



# Plant Chemiluminescence as a Physical Phenomenon and its Agriculture Applications on Plant Seedlings: A Comprehensive Review

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## Abstract

This paper provides a general review of the findings published in research papers from 1955 to the present on the history of plant chemiluminescence (CL) studies and their agriculture applications on plant seedlings. The CL technique has become a very duty tool in agricultural application fields due to its high sensitivity, simplicity, and low cost. As a result, CL's study of plants and their applications in agriculture has carried out great progress. There is a possibility that an easy new way to check environmental changes and oxidative stress and to determine a plant's overall health may be to measure the CL emitted from a plant. I have selected and summarized some interesting CL application techniques in this review, such as the degree of accelerated aging seeds under stress and storage conditions and also the study of mechanical injury to plant tissue. Besides, I found several dedicated reports in this review that include the CL method for detecting or monitoring the plant's capacity to produce reactive oxygen species (ROS) or to detect compounds that present scavenger effects over these radicals.

**Keywords:** chemiluminescence, ageing, imbibition, reactive oxygen species, radical scavengers

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## 1. Introduction

Luminescence is the most convenient term for light-producing processes. Luminescence could be classified into three types as fluorescence, bioluminescence, and chemiluminescence (CL), according to the source of excitation. In all cases mentioned above, light is produced when molecules in an electronically excited state decay into a stable ground state. Light is the energy

source for fluorescence, and chemical reactions such as oxidation are the energy source of bioluminescence and chemiluminescence (Fereja *et al.*, 2013; Jimenez & Navas, 2002; Abeles, 1986). Some of the other terms used in the literature to describe the same phenomenon are ultraweak photon emission (UPE) and ultraweak chemiluminescence (UCL). UBE in the

visible region is low-level chemiluminescence from biological systems such as microorganisms, plants, and animals, in originates from the relaxation of electronically excited species during the oxidative metabolic and stress processes (Prasad *et al.*, 2020; Pospisil *et al.*, 2014; Kato *et al.*, 2014; Bertonha *et al.*, 2010). Due to its very significant activity, UPE researchers have a wide range of potential applications in agriculture. It is well known those plant seedlings, as they grow emit a very low amount of light, the so-called ultraweak photon emission (UPE). From the first discovery of ultra-weak photon emission (UPE) in seeds measurements by Coli in 1955, there has been a lot of evidence that plants change the intensity of UPE depending on changes in their condition and circumstances (Kato *et al.*, 2014). Those authors have been researching and developing techniques using UPE to non-invasively test plant states in response to stress since the 1990s. The study of UCL from plants is a new technique for plant micro-analysis that has been in place since the 1980s. The term chemiluminescence (CL) is identified as the light emission, of ultraviolet, visible, or infra-red radiation from a molecule or atom as a result of the transition to an electronically excited state decay to a stable ground state, as a consequence of a chemical reaction, (Jimenez & Navas, 2002). It is possible to split studies on CL into three parts. The first is oxidative chemistry, which is the driving force behind CL; the second is plant endogenous CL and the factors affecting its production; and finally, experiments using CL probes, which are light-producing compounds when exposed to H<sub>2</sub>O<sub>2</sub> or superoxide anion. Via two mechanisms, plants generate light: fluorescence and chemiluminescence. Fluorescence energy comes from light, while CL energy comes from superoxide radical oxidative reactions and H<sub>2</sub>O<sub>2</sub> (Moraes *et al.*, 2010). The best chemiluminescent probes that have been

