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Functional imaging of biophoton responses of plants to fungal infection

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Abstract Each living cell of a plant produces photons in certain conditions. Under normal physiological conditions, cell photon emission is stationary and minimal. Disturbance in the oxidative homeostasis by biotic stress is manifested by increased 'biophoton' production. Such biophoton responses of plants may be used as an integral indicator of the degree of oxidative homeostasis misbalance. Our results demonstrate that biophoton generation has been much higher in a resistant potato variety than in a susceptible one till 10 h after *Phytophthora infestans* inoculation. In contrast, ultra-weak luminescence from detached susceptible potato and moderately resistant pelargonium leaves increased from 1–4 to 4–5 days after inoculation with *Phytophthora infestans* or *Botrytis cinerea*, respectively. Pre-treatment of susceptible potato leaves with a defence inducer, arachidonic acid, resulted in a transient burst of light in response to *P. infestans* lasting for 30–45 h post inoculation (hpi). This study presents the potential adaptation of functional imaging of ultra-weak luminescence to monitor time-dependent free radical processes during disease development and its application to draw conclusions on plant resistance to pathogens of different lifestyle. Moreover, it has been shown that imaging of temporal biophoton generation from potato leaves treated with arachidonic acid might be a helpful marker in mapping oxidative changes leading to systemic acquired resistance (SAR).

Keywords Defence response · Plant-pathogen interaction · Spontaneous biophoton emission

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Introduction

Plant cells, after receiving and synchronizing signals originating from a pathogen, the environment and the plant itself, are capable of activating signalling pathways, which through induced events may lead to the state of defence potentiation. A key role in plant response to pathogen attack is played by mechanisms of intracellular regulation of redox metabolism. Each stress, depending on the force of its action, modifies or causes a significant misbalance in oxidative cell homeostasis, which leads to

the stimulation of signalling pathways (Grant and Loake 2000; Arasimowicz and Floryszak-Wieczorek 2007; Miller et al. 2008), the activation of redox-sensitive transcription factors, the expression of genes responsible for pathogenesis related proteins (PR) synthesis (Gòmez-Lim 2002; Apel and Hirt 2004; Zago et al. 2006), and phytoalexin accumulation or elicitation of hypersensitive response (HR) cell death (Overmyer et al. 2003; Zhao et al. 2005). These and other defence responses against pathogen invasion activated by the plant on molecular or structural levels (reorganization of the cellular wall, formation of papillae, apposition of callose, etc.) usually require an effective reaction of the host plant (Schulze-Lefert 2004). Promptness and speed of activated responses determine the success of plant defence or pathogen offence strategies. For this reason, in the case of biotic stresses, an early generation of reactive oxygen forms is usually observed (especially superoxide anion radicals and hydrogen peroxide), as well as generation of nitric oxide (Garcia-Brugger et al. 2006). The quantitative and qualitative nature of this overproduction seems to be dependent on the genetic make-up of the host plant and on the biology of the pathogen (e.g. it being a biotroph or necrotroph) (Glazebrook 2005; Mur et al. 2006; Spoel et al. 2007). In order to gain insight into post-stress regulation mechanisms of the intracellular redox potential connected with defence responses of the plant, attempts should be made to maintain natural intracellular parameters instead of using drastic and invasive free radical detection methods. On the other hand, the immense diversity of data obtained so far by research makes it necessary to find convenient methods for their ordering and presentation. Since humans are physiologically prepared to perceive and process information contained in images, hence—both in the past (picture writing) and at present—techniques based on these forms of data transmission (video, confocal and fluorescence microