A TECHNICAL SEMINAR REPORT

ON

BIOLUMINESCENCE

BACHELOR OF TECHNOLOGY

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Submitted by

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Certified that this technical seminar report **"BIOLUMINESCENCE"** is the bonafide work of "**K. SIDDHARTHA"** who carried out the seminar presentation under my supervision.

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Abstract

Bioluminescence is a natural light emitting phenomenon that is emitted by the several terrestrial, marine and some fresh water organisms. This phenomenon could be found in all the major taxonomic groups that are known to comprise around 700 genera of bioluminescent organisms. Though their luminescence emissions are due to two principal components i.e. luciferin and luciferase, yet for many bioluminescent organisms the chemistry involved in the luminescence emission is remains unknown.

Over all the bioluminescent organisms, luminescent bacteria are mostly scrutinized paradigm for bacterial and host symbiosis that led researchers to find many genes which involved in luminescence control and symbiosis. However, the principal proteins and genes involved in different luminescent organisms including bioluminescent bacteria are being mostly used for biotechnological applications such as detection of environmental pollutants, contaminants in foods, and more significantly as reporter genes.

The motif of this report is to address the knowledge on current studies on bioluminescence aspect and its applications in real time environment.

KEYWORDS: Bioluminescence distribution, Bioluminescence applications and Genetic engineering.

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Chapter 1

INTRODUCTION

1.1 A Sneak Peek of Bioluminescence

Bioluminescence is a chemical reaction that takes place in an organism and produces detectable light. These organisms use a variety of body parts to emit light in different colors and for different purposes. This chemical process is different from fluorescence, another process that can cause things to emit light. In few organisms, bioluminescence and fluorescence both occur.

Figure 1.1

Bioluminescence is an interesting chemical reaction whereby a conspicuous visible light is emitted by the several luminescent organisms. The history of this phenomenon could be found in terrestrial, freshwater and particularly marine environments. Yet, all most of all the luminous organisms share similar chemical components involved in the luminescence emission refer to as luciferin and luciferase. Particularly this phenomenon is enormously common in deep sea, especially from aphotic zone to till the bottom of the sea. This emission could be found over all the major phyla which represent at least one genus, except few groups refer to plants, birds, amphibians, and mammals. This phenomenon covers diverse hues, reactions and emission patterns. The proteins and genes involved in the luminescence of some organisms have a wide importance in medical and biotechnological applications. In this review we shall devote to talk very briefly about bioluminescence of different organisms, current aspects, and applications.

1.2 History of Bioluminescence

Pliny the Elder (23-79 CE) in his *Naturalis Historia*, wrote quite detailed descriptions of many bioluminescent animals: glowworms and fireflies, the luminous mollusk *Pholas dactylus* (a Roman delicacy), the purple jellyfish *Pelagia noctilus* common in the Mediterranean, the lantern fish, and the fungi, glowing wood and mushrooms (Fig. 1.2).