used extensively in plant and animal research are luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) or its derivatives and lucigenin (bis-N-methylacridinium nitrate) (Al-Hashimi *et al.*, 1997; Jimenez & Navas, 2002). The electronically excited state in these compounds returns to their ground state with the emission of energy as light (CL), which can be detected by the photomultiplier tube detectors (Moraes *et al.*, 2010). It is well known that photon emission intensity measurement is useful for detecting physiological changes and determining the of these changes before harm takes place on plants (Lida *et al.*, 2000). In plants, CL has been emitted in response to environmental changes and stress, such as wounding plants mechanically (Lida *et al.*, 2000; Salin & Briges, 1981); oxidative stress (Beals & Byl, 2014); drought damage (Ohya *et al.*, 2002), and temperature and humidity changes (Footitt *et al.*, 2016; Chen *et al.*, 2006a; Boveris *et al.*, 1984). Several reports on the early studies of CL in plants have been published. In the early 1950s, for the first time, Colli and collaborators discovered that plants produce CL (Colli *et al.*, 1954; Colli *et al.*, 1955). These authors discussed some properties of chemiluminescence from wheat, bean, lentil, and corn seedlings, giving a quantitative comparison of the intensity of chemiluminescence for different plants including some plant parts, and at various stages during germination. In the 1960s, several Russian groups managed by Vladimirov (1966) and Agaverdiyev & Tarusov (1965) studied the visible region luminescence from many plants. Then, from the early 1970s to the present, several reports of chemiluminescence from different parts of plant tissues began to appear. For example, CL has observed with seeds (Gallep & Robert, 2020; Saeidfirozeh *et al.*, 2018; Lida *et al.*, 2000; Saeki *et al.*, 1989; Boveris *et al.*, 1984); with roots (Wany *et al.*, 2016; Chen *et al.*, 2010; Zielinska *et al.*, 2008; Rogovin *et*

*al.*, 2001; Salin & Briges, 1981; Abeles, 1978; Vartapetian *et al.*, 1974; and Colli *et al.*, 1955); with stems (Lida *et al.*, 2000; Salin & Briges, 1981; Abeles *et al.*, 1978; Agaverdiyev & Tarusov, 1965; Colli *et al.*, 1955), and with leaves (Wany *et al.*, 2016; Miladinovic *et al.*, 2013; Chen *et al.*, 2010; Sakharov, 2001; Lida *et al.*, 2000; Skotnica *et al.*, 1999; Butt & Bestwick, 1997; Roschger *et al.*, 1993). It has well known that all stressed and unhealthy cells emit more photons than healthy cells (Morales *et al.*, 2010; Salin & Briges, 1981). Many research groups have studied the CL emission from plants and seeds, indicated a correlation with metabolic changes and oxidative stress (Albert *et al.* 2015; Miladinovic *et al.*, 2013; Jantan *et al.*, 2011). Other studies were found that light levels rise when organisms are injured (Yoshinaga & Kato, 2006; Chen *et al.*, 2006 b; Chen *et al.*, 2003b; and Abeles *et al.*, 1978); or when the early imbibition of seeds (Chen *et al.*, 2010; Chen *et al.*, 2006a; Chen *et al.*, 2003a; Saeki *et al.*, 1989; Boveris *et al.*, 1984). Because of its variety of practical applications in industries, agriculture, and medicine, the chemiluminescence phenomenon in transforming chemical energy into light emission has been an attractive subject of intensive research over the years (Yeh & Ai, 2019; Liu *et al.*, 2010; Roda *et al.*, 2004; Lida *et al.*, 2000; Chen *et al.*, 2003a; Duong *et al.*, 2020; Khan *et al.*, 2014). In plants, the study of CL and their agriculture applications has gained significant progress due to their simplicity, low cost, and high sensitivity (Pandey *et al.*, 2017; Amarowicz & Raab, 1997; Perez & Rubio, 2005; Pulgarin *et al.*, 2012). Agriculture is affected by many problems of environmental and oxidative stress. The photon emission intensity of CL from damaged plants may be to hints as to some physiological and biochemical response to external stress. It's possible that measuring the chemiluminescence emitted from a plant could be a practical application and maybe an easy way to

measuring the overall health of a plant. Therefore, this review article focused on CL emission as a significant signal from plants undergoing physiological changes.

