Journal of Photochemistry and Photobiology 19 (2024) 100224

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology

journal homepage: www.sciencedirect.com/journal/journal-of-photochemistry-and-photobiology

Rooting out ultraweak photon emission a-mung bean sprouts

Alasdair M. Mackenzie [a,*], Holly E. Smith [a], Rhys R. Mould [b], Jimmy D. Bell [b], Alistair V. W. Nunn [b,c], Stanley W. Botchway [a,*]

[a] OCTOPUS, Central Laser Facility, Science and Technology Facilities Council, Oxfordshire, UK
[b] Research Centre for Optimal Health, School of Life Sciences, University of Westminster, London, UK
[c] The Guy Foundation, Chedington, Dorset, UK

ARTICLE INFO

Keywords:
Biophoton
Ultraweak Emission
Reactive Oxygen Species
Microscopy
Biophotonics
Metabolism
Mitogenic Effect
UPE
Ultraviolet
BAL
Biological Autoluminescence

ABSTRACT

It is well known that life has evolved to use and generate light, for instance, photosynthesis, vision and bioluminescence. What is less well known is that during normal metabolism, it can generate 1–100 photons s^{-1} cm^{-2} known as ultra-weak photon emission (UPE), biophoton emission or biological autoluminescence. The highest generation of these metabolic photons seem to occur during oxidative stress due to the generation and decay of reactive oxygen species (ROS), and their interaction with other components of the cell. To study this further, we have configured a sensitive detection system to study photon emission in germinating mung beans.

Here we investigated growing mung beans over 7 days at a constant temperature of 21 ± 1 °C in a light tight box, using dual top and bottom opposing photomultiplier tubes. Over this time period we showed that in total, mung beans grown from seeds generated an average of 5 ± 1 counts s^{-1} above background. As the new bean stems grew, they showed a gradual linear increase in emission of up to 30 ± 1 counts s^{-1}, in agreement with previous literature. In addition to this "steady-state" emission we also observe delayed luminescence and drought-stress response emission previously observed in other species. Finally, we also observe episodic increased emission events of between 2 and 15 counts s^{-1} for durations of around 3 h detected underneath the sample, and assign these to the growing of secondary roots.

We then induce secondary root formation using aqueous solutions of growth hormones hydrogen peroxide (H_2O_2, 167 μM) or 3-indole acetic acid (IAA, 0.5 μM) for watering. Both hormones show prolonged increase in emission above steady-state, over days 3–5 with at least 3 times the number of secondary roots formed compared with water alone. We also observed a significant peak increase in photon emission (474 and 1738 cps vs. 28 and 55 cps for water alone) for the H_2O_2 which we attribute to direct ROS reaction emission as confirmed by measurement on dead plants.

Altogether we have expanded upon and demonstrated an instrument and biological system for reliably producing and measuring intrinsic metabolic photons, first observed 100 years ago by Alexander Gurwitsch.

Introduction

Understanding the origins of life is one of the great missions in biology. The mix of prebiotic chemicals and ions, are well described, but the role of heat, light, electric and magnetic fields have all been suggested as important factors [1–4]. Prior to the evolution of photosynthesis, it is unknown to what extent light may have been a crucial factor or hindrance in early development of proto-cells. Today, not only do we find cells filled with molecules which can absorb light at wavelengths from the ultraviolet (UV) to the near infra-red (NIR), but that metabolism can also produce low levels of photons of many different wavelengths, for instance, during oxidative stress.

One possible role of these "metabolic photons" in biology is thought to be non-chemical communication between cells. This was first demonstrated in the 1920s by Alexander G. Gurwitsch who placed two onion roots in sealed quartz tubes and showed increased mitosis on the side of the root exposed to the other, naming this the mitogenic effect (MGE) as the effect could not have been chemically transmitted [5]. Further work showed that although the MGE largely seemed to be due to light in the UV-B & C (~200–315 nm) region, sensitive detectors indicated that a broader spectrum of light was being emitted [5]. This light was later called biophotons (BP), metabolic photon emission (MPE),

* Corresponding authors.
E-mail addresses: alasdair.mackenzie@stfc.ac.uk (A.M. Mackenzie), stan.botchway@stfc.ac.uk (S.W. Botchway).

https://doi.org/10.1016/j.jpap.2023.100224

Available online 5 December 2023

Biological autoluminescence (BAL) or ultra-weak photonic emission (UPE), due to the extremely low number of photons, which is in the order of 1–100 photons per second per cm^2. BP have been discussed in several recent reviews [5–11]. "Spontaneous UPE" has been found to be produced in all kingdoms and biological systems, including plants, humans and their cells, bacteria, yeast and even isolated mitochondria [12–17]. Interestingly, a common finding is that biosystem photonic emission can be increased by stressors. Although this "induced emission" has been observed many times it is not clear if these are the result of the same biological process(s) and can complicate the field [18–22]. It is worth noting that when plants are damaged, for example by cutting leaves, this leads to an order of magnitude increase in photon emission. It is not clear whether the photon emissions from the plant wounding and metabolic emission comes from the same mechanism [21].

It is generally thought that UPE is generated from chemical reactions in cells, such as lipids reacting with reactive oxygen species (ROS) to generate excited radicals which can further react with nearby molecules and excite them. The decay of these electronic excited states by the emission of a photon, can either be detected as UPE or be absorbed then emitted at a lower wavelength by other chromophores in the cell and this is detected as UPE. The radical chain reactions and photon emission chains can be complex and are summarised in the literature [9]. This underlying mechanism has not been definitively proven however, due to limited resolution of spectroscopy and the very low numbers of photons involved.

Thus, the core challenge of studying UPE is the very low flux of light. This leads to related challenges of discriminating it from other sources of photons, such as environmental light from sunlight or indoor lighting, which are orders of magnitude brighter, or even photons produced by instrumentation itself. A further challenge is also finding a reliable biological system that can emit enough light repeatedly under controlled conditions to provide consistent data. As a rule, studies have been performed with sample and detector inside a sealed light-tight container, which is itself placed inside in a pitch-black room with no sources of light. Often the room or hall outside this room is also kept pitch black, as the detectors used are so sensitive. In fact, the standard procedure for photomultiplier tubes is to not expose them to bright light before they are turned on as they will have a diminished response [23]. Furthermore, it is also crucial to not expose the dark chamber to light for this period as long-lived phosphorescence of the chamber walls can persist for hours, even though it is not visible by eye.

Another big challenge is the phenomenon of delayed luminescence (DL), one cause of which is where a molecule in a lowest triplet state undergoes intersystem crossing into an excited singlet state which then fluoresces. This is different to a triplet state emission of phosphorescence that decays on a microsecond and millisecond time scales. It can result in an intensity of emission that is orders of magnitude brighter than UPE and can last for hours before it is minimal and days before it is undetectable. It has been shown to occur in agar and cell media but is particularly strong in plants due to a stable dark state of chlorophyll [24]. Many studies claiming to image UPE only leave 30 min after light exposure before recording, while DL takes hours to diminish and is likely being recorded instead. Although UPE may be present in such systems, they will be overwhelmed by the DL which renders such studies and data difficult to interpretate.

Chloroplasts can be a main source of DL but also of UPE. The other leading location of UPE is thought to be the mitochondria, which is present in the majority of species that show it. Mitochondria isolated from spinach leaves were shown to emit UPE light when given metabolic food sources [17]. Previous work has shown that as plants grow, the light emission increases linearly in relation to their size, which is attributed to the doubling of the number of cells, we refer to this as "steady-state" UPE[25]. Imaging of growing roots shows that the majority of UPE comes from the primary root meristem, even though they make up only 20 % of the volume of the root [26]. It has been suggested that this is because during cell division there is increased mitochondrial activity and therefore periods of growth should show increased periods of UPE above the steady state emission. There is also evidence that by cutting a leaf there is an increased emission at the wound site [20,27]. This could be a due to a ROS-related stress response. However, for understanding intrinsic UPE, due to the invasive and potentially variable nature of the wounding protocol, it is probably not an ideal starting point. Work on red beans has shown that when they have not been watered and start to undergo drought stress, their UPE increases[28]. Mitochondria are essential in the plant's drought stress response [29]. Upon re-watering there is also a spike of emission that decays down to the steady-state rate of emission before the drought stress. Stress is well known to enhance mitochondrional ROS production which adds to the hypothesis that they can be a source of UPE. Therefore, we want to tailor our experiments to measure mitochondrial emission over that in chloroplasts.

For our experiments, we still needed a reliable and reproducible biological system. Research led us to the mung bean, which is normally used to produce bean sprouts. These plants have previously been used in both delayed luminescence and UPE studies [25,30,31]. They are a good candidate because they grow in complete darkness with only the addition of water (stimulation of germination using water is known as imbibition). This minimises other sources of light that could confound the study of UPE. They are also large enough to provide measurable emission from a single growing bean.

This work then seeks to test if it is possible to repeatably detect intrinsic UPE at a rate high enough for further characterisation and to give insight into their origin and relationship to the stages of plant germination:

We first seek a source of increased emission by measuring individual germinating mung beans, which were grown for different periods of time. These initial measurements showed the dominance of delayed luminescence, but also the indication of events of increased ultra-weak photon emission coinciding with secondary root growth.

