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Application of ultra-weak photon emission measurements in agriculture

Kimihiko Kato [a], Hiroyuki Iyozumi [a,*], Chizuko Kageyama [a], Hidehiro Inagaki [a,b], Akira Yamaguchi [a], Hideki Nukui [a]

[a] Shizuoka Prefectural Research Institute of Agriculture and Forestry, 678-1 Tomigaoka, Iwata, Shizuoka 438-0803, Japan
[b] Faculty of Agriculture, Shizuoka University, 63 Kariyado, Fujieda, Shizuoka 426-0001, Japan

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ABSTRACT

Here we report our two applications of ultra-weak photon emission (UPE) measurements in agriculture. One is to find new types of agrochemicals that potentiate plants' defense, so-called "plant activator". We first analyzed the relation between plant defense and Elicitor-Responsive Photon Emission (ERPE) using a combination of rice cells and a chitin elicitor. Pharmacological analyses clarified that ERPE was generated as a part of the chitin elicitor-responsive defense in close relation with the generation of reactive oxygen species (ROS). Then we successfully detected the activity of plant activators as the potentiation of ERPE, and developed a new screening system for plant activators based on this principle. Another UPE application is to distinguish herbicide-resistant weeds from susceptible ones by measuring UPE in weeds. In our study, it was revealed that the weed biotypes resistant to sulfonylurea (SU) herbicides, one of the major herbicide groups, showed stronger UPE than susceptible ones after an SU herbicide treatment. By further analysis with a pharmacological and RNAi study, we found that the detoxifying enzyme P450s contributed to the UPE increase in SU herbicide resistant weeds. It is considered that weeds resistant to herbicides other than SU might also be able to be distinguished from susceptible ones by UPE measurement, as long as the herbicides are subject to detoxification by P450s.

1. Introduction: ultra-weak photon emission in plants responding to stresses

From the first report of ultra-weak photon emission (UPE) measurements by Coli in 1954, there have been a lot of evidence that plants change the intensity of UPE depending on changes in their condition and circumstances [1–16]. From the facts that UPEs have been observed in intact cells and plants have evolved the mechanisms to survive a variety of threats in nature, it has been expected that the changes in UPE in plants would include "the responses" to threats. From 1990s, we have been studying "how to apply UPE measurements in agriculture", and have developed techniques utilizing UPE to measure noninvasively the states of plants in response to stresses. Until now, we have found new UPE phenomena in plants responding to fungal pathogen [17,18], nematode [19], herbivore [20], plant hormones [18], temperature changes [18], and herbicide treatments [21–23]. For example, in sweet potato root disk-fungus interaction, the transient increase and dynamic spectral shift of UPE were observed after fungus infection [17,18]. The increase of UPE was observed at the site of fungus inoculation, and the fungal growth was limited in the same area on the root disk (Fig. 1a) [17]. The rise of photon counts was apparent about 6–20 h after inoculation, when most of the fungal conidia germinated (Fig. 1b) [18]. The spectral shift of UPE to a shorter range occurred 2–10 h after inoculation and was maintained for more than a day (Fig. 1c) [18]. These phenomena were thought to be associated with defense responses including lipoxygenase activities leading to the anti-fungal phytoalexin synthesis and cell death (Fig. 1) [17,18,24].

Among these findings, we focused our research resources on two big threats to global food security; plant disease and herbicide-resistant weeds. Here we report our two applications of UPE measurements in agriculture. One is to find new types of agrochemicals that potentiate plants' defense activity, based on the measurement of UPE in plants responding to pathogen infection; another is to distinguish herbicide-resistant weeds from susceptible ones by measuring UPE in the detoxification of herbicides by weeds.

2. Application of UPE measurement to screen chemicals for plant defense activation

A new type of agrochemicals, called plant activators, has been developed and used for disease control recently [25,26]. These

* Corresponding author. Tel.: +81 538357211; fax: +81 538378466.
E-mail address: hiroyuki1_iyozumi@pref.shizuoka.lg.jp (H. Iyozumi).

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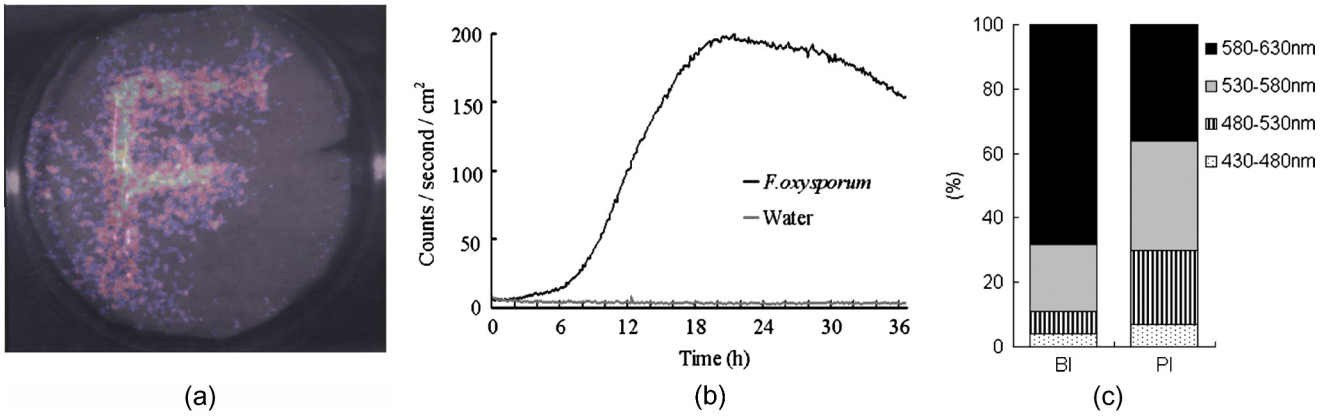

