SEMINAR REPORT

Glowing Plants - Synthetic Biology

By

Bharde Pranali Rajendra

2018-11-140

Presented on 08/11/2019

Submitted in partial fulfilment of requirement of the course

MBB 591 Masters' Seminar (0+1)

CENTRE FOR PLANT BIOTECHNOLOGY AND MOLECULAR BIOLOGY

COLLEGE OF HORTICULTURE

KERALA AGRICULTURAL UNIVERSITY

VELLANIKKARA

THRISSUR, KERALA- 680656

DECLARATION

I, Bharde Pranali Rajendra (2018-11-140) declare that the seminar entitled **"Glowing Plants - Synthetic Biology"** has been prepared by me, after going through various references cited at the end and has not been copied from any of my fellow students.

Vellanikkara — Bharde Pranali Rajendra

27-01-2020 — (2018-11-140)

CERTIFICATE

This is to certify that the seminar report entitled **"Glowing Plants - Synthetic Biology"** has been solely prepared by **Bharde Pranali Rajendra (2018-11-140)**, under my guidance and has not been copied from seminar reports of any seniors, juniors or fellow students.

Vellanikkara

27/01/2020

Dr. Rose Mary Francies
Professor and Head

(Plant Breeding and genetics)
Department of Seed Science and Technology

College of Horticulture,

KAU, Vellanikkara - 680656

CERTIFICATE

This is to certify that the seminar report entitled **"Glowing Plants - Synthetic Biology"** is a record of seminar presented by **Bharde Pranali Rajendra (2018-11-140)** on **08th November, 2019** and is submitted for the partial requirement of the course MBB 591.

Dr. Anil Kuruvila

Professor

Dept. of Agricultural Economics

College of Horticulture Vellanikkara

Dr. Reshmy Vijayaraghavan

Assistant professor

Dept. of Plant Pathology

College of Horticulture Vellanikkara

Dr. Sangeeta Kutty M.

Assistant professor

Dept. of Vegetable Science

College of Horticulture Vellanikkara

INDEX

LIST OF PLATES

I. Introduction

Glowing plants or Glow- in- the- dark plants or Autoluminiscent plants are capable of emitting light. These are not of natural occurrence. Researchers have genetically engineered plants like tobacco, water cress using synthetic biology techniques to glow in the dark. Bioluminescence is one of nature's spectacular tricks. The glow in the plants is due to the phenomenon of '**Bioluminescence**' meaning light emission by biological organisms. Bioluminescence is an inner glow exhibited by many organisms in nature. Light is produced by a chemical reaction occurring within a living organism (Pandey and Sharon, 2017).

The natural bioluminescent organisms include various terrestrial creatures such as beetles, fungi and bacteria. These organisms develop the capacity of bioluminescence as per their habitat and survival needs. For example, many luminescent bacteria exist planktonically, as gut symbionts, as saprophytes or parasites or in specialized light organs of certain fishes and animals in marine environments (Pandey and Sharon, 2017). Bioluminescence is a type of chemiluminescence, which produces cold light and a very little heat is generated in the process. It is based on a specific molecule called luciferin. It was the French pharmacologist Raphael Dubois who first initiated the research in this direction.

2. Biochemical mechanism leading to bioluminescence

It is basically an enzyme substrate interaction. All bioluminescence reactions involve oxidation of an organic molecule (called the luciferin). The reaction is catalyzed by an enzyme called a luciferase and in many cases, the bioluminescence intensity is assumed to reflect the velocity of the enzyme-substrate reaction, and this intensity is used to analyze the kinetics on the Michaelis-Menten model.

2.1 Reaction

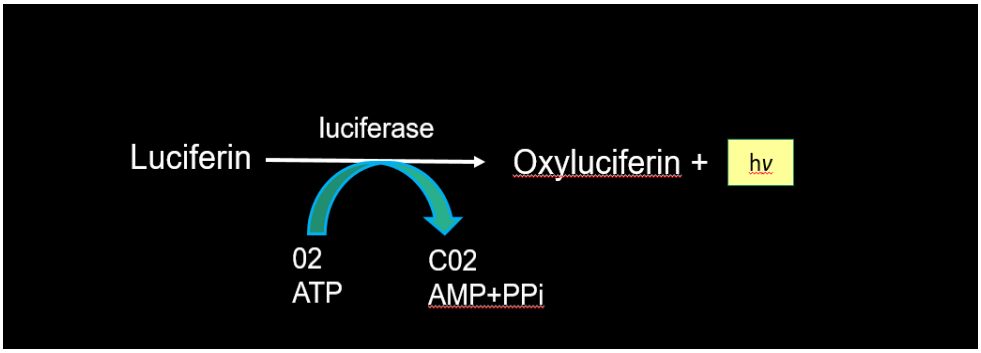


Plate: 1 The reaction leading to the glow

Luciferin acts as a substrate. Oxidation of luciferin is catalysed by enzyme luciferase which results in the production of oxyluciferin and light emission.