Next, we measured emission from a whole petri dish of twenty beans grown at different times. This showed a strong UPE signal above DL, and a more homogeneous response. Using our opposing detector configuration, secondary roots are shown developing and are correlated in measurement of more emission features underneath as compared to above the sample.

Then we measure emission from petri dishes of beans grown from the first stage of germination: imbibition of water, in the presence of hormones and observed significantly increased light emission confirming our hypothesis that secondary root growth leads to UPE.

Materials and methods

Mung beans and chemicals

Dry mung beans (*Vigna radiata*) were sourced from a local food shop (Honor foods, imported to UK by Interlink Direct Ltd, IG8 8EY, UK) and were stored dry at room temperature until used for the experiments.

All water used for watering and chemical preparations was 18.2 MΩ reverse osmosis deionised water. This reduces the chance of impurities causing emission.

3-indole acetic acid sodium salt (IAA) was purchased from Insight Biotechnology Limited. Hydrogen peroxide (H_2O_2 30 % w/w in water) was purchased from Merck. Chemicals were dissolved in 18.2 MΩ reverse osmosis deionised water and used immediately without further purification.

Photography

All plant samples were photographed before and after their growth in the dark chamber using a mobile phone camera (Poco M4 Pro 5G - 21091116AG, Xiaomi). Dry beans were not photographed before the experimental run.

Instrumentation for measuring ultra-weak photon emission

Photon emission was measured using the setup illustrated in Fig. 1. A light-tight dark box (30 × 40 × 50 cm) was made from black aluminium frames and panelling (custom built by Engineering Department, Central Laser Facility, Rutherford Appleton Laboratory, Oxfordshire, UK). Power and data wires were passed through a 50 mm PVC pipe chicane blocked with blackout cloth and liquid tubes passed through a 25 mm hole shielded with two flexible black-anodised aluminium sheets. Over the top of the box was placed a blackout cloth (BK5, ThorLabs) and the box was kept inside a dark room with a sealed door.

Inside the dark box were two photomultiplier tube (PMT)-based photon counting heads (H11870-01, Hamamatsu Photonics, Japan) which send digital TTL pulses to a time-tagged time-resolved (TTTR) pulse counting box (Picoharp 300, Picoquant, Germany) interfaced with a PC using Qucoa or SymPhoTime64 software (Picoquant, Germany). The two PMTs had an iris aperture to protect them from light (SM1D25, Thor Labs, maximum diameter 25 mm) and were placed facing each other with a sample holder in between. The sample was contained inside a cuvette (box horizontal) or petri dish (box vertical). The PMT detector surfaces were positioned 30 mm from the respective front surface of the cuvettes or 28 and 64 mm from the front surfaces of the petri dish (bottom and top respectively). A Raspberry Pi – based temperature and humidity sensor recorded the inside of the chamber as 21–22 ± 0.01 °C and 60–80 ± 0.1 %RH throughout the experiment. Although the PMTs were not cooled and the dark count matched that as specified by the manufacturer at this temperature.

Emission data was measured for up to 100 h uninterrupted at a time. Longer experiments were performed by restarting the measurements and concatenating together 1 s buckets of TTTR counts. The missing time was < 5 min if hand-watering or < 60 s if not. The 1s data was then averaged over 1,000 s and the detector dark count (Fig. 2) was subtracted from this. Due to the nature of the growing plant, a direct distance correction to intensity was not meaningful, therefore a ratio of detector signal is presented to show the variation of the relative intensities.

Bean imbibition

Dry mung beans (20) were checked for mould or damage and then placed into a 60 mm polystyrene petri dish (150340, Nunc, Thermo Scientific). To initiate imbibition (the first stage of seed growth), 5 ml of water was added, which was designated as day 0 for all experiments.

Measuring emission at different stages of growth

A petri dish of 20 imbibed beans were covered with aluminium foil and grown in the dark inside a covered cardboard box at room temperature. Every 3–4 days the seeds were watered with a further 5 ml before going back into their covers. After the desired number of days growth, the dish was photographed and exposed to diffuse daylight outside for 60 s to set delayed luminescence at a similar level. It was then placed into the dark chamber and emission recorded. After 3–4 days the dish was removed and photographed. A dish containing only water was also recorded similarly as a DL control.

Emission from single beans

Petri dishes containing mung beans were grown as above. Following 3 days growth the straightest growing bean was selected from the dish, placed into a cuvette (held in place using filter paper), and allowed to grow, all in ambient light. The cuvette was refilled with water up to the bean seed every 3–4 days. The cuvette was partially covered with parafilm to reduce evaporation. After a total growth of 11 or 16 days the cuvette was taken outside and exposed to diffuse daylight for 60 s (to mitigate DL differences due to room light), before being placed inside the measurement apparatus.

Emission measurement from growing seeds

A petri dish of beans was imbibed as above, but inside the dark chamber. Watering from a 5 ml syringe was performed using a weak "white LED" for vision at day 0, 3 and 6. (It is worth noting that the white LED used here contains a mixture of a blue LED and a yellow phosphor as confirmed by a spectrometer). Emission measurements were stopped for the watering process, to protect the PMT, and resumed as soon as possible, no later than 60 s after watering.

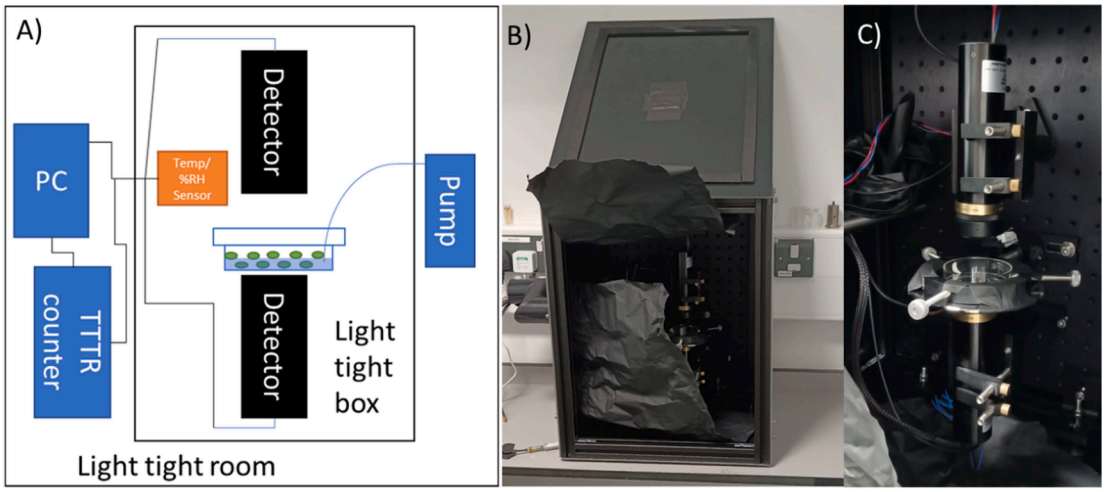

Fig. 1. Set up for photon emission detection in petri dish configuration. A) Schematic of the apparatus: two PMT detectors were orientated above and below the sample. A syringe pump remotely added water or solutions with dissolved chemicals mid-way through the experiment. A temperature and humidity sensor verified the experimental conditions. The detector TTL pulses were measured on a time-tagged time-resolved counter and recorded on a PC. B) Image of the apparatus inside the box: aluminium foil minimizes any accidental exposure to light when open and the lid features a foam seal. C) A close image of the dual photon counting units with an empty petri dish, the temperature and humidity sensor can be seen behind the dish. Further details of the dish and cuvette setup can be found in the Supporting Information.

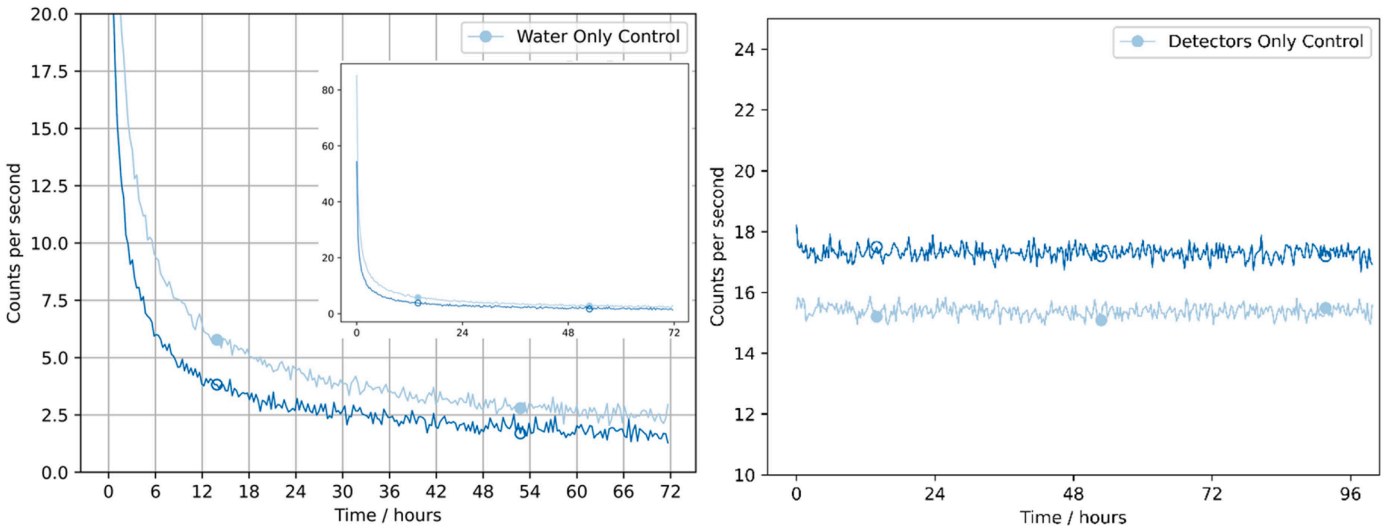

Fig. 2. (left) DL over time in a dish containing only water, with full measurement in inset. (right) Detectors in an empty chamber showing an average dark count of 15.4 and 17.3 cps over 4 days. Dark lines, open circles are detector below and light lines, closed circle are detector above.