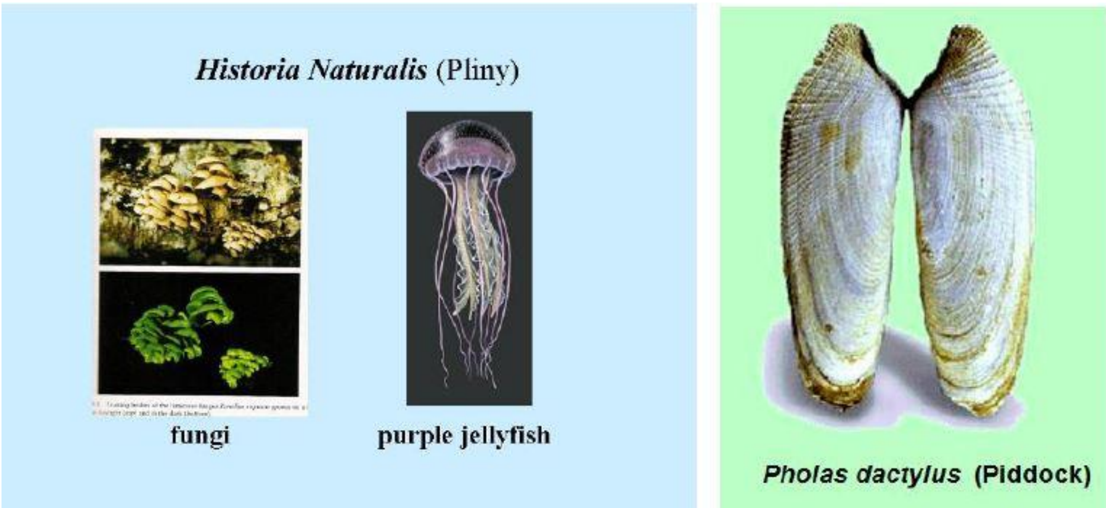

Figure 1.2

The 17th Century is known as the age of the "Science Revolution". Up till 1600 CE, as a consequence of the increase in exploration and commerce, there was a demand for reliable and predictive knowledge that required development of an objective methodology for discovery. Also, there were advances in technology, such as the invention of the telescope and the microscope, enabling the astronomical discoveries of Galileo (1564-1642) and of van Leeuwenhoek (1632-1723) in microbiology. The air pump, improved by Boyle (1627-1691) and his able assistant Hooke (1635-1703), led to landmark observations in bioluminescence and many related phenomena.

By the end of the 17th Century the Theory of Light had divided into opposing views, the wave theory of Hooke and Huygens, and that light consisted of particles, an idea more associated with Newton (1642-1727) than Descartes. Due mainly to the dominant reputation of Newton, his corpuscular theory remained unchallenged for almost 150 years, when it was clearly incompatible with the diffraction experiments of Young and the work of Fresnel.

Kircher (1602-1680), a German Jesuit priest, applying the Baconian principle, collected and tabulated many new observations of bioluminescent phenomena. He noted that in fireflies, the light would dim on handling the insect, and then reappear if left alone. Many of the insects placed together would display "ostentatiously". Kircher conjected (the axiom) that the light was in order to be seen and extended this idea to the function of bioluminescence in fish in the darkness of the ocean, that it was similar to the function in fireflies, for communication. Kircher's suggestions we now know to be essentially correct. He even made experimental observations showing how the luminescence on some organisms such as the clam *Pholas dactylus*, could be rubbed off on a stick. He also was unable through many variations, to produce an indefinitely glowing extract from firefly light organs (photophores), concluding that the *Liquor Lucidus* claim of Magnus was demonstrably false. In 1798, Carradori observed that the firefly photophore could be excised and dried whereupon the emission ceased, but would revive again with water. Later workers reported that the dried material could be kept for long periods and emission could occur on moistening. Perhaps we can give some credit to Magnus for making the first extract of bioluminescence, but not for the claim of a permanent glow.

1.3 Usage of Bioluminescence by the Organisms

Scientists have observed organisms using bioluminescence in many different ways. These include self-defense, illuminating or luring prey, camouflage, and attracting mates. For example, the vampire squid squirts out a cloud of bioluminescent fluid that may confuse predators. Glowing spots on the "shoulders" of the click beetle give the impression of a much larger animal crawling at night. Some species of fireflies communicate with flash patterns to signal their availability and attract potential mates. Dinoflagellates light up when disturbed, perhaps to startle predators — or to attract animals that may eat their attackers. Rows of light organs on the undersides of the hatchet fish help it blend with light from above, making it barely visible to a predator looking up from below. The larvae of fungus gnats glow to attract insects to their sticky “fishing lines,” while biologists think that the deep-sea stoplight loose jaw fish uses its red light (a color invisible to most deep-sea organisms) to illuminate its prey. While we know a lot about how bioluminescence works and how organisms use it, we have a lot more to learn. Many intriguing bioluminescent organisms await discovery as we explore Earth’s final frontier, the deep sea.

1.4 Chemistry involved in Bioluminescence

This chemical reaction requires at least three ingredients. An enzyme known as luciferase acts upon an organic molecule called luciferin in the presence of oxygen. The reaction produces a molecule called oxyluciferin, and energy. The energy takes the form of photons, units of light.