2.2 Mechanism

Plate: 2 It is basically an enzyme substrate interaction. In bioluminescence a luciferin produce light and luciferase allow the light producing chemical reaction takes place

Plate: 3 In this reaction luciferase act as a catalyst

Plate: 4 The luciferase allows the oxygen to combine the luciferin

Plate: 5 This reaction produces photons of light

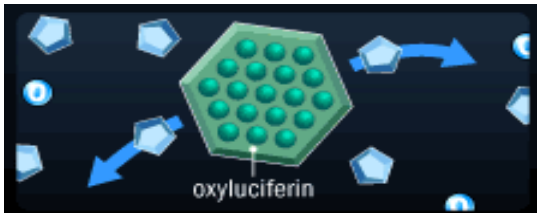


Plate: 6 The oxidized luciferin becomes inactive oxyluciferin

3. Bioluminescence and its occurrence:

Bioluminescent organisms are widely distributed, mostly inhabiting terrestrial and marine ecosystems. These organisms develop the capacity of bioluminescence as per their habitat and survival needs. For example, many luminescent bacteria exist planktonically, as gut symbionts, as saprophytes or parasites or in specialized light organs of certain fishes and animals in marine environments. These bacteria whether they are free living or in association with higher organisms, utilize bioluminescence for vital functions ranging from defence to reproduction. So far as terrestrial or higher bioluminescent organisms are concerned they use this property mainly for attracting prey, intra-species communication and escape from predators. However, this phenomenon is not reported to exist normally in plants, amphibians, reptiles, birds and mammals.

Few examples of organism that glow in the dark:

3.1 Firefly bioluminescence:

Firefly is an insect belonging to the family *Lampyridae* with about 2000 species. They are found in habitats like wet decaying wood, marshes, river side etc. of tropical and temperate areas. They produce yellow, green or light red luminescence from their lower abdomen to attract mates and prey of the known bioluminescent systems. Firefly luciferase is particularly well studied and characterized due to their common use as reporter genes. The luciferase from *Photinus pyralis* was the first to be chosen for creating glowing transgenic plant. The protein was expressed in tobacco plants, from a cauliflower mosaic virus promoter, and the resultant genetically modified organisms (GMOs) were then treated with luciferin. Light emission was seen when plants were placed on photographic film with the brightest light tracing out of the water transporting vessels of the leaves and stem (Pandey and Sharon, 2017).

Plate: 7 Firefly

3.2 Deep-Sea Bioluminescence Blooms:

The deep ocean is the largest and least known ecosystem on Earth. It hosts numerous pelagic organisms, most of which are able to emit light. Luminous bacteria most likely are the main contributors to the observed deep-sea bioluminescence blooms. Many observations provide a consistent and rapid connection between deep sea convection and bathypelagic biological activity, expressed by bioluminescence. Deep-sea bioluminescence is also viewed as an expression of abundance and adaptation of organisms to their environment. Marine bioluminescent organisms include a variety of distinct taxa. When stimulated mechanically or electrically, eukaryotic bioluminescent organisms emit erratic luminous flashes, and also spontaneous flashes to attract prey and mates for recognition of congeners or for defence purposes. In contrast, luminescent bacteria are unaffected by mechanical stimulation and can glow continuously for many days under specific growth conditions. Bioluminescent bacteria are observed in marine waters as free-living forms, as symbionts in luminous organs of fishes and crustaceans and attached to marine snow aggregates sinking through the water column. A strong bioluminescence is observed by colonies of bacteria during micro algae blooms.

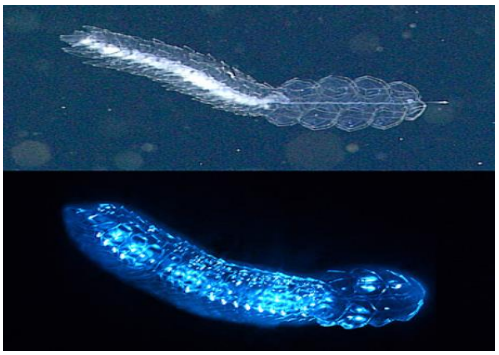
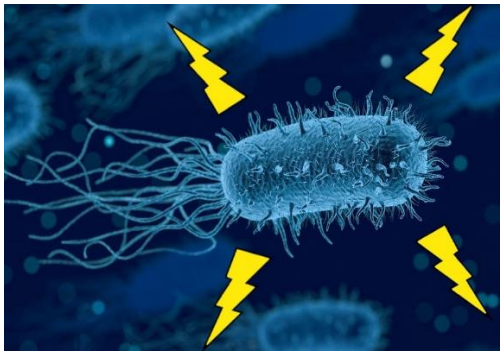


Plate: 8 Dinoflagellates



Plates: 9 *Shewanella*

Dinoflagellates are unicellular organisms with two flagella. Next to fireflies, dinoflagellates are the most commonly encountered bioluminescent organism. They typically cause the sparkling lights in the water seen by sailors, swimmers, and beachgoers, and they produce the "bioluminescent bays" which are tourist destinations in Puerto Rico and Jamaica. These protists can be autotrophic (photosynthetic) or heterotrophic, feeding on other phytoplankton and prey. In large numbers, some species may form potentially toxic red tides, typically during a stratified calm period after an influx of nutrients. Dinoflagellates including *Gonyaulax, Noctiluca, Protoperidinium* and *Pyrocystis* exhibit their ability to luminescence, and allocate energy to bioluminescence before growth, although luminescence serves secondary to the ability to swim.