Emission measurements with remote addition of plant hormones

A petri dish of dry beans was imbibed in the dark chamber as above using an attenuated white LED for vision. A syringe pump was connected to 3 ml (internal) PTFE & PET tubing and attached to a modified petri dish lid. Photon measurement was performed as soon as possible after imbibition. At day 3 the addition of liquid (5 ml of water, 167 μM H_2O_2 or 0.5 μM IAA, plus 3 ml priming draining the tubes) via syringe pump was performed while measuring emission. After 7 days total emission measurement the number of secondary roots were measured as below.

A control experiment was performed to test chemiluminescence from dead beans. Addition of 167 μM H_2O_2 was performed as above, replacing the fresh bean seeds with 6 day grown plants which had then been left to dry and die for 3 months.

New primary and secondly root growth measurement

Plants from one petri dish were spread on square millimetre paper and photographed from above. The photographs were then analysed using FIJI (ImageJ, National Institute of Health, USA) to count and measure the visible secondary roots [32].

The effect of hydrogen peroxide on secondary roots

Twenty dry beans were placed into a 60 mm petri dish and immersed in 5 ml water. Two dishes per concentration (between 30 % w/w and 1 μM) were placed in aluminium foil and placed in the incubator for 3 days. Each dish then received 5 ml of the relevant concentration of H_2O_2 and was placed back into the incubator for 3 more days. The dishes were imaged and secondary roots measured as described above before being given 5 ml of DI water and placed back into the dark incubator for 3 days. The roots were imaged and counted again as above (See SI Table S1).

Results

Characterisation of equipment

Prior to UPE measurements from growing seeds, the experimental chamber as characterised to establish the level and decay of the DL and detector dark count. The detectors were opened to the chamber in the dark and without any sample inside for 4 days and recorded. The average counts over this period were taken as the dark count: 15.4 and 17.3 cps for top and bottom detector respectively (Fig. 2). A petri dish containing only 5 ml water was measured for DL to estimate the effect of delayed luminescence from the holder.

Photon emission during different stages of plant growth

In these studies, we aimed to initially establish a suitable growth regime that could provide measurable photon emission of sufficient intensity to give a good signal to noise ratio. Photon emission measurements on mung beans after different stages of growth [0, 3, 6, 10 days after imbibition and a water-only control] are shown in Fig. 3. The dishes with more mature beans showed more emission, possibly due to a greater tissue mass. The more mature dishes also showed a higher DL peak and decay, which started to overlap with UPE emission features. All dishes always showed more emission from the bottom detector, except day-6, which showed exceptional growth and grew inside the iris of the top detector; the observed increased signal of top vs bottom detectors is due to its closer proximity to the detector as well as the origins of the photon emission, i.e., roots versus shoots/leaves.

Spikes of emission lasting about 3 h were detected by the bottom PMT: day-3 (at 22, 58 h, and smaller spikes at 6–12 h), day-6 (at 72 h), day-10 (at 6 h). No spikes were seen in the top detectors. Increased broad emission events can be seen in both detectors, although not necessarily at the same time in almost every dish. Therefore, these broad emission events could be attributed to general plant growth, whereas the emission spikes can be attributed to events primarily observed underneath the dishes – i.e. root growth. Due to the density of plant material in the dish it is difficult to attribute this to solely secondary root events as the primary root also grows.

Emission during watering: comparing with and without light

To minimise contribution from delayed luminescence while measuring emission spikes from later stages of growth, emission was measured from dishes containing 20 beans from the point of imbibition - Fig. 4. During hand watering on day 3 and 6 using an attenuated white LED to aid vision, spikes of emission can be seen immediately after. The day 3 spike decays over about 3 h. We also note that after watering there is a prolonged decay in emission. The reason for this is currently unknown, however, we speculate the following may be happening: 1) it could be delayed luminescence from the photon generated from internal emission; 2) prior drought emission process continuing to diminish; 3) metabolic activity of the plant diminishing. The day 6 spike shows an

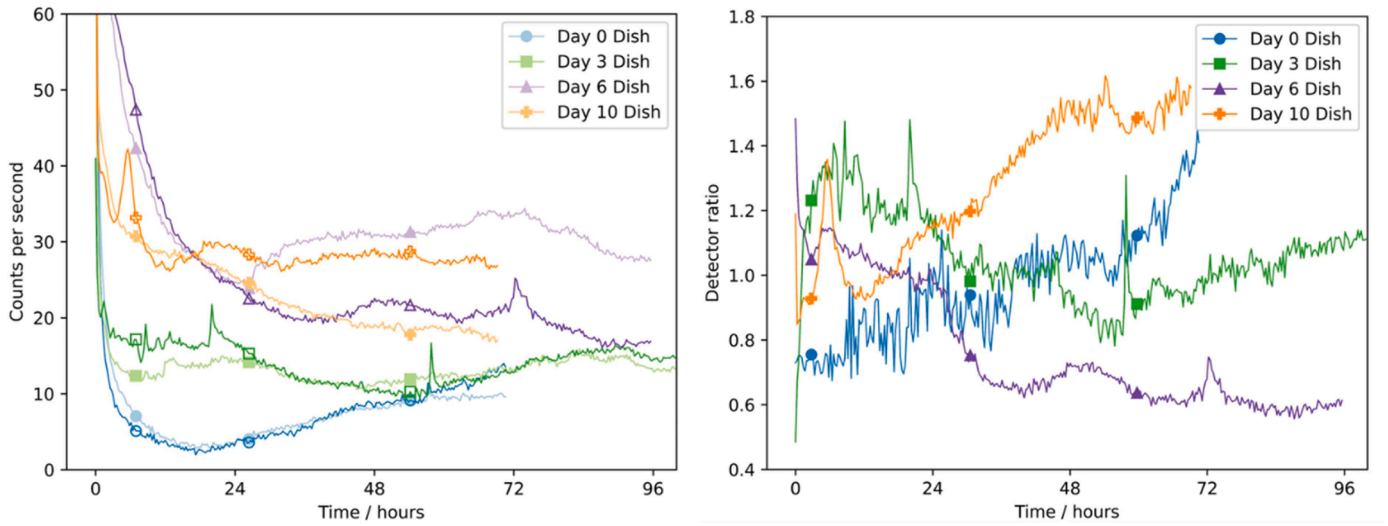

Fig. 3. (left) Photon emission profiles of dishes of twenty growing mung beans, measured after different amounts of growth. Dark lines, open symbols show detector below the dish and light lines, closed symbols the detector above the dish. Delayed luminescence is stronger for the plants grown for longer, but spontaneous UPE then becomes dominant. Spikes of emission from the roots can be seen on the bottom detectors but not on the top detectors. (right) the ratio of bottom to top emission, with increases showing more intensity measured on the bottom detector from root events and decreases showing more intensity on the upper detector, mostly from plant growth towards the detector.