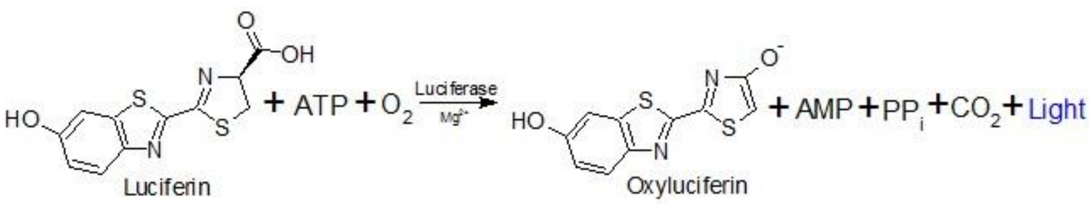

Figure 1.3

Some bioluminescent organisms produce their own light, either making all of the ingredients themselves or making everything but luciferin, which they take in through their diet. Other bioluminescent organisms, such as the flashlight fish, do not produce their own light. Instead, they have a **symbiotic** relationship with bioluminescent bacteria that live inside their bodies.

Chapter 2

SOURCES OF BIOLUMINESCENCE

2.1 Land Species

Bioluminescence is rare on land, which makes it seem even more surprising when we do come across it. Scientists are just beginning to delve into the mystery of why these unusual terrestrial organisms glow. Some examples of species exhibiting bioluminescence on land are as follows:

- **Woods Mushrooms:** In the forests of eastern North America, bioluminescent mushrooms grow on decaying wood.

Figure 2.1.1

- **A Summer's Night Fireflies:** In a grassy clearing in eastern North America, fireflies are using a system of flashes to communicate: to attract mates and also to lure other firefly species close enough to catch and eat. The light may also discourage predators by signaling that the firefly will taste bad.

Figure 2.1.2

- **A Mysterious Cave Glowworms:** On the ceiling of New Zealand's Waitomo Cave, glowworms secrete threads studded with adhesive droplets that reflect light from their bioluminescent tails. These tails glow brighter when the animals are hungry. When aquatic insects from the stream below fly toward the light, they become tangled in these lines. The glowworms then reel in their catch.

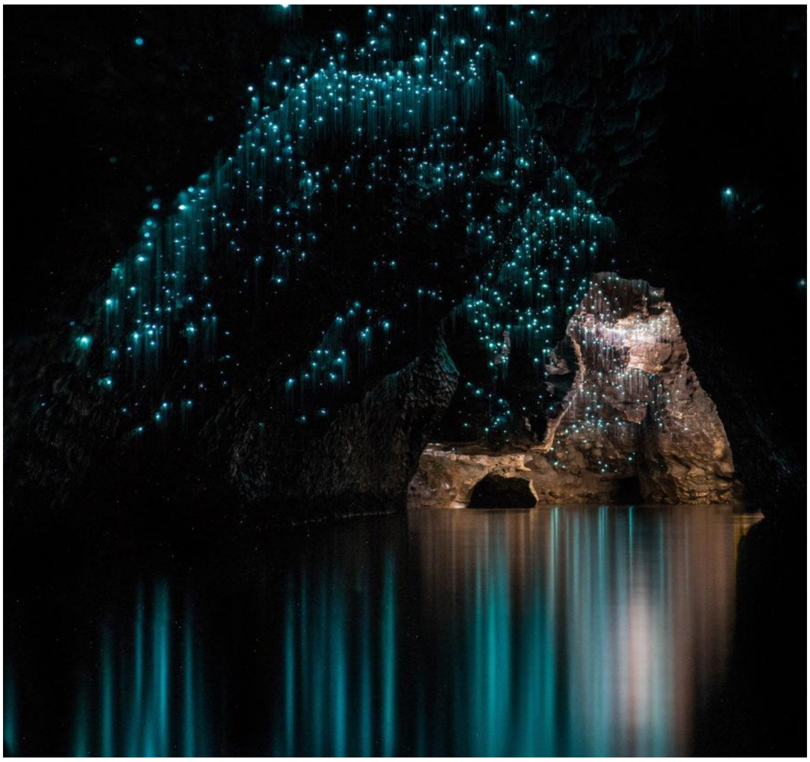

Figure 2.1.3

2.2 Water Species

Bioluminescence is much more common in water than on land. By far the greatest diversity of bioluminescent live in the deep sea, where upwards of 80% of organisms exhibit the phenomenon. Some examples of species exhibiting bioluminescence in water are as follows:

- **A Sparkling Sea Dinoflagellates:** It's nighttime at a quiet lagoon in Vieques, Puerto Rico. The bay is full of marine organisms known as dinoflagellates, each the size of a pinprick. When something bumps into a dinoflagellate, the impact triggers a chemical reaction that ends in a burst of light.