Fig. 1. Characteristics of UPE from storage root disk of sweet potato responding to fungus (*Fusarium oxysporum*). (a) Imaging of UPE from sweet potato–Fusarium interaction. Photon counts were accumulated using an ARGUS-50 (Hamamatsu Photonics, Japan) for 0.5 h starting from 12 h after inoculation. (b) Increase of UPE in sweet potato disk inoculated with conidia of *F. oxysporum* at 0 h in figure. UPE was measured using the photon counting system MSPCII (Hamamatsu Photonics, Japan). (c) Spectral shift in UPE between before inoculation (BI) and 12 h after inoculation (PI). The spectral composition of BI was equal to that in a water-treated disk [18].

plant activators can help plants overcome pathogen attacks by potentiating plant defense responses just after the recognition of pathogens [27]. So far the development of plant activators had been restricted because the lack of high throughput and a less-expensive screening method. We have successfully detected the activity of plant activators by observing the potentiation of Elicitor-Responsive Photon Emission (ERPE). Based on this principle, a plant activator screening system with high throughput and lower costs was developed.

2.1. Elicitor-responsive photon emission in plants

Plant defense responses are triggered by microbes' cell components (Microbe-Associated Molecular Patterns: MAMPs) [28,29], pathogens' secreted proteins (Effectors) [29,30], inorganic substances [30,31] or ultraviolet radiation [32], these are generally called "Elicitors" [33]. We found UPEs in leaf segments and cultured cells of rice treated with microbial elicitors (MAMPs) [34,35] and inorganic substances such as dipotassium hydrogen phosphate or copper chloride [36], and named them Elicitor-Responsive Photon Emissions (ERPEs). The UPEs in plants induced by pathogen derived molecules have also been noted in other reports [14,15], and ERPEs are thought to be common in plant–pathogen interactions.

To investigate further, we used a combination of rice cells and a chitin elicitor (Fig. 2) as a model for analyzing the relation between plant defense and ERPE [35]. Suspension cultured rice cells have commonly been used to analyze cellular elicitor responses because of their uniformity and ease of sample preparation [37–39]. Meanwhile, chitin oligomers are best characterized as MAMP of fungi, the massive majority in plant pathogens [40]. The signaling cascade of the chitin-triggered defense has been elucidated in rice, from the reception of chitin by a receptor until the activation of defense responses [41,42]. Our pharmacological analyses clarified that chitin ERPE was generated through the signaling cascade of chitin induced defense in the downstream of phosphatidic acid (PA), the messenger molecule leading to the generation of reactive oxygen species (ROS) (Fig. 3) [35]. In addition, we found that the pattern of chitin ERPE was almost identical to that of chitin induced hydrogen peroxide generation, suggesting that ERPE is closely associated with the ROS generating system used in defense [43,44].

Recently, another type of ERPEs that are triggered by effector proteins has been reported [45]. These ERPEs were not affected by ROS generation inhibitors, but by nitric oxide synthase inhibitors [45]. Different from the chitin triggered defense, the effector-triggered defense leads to a hypersensitive reaction (or programmed cell death) in plants. The difference between their signaling cascades is thought to be reflected in the sources of the ERPEs [35,45].

2.2. A new evaluation method for plant activators based on potentiation of ERPE

The induced defense of plants to pathogenic organisms has been of interest in the study of crop protection. Especially in the last few decades, the induction of defense in plants as a practical method of crop protection has involved the investigation of several chemicals that induce systemic defense in plants, so-called "plant activators" [25,26]. Rapid and enhanced defense responses to pathogens/elicitors are characteristic cellular events in plants treated with plant activators. This phenomenon has been called "priming" [27,46]. The priming of several defense responses has been reported, including phytoalexin accumulation [47,48], defense-related gene/protein accumulation [47–51], and ROS generation [52,53]. Priming phenomena should be useful markers of defense activation in plant cells [27,46].

Based on the studies of ERPE, we proposed a pilot system for the screening of plant defense activators (Fig. 4) [34]. It takes less than half a day to judge the plant activator ability of a tested compound. It is quite shorter than conventional pathogen inoculation-based tests (1–2 weeks).

Plant hormones, such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are known to be key substances of systemic disease resistance in plants [54–56]. Plants employ these hormones differently to different kinds of threats. SA is used for biotrophic pathogens, JA and ET are used for necrotrophic pathogens and herbivores [54–56]. We tested such conflicting hormones to evaluate their priming effect using the pilot system with a combination of rice cells and a chitin elicitor. Interestingly, our pilot system could detect the priming effect of all three hormones against chitin induced ERPE (Fig. 5). Commercially available plant activators Probenazole and Acibenzolar-S-methyl (syn. benzothiadiazole), Thiadinil, the functional agonists of SA, and Carpropamid, the functional agonist of JA also potentiate chitin induced ERPE [36]. These results indicated that our screening system is capable of screening for several different and major groups of plant activating compounds.

We have screened about ten thousand compounds for plant defense activation by this method in collaboration with a chemical