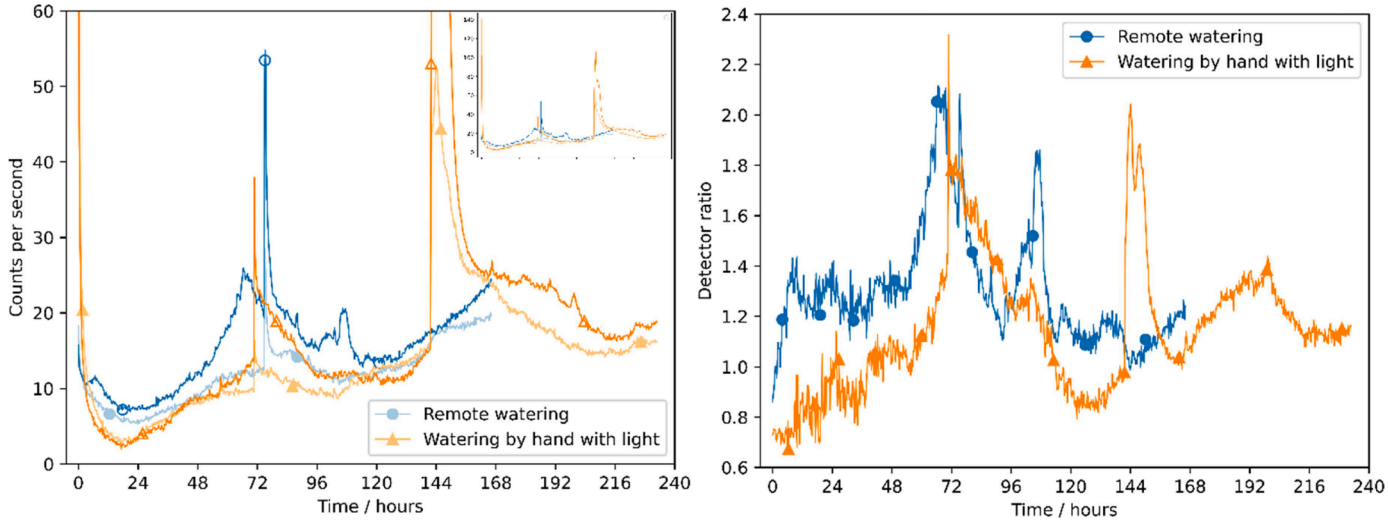

Fig. 4. (left) Emission measured from above (light, solid symbols) and below (dark, open symbols) after initial imbibition of 20 seeds in a petri dish. Orange lines (triangles) show dish watered with a weak white LED light; blue lines (circles) are plants watered by remote pump without light. Inset show full height of peaks. Watering was performed at day 3 (72 h) for both conditions and only by hand on day 6 (144 h). (right) the ratio of intensity of bottom and top detectors highlighting continuous steady-state growth and events unique to the roots (larger number = more light underneath).

increase in emission after removal of light for a more prolonged emissive period of around 15 h. This may be due to the development of cotyledon leaves not present on day 3 reacting to the light.

To test the effect of light on the day 3 (72 h) watering, the experiment was repeated using a syringe pump system, which did not require exposure of the growing plants to light. The emission is also plotted in Fig. 4 for comparison. The day 3 (72 h) spike upon watering shows a similar intensity whether light was used or not. This suggests that this increased photon emission is highly likely due to a response to the watering process and not the light itself. Whether this is through a relief of drought stress as suggested previously [28], or an increased growth activity caused by access to more water is not clear. The plants on day 6 grew to such an extent that they forced the lid of the petri dish off, which prevents remote addition of water through this method for watering on later days.

Photon emission from single plant roots

To investigate the cause of photon emission, measurement was made on single beans placed in the dark chamber after growing for a different number of days (Fig. 5). All samples showed initial delayed luminescence, which then decayed, which was of higher intensity and lasted longer in the samples containing plants compared with the water controls. The plant grown for 16 days before measurement showed very little variation in signal compared with a delayed luminescence decay after 11.1 h, whereas that grown for 11 days showed an increased emission event at 14–33 h and at 64–70 h.

When comparing the images of day 11 and 16 plants (Fig. 6 below), the day-16 plant changed very little in size or composition during the two days of measurement whereas the day-11 plant grew longer roots and shoots. It also developed leaves and grew secondary roots in the 72 h measurement period. Since the detector was only capturing the area of

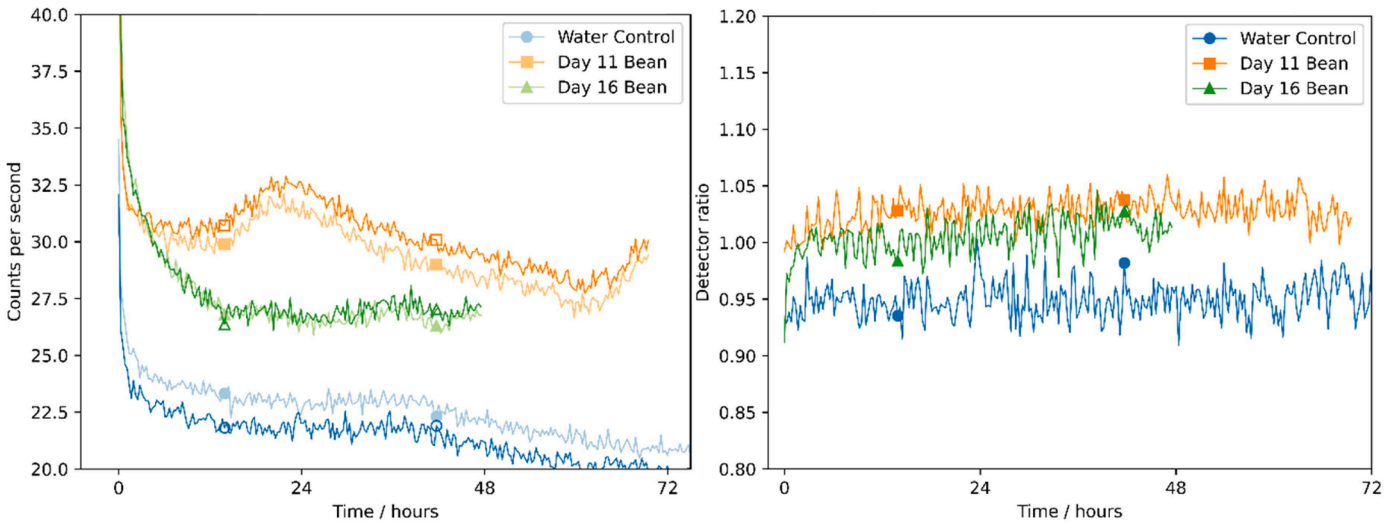

Fig. 5. (left) Single mung bean measured emission from two equi-distant detectors (left detector: dark, open symbols and right detector: light, closed symbols) of 3 samples: a 16-day grown bean (green triangles); an 11-day grown bean (orange squares); and a water-only blank (blue circles). DL in plant and water show decay whereas UPE shows increase in emission signal. (right) The difference in intensity of the detectors expressed as a ratio. This is due to the plant being positioned closer towards one side of the cuvette, as can be seen from the ratio remaining the same over the course of the experiment.

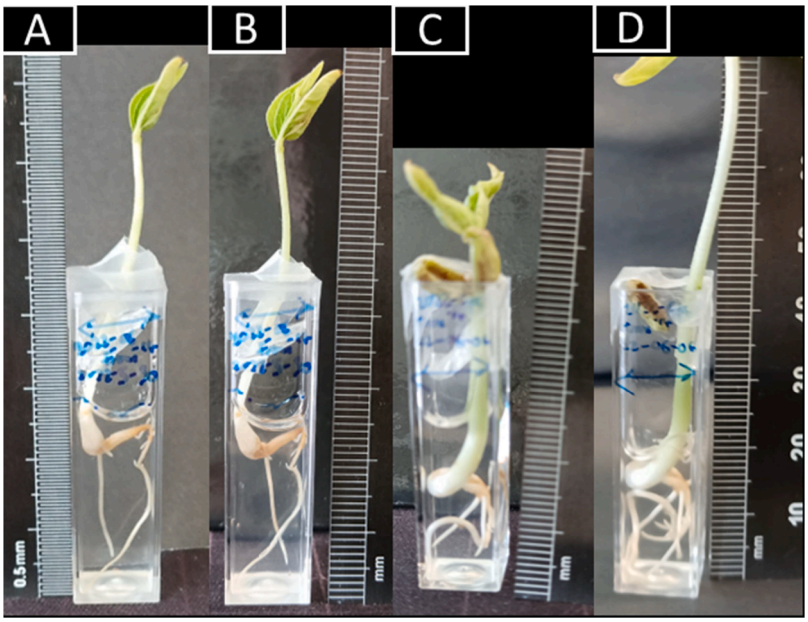

Fig. 6. Single mung bean plants grown for 16 (A, B) and 11 (C, D) days then placed into the dark chamber. Images show before (A, C) and after (B, D) measurement. The growth from A to B showed little change, however C -D showed an increased root length, shoot length, development of leaves and secondary roots appearing 9 mm from base of cuvette. These were accompanied by increased photons from the detector capturing emission from below the stems.

the plant inside the cuvette up to 30 mm from the base, it is highly unlikely that the photon emission from the leaves would provide any meaningful contribution to the signal. Therefore, the source of this emission is clearly from the roots or base of the stem. This lends support to root growth as the main source of emission in our studies in this configuration.

Increasing secondary root formation using hormones

To test for the effect of secondary root formation on photon emission in bean dishes, the remote watering in the dark on day 3 (72 h) was supplemented with a plant growth hormone. Concentrations were chosen of H_2O_2 [167 μM] and sodium indole-3 acetate (IAA) [0.5 μM] which induce increased secondary root formation (see SI) of approximately 3 times that of water alone. This was added on day 3 (72 h), when the dish is dry, to compare with the water only addition and to avoid dilution. The results are shown in Fig. 7.