No one really knows why dinoflagellates flash on contact, but scientists think it may startle or expose predators, or help remove toxic oxygen from their bodies.

Figure 2.2.1

- **Sea Shores Corals, Jellies, & Fishes:** Fluorescence can also cause organisms to glow by transforming and reemitting light from an external source. In the Caribbean Sea, shining a blue or violet light onto corals makes them glow in neon shades of pink, orange, and green. In the Pacific Ocean, spots on the rim of a jellyfish light up when touched. Its mysterious green glow is the product of both bioluminescence and fluorescence.

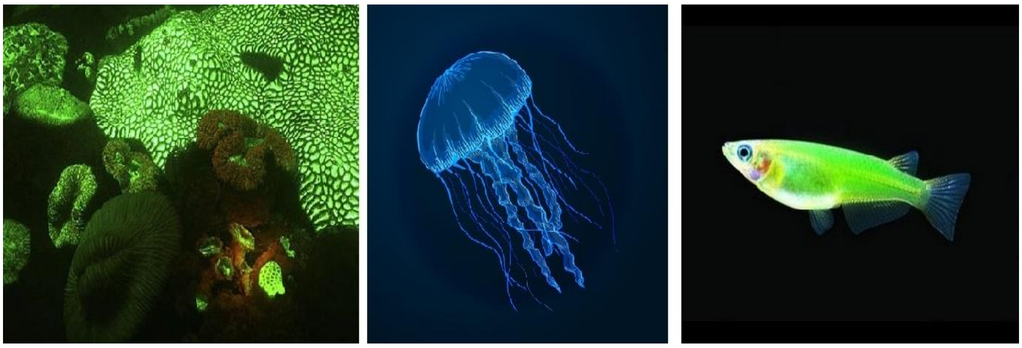

Figure 2.2.2

Chapter 3

GENETIC ENGINEERING

3.1 Introduction

Genetic Engineering is manipulation/alteration of structure of a gene to create a desired characteristic in an organism. A Gene is a fundamental, physical and functional unit of heredity. It is responsible for the physical and inheritable characteristics of an organism. If genetic material from another species is added to the host, the resulting organism is called transgenic. Genetic engineering can also be used to remove genetic material from the target organism, creating a knock out organism. There are many advantages of genetic engineering and some of them are as follows:

- To improve agricultural, horticultural or ornamental value of a plant (much faster than conventional plant breeding)
- To serve as a living bioreactor for production of economically important proteins or metabolites
- To produce vaccines or antibodies for human health
- To provide a renewable source of energy
- To study the role of genes (and gene products) in plant growth and development

3.2 History

The term “Genetic Engineering" was first coined by Jack Williamson in his science fiction novel. James Watson and Francis Crick showed that the DNA molecule has a double-helix structure.

In 1972, Paul berg created the first recombinant DNA molecules by combining DNA from the monkey virus SV40 with that of the lambda virus. In 1973 Herbert Boyer and Stanley Cohen created the first transgenic organism by inserting antibiotic resistance genes into the plasmid of an E.coli bacterium.

The first trials of genetically engineered plants occurred in France and the USA in 1986, tobacco plants were engineered to be resistant to herbicides.

3.3 Ways To Develop Genetically Modified Plants

There are three ways to create new plants:

3.3.1 Grafting

Grafting is, at its simplest, wounding and healing. It's the gardener's way of taking advantage of a tree's natural repair mechanisms. The growth of a tree's trunk and branches occurs in a layer called the cambium, a greenish tissue that lies just under the tree's protective bark. One unique talent of the cambium layer is that if it's cut or torn, the two pieces will grow back together again. Grafters make use of this by cutting parts of the trees they want to graft together taking a branch cut from one tree and putting it over the spot where a branch has been removed from another and securing them in such a way that their cambium layers are in contact with each other. Over time, these layers will grow together, and the grafted part will become just another branch of the tree.