Fish bioluminescence is observed in at least 42 families across 11 orders of bony fishes, including one family of sharks. In contrast with invertebrate taxa, most of these groups use bacterial symbionts for light production, including the angler fishes, flashlight fish like *Photoblepharon* spp, and shallow ponyfishes like *Leiognathus* spp (Pandey and Sharon, 2017).

3.3 Self-luminescence in marine organism

Plate: 10 Jelly fish

Plate: 11 Angler fish

In Jelly fish bioluminescence is a common phenomenon, particularly in marine organisms, that arises from the oxidation of a substrate (a luciferin) by an enzyme (a luciferase), usually in the presence of molecular oxygen. *A. victoria* is a hydromedusan jellyfish that

belongs to the coelenterates and is commonly found in the sea off the north-eastern USA. Apart The classical luciferin–luciferase mechanism; the green luminescence arises from a photoprotein called the green-fluorescent protein (GFP), which is found in specialized organs situated in the umbrella of the jellyfish (Jones, K.,1999).

3.4 Bioluminescence through symbiosis

Deep sea fish consists of essentially monochromatic light from two sources: first dim down welling light with a chromatic spectrum centering on 475 nm and second bioluminescence, with spectra characteristically peaking at 460-490nm (Widder, *et al*., 1983).

The **firefly squid** *Watasenia scintillans* lives deep (below 250m in the daytime) in the open ocean around Japan. The mature squid has a mantle length of about 6 cm. This species is known as the firefly squid because of its intense bioluminescence emitted from three large photophores at the tips of the fourth arms (Tsuji, 1985). It also has five relatively large photophores on the ventral side of each eye and numerous small photophores distributed mainly over the ventral surface of the body (Inamura et al. 1990), which provide a counter-illumination system to camouflage the silhouette of the body against downwelling light (Herring, 1988).

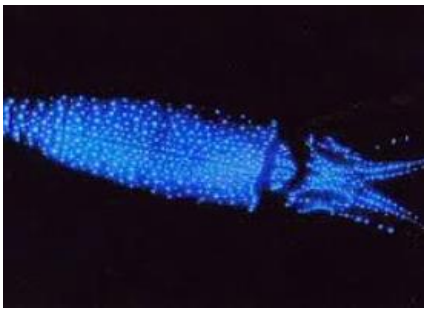


Plate: 12 Deep sea fish

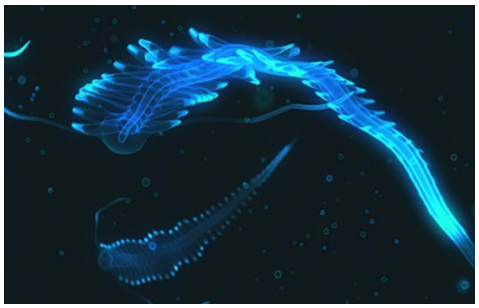


Plate: 13 Deep sea firefly squid

3.5 Fungal Bioluminescence:

Like firefly, bioluminescent fungi are also found in both tropical and temperate zone. Fungal bioluminescence, colloquially known as `foxfire' is an impressive sight in wet woodland. More than 75 terrestrial bioluminescent fungal species have been described. All of them belong to order Agaricales, the order of Mushrooms. Despite Aristotle's and Pliny's description on bioluminescent mushrooms and reports by botanists on the distribution of

luminous mushrooms, early attention was focused mainly on the light emitted from rotten wood instead on the fungi (Pandey and Sharon, 2017).

The biochemistry is relatively poorly understood. Bioluminescence fungi leading to constant light emission with maximum intensity in the range of 520–530 nm whose chemiexcitation step is catalyzed by an enzyme generally called luciferase. This phenomenon should not be confused with transient, low-level or ultraweak chemiluminescence that often increases in response to oxidative stress (e.g., elevated O2 concentrations or introduction of ROS-generating compounds) wherein light may be emitted from singlet oxygen, triplet excited states, reactions of ONOO−, lipoxygenase activity, heme protein-peroxide reactions. Moncalvo and his coworkers recognized 64 species of luminescent fungi. In Brazil they discovered eight luminescent taxa from a single site in primary Atlantic Forest habitat in Sao Paulo State, and *M. lacrimans* from Amazonas State (Dennis, E., *et al* 2008).

Plate: 14 Foxfire

4. Timeline:

- A glow in the dark tobacco plant was first engineered in 1980's.
- In 1986 Ow and coworkers used complementary DNA clone of firefly luciferase gene introduced it into protoplast cells of carrot through electroporation. They also produced glowing transgenic tobacco plants through indirect gave transfer method using Agrobacterian tumifaciens.
- In 1987, scientists sequenced the gene encoding firefly luciferase enzyme.
- In 1989, the gene encoding bacterial luciferin-luciferase system was sequenced.
- In 2008, the US scientists discovered GFP i.e., green fluorescent protein in jelly fish. The GFP gene is widely used in genetics as a marker; an indicator that allows for easy verification of genetic transformation success. And though it is now supplied to genetic

laboratories without problem, the natural source of this gene was originally a jellyfish called *Aequorea victoria*, from the Northeast Pacific, in which GFP glows in the dark. After the transformation of the cells that have been required for each case, the gene synthesizes the protein, which allows the cells to emit a bright green color when exposed to blue or black light. However, GFP is also present in hundreds of sea species, with green, orange, and red colors, as in sharks, eels, seahorses, fish, coral, etc. This discovery has recently given rise to fluo diving, night diving in fluorescent underwater marine life.

