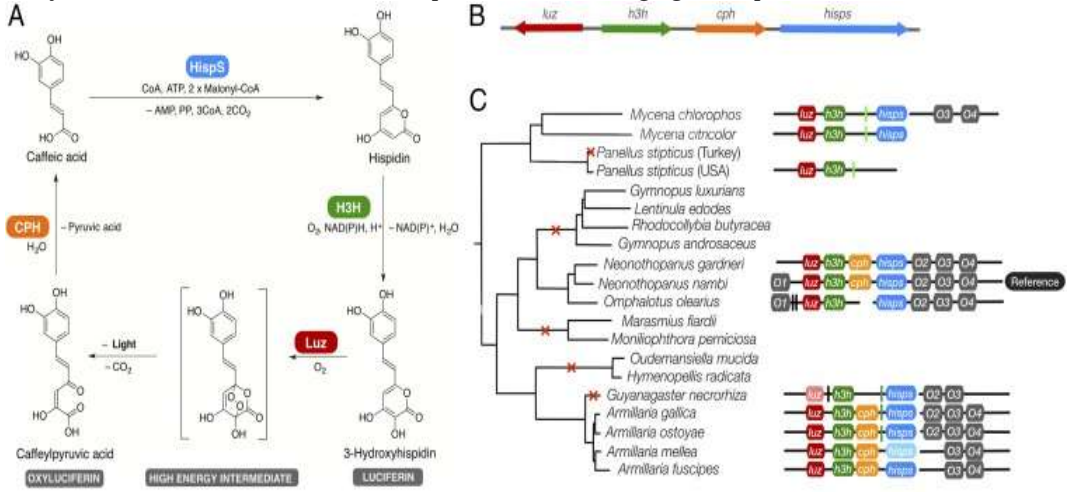


Figure: Showing luciferin biosynthetic pathway and gene clustering in Neonothopanus nambi

The wild type Neonothopanus nambi luciferase enzyme is functional in heterologous systems, as a promising reporter gene, and fungal luciferin, a water-soluble and cell-permeable compound can also be synthesized from aromatic amino acids in other eukaryotes and its light-emitting reaction is not dependent on the availability of ATP, making the fungal bioluminescent system more interesting for numerous applications in genetic engineering and molecular biotechnology.

Among the various ornamental plants having different colors of flowers, those with white flowers contain less petal pigments, indicating that less excitation of light and fluorescence would be absorbed, so mostly the plants with white flowers were chosen in the experimental trials (Chin et al., 2018). Some of the commercial plants were transformed with CpYGFP, and green fluorescence, which was observed from the flowers when emission filter was, used (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.

Table-1: Showing the list of ornamental plants developed from the bioluminescent system of fungi Aspergillus nidulans and Neonothopanus nimbi (Khakhar et al., 2020)

S. No.	Plants developed through genetic engineering technique	Gene of interest Introduced into the plants
1.	Dahlia pinnata	NPGA, H3H, Hisps, Luz and CPH
2.	Petunia hybrida	NPGA, H3H, Hisps, Luz and CPH
3.	Solanum lycopersicum	NPGA, H3H, Hisps, Luz and CPH

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 930-933 www.ijprajournal.com ISSN: 2249-7781

4.	Catharathus roseus	NPGA, H3H, Hisps, Luz and CPH
5.	Nicotiana benthamiana	NPGA, H3H, Hisps, Luz and CPH
6.	Arabidopsis thaliana	NPGA, H3H, Hisps, Luz and CPH

II. CONCLUSION:

The enzymatic cascade that leans to emit light by fungi is a eukaryotic bioluminescence system with known biosynthesis of luciferin and it is shown that the luciferin is synthesized from its precursor hispidin by N. nambi H3H and that hispidin can be directly synthesized by hispidin synthase from caffeic acid, a widespread cellular metabolite. Attempts to develop autoluminescent plants have been constrained by the poor performance in eukaryotes by bacterial bioluminescent system are the only system for which luciferin biosynthesis was known. Reconstitution of fungal bioluminescent pathway in eukaryotic organisms especially plants might be used in the applications where organisms are genetically engineered in their physiological state with autonomous light emission to develop next generation glowing plants.

REFERENCES

[1]. Airth, R. L. and Mcelroy, W. D. 1959. Light emission from extracts of luminous fungi. Journal of Bacteriology. 77(2):249-50.
[2]. Chin D. P., Shiratori, I., Shimizu, A., Kato, K., Mii, M. and Waga, I. 2018. Generation of brilliant green fluorescent petunia plants by using a new and potent fluorescent protein transgene. Scientific Reports. 8(1): 16556.
[3]. Grigoriev, Nikitin, R., Haridas, S., Kuo, A., Ohm, R., Otillar, R., Riley, R., Salamov, A., Zhao, X., Korzeniewski, F., Smirnova, T., Nordberg, H., Dubchak, I. and Shabalov, I. 2014. MycoCosm portal: gearing up for 1000 fungal genomes. Nucleic Acids Res. 42: D699-704.
[4]. Haddock, S. H., Rivers, T. J. and Robison, B. H. 2001. Dietary requirement for luciferin in cnidarian bioluminescence. Proc. Natl. Acad. Sci. pp: 11148–11151.
[5]. Keller, N. P., Turner, G. and Bennett, J. W. 2005. Fungal secondary metabolism from biochemistry to genomics. Nat Rev Microbiol. 3(12): 937-47.
[6]. Khakhar, A., Leydon, A. R., Lemmex, A. C., Klavins, E. and Nemhauser, J. L. 2018. Synthetic hormone-responsive transcription factors can monitor and re-program plant development. eLife 7:e34702.
[7]. Khakhar, A., Starker, C. G., Chamness, J. C., Lee, N., Stokke, S., Wang, C., Swanson, R., Rizvi, F., Imaizumi, T. and Voytas, D. F. 2020. Building customizable auto-luminescent luciferase-based reporters in plants. eLife. 9: e52786.
[8]. Kishi-Kaboshi, M., Aida, R. and Sasaki, K. 2017. Generation of gene-edited chrysanthemum morifolium using multicopy transgenes as targets and markers. Plant and Cell Physiology. 58(2): 216–226.
[9]. Li, B., Chen, R., Zhu, C. and Kong, F. 2021. Glowing plants can light up the night sky? A review. Biotechnology and Bioengineering. 118(10): 3706–3715.
[10]. Shimomura, O. 2006. Bioluminescence: Chemical Principles and Methods. World Scientific Publishing Co.
[11].