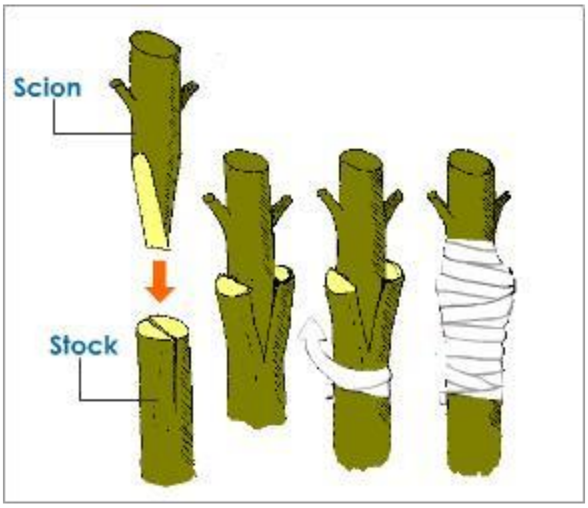

Figure 3.3.1

There are some good reasons to put a tree through all this injury (besides getting it to grow three different fruits). Consider orange trees: the roots of the tree that produce the sweet orange that we know and love can take up a virus that affects the growth of tree bark. The roots of the less-loved sour orange, however, won't accept this virus. So by grafting sweet orange buds onto sour orange trees, growers in places like Texas protect their crop from disease. Grafting can make trees resistant to a variety of factors, including cold, drought, and microbes.

A second reason to graft is that some tree families, such as citrus, don't reproduce reliably. Oranges, for example, are either seedless, or are hybrids that may produce a tree that is somewhat different from its parent. Grafting gives growers a simple way to reproduce fruit trees without having to rely on seeds.

3.3.2 Hybrids

These variations between individuals in a species are one of the driving forces behind evolution: The plants (or animals or other living things) best adapted to their environments are the most likely to survive and reproduce their traits. The enormous range of environments on earth has produced an even more impressive range of adaptations. The result, after millions of years, is the biodiversity we see today.

Figure 3.3.2

In nature, the highest priority of a plant is to reproduce itself. In the garden, the highest priority for that plant will be determined by the gardener's intentions, which she'll fulfill by taking advantage of how the plant reproduces itself. If she wants taller stalks, for instance, she'll force the plant to breed with another of its kind that's tall. The likely result will be offspring known as hybrids with taller stalks than the original plant.

For millennia, farmers and gardeners without any knowledge of genetics have used this forced breeding to bring out plant traits that are useful for humans. They then save the seeds from the plants they like best, in effect passing along those qualities to future generations. The people performing these unnatural plant crosses 10,000 years ago were the original genetic manipulators. Their plant hybridization was the earliest practice of agriculture.

3.3.3 Transgenics

Scientists have done this with the bacterium *Bacillus thuringiensis*, or Bt for short. Bt, which is found naturally in soil and on the leaves of many plants, is toxic to many plant pests. The genes that give it that toxicity were first put into potatoes in 1997. Now many vegetables grown commercially have the Bt genes in them. This gene adding process is called "genetic engineering" (GE). GE is one form of what's called "genetic modification" (GM). Though GE and GM are related, they are not the same. "Genetically modified" refers to any plant (or other organism) whose DNA has been manipulated by humans. This could happen through artificial selection and hybridization or through engineering. "Genetically engineered" refers specifically to one or more extra genes that have been inserted into a plant's genome from an organism it can't mingle genes with via sexual reproduction. The products of such engineering feats like Bt tomatoes are called "transgenic."

Intuitively, GE doesn't seem like a very "natural" process. After all, genes in nature don't cross so many boundaries. But the line between natural and unnatural may be grayer than you think.

In the wild, most plants (actually, most living things) share genes only with members of their own or very closely related species living in nearby environments. Hybridization allows plant breeders to go beyond these limitations that exist in nature. Suppose a wild tomato exists that seems particularly resistant to cold. A breeder can cross this plant with one that produces store-bought tomatoes in order to impart these useful ancestral genes. The resulting tomatoes may look and taste like the ones we're familiar with, and their DNA doesn't contain any "nontomato" genes. But this cross isn't natural: these two plants would never have found each other in the wild. In fact, the grocer's tomato doesn't even exist in nature. It's the product of hundreds of similar hybridizations.

The main difference between a hybridized tomato and a transgenic one is that the latter has additional bits of DNA that originally came from another species. It has an altered genome. Critics of genetic engineering have argued that this process may have unintended consequences: They are concerned that seeds from GE plants could carry their genetic alterations into the environment and the food supply, or that untested GE products may pose health risks for consumers. Supporters of genetic engineering counter that the benefits of GE plants with added nutrition, higher yields, or the ability to produce pesticides or drugs outweigh the risks, and that a world with rapidly increasing population can't be fed without them.