- In 2010, Cambridge University scientists introduced firefly luciferase in bacteria to make it glow.
- Krishevsky and coworkers in 2010 engineered a glowing tobacco using bacteria lux operon.
- Glowing tobacco plants containing genes from firefly and jellyfish were developed by Callaway in 2013.
- Nanobionic light emitting plants were developed by Kwak and coworkers in 2017 at MIT, US.
- Roots of globe arthichoke was made Luminescent by feeding with nutrients (strontium aluminate pigment phosphour doped with Europium and dysprosium by Khattab and coworkers in 2019.

5. Why we need glowing plants:

- Glowing plants do not pollute the environment
- There is no emission of toxic gases and so they can be considered as a clear case of **"Green energy"**. Hence, it is an environment friendly technology that can contribute to reducing Global warming
- It also reduces the dependence on Natural resources for energy production
- Since it encourages re-forestation, we can assume that it can purify the air and replenish Oxygen in the atmosphere.It can save electricity
- It reduces the transmission loss and therefore is a cheap and better renewable source of energy
- It is a self-powered and self- driven light source

6. Case study:

Plants with self-sustained luminescence

Developing glowing plants by engineering firefly luciferase gene into plants require the external application of substrate Luciferin. Although in case of bacterial biolumiscence, the lux operon 5 genes is sufficient to produce light autonomously. However, there are a lot of limitations in using Lux operon system in eukaryotes. Michoichkina & Co-workers in 2019 **tried to incorporate the fungal bioluminescence system into tobacco plants** and make self glowing without addition of luciferin externally.

6.1. Materials and methods:

6.1.1 Plasmid assembly:

Coding sequences of the nnLuz, nnHispS, nnH3H and nnCPH genes were optimized for expression in *Nicotiana tabacum* and ordered synthetically from Evrogen (Moscow, Russia). Genes were later assembled into vectors for Golden Gate cloning following MoClo standard. Each transcriptional unit contained 35s promoter, a fungal gene and ocs terminator. Final vectors for plant transformation contained kanamycin resistance cassette as well as transcriptional units for expression of fungal genes.

All Golden Gate cloning reactions were performed in 1X T4 ligase buffer (ThermoFisher) containing 10U of T4 ligase, 20U of either BsaI or BpiI (ThermoFisher), and 100ng of DNA of each DNA part. Golden Gate reactions were performed according to "troubleshooting" cycling conditions described in ref. 33: 25 cycles between 37°C and 16°C (90 sec at 37°C, 180 sec at 16°C), then 5 min at 50°C and 10 min at 80°C.

6.1.2 Agrobacterium-mediated transformation:

Assembled plasmids were transferred into Agrobacterium tumefaciens strain AGL0.

Bacteria were grown on a shaker overnight at 28°C in LB medium supplemented with 25 mg/l rifampicin and 50 mg/l kanamycin. Bacterial cultures were diluted in liquid Murashige and Skoog (MS) medium to the optical density of 0.6 at 600 nm.

Leaf explants used for transformation experiments were cut from two-week-old tobacco plants (*Nicotiana tabacum* cv. *Petit Havana* SR1, *Nicotiana benthamiana*) and incubated with bacterial culture for 20 minutes. Leaf explants were then placed onto filter paper overlaid on MS medium (MS salts, MS vitamin, 30 g/L sucrose, 8 g/L agar, pH 5.8) supplemented with 1 mg/L 6-benzylaminopurine and 0.1 mg/L indolyl acetic acid. Two days after inoculation, explants were transferred to the same medium supplemented with 500 mg/L cefotaxime and 75 mg/L kanamycin. Regeneration shoot were cut and grown on MS medium with antibiotics.

6.1.3 Molecular analysis of transgenic plants:

Genomic DNA was extracted from young leaves of greenhouse-grown plantlets using cetyltrimethylammonium bromide method. The presence each of the transferred genes was confirmed by PCR with gene-specific primers. For Southern blot, 30 μg of plant genomic DNA was digested overnight at 37°C by 100U of EcoRV, a restriction enzyme that cuts T-DNA constructs used in this study at a single position inside nnHispS coding region. After gel electrophoresis, digestion products were transferred onto Amersham Hybond-N+ membrane (GE Healthcare, UK) and immobilized. The DNA probe was constructed by PCR using cloned synthetic nnLuz gene as the template and nnLuz-specific primers. Probe DNA was labeled with alkaline phosphatase using the AlkPhos Direct Labeling Kit (GE Healthcare, UK). Prehybridization, hybridization (overnight at 60°C) with alkaline phosphatase-labeled probe, and subsequent washings of the membrane were carried out according to the AlkPhos Direct Labeling Kit protocol. Detection was performed using Amersham CDP-Star detection reagent following the manufacturer's protocol (GE Healthcare, UK). The signal from the membrane was accumulated on X-ray film (XBE blue sensitive, Retina, USA) in film cassette at room temperature for 24 hours. X-ray films were scanned on Amersham imager 600 (GE Healthcare Life Sciences, Japan).