## **2. Chemiluminescence Under Wounded Plant Tissue**

It is well known that when an animal or plant materials are stimulated by external injuries, they will instinctively protect themselves against damage (Sitprija & Sitprija, 2012; Chen *et al.*, 2006b; and Salin & Briges, 1981). All plant species are vulnerable to mechanical damages during their life by injury, which may lead to cause both losses of nutrients and entry of microbes (Piesik *et al.*, 2006). Plant tissue responds to environmental changes and stress factors, such as other mechanically injured, results in increased CL as a defense mechanism against wounding (Winkler *et al.* 2009; Piesik *et al.*, 2006; Chen *et al.*, 2006b; Salin & Briges, 1981). Many studies have found more photons emission near an injury from stress and damaged plant parts than from unstressed (Winkler *et al.*, 2009; Kokubo & Yamamoto, 2008; Yoshinaga & Kato, 2006; Suzuki *et al.*, 1991). These authors found that spectral characteristics and decay of the photon emission from wound plants depend on the state of health of the tissue. Furthermore, the authors suggested that time-dependent variations in emission intensity suggest that some physiological and biochemical changes, maybe have taken place in the wounded tissues. Thus, the photon emission intensity measurement is beneficial for detecting physiological and biochemical changes in the wounded tissue. Plants respond to self-defense mechanisms in response to injury, either to repair damaged tissues or defense against pathogens and herbivores (Pandey *et al.*, 2017; Yoshinaga & Kato, 2006). Consequently, the phenomenon of enhanced light emission upon wounding may be part of an over-all defensive response by plants to seal off the wound

and generate new tissue (Creath, 2008). Several previous reports had shown that stress resulting from wounding (mechanical injury) increases CL or ultraweak photon emission from various parts of plants. Abeles (1978) has recorded a light production from the root and stems tissue of peas (*Pisum sativum*), beans (*Phaseolus Vulgaris*), and corn (*Zea mays*) by measuring chemiluminescence in a scintillation spectrophotometer. Salin & Bridges (1981) observed an enhanced CL upon mechanical injury stem and root segments from soybean seedling. Suzuki *et al.* (1991) applied a photon counting system to detect ultraweak light originating from physically injured adzuki bean and soybean seedlings. As well as, the authors found that possible to determine accurately the wounded regions of the seedlings which exhibit strong emission. Also, Lazim (2005) observed the light emission by measuring CL from roots of corn (*Zea mays* L.) after mechanical injury and was found that emission increased by the addition of luminol or lucigenin. Chen *et al.* (2006b) used a sensitive single-photon counter system to identify ultraweak bio-chemiluminescence (UBC) from germinating soybean cotyledon under mechanical wounding. Yoshinaga & Kato (2006) reported ultraweak photon emissions from herbivore-damaged maize plants, concluding that caterpillar regurgitation is a significant behavior for releasing photon emissions from cut corn leaves. Kokubo & Yamamoto (2008) used the cucumber plant's ability to emit biophotons to investigate the effects of non-contact healing known as laying-on-of-hand. Also, Winkler *et al.* (2009) measured the ultraweak and induced photon emission from wounded Cucurbita pepo seedling using single-photon counting devices. As well as, the authors observed an increase in the ultraweak photon emission depends on the kind of wounding and its localization on the plant. A recent study showed that *Arabidopsis* subjected to mechanical injury

showed enhanced photon emission, which led to changes in the spectral distribution of the emitted photons (Prasad *et al.*, 2020). Some hypotheses were proposed from earlier chemiluminescence studies of seedlings, focusing on chemiluminescence wounded tissue, which produces higher photon emission than normal tissue. It is known that, whether animal or plant matter that when their bodies are stimulated by external stress or injuries, they will respond instinctively to defend. For example, polymorphonuclear leukocytes emit photons during the process of phagocytosis (Prilutsky *et al.*, 2011; Lilius & Marnila, 1992). The peroxidase system in the animal cells may be responsible for light production by actively phagocytizing leukocytes (Bedouhene *et al.*, 2017; Schadelin *et al.*, 1980). Peroxidase is one of the main wound-inducible proteins in different plants upon mechanical wounding (Pandey *et al.*, 2017). It seemed appropriate to examine whether plant CL is affected by a mechanism similar to that of animal cells. The similarities between emission from plant and root tissue and emission from leukocytes in animal cells have described by Salin & Bridges (1981); Abeles (1978). Moreover, the author suggested that a peroxidase enzyme system upon mechanical injury has contributed to the production of chemiluminescent-excited species. This system is known to be responsible for chemiluminescence in leukocytes, and it appears that plants have a similar system. According to Saeki *et al.* (1989), mechanical injury enhanced the chemiluminescence of root and stem segments of soybeans. And also, they suggested that peroxidases, found abundantly in roots predominantly, contribute to light emission in wounded plant tissue. On the other hand, Chen *et al.* (2003b) suggested that ROS reaction induced by chloroplast and mitochondria damage due to wounding could be the primary step that initiates a stimulating burst of lipid peroxidation. Which leads to