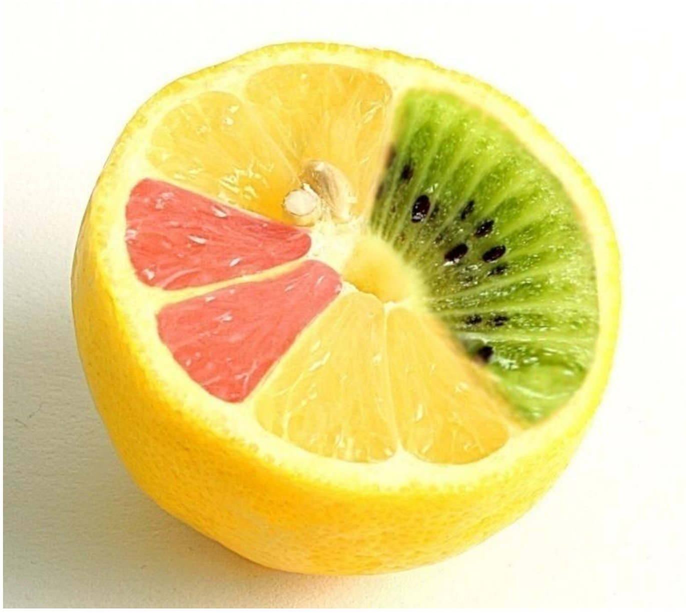

Figure 3.3.3

As a backyard gardener, you're not likely to be planting any transgenics. These engineered plants have been developed for agriculture. They are patented, and the companies that sell them would make you aware of that when you buy them. That doesn't mean, however, that the plants you grow in your garden are the products of completely natural processes. There are manipulations everywhere out there.

3.4 Transgenics in Animals

Some examples of animals which are genetically modified are:

- The GloFish was the first genetically modified animal to become available as a pet. It is a natural Zebrafish which has genetic information from bioluminescent jellyfish added to its DNA.

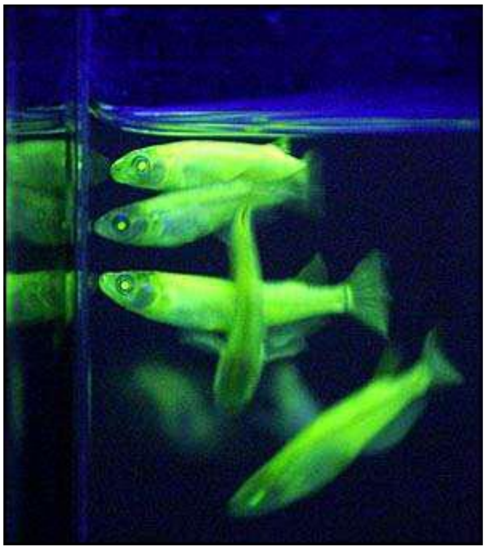

Figure 3.4.1

- Using Modern DNA and cross fertilization techniques; the Dolion is a cross between a lion and a dog.

Figure 3.4.2

- The Zorse is a cross between a zebra and a domestic horse. The crosses were originally done in England and Africa to try to produce a domestic horse like animal that was resistant to diseases spread by a fly in Africa.

Figure 3.4.3

- The transgenic mice have been genetically modified so that they carry a green fluorescent protein which glows green under blue light

Figure 3.4.4

CHAPTER 4

BIOLUMINESCENT PLANTS

4.1 Introduction

Imagine that instead of switching on a lamp when it gets dark, you could read by the light of a glowing plant on your desk. MIT engineers have taken a critical first step toward making that vision a reality. By embedding specialized nanoparticles into the leaves of a watercress plant, they induced the plants to give off dim light for nearly four hours. They believe that, with further optimization, such plants will one day be bright enough to illuminate a workspace.

“The vision is to make a plant that will function as a desk lamp — a lamp that you don’t have to plug in. The light is ultimately powered by the energy metabolism of the plant itself,” says Michael Strano, the Carbon P. Dubbs Professor of Chemical Engineering at MIT and the senior author of the study.

This technology could also be used to provide low-intensity indoor lighting, or to transform trees into self-powered streetlights, the researchers say.