6.1.4 Plant growth conditions:

Tobacco plants were propagated on Murashige and Skoog (MS) medium supplemented with 30 g/l sucrose and 0.8 w/v agar (Panreac, Spain). In vitro cultures were incubated at 24±1°C with 12-16-day photoperiod, with mixed cool white and red light (Cool White and Grolux fluorescent lamps) at light intensity 40 μmol / sec*m2. After root development, plantlets were transferred to 9 cm pots with sterilized soil (1:3 w/w mixture of sand and peat). Potted plants

were placed in the greenhouse at 22±2°C under neutral day conditions (12h light / 12h dark; 150 μmol m-2s-1) and 75% relative humidity.

6.1.5 Plant imaging setup with photo cameras:

They used Sony Alpha ILCE-7M3 camera to capture all photos and videos presented in this paper, except those taken on a smartphone and a long-term time-lapse filmed on Nikon D800. Depending on the experimental setup, lens aperture and other considerations, a range of ISO values from 6400 to 40000 was used, with exposure times from 5 seconds (leaf injury) to 20 minutes (root microscopy). Most of the photos were captured with 30-second exposure time.

They used SEL50M28 lens (Sony, f/2.8), or 35mm T1.5 ED AS UMC VDSLR lens (Samyang, ~f/1.4). Long-term timelapse of growing tobacco plants was captured with Nikon D800 camera and Sigma AF 35mm f/1.4 DG HSM Art at ISO 8063 and 30-second shutter speed. Root microscopy was performed with Sony Alpha ILCE-7M3 camera with Meiji MA833 U. Plan 20X Objective lens was mounted on the camera via a custom-made adaptor.

The photos were then processed in the following way. First, a raw photo obtained in the dark with the same settings was per-channel subtracted from a raw photo of plants (LibRaw version 0.19.2, 4channels tool) to remove hot pixels and reduce noise. Then, an ImageJ plugin was applied sto remove outliers (hot pixels). For most photos, only the green channels (G and G2) were kept in the final image.

6.1.6 Absorption spectra of tobacco leaves:

The leaves from adult wild type *Nicotiana tabacum* plants were collected and measured directly by spectrophotometer Cary 100 Bio (Varian).

6.1.7 Treatment with methyl jasmonate:

3 week old transgenic bioluminescent Nicotiana tabacum plants were treated with methyl jasmonate (5 mM in 10 mM MES buffer pH 7.0) by spraying. Control plants were treated with buffer (10 mM MES buffer pH 7.0). Plants were then imaged in closed glass jars for 3 days in the dark.

6.1.8 Incubation with banana skin:

Three week old transgenic bioluminescent *Nicotiana tabacum* plants were imaged with ripe banana skin in closed glass jars for 24 hours.

6.1.9 Quantitative PCR:

They collected the third leaves from twenty seven 25-day-old transgenic glowing plants. The leaves were collected with three-hour intervals during 24 hours, and to reduce the biological noise, leaves from three plants were combined. All leaves were flash-freezed in liquid nitrogen and homogenized for RNA extraction with TRIzol kit (Thermo Fisher Scientific, USA). Synthesis of the first cDNA strand was carried out with MMLV kit (Evrogen, Russia). Quantitative PCR was performed with qPCRmix-HS SYBR+LowROX kit (Evrogen, Russia) on 7500 Real-Time PCR machine (Applied Biosystems, USA) with primers annealing at nnluz transcript: GGACCAGGAGTCCCAGGC and CTTGGCATTTTCGACAATCTTA.

6.1.10 LC-MS/MS analysis:

Analytical standard (≥98.0) caffeic acid and acetic acid were purchased from Sigma-Aldrich. Hispidin was synthesised by Planta (≥95.0 %). HPLC-grade-acetonitrile was purchased from J.T. Baker. Deionized water was obtained from a Milli-Q System (USA).

They analysed several groups of samples: leaves and flowers of the wild type *Nicotiana tabacum* (NT000) and two transgenic lines of plants (NT001, NT078). Immediately after collection, the samples were frozen in liquid nitrogen and manually ground in a mortar. To reduce biological variability, they mixed plant material from three different organisms of the same group. For each sample, about 1 g of the frozen tissue was lyophilized in 50 ml falcon, and freeze-dried material was stored at -20C. Each sample was prepared and analyzed in three replicates.

For the analysis, about 50 mg of lyophilized powder was weighed and treated with 7 ml 70% methanol for 30 min in an ultrasonic bath, then centrifuged for 10 min at 4,000 rpm. The supernatant was collected, filtered with Phenex GF/PVDF syringe filter (Ø 30 mm, 0.45 μm) and analysed on LCMS instrument. Analyses were performed by Shimadzu 8030 system consisting of HPLC coupled to PDA and triple quadrupole mass spectrometer (HPLC-DAD-ESI-TQ MS). The chromatographic separation was performed on Discovery C18 column

4.6×150 mm, 5 μm in a gradient mode with mobile phase components A (0.3% acetic acid in water) and B (acetonitrile). The gradient run was performed in the following way: 0 – 4 min 10–40% B, 4 – 5 min 40-80%, 5 – 10.5 min, isocratic elution with 100% B, and then returned to the initial condition. The column temperature was 40C, the flow rate was 1 ml / min, the sample injection volume was 20 μL.