Days 0–3 (0–72 h) of both dishes showed similar emission profiles when compared with pure water, except IAA which shows less drought stress emission. It can be seen that above the delayed luminescence decay signal an increase on day 0 (4–7 h) is seen in water and IAA dishes. This could be the emergence of the first roots from the beans or breaking of the seed skin. IAA also shows a smaller watering-response peak than water alone (11 & 28 vs 28 & 55 cps: top and bottom detectors respectively), probably since less drought stress was relieved. For H_2O_2 addition, emission peaks of 474 and 1738 cps were recorded at the top and bottom detectors respectively (16–31 times that of water alone). It is likely that the H_2O_2 is not only acting as a biological growth stimulant

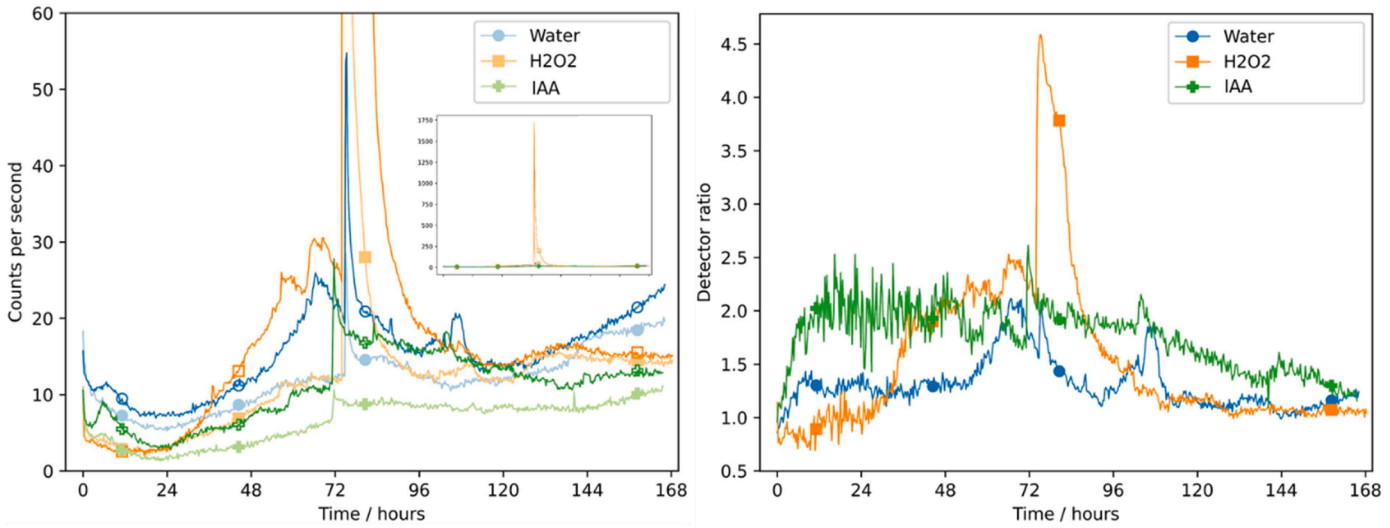

Fig. 7. (left) Photon emission during remote addition on day 3, (~72 h) of water (blue circles), IAA (0.5 μM, green crosses) or H_2O_2 (167 μM, orange squares) to a dish of twenty growing mung beans measured both above (light, closed symbols) and below (dark, open symbols). Common features across treatments are seen in the drought stress (24–70 h) and stress relief (72–80 h) shapes. A gradual steady state emission can be seen as a baseline increase of emission in all the dishes from 24 to 168 h. Unique peaks are discussed in text body. Inset shows full height of H_2O_2 peaks (474 and 1738 cps). (right) ratio of bottom to top detector emission highlighting steady-state growth appearing as a smooth gradient and root favoured events (larger number).

but also as a direct chemiluminescent initiator, which would obscure the biophoton emission signal.

All three dishes showed an elevated emission between day 3 to 4.5 (80–100 h) and a more defined peak in IAA and water on the top detectors at day 4.5 (96–120 h) it is unknown what caused this. This defined peak seems to follow time from syringe watering rather than time since imbibition. Water and peroxide show a steady state rise from day 5 (120 h) and IAA from day 6 (144 h) which is probably due to further drought stress.

If secondary root growth causes emission of photons, this would be expected to be seen as a difference of emission above steady state occurring between day-3 addition of liquid and day-7 termination of experiment. Although the drought-relief emission peaks which occur after liquid addition are all different heights, the water emission peak decays to steady-state emission faster than both growth hormones. This suggests that the emission from secondary root growth (the key difference between treatments) prolongs the decay to steady state.

Application of H_2O_2 to biological samples can result in chemiluminescence [33,34]. To determine the non-biological chemiluminescence component of H_2O_2 on the mung bean emission, a measurement was performed on grown beans which had dried out and were no longer viable (Fig. 8). This showed increased DL on initial measurement as compared to the water-only control, but otherwise was very similar in photon output characteristics to a background DL. There was no increase in emission following DL decay (day 0–4), since the plant was not alive. At day 4, upon addition of H_2O_2 in the dark, a spike of emission was observed which underwent rapid decay and showed no further increase, therefore showing no induced photon emission due to hormone activity. We attribute the smaller peak to the chemiluminescence reactants being used up as the plant dries out, as well as

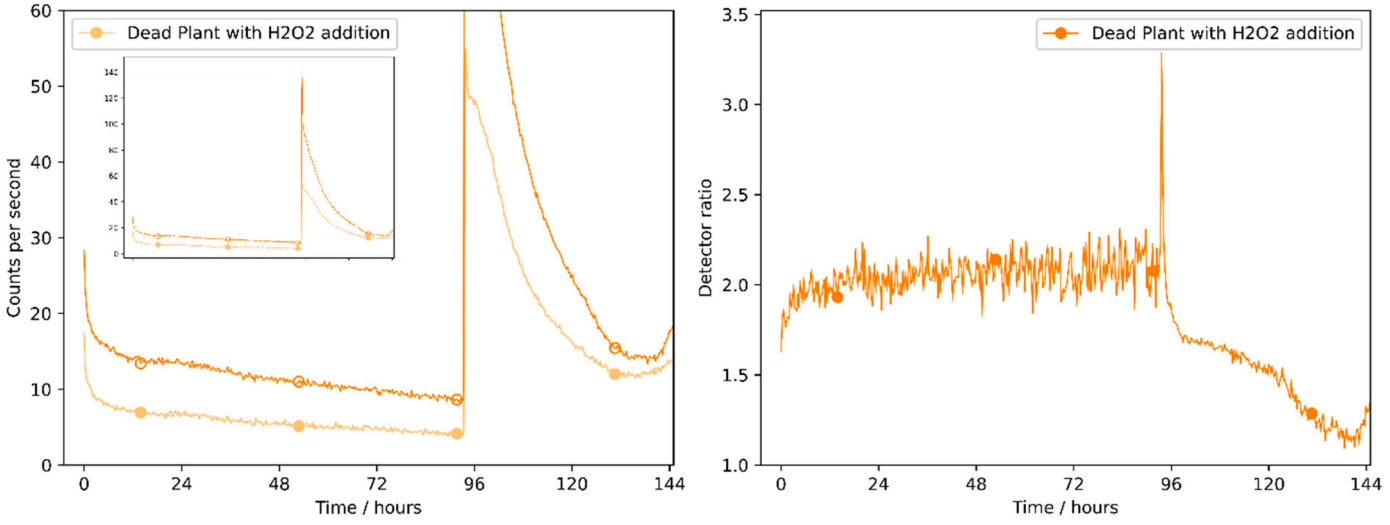

Fig. 8. (left) Emission from dead, dried bean plants (6-day growth) with addition of H_2O_2 167 μM on day 4 (96 h) upper detector (light, solid circles) and lower detector (dark, open circles). Initial delayed luminescence can be seen decaying for the first 4 days. Addition of H_2O_2 can be seen to cause a large increase in emission (full height in inset), which is due to direct chemical excitement of the dead plant matter. At 140 h emission from growing mould on the plant can be seen. (right) The ratio of bottom to top emission, showing no variation during delayed luminescence decay and a very short, sharp peak favouring emission underneath as the liquid reacts here first. The reaction is over quickly and all that remains are slow decay of excited species in the plant which emits equally above and below.

the lack of contribution from metabolic emission or secondary root growth. The long decay of emission may be due to the action of H_2O_2 molecules over time before their complete degradation. At day 5.5 emission from mould growing on the newly-wet plant sample can be observed. The presence of peaks in our studies correlates with plant growth activity, as there are no peaks observed within the instrumentation alone. The only increases in emission observed are due to living processes or direct chemical stimulation, every other process shows a decay or diminishing of emission.

Discussion

To date, studies that aim to detect and characterise UPE are growing but still not extensive in the literature. This has been largely due to technical limitations of older technology, a limited acceptance that metabolism can indeed produce photons and that they may have a homeostatic function, as well as testable hypotheses on how they are produced. Setting up an UPE detection system needs three key things. First, an instrumentation system which can both exclude external light and can detect the ultra-low flux of photons, secondly, a biological system which can reliably and consistently produce enough photons to detect, and thirdly, a testable scientific hypothesis.