$^1\text{O}_2$  generation, and when singlet oxygen and ROS decay from excited states, photon emission has generated. While Salin and Bridges (1981) suggested that increased CL in wounded root tissue is associated with increasing peroxidase activity, hydrogen peroxide generation, and singlet oxygen production. Additionally, oxidative injury in response to a wound involves the initial formation of activated oxygen species and the consequent interaction with macromolecules such as proteins, fats, sugars, and nucleic acids, leading to its damage (Thompson *et al.*, 1987). Finally, the wound-healing response, involving peroxidative reactions, and the enhanced light emission response to wounding may be part of a wider protective response by plants to close the wound and regenerate new tissue.

### 3. A Chemiluminescence Technique to Determine the Degree of Ageing Seeds

The crop quality study is one of the most critical parts of seed science. Preservation of viability of seeds without losing their standard vigour is a problem in agriculture and horticulture. Abiotic stress conditions, such as high temperature and relative humidity or, storage conditions cause seeds to lose vigour and their viability of germinability (Sharma *et al.*, 2021 & Chen *et al.*, 2003a). Seeds usually lose viability after a few days or weeks under such storage conditions (Murthy *et al.*, 2003). Accelerated ageing is an actual test sign of seed vigor and storability, where it helps to understand the seed degradation processes that occur during ageing (Patil *et al.*, 2019; Barreto & Garcia, 2017). The spontaneous CL technique has known as a possible way of developing a fast, quantitative, and noninvasive method for the rapid measurement of the degree of ageing seed (Chen *et al.*, 2003a). The chemiluminescence method may be provided new information on biochemical and physical changes in seeds during germination and help estimate the seed age

(Saeki *et al.*, 1990). It is well known that the interactions between water and cereal have a significant impact on cereal crop, storage, and quality (Sharma *et al.*, 2020; Chen *et al.*, 2002a). Several studies have been observed low-level chemiluminescence with sharply increases upon imbibition on different seeds, such as macaw palm (Barreto & Garcia, 2017); mung bean (Rafieiolhosseini *et al.*, 2016); rice (Chen *et al.*, 2006 a); soya (Triglia *et al.*, 1993); and soybean (Boveris *et al.*, 1984, 1983). There are several reports and reviews on seed ageing that showed seeds with a longer storage time had a lower intensity of CL during early imbibition. For example, some studies in storage rice seeds have confirmed that chemiluminescence emission had an attachment with the degree of seed aging during early imbibition (Chen *et al.*, 2003a; Chen *et al.*, 2002 a, b). Likewise, the same results were recorded in rice by Chen *et al.* (2000) when they note storage time of rice seeds was related to the ultra-weak biophoton emission characters during the early imbibition period. As well, they mentioned that emission might be a way to examine new and aged seeds. On the other hand, the connection between chemiluminescence intensity and water content of soybean seeds after the storage has been established through Saeki *et al.* (1990). While, Puntarulo & Boveris (1990) shown that the spontaneous and imbibition chemiluminescence upon accelerated aging of soybean embryonic axes was similar to those measured after natural aging. The CL studies contributed significantly to the knowledge of seed ageing mechanisms during early seed imbibition. Several mechanisms to understand seed ageing during early imbibition have been suggested by some authors. Chen *et al.* (2002 a) suggested that CL in rice seeds during early imbibition has mainly linked to single oxygen ( $^1\text{O}_2$ ), also it depended upon the aging of degree seeds. Chen *et al.* (2006 a) also suggested that CL of rice seed during

early imbibition arises partially from enzyme-catalyzed reactions. Whereas Wang *et al.* (1990) suggested that enzymic lipid peroxidation may play a remarkable role in CL of Lipoxygenase-lacking soybean seeds during an accelerated aging process. A number of other studies have shown that aging in seeds during early imbibition has closely linked with reactive oxygen species (ROS), which is also responsible for CL (Liu *et al.*, 2007; Chen *et al.*, 2003a; Chen *et al.*, 2002 a; Boveris *et al.*, 1984). Chemiluminescence in all biological tissues reflects the production of free radicals accompanied by lipid peroxidation. On the other side, the lipid peroxidation reaction involved in seed aging is considered a factor in plant seed CL (Saeki *et al.*, 1990). Seed ageing during storage under accelerated ageing conditions has primarily been caused by lipid peroxidation and the accumulation of free radicals, which is the most common cause of accelerated damage seed. As well as, oxidative during aging involves the initial formation of activated oxygen species and the consequent interaction with macromolecules (Thompson *et al.*, 1987), and it can then cause damage to proteins, fats, sugars, and nucleic acids. There are many studies and reviews that have confirmed that seed aging and germination capability loss are due to the accumulation of free-radical lipid peroxidation products (Puntarulo & Boveris, 1990; Murthy *et al.*, 2003; Veselova *et al.*, 2015; Patil *et al.*, 2019).

#### **4. Monitoring ROS formation and Antioxidant Defense Machinery in Plants using Chemiluminescence Method**

There are many sources of reactive oxygen species (ROS) and antioxidative defense in plants, including various environmental and biological factors, such as drought, high salinity, external injuries, and aging of plant organs. It is well known, chloroplasts, mitochondria, cell walls,

endoplasmic reticulum, and plasma membrane all contain ROS in plant cells (Pandey *et al.*, 2017). Also, ROS arises during the fundamental metabolic process such as mitochondrial aerobic respiration and photosynthesis (Ohya *et al.*, 2002). The reactive oxygen species (ROS) involves both non-radical forms (hydrogen peroxide, H<sub>2</sub>O<sub>2</sub>; and singlet oxygen, <sup>1</sup>O<sub>2</sub>), and free radicals such as superoxide radicals (O<sup>\*-2</sup>); hydroxyl radical (OH); perhydroxy radical (HO<sub>2</sub>); and alkoxy radical (RO) (Gill & Tuteja, 2010). These reduced species of oxygen are linked with membrane lipid peroxidation, protein oxidation, and enzyme inhibition leading to Cell Death (Pandey *et al.*, 2017). When plants are exposed to biotic and abiotic stress factors, oxidative bursts of ROS are generated either during the response to a typical cellular stress reaction or during metabolic processes linked to life-sustaining enzyme-catalyzing reactions (Albert *et al.*, 2015; Pospisil *et al.*, 2014). Excessive concentrations of ROS can be toxic by the damage of the chloroplasts and mitochondria through oxidative destruction to lipids, proteins, and nucleic acid. Oxidative injury during aging or in response to a wound involves the initial formation of activated oxygen species and the consequent interaction with macromolecules (Thompson *et al.*, 1987), and it can then cause damage to proteins, fats, sugars, and nucleic acids. Antioxidant protection systems, both enzymatic and non-enzymatic, can prevent stress-induced ROS accumulation. They include a variety of scavengers, such as superoxide dismutase, SOD; ascorbate peroxidase, APX; glutathione peroxidase GPX; and catalase, CAT; and non-enzymatic low molecular metabolites, such as ascorbic acid, ASH; glutathione, GSH; and flavonoids (Gill & Tuteja, 2010). Thus, the degree of physiological damage to the stressed plant depends on the ROS concentration. As a result, knowing the ROS concentration in a stressed plant aids in determining the extent of harm caused

by environmental stress (Ohya *et al.*, 2003). CL is widely used to measure ROS in a noninvasive, direct manner (Kim *et al.* 2019; Haklar *et al.* 1998). Moreover, it is a sensitive, simple, and rapid method for monitoring these radicals, or compounds that have scavenging properties over them (Jimenez & Navas 2002). When ROS interacts with biomolecules, they produce unstable bimolecular intermediates, which decompose into exciting electron species. Consequently, excited states decay radiatively by the emission of photons (Saeidfirozeh *et al.*, 2018). Depending on the ROS reacts to a biomolecule, a part of the reaction energy is used to emit photons whose wavelengths range from far infrared to ultraviolet (Ohya *et al.*, 2003). Ultra-weak photons (CL from biological systems) are released automatically by the relaxation of electronically excited species when ROS are produced via oxidative metabolic processes and oxidative stress reactions (Cifra & Pospisil, 2014; Pospisil *et al.*, 2014). Moreover, ROS is one of the most toxic factors in the emission of ultra-weak photons in plant systems. According to this factor, biophoton emission occurs as a result of ROS formation during plant damage caused by various environmental stresses (Ohya *et al.*, 2003). Several studies showed a growing interest in the possible association of oxidative free radicals in some plant processes like wound healing (Pandey *et al.*, 2017; Bryan *et al.*, 2012; Lazim, 2005) and seed aging (Kurek *et al.*, 2019; Wiebach *et al.*, 2019). Several authors studied the ROS involvement in the CL emission. For example the involvement of free radical traps and lipoxygenase inhibitors was noted in CL emission for soybean embryonic axes by Boveris *et al.* (1984). Albert *et al.* (2015) used a luminol-enhanced chemiluminescence assay to demonstrate the ROS-burst in plant leaf pieces of Arabidopsis plant. Yoshioka *et al.* (1990) studied the CL emission and formation of radicals from tea treatment leaves in various methods. They suggested

that the chemiluminescence becomes more stimulating as the quantity of the free radical increases. They also suggested that the radical generated by enzymatic oxidation of polyphenols is the source of CL in the tea leaf. The Ultra-weak CL from germinating soybean cotyledon under mechanical wounding has been documented by Chen *et al.* (2002c). They suggested that the main process was probably that chloroplast and mitochondria damage at the wounded place and led to ROS generation. The chemiluminescence of extracts from leguminous seedlings of adzuki bean and soybean has been investigated by Lida *et al.* (2000). And as described by these authors, the defense against reactive oxygen metabolites can lead to the formation of chemiluminescence substances such as flavonoids, phenolic acid, and peroxidase during germination. Using the flow injection—CL system, Choi *et al.* (2000) demonstrated the high radical scavenging effect of Chinese herbal components and the structures of flavonoids. On other hand, Veselova *et al.* (2004) have observed an increase in ROS generated from embryonic axes of dry pea seeds by chemiluminescence. They suggested that post-hypoxic oxidative stress was the cause for the damage of cell division. While, Ohya *et al.* (2003) have recorded the correlation between the physiological damage of ROS to adzuki bean seedlings and biophoton emission from the root. They have found that photon measurement has very useful for estimating physiological damage to plants due to environmental stress. On the other hand, the interaction of luminol with ROS was investigated by Papuc *et al.* (2012) to see whether celandine polyphenol extracts could remove any reactive oxygen species formed. They found that celandine leaf extract had the best ability to clean out the hydrogen peroxide. Rastogi & Pospisil (2010) also found that ROS, especially singlet oxygen, plays a role in biophoton emission in radish root plants. Lastly, CL

may serve as a simple, fast, sensitive, and non-destructive way to monitor the amount of ROS capable of interacting with peroxidase in plant tissue.

## 5. Conclusions

In conclusion, this paper present a general review of the history of chemiluminescence (CL) in plant studies and their applications in agriculture. Chemiluminescence methods have become a valuable tool due to their numerous advantages, such as low cost, high sensitivity, and simplicity. In addition, their use of easy and inexpensive instrumentation for monitoring emission. All these advantages have been allowed the method that has conveniently used in the agricultural application fields. There are many possible applications of CL based on the relationship between photon emission and environmental changes and oxidative stress indicators in plants. Lastly, new information about the mechanism of physiological changes and damage can be acquired through CL measurements.

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