The ESI source was set in negative ionization mode. Multiple reaction monitoring was used to perform mass spectrometric quantification. MS conditions: interface voltage 3500V (ESI−), nebulizer gas (nitrogen) flow 2.5 l/min, drying gas (nitrogen) flow 15 l/min, CID gas pressure 60 kPa, DL temperature 250C, heat block temperature 400C. High purity argon was used as collision gas. The precursor and product ions (m/z) of target analytes were 178.95 and 134.95 for caffeic acid, 245.05 and 159.00 for hispidin; collision energy was 35V for both compounds.

Due to the lack of isotope-labeled standards, we added standards to samples to account for substantial matrix effect. Each sample was analysed twice, with and without the addition of standards. After the first analysis, a solution with known amount of caffeic acid and hispidin was added. Assuming a linear relation between the observed signal and concentration of compounds, concentration of the extract was calculated as Cextr = Cad * Sextr / (Stot – Sextr), where Cad – concentration of the added compound in the extract, Sextr and Stot t – analyte peak area in the first and second analyses.

6.2 Result:

The activities of four enzymes: luciferase Luz; two enzymes of luciferin biosynthesis, hispidin synthase HispS and hispidin-3-hydroxylase H3H; and a putative oxyluciferin recycling enzyme CPH. The transgenic **expressing the incorporated 4 genes emitted** at all developmental stages without application of Luciferin

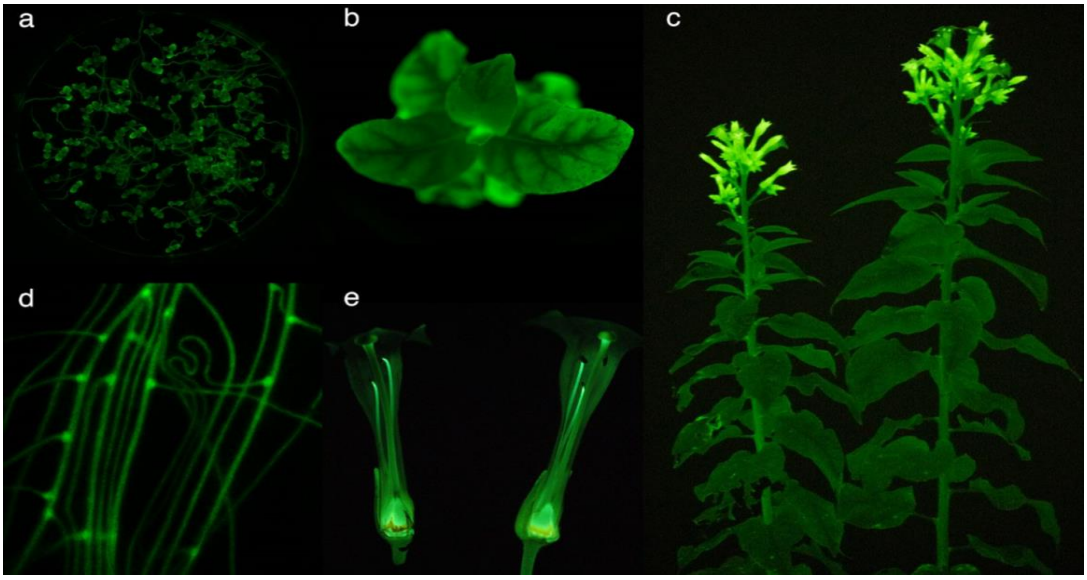


Plate: 15 Bioluminescent *N. tabacum* at various stages of development**.**

a) Vegetative stage
b) Flowering stage
c) Roots
d) Cross section of flowers
e) Photos were captured on Sony Alpha ILCE-7M3

Seeds of transgenic tobacco lines started glowing upon germination (Figure 15(a), with major light sources being the tips of cotyledons and roots. As the plant grew, luminescence was found to be uneven in different plant parts. The brightest being, the zone between root and stem. At flowering, the glow of buds surpassed the glow from other parts of the plant (Figure 15c). The roots glowed particularly bright at branching points (Figure 15d). At the flowering stage, the glow of flower buds surpassed the luminescence from other parts of the plant. Luminescence was strikingly brighter in the petals and particularly the ovary, and apical portions of the style and stamen filaments (Figure 15e).

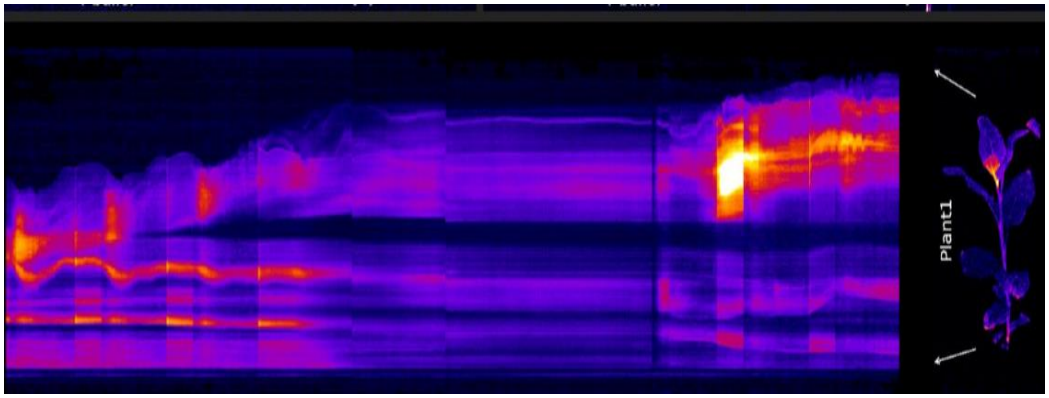


Plate: 16 Dynamics of luminescence in living plants.