The instrumentation we have built successfully demonstrated the ability to detect changes of 0.1 average cps. In our studies, the PMTs used were rated as having an average dark count of 15 & 17 cps at 20 °C, which we confirmed prior to any measurements. They have a second-to-second reading of between 10–80 cps, therefore only upon averaging 1000 s of data could we measure signal changes of fractions of a count per second. The PMTs are specified sensitive between 300–650 nm. However, they record $>$ 0.01 quantum efficiency (QE) from the range of 620 nm to below 270 nm (with a max QE of 0.182 at 375 nm, see Fig. SI-4). Therefore, each cps of signal represents between 5.5 and 100 photons s^{-1} within this spectral range. The limiting factor in these experiments is almost always the lack of photons which are available to measure during relevant biological conditions. To achieve detection of experimental photons with good signal-to-noise ratio (SNR), we also had to ensure the equipment was thoroughly light tight in our experimental box, which we managed to achieve successfully.

The mung beans themselves represent a very repeatable biological system of study. Using 20 beans instead of 1 when given the same amount of water on the same days showed a similar average growth regime for each condition, where variation between each bean seems to average out. Our photographic image of twenty growing mung beans showed similar growth characteristics (Fig. SI-2 for typical growth). This is contrasted with attempts to grow single beans or dishes in ambient light and temperature conditions, which showed similarly treated beans grow to different sizes and condition. Attempting to measure these showed large variations in delayed luminescence and emission events. The reasons for such variations in a single bean growth condition is unknown, however work by Gallep & Robert used repeatable growth in the first few days of single beans to correlate size to emission data [25]. Fig. 3 shows emission peaks and humps at different growth stages, yet the absolute intensity of DL and UPE emission is a function of plant size. This is why we moved away from single beans or more mature plants and towards measuring 20 seeds in a dish. Since adding food or other growth media could be a potential source of emission, only water was added and the store of nutrients inside the dry bean is what allowed growth. We observed that the nutrients in the mung bean seem to enable growth in the dark for at least 9 days without any obvious impact on development of the plant.

Previous work on UPE showed three time-resolved emission events, which we also observe: 1) The initial delayed luminescence from bringing a sample from the light into the dark chamber is related to not only the size of the plant, but also the development of the leaves, since chlorophyll is such a strong source of DL [24]. By starting from dry seeds both of these aspects are minimised.

2) The drought and drought-relief response [35]. Watering only on day 3 allowed us to observe similar build up and relief emission peaks each time. Adding a weight on top of the petri dish helped combat accelerated loss of water due to plant growth pushing off the lid and allowing evaporation.

3) The linear increase of emission due to increased size of plant: “steady-state emission” [25]. For each experiment we observe a minimum count at all points in time which increases as the plant grows. This can be understood that as the number of cells in the field of view increases and they all have a fixed chance of emitting light during normal metabolism, then we should measure a contribution to total emission which is a direct function of plant size. This was previously shown to be dominated by growth of the primary root tip meristem [26]. Since during these experiments the primary root grows continuously, this contribution would be encompassed by the steady-state emission. Using our methodology, the time-resolved emission data shows these events with the same features in agreement with these previous reports.

In addition to these features, we observe two new features not previously reported. For beans germinated in the dark, we observe a peak in emission at around 5.5 h. This aligns with the period of germination where the seed coat splits allowing the root to emerge. We assign this to the rapid growth of the primary root through the process of cell division at this growth stage contributing more emission [38,38], before it slows to steady state emission levels. The other feature is both broad and sharp peaks after watering on days 3–7. We assign these to the growth of secondary roots, first seen during this time period, causing emission above steady-state due to emergence of several growing root tips in a short amount of time. Our evidence for this is three-fold. First, in the single-bean study the plant which showed an increased emission peak also showed development of secondary roots. Since the detectors only measure from below the stem, the source of the emission most likely originated from the roots. Second, the peaks are seen clearly on the bottom detectors, but not on the top ones. This is because the growing stems and leaves obscure most of the roots from the upper detector, even taking into account the difference in distance from the plants. Third, on addition of plant growth hormone during watering, we see increased emission at the same time as increased secondary roots.

To fully resolve the secondary roots as the source of this emission one would need to add imaging capabilities to the setup. However, now that we can indicate a point in experimental time which shows increased emission without contributions from DL or unusual stress, we can measure this in future studies.

When growing 20 beans in a single petri dish, we observe a similar average growth per dish, however we suspected the environment of each bean is affected by the surrounding plants. Competition for water may cause a distribution of growth rates within the dish. It therefore follows that the emission from secondary roots may be spread out in time as each bean independently grows its multiple secondary roots. By addition of a hormone to initiate the process we hypothesised we would observe a higher and briefer peak as all the seedlings create their roots at once.

The initial choice of H_2O_2 as an additive was informed from several factors. H_2O_2 is part of the auxin hormone pathway and contributes to ROS generation which stimulates growth [37,38]. At low concentrations, it stimulates growth in all forms of life, supporting the redox theory of cellular signalling and control of the cell cycle and it may have even been pivotal at the origins of life [39,40]. Adding H_2O_2 is likely to trigger several different processes at once. At the concentration used in this study (167 μM), it is expected to induce the secondary root growth process, which we verified (Fig. SI-1) [37]. However, it will also directly cause radical generation, cause chemiluminescence from its own degradation and react with numerous other chemicals as a peroxidiser/oxidiser [33]. This is similar to other “induced” biophoton emission in perturbing the biological system to generate photons and obfuscates the role of secondary roots. A previous study which added H_2O_2 to radish cells observed increased emission which they attributed to it generating organic peroxides inside the cell via OH radical

degradation [34]. Mitochondria are implicated here as they both generate and destroy hydrogen peroxide using anti-oxidants as a pivotal role in plant homeostasis and in the response to stress [41–45].

As a control experiment, we added H_2O_2 to dishes containing dead/non-viable, dry mung plants. This showed emission from H_2O_2, at about $1/10^{th}$ the photon emission intensity of equivalent living plants (Fig. 8). Since the metabolic activity is ceased, but likely some of the chemicals involved in peroxide-breakdown would still be present, this could account for the sharp peak and decay of emission, although further studies would be needed for quantitative attribution. We believe this non-biological chemiluminescence may be seen in other studies involving direct addition of H_2O_2 and suggest further thought and care on its use [34,46,47].

We next added to live beans IAA, a hormone which controls cell growth as well as root formation. Although IAA can induce ROS formation, it does so only via enzymes catalysis in the process of initiating cell growth [48]. Addition of IAA showed a relatively small drought relief peak similar to that of water, but then maintained a higher emission above steady-state for a period of 3 days (Fig. 7). The increased emission is further contrasted against the water control as the dish showed a lower drought response peak.

This work aimed to further understand the origins of UPE through development of a repeatable expression of intrinsic emission and inducing the underlying mechanism by targeted hormonal addition. To understand how this reveals the origin of UPE we will briefly discuss the overall findings thus far.

The general mitogenic effect as previously described in the literature showed an increased cell division and growth induced by non-chemical plant communication through quartz [5]. It was established that this is due to UV light emission which has a peak action wavelength below or around 220 nm and is almost completely diminished by 300 nm [49]. Although we have not established the emission wavelength from our current studies, the sensitivity of our detectors falls within this range as well as the visible spectrum. Spontaneous ultra-weak intensity emission from not just plants but yeast, bacteria, humans and other life forms have been previously reported. Although the emission varies slightly between species, in plants the emission attributed to ROS decay, reactant decay and chlorophyll fluorescence in UV-C, visible and NIR wavelengths have been implicated [9]. ROS can be generated by light in the UV to NIR wavelengths [50,51]. It is known that ROS (such as H_2O_2) can help to govern plant growth and increasing ROS with small amounts of auxin hormones increases growth [48]. Furthermore, plants regulate their own growth with antioxidants and that various forms of stress can increase the concentration of ROS and thus induce growth [52]. Whilst radical reactions and decomposition of ROS may lead to light emission from chain reactions which generate further ROS, even multiplying over time if the concentration of peroxides are maintained. It has also previously been shown that the greatest spontaneous emission of UPE in certain plants occurs in the rapidly dividing cells of the apical meristem [13,36].

Therefore, we propose that the part of the general mitogenic effect as described in the literature may be caused by ultraviolet absorption of H_2O_2 leading to homolytic fission. The absorption spectrum of H_2O_2 matches very closely with the action spectrum of UPE (peaking at 190 nm and almost 0 by 300 nm). Large amounts of UV-C would be damaging to the organism. However, since radical formation is a chain-reaction, even a small amount of UV-C light would lead to a significant increase in the rate of ROS production over spontaneous decay. This then leads to an increase in growth of the plant roots before anti-oxidants are produced to lower this rate back to normal steady-state levels. Due to the spherical emission (4π) of the light most of it will be absorbed by the same root, however cells near the surface will also have a portion of light emit externally.

Peroxide and ozone absorption in the atmosphere will mean the amount of solar radiation reaching sea level at wavelengths below 300 nm is relatively small. Soil, a complex mixture of organic and inorganic material, would further attenuate this light dramatically therefore it would have significant effect only on the order of millimetres.