4.2 Nanobionic Technology

Plant nanobionics, a new research area pioneered by Strano’s lab, aims to give plants novel features by embedding them with different types of nanoparticles. The group’s goal is to engineer plants to take over many of the functions now performed by electrical devices. The researchers have previously designed plants that can detect explosives and communicate that information to a smartphone, as well as plants that can monitor drought conditions.

Lighting, which accounts for about 20 percent of worldwide energy consumption, seemed like a logical next target. “Plants can self-repair, they have their own energy, and they are already adapted to the outdoor environment,” Strano says. “We think this is an idea whose time has come. It’s a perfect problem for plant nanobionics.” To create their glowing plants, the MIT team turned to luciferase, the enzyme that gives fireflies their glow. Luciferase acts on a molecule called luciferin, causing it to emit light. Another molecule called co-enzyme A helps the process along by removing a reaction byproduct that can inhibit luciferase activity.

The MIT team packaged each of these three components into a different type of nanoparticle carrier. The nanoparticles, which are all made of materials that the U.S. Food and Drug Administration classifies as “generally regarded as safe,” help each component get to the right part of the plant. They also prevent the components from reaching concentrations that could be toxic to the plants. The researchers used silica nanoparticles about 10 nanometers in diameter to carry luciferase, and they used slightly larger particles of the polymers PLGA and chitosan to carry luciferin and coenzyme A, respectively. To get the particles into plant leaves, the researchers first suspended the particles in a solution. Plants were immersed in the solution and then exposed to high pressure, allowing the particles to enter the leaves through tiny pores called stomata.

Figure 4.2

Particles releasing luciferin and coenzyme A were designed to accumulate in the extracellular space of the mesophyll, an inner layer of the leaf, while the smaller particles carrying luciferase enter the cells that make up the mesophyll. The PLGA particles gradually release luciferin, which then enters the plant cells, where luciferase performs the chemical reaction that makes luciferin glow. The researchers’ early efforts at the start of the project yielded plants that could glow for about 45 minutes, which they have since improved to 3.5 hours. The light generated by one 10-centimeter watercress seedling is currently about one-thousandth of the amount needed to read by, but the researchers believe they can boost the light emitted, as well as the duration of light, by further optimizing the concentration and release rates of the components.

4.3 Plant Transformation

Previous efforts to create light-emitting plants have relied on genetically engineering plants to express the gene for luciferase, but this is a laborious process that yields extremely dim light. Those studies were performed on tobacco plants and *Arabidopsis thaliana*, which are commonly used for plant genetic studies. However, the method developed by Strano's lab could be used on any type of plant. So far, they have demonstrated it with arugula, kale, and spinach, in addition to watercress. For future versions of this technology, the researchers hope to develop a way to paint or spray the nanoparticles onto plant leaves, which could make it possible to transform trees and other large plants into light sources.

Figure 4.3

"Our target is to perform one treatment when the plant is a seedling or a mature plant, and have it last for the lifetime of the plant," Strano says. "Our work very seriously opens up the doorway to streetlamps that are nothing but treated trees, and to indirect lighting around homes." The researchers have also demonstrated that they can turn the light off by adding nanoparticles carrying a luciferase inhibitor. This could enable them to eventually create plants that shut off their light emission in response to environmental conditions such as sunlight, the researchers say. The research was funded by the U.S. Department of Energy.

CHAPTER 5

ADVANTAGES AND LIMITATIONS

5.1 Advantages

- Energy can be saved.
- It provides scenic beauty.
- It produces oxygen in the day.
- It helps to reduce light pollution due to street lights in major cities.
- It is mostly natural so cost of implementation will be very low.
- Enhancing living standards and preserving biodiversity.
- Replacement of traditional street lights.
- Creating an energy neutral environment.

5.2 Limitations

- Intensity of the light produced is very low.
- Genetic Structure of each and every plant should be modified.
- Lot of insects and flies are attracted to such lights.
- Controlling or switching of the light is difficult.
- Color of the light is mostly blue or green.

CHAPTER 6

CONCLUSION AND FUTURE SCOPE

We are cutting down trees to facilitate ourselves but now we can engineer them in a way that they are beneficial and also can be adjusted with our urban lifestyle. We also get to accommodate nature in our society. This idea also helps to reduce many of the main global problems due to deforestation. In future we can expect these bioluminescent plants can replace traditional street lights and domestic lights.

CHAPTER 7

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