It was observed that when the transgenes were placed under a normal day-night cycle for 3 days followed by a steady dark period for the next 4 days and again exposed to normal day-night cycle for another 3 days, the glow in plant parts exhibited circardian oscillation during the 1st 3 and last 3 days while, during the constant dark period, light emission was steady.

7. Summary:

Glowing plants have the ability to emit soft light. The glow is due to the phenomenon of bioluminescence. Interaction between substrate Luciferin and enzyme Luciferase is responsible for this bioluminescence in most organisms. Bioluminescence is common in bacteria, fungi and many marine organisms but not reported in plants, birds, mammals. Bioluminescence is common in bacteria, fungi and many marine organisms but not reported in plants, birds, mammals. Transgenic glowing tobacco was the first glowing plant developed. Genetic transformation of plant like tobacco has been attempted through *Agrobacterium* mediated as well as gene gun method and found successful in making plants glow. The concept is safe, sustainable, energy saving and Green technology.

8. Conclusion:

- The transgene tobacco plants were capable of autoluminiscence
- No external application of substrate luciferin was required to make the transgene glow
- The intensity of luminescence was dependent on the developmental stage of the crop
- A definite circardian rhythm existed in gene expression

9. Discussion:

1) The signal for making in insects are given by male or female?

Ans: Female fireflies are giving the signals for mating and they emit yellow green or light red glow.

2) How long the bioluminescence is effective in plants? How long in plants grow?

Ans: Based on the studies researchers are found that bioluminescence plant can glow up to six to eight hours.

3) In which growth stage bioluminescence is maximum in plants?

Ans: In flowering stage bioluminescence is maximum in plants.

4) In which area bioluminescence mushrooms are found?

Ans: In Brazil Atlantic Forest habitat in Sao Paulo State, and *M. lacrimans* from Amazonas State are found.

5) Is there any studies based on replacement of bioluminescence plants with street light to save electricity?

Ans: Yes, in 2019 Ardavani and Co-workers conducted a study on" redesigning the exterior lighting as a part on urban landscape: the role of bioluminescent plants in Mediterranean urban and suburban lightning environment.

6) Which season bioluminescence show in plants?

Ans: Mostly in winter season bioluminescence show in plants.

7) What is chemioluminescence?

Ans: The emission of cold light during chemical reaction which does not produce significant quantities of heat.

8) Is there any study on switch off mechanism study are available?

Ans: Switch off mechanism work is going on currently but not completed so studies are not available.

10. References:

Ardavani, O., Zerefos, S., and Doulos, L.T. 2020. Redesigning the exterior lighting as part of the urban landscape: The role of transgenic bioluminescent plants in Mediterranean urban and suburban lighting environments. *J. Cleaner Prod. 242*: 118477.

Callaway, E. 2013. Glowing plants spark debate. *Nature News*. 498(7452): 15.

Ckurshumova, W., Caragea, A. E., Goldstein, R. S., and Berleth, T. 2011. Glow in the dark: fluorescent proteins as cell and tissue-specific markers in plants. *Mol. Plant*. 4(5): 794-804.

Desjardin, D. E., Oliveira, A. G., and Stevani, C. V. 2008. Fungi bioluminescence revisited. *Photochem. Photobiology. Sci*. 7(2): 170-182.

Jones, K., Hibbert, F., and Keenan, M., 1999. Glowing jellyfish, luminescence and a molecule called coelenterazine. *Trends Biotechnol*. 17(12): 477-481.

Khakhar, A., Starker, C., Chamness, J., Lee, N., Stokke, S., Wang, C., Swanson, R., Rizvi, F., Imaizumi, T., and Voytas, D. 2019. Building customizable auto-luminescent luciferase-based reporters in plants. *BioRxiv.* p.809533.

Koncz, C., Langridge, W. H., Olsson, O., Schell, J., and Szalay, A. A. 1990. Bacterial and firefly luciferase genes in transgenic plants: advantages and disadvantages of a reporter gene. *Dev. Genet*. 11(3): 224-232.

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. *et al.* 2018. Genetically encodable bioluminescent system from fungi. *Proceedings of the National Academy of Sciences*. 115(50): 12728-12732.

Krichevsky, A., Meyers, B., Vainstein, A., Maliga, P., and Citovsky, V. 2010. Autoluminescent plants. *PloS one*. *5*(11): 1-7.

Pandey, A. and Sharon, M. 2017. Bioluminescent Organisms. BAOJ. *Chem.* 3: 029

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. *et al*. 2019.