We have shown that an increase in secondary root correlates well with increased photon emission. We therefore speculate the biological advantages of this could be as follows:

- Help roots in close proximity grow away from each other for efficient resource gathering.
- When a root is in drought stress signal to nearby roots to grow lower for water.
- When a root is undergoing disease or poison stress, keep other roots away from the source.

Since our detectors are sensitive over a large range of wavelengths, it is likely the majority of the signal is UV to visible emission from possible ROS decay products or fluorescence induced by this light. With further work, we shall test whether enough overall light is produced during these peaks of emission that enough UV light can be detected: either through the established method using filters, or through a highly-sensitive and high-resolution spectrometer [16,53,54]. Further, by using the same growth regime in this work and pairing up with PMT time-resolution, we hope to capture time-resolved spectroscopy of each emission event measured herein. Finally, replacing the spectrometer with a highly sensitive imaging camera would allow us to spatially resolve the location of emission at each of these events.

Conclusions

We have established a highly sensitive experimental setup using opposing single photon detectors to study spontaneous and induced UPE in a model plant system. Using this method, we have shown that the photon emission from plant roots can be separated or differentially observed away from those potentially produced in the upper structures, such as the leaves. We have shown that the emission correlates with secondary root formation.

The addition of the ROS-generating compound H_2O_2, a key signalling molecule found in life, showed an increase in the emission which also correlated with secondary root formation. Addition of H_2O_2 to dead plants showed a diminished signal of similar characteristics, indicating a large part of the measured signal is from the direct emission of ROS decay products. The use of IAA, a key plant hormone that induced an increase in new secondary roots, also led to increase photon emission, attributed to enzyme-regulated generation of ROS. Short periods of increased emission lasting a few hours were observed in plants which produced secondary roots over the course of the experiment (0-3 days and 3-8 days after imbibition of the seeds).

In this study, we have identified and confirmed characteristics of light emission from mung beans which suggests a potential photonic hormesis system in plants, and possible other life forms (due to the mode of photon production from ROS). Our data can be summarised as: 1) a steady state photonic emission directly related to plant size 2), a temporary increase in emission in relation to dehydration and then rehydration and 3), an increase in photonic emission related to secondary root growth, possibly caused by increased cell division. Our data therefore adds to the investigation of UPE which originates from the studies of the mitogenic effect first observed almost exactly a hundred years ago by Alexander Gurwitsch.

CRediT authorship contribution statement

Alasdair M. Mackenzie: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Holly E. Smith:** Investigation, Formal analysis. **Rhys R. Mould:** Conceptualization, Writing – review & editing. **Jimmy D. Bell:** Conceptualization, Supervision, Funding acquisition. **Alistair V.W. Nunn:**

Conceptualization, Funding acquisition, Writing – review & editing. **Stanley W. Botchway:** Conceptualization, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This project was funded by a grant from the Guy Foundation with facilities provided by Science and Technology Facilities Council. Help on the plant growth was provided by Ellen Clancy and Rosie Sanders. We thank Daniel Robert and Imogen Sparkes for their very helpful discussion about the data and Verena Kriechbaumer for the suggestion of IAA addition. AMM thanks Timur Avni for help with Python and Tony Parker for helping with the writing process.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jpap.2023.100224.

References

[1] E. Smith, H.J. Morowitz, The Origin and Nature of Life on Earth: The Emergence of the Fourth Geosphere, Cambridge University Press, New York, NY, 2016.
[2] A.Y. Mulkidjanian, On the origin of life in the Zinc world: 1. Photosynthesizing, porous edifices built of hydrothermally precipitated zinc sulfide as cradles of life on Earth, Biol. Direct 4 (1) (2009) 26, https://doi.org/10.1186/1745-6150-4-26.
[3] A.Y. Mulkidjanian, M.Y. Galperin, On the origin of life in the Zinc world. 2. Validation of the hypothesis on the photosynthesizing zinc sulfide edifices as cradles of life on Earth, Biol. Direct 4 (1) (2009) 27, https://doi.org/10.1186/1745-6150-4-27.
[4] A.V.W. Nunn, G.W. Guy, J.D. Bell, Bioelectric fields at the beginnings of life, Bioelectr. 4 (4) (2022) 237–247, https://doi.org/10.1089/bioe.2022.0012.
[5] E.v. Naumova, Y.A. Vladimirov, L.v. Beloussov, V.v. Tuchin, I.v. Volodyaev, Methods of studying ultraweak photon emission from biological objects: I. History, types and properties, fundamental and application significance, in: Biophysics (Russian Federation), 66, Pleiades journals, 2021, pp. 764–778, https://doi.org/10.1134/S0006350921050158.
[6] I.v. Volodyaev, L.v. Beloussov, I.I. Kontsevaya, A.E. Naumova, E.v. Naumova, Methods of studying ultraweak photon emissions from biological objects. II. Methods based on biological detection, Biophys. (Russ. Feder.) 66 (6) (2021) 920–949, https://doi.org/10.1134/S000635092106021X.
[7] E.v. Naumova, Y.A. Vladimirov, V.v. Tuchin, V.A. Namiot, I.v. Volodyaev, Methods of studying ultraweak photon emission from biological objects: III. Physical methods, Biophys. (Russ. Feder.) 67 (1) (2022) 27–58, https://doi.org/10.1134/S0006350922010109.
[8] I. Volodyaev, L.v. Beloussov, Revisiting the mitogenetic effect of ultra-weak photon emission, Front. Physiol. 6 (September) (2015) 1–20, https://doi.org/10.3389/fphys.2015.00241.
[9] M. Cifra, P. Pospíšil, Ultra-weak photon emission from biological samples: Definition, mechanisms, properties, detection and applications, J. Photochem. Photobiol. B 139 (2014) 2–10, https://doi.org/10.1016/j.jphotobiol.2014.02.009.
[10] J.-J. Chang, J. Fisch, F.-A Popp, Biophotons, 1st ed., Springer Netherlands, 1998 https://doi.org/10.1007/978-94-017-0928-6.
[11] C. De Mello Gallep, Ultraweak, spontaneous photon emission in seedlings: toxicological and chronobiological applications, Luminescence 29 (8) (2014) 963–968, https://doi.org/10.1002/bio.2658.
[12] M. Havaux, C. Triantaphylidès, B. Genty, Autoluminescence imaging: a non-invasive tool for mapping oxidative stress, Trend. Plant. Sci. 11 (10) (2006) 480–484, https://doi.org/10.1016/j.tplants.2006.08.001.
[13] S. Kai, T.O.K. Moriya, T. Fujimoto, Growth control and biophoton radiation by plant hormones in red bean, Jpn. J. Appl. Phys. 34 (Part 1, No. 12A) (1995) 6530–6538, https://doi.org/10.1143/JJAP.34.6530.
[14] K. Tsuchida, M. Kobayashi, Oxidative stress in human facial skin observed by ultraweak photon emission imaging and its correlation with biophysical properties of skin, Sci. Rep. 10 (1) (2020) 9626, https://doi.org/10.1038/s41598-020-66723-1.
[15] A. Prasad, P. Pospíšil, Towards the two-dimensional imaging of spontaneous ultra-weak photon emission from microbial, plant and animal cells, Sci. Rep. 3 (1) (2013) 1211, https://doi.org/10.1038/srep01211.
[16] R.N. Tilbury, T.I. Quickenden, Luminescence from the yeast Candida utilis and comparisons across three genera, J. Biolumin. Chemilumin. 7 (4) (1992) 245–253, https://doi.org/10.1002/bio.1170070404.
[17] È. Hideg, M. Kobayashi, H. Inaba, Spontaneous ultraweak light emission from respiring spinach leaf mitochondria, BBA - Bioenerget. 1098 (1) (1991) 27–31, https://doi.org/10.1016/0005-2728(91)90005-9.
[18] M. Flor-Henry, T.C. McCabe, G.L. de Bruxelles, M.R. Roberts, Use of a highly sensitive two-dimensional luminescence imaging system to monitor endogenous bioluminescence in plant leaves, BMC Plant Biol. 4 (2004), https://doi.org/10.1186/1471-2229-4-19.
[19] C.L. Oros, Biophoton Emission From Plants: A Contribution to the Understanding of Plant Signaling, Naval Postgraduate School, Monterey, California, 2018.
[20] A. Prasad, P. Gouripeddi, H.R.N. Devireddy, A. Ovsii, D.P. Rachakonda, R. van Wijk, P. Pospíšil, Spectral distribution of ultra-weak photon emission as a response to wounding in plants: An in vivo study, Biology 9 (6) (2020) 1–14, https://doi.org/10.3390/biology9060139.
[21] M. Havaux, B. Ksas, Imaging of lipid peroxidation-associated chemiluminescence in plants: spectral features, regulation and origin of the signal in leaves and roots, Antioxidants 11 (7) (2022), https://doi.org/10.3390/antiox11071333.
[22] K. Creath, A look at some systemic properties of self-bioluminescent emission, Nat. Light: Light Nat. II 7057 (2008), 705708, https://doi.org/10.1117/12.800667.
[23] H11870-01 Photon counting head manual, Hamamatsu Photonics, 2014.
[24] J.M. Ortega, M. Roncel, The Afterglow Photosynthetic Luminescence, Physiol. Plant. 171 (2) (2021) 268–276, https://doi.org/10.1111/ppl.13288.
[25] C. Gallep, M. de, D. Robert, Time-resolved ultra-weak photon emission as germination performance indicator in single seedlings, J. Photochem. Photobiol. 1 (July) (2020), 100001, https://doi.org/10.1016/j.jpap.2020.100001.
[26] S. Kai, T. Mitani, M. Fujikawa, Morphogenesis and bioluminescence in germination of red bean, Physica A 210 (3–4) (1994) 391–402, https://doi.org/10.1016/0378-4371(94)90087-6.
[27] W.L. Chen, D. Xing, S.C. Tan, Y.H. Tang, Y. He, Imaging of ultra-weak bio-chemiluminescence and singlet oxygen generation in germinating soybean in response to wounding, Luminescence 18 (1) (2003) 37–41, https://doi.org/10.1002/bio.703.
[28] T. Ohya, S. Yoshida, R. Kawabata, H. Okabe, S. Kai, Biophoton emission due to drought injury in red beans: possibility of early detection of drought injury, Jpn. J. Appl. Phys. 41 (1) (2002) 4766–4771, https://doi.org/10.1143/JJAP.41.4766. PartNo. 7A.
[29] O.K. Atkin, D. Macherel, The crucial role of plant mitochondria in orchestrating drought tolerance, Ann. Bot. 103 (4) (2009) 581–597, https://doi.org/10.1093/aob/mcn094.
[30] N. Rafieiolhosseini, M. Poplová, P. Sasanpour, H. Rafii-Tabar, M.R. Alhossaini, M. Cifra, Photocount statistics of ultra-weak photon emission from germinating mung bean, J. Photochem. Photobiol. B 162 (2016) 50–55, https://doi.org/10.1016/j.jphotobiol.2016.06.001.
[31] M. Dlask, J. Kukal, M. Poplová, P. Sovka, M. Cifra, Short-time fractal analysis of biological autoluminescence, Plos One 14 (7) (2019), e0214427, https://doi.org/10.1371/journal.pone.0214427. Edited by M.A. Sánchez Granero.
[32] J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D.J. White, V. Hartenstein, K. Eliceiri, P. Tomancak, A. Cardona, Fiji: an open-source platform for biological-image analysis, Nat. Method. 9 (7) (2012) 676–682, https://doi.org/10.1038/nmeth.2019.
[33] A.A. Gurwitsch, V.F. Eremeyev, Y.A. Karabchievsky, Ultra-weak emission in the visible and ultra-violet regions in oxidation of solutions of glycine by hydrogen peroxide, Nature 206 (4979) (1965) 20–22, https://doi.org/10.1038/206020b0.
[34] A. Rastogi, P. Pospíšil, Effect of exogenous hydrogen peroxide on biophoton emission from radish root cells, Plant Physiol. Biochem. 48 (2–3) (2010) 117–123, https://doi.org/10.1016/j.plaphy.2009.12.011.
[35] S. Komatsu, A.H.M. Kamal, T. Makino, Z. Hossain, Ultraweak photon emission and proteomics analyses in soybean under abiotic stress, in: Biochimica et Biophysica Acta - Proteins and Proteomics, 1844, Elsevier, 2014, pp. 1208–1218, https://doi.org/10.1016/j.bbapap.2014.04.002.
[36] T.O. Tomoyuki Ohya, H.K. Hideaki Kurashige, H.O. Hirotaka Okabe, S.K. Shoichi Kai, Early detection of salt stress damage by biophotons in red bean seedling, Jpn. J. Appl. Phys. 39 (6R) (2000) 3696, https://doi.org/10.1143/JJAP.39.3696.
[37] S.W. Li, L. Xue, S. Xu, H. Feng, L. An, Hydrogen peroxide acts as a signal molecule in the adventitious root formation of mung bean seedlings, Environ. Exp. Bot. 65 (1) (2009) 63–71, https://doi.org/10.1016/j.envexpbot.2008.06.004.
[38] V.D. Petrov, F. Van Breusegem, Hydrogen peroxide—a central hub for information flow in plant cells, AoB PLANTS 2012 (2012), https://doi.org/10.1093/aobpla/pls014.
[39] S.G. Menon, P.C. Goswami, A redox cycle within the cell cycle: ring in the old with the new, Oncogene 26 (8) (2007) 1101–1109, https://doi.org/10.1038/sj.onc.1209895.
[40] R. Ball, J. Brindley, Toy trains, loaded dice and the origin of life: dimerization on mineral surfaces under periodic drive with Gaussian inputs, R. Soc. Open Sci. 4 (11) (2017), 170141, https://doi.org/10.1098/rsos.170141.
[41] D. Munro, J.R. Treberg, A radical shift in perspective: mitochondria as regulators of reactive oxygen species, J. Exp. Biol. 220 (7) (2017) 1170–1180, https://doi.org/10.1242/jeb.132142.

[42] C. Kamunde, M. Sharaf, N. MacDonald, H2O2 metabolism in liver and heart mitochondria: Low emitting-high scavenging and high emitting-low scavenging systems, Free Rad. Biol. Med. 124 (2018) 135–148, https://doi.org/10.1016/j.freeradbiomed.2018.05.064.
[43] M.J.C.D.H. Millar, Unraveling the role of mitochondria during oxidative stress in plants, IUBMB Life (Int. Union Biochem. Molecul. Biol.: Life) 51 (4) (2001) 201–205, https://doi.org/10.1080/152165401753311735.
[44] S. Saroussi, D.A.J. Karns, D.C. Thomas, C. Bloszies, O. Fiehn, M.C. Posewitz, A. R. Grossman, Alternative outlets for sustaining photosynthetic electron transport during dark-to-light transitions, Proc. Natl Acad. Sci. 116 (23) (2019) 11518–11527, https://doi.org/10.1073/pnas.1903185116.
[45] X. Pu, X. Lv, T. Tan, F. Fu, G. Qin, H. Lin, Roles of mitochondrial energy dissipation systems in plant development and acclimation to stress, Ann. Bot. 116 (4) (2015) 583–600, https://doi.org/10.1093/aob/mcv063.
[46] A. Prasad, P. Pospišil, Two-dimensional imaging of spontaneous ultra-weak photon emission from the human skin: role of reactive oxygen species, J. Biophoton. 4 (11–12) (2011) 840–849, https://doi.org/10.1002/jbio.201100073.
[47] M. Poplová, A. Prasad, E. Van Wijk, P. Pospíšil, M. Cifra, Biological auto(chemi) luminescence imaging of oxidative processes in human skin, Anal. Chem. 95 (40) (2023) 14853–14860, https://doi.org/10.1021/acs.analchem.3c01566.
[48] T. Kawano, Roles of the reactive oxygen species-generating peroxidase reactions in plant defense and growth induction, in: Plant Cell Reports, 21, Springer Verlag, 2003, pp. 829–837, https://doi.org/10.1007/s00299-003-0591-z.
[49] Y.A. Vladimirov, Ultraweak Luminescence Accompanying Biochemical Reactions, NASA, C.F.S.T.I., Springfield, Vermont, 1967.
[50] S.W. Botchway, A.G. Crisostomo, A.W. Parker, R.H. Bisby, Near infrared multiphoton-induced generation and detection of hydroxyl radicals in a biochemical system, Arch. Biochem. Biophys. 464 (2) (2007) 314–321, https://doi.org/10.1016/j.abb.2007.04.026.
[51] S.W. Botchway, S. Chakrabarti, G.M. Makrigiorgos, Novel visible and ultraviolet light photogeneration of hydroxyl radicals by 2-methyl-4-nitro-quinoline-N-oxide (MNO) and 4, 4'-dinitro-(2, 2') bipyridinyl-N, N'-dioxide (DBD), Photochem. Photobiol. 67 (6) (1998) 635–640, https://doi.org/10.1111/j.1751-1097.1998.tb09105.x.
[52] H. Tsukagoshi, Control of root growth and development by reactive oxygen species, Curr. Opin. Plant Biol. 29 (2016) 57–63, https://doi.org/10.1016/j.pbi.2015.10.012.
[53] B.T. Dotta, N.J. Murugan, L.M. Karbowski, R.M. Lafrenie, M.A. Persinger, Shifting wavelengths of ultraweak photon emissions from dying melanoma cells: Their chemical enhancement and blocking are predicted by Cosic's theory of resonant recognition model for macromolecules, Naturwissenschaften 101 (2) (2014) 87–94, https://doi.org/10.1007/s00114-013-1133-3.
[54] H. Iyozumi, K. Kato, T. Makino, Spectral shift of ultraweak photon emission from sweet potato during a defense response, Photochem. Photobiol. 75 (3) (2007) 322–325, https://doi.org/10.1562/0031-8655(2002)0750322SSOUPE2.0.CO2.