Plants with self-sustained luminescence. 2019. bioRxiv Available : http://dx.doi.org/10.1101/809376 [05 Nov. 2019].

Ow, D.W., De-Wet, J.R., Helinski, D.R., Howell, S.H., Wood, K.V., and Deluca, M. 1986. Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. Science 234: 856-859.

Rees, J. F., De Wergifosse, B., Noiset, O., Dubuisson, M., Janssens, B., and Thompson, E. M. 1998. The origins of marine bioluminescence: turning oxygen defence mechanisms into deep-sea communication tools. *J. of Exp. Biology* 201(8): 1211-1221.

Stewart, C. 2001. The utility of green fluorescent protein in transgenic plants. *Plant cell Reports*. *20*(5): 376-382.

Widder, E. A. 2010. Bioluminescence in the ocean: origins of biological, chemical, and ecological diversity. *Sci.* 328(5979): 704-708.

Wong, M. H., Giraldo, J. P., Kwak, S. Y., Koman, V. B., Sinclair, R., Lew, T. T .S., Bisker, G., Liu, P., and Strano, M. S. 2017. Nitroaromatic detection and infrared communication from wild-type plants using plant nanobionics. *Nature Mater*. 16(2): 264-272.

KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF HORTICULTURE, VELLANIKKARA

Department of Plant Biotechnology

MBB 591: Masters Seminar

Name : Bharde Pranali Rajendra

Admission No : 2018 - 11- 140

Major Advisor: Dr. Rose Marry Francis

Venue : Seminar Hall

Date : 08- 11- 201

Time : 9:00 am

Glowing Plants - Synthetic Biology

Abstract

Glowing plants or 'Glow-in-the-dark' plants or 'Autoluminescent' plants refer to those plants which are capable of emitting light. The glow or bioluminescence of organisms is essentially an enzyme-substrate chemical reaction that involves the light-emitting organic molecule termed 'luciferin' and the enzyme 'luciferase'. Oxidation of the substrate luciferin by molecular oxygen, mediated by the enzyme luciferase results in the emission of light. The phenomenon is catergorised as a form of chemiluminescence as it involves the conversion of chemical energy to radiant energy. The light thus emitted is called cold light or luminescence since very little heat is given off in the process.

Bioluminescence occurs sporadically in nature. Typically the organisms use bioluminescence to warn or evade predators, to lure or detect prey, and for communication between members of the same species. A wide range of protists and animals, from bacteria and fungi to insects, marine invertebrates, and fish, is known to emit light. The flickering signals of fireflies and the shimmering radiance of protozoans in tropical seas are classic examples of bioluminescence. However, the phenomenon is not known to exist naturally in plants, amphibians, reptiles, birds, or mammals.

The concept of 'Glowing plants' has been gaining momentum in the recent past. Plants are engineered or redesigned to emit light using synthetic-biology techniques. A glow-in-

thedark tobacco plant was first engineered by scientists in the 1980s through introduction of the gene encoding the firefly enzyme luciferase into the tobacco plant (Callaway, 2013). The luciferase gene from the firefly, Photinus pyralis, is widely used as a reporter of gene expression by light production in transfected plant cells and transgenic plants. Ow et al. (1986) used a complementary DNA clone of the firefly luciferase gene under the control of a plant virus promoter (cauliflower mosaic virus 35S RNA promoter). It was introduced it into protoplast cells of carrot (Daucus carota) by electroporation and into tobacco plants by using the Agrobacterium tumefaciens tumor-inducing plasmid. Extracts from electroporated cells (4 hours after the introduction of DNA) and from transgenic plants produced light when sprayed with luciferin making the plant glow temporarily. Instead in 2010, another group engineered glowing tobacco plants using the lux operon from bacteria (Photobacterium leiognathi) (Krichevsky et al., 2010).

Light emission from luciferase reporters requires exogenous addition of a luciferin substrate. Mitiouchkina et al. (2019) engineered autonomously glowing tobacco plants by constitutively expressing enzymes of the caffeic acid cycle, derived from the fungus Neonothopanus nambi. Transgenic plants expressing these genes emitted light at all developmental stages, without the need for externally supplied substrates and with sufficient brightness to be visible to the naked eye.

Glowing plants are no more a fantasy. The concept of glowing plants can be used to develop cheap and durable lighting. Plants with luminescent properties offer a wide scope for replacing the street lights with glowing avenue trees and for lighting country backroads. This will help replace and save electricity. Glowing plants, are therefore, a clean ‘Green technology’ providing a safe alternative non-conventional source of lighting. It can also improve the aesthetics in indoor and outdoor landscaping without the burden of using electricity.

References

Callaway, E. 2013. Glowing plants spark debate. Nature 498:15.

Krichevsky, A., Meyers, B., Vainstein, A., Maliga, P., and Citovsky, V. Autoluminescent plants. 2010. PLoS One 5(11): 1-7.

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. *et al*. 2019.

Plants with self-sustained luminescence. 2019. bioRxiv Available : http://dx.doi.org/10.1101/809376 [05 Nov. 2019].

Ow, D.W., De-Wet, J.R., Helinski, D.R., Howell, S.H., Wood, K.V., and Deluca, M. 1986. Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. Science 234: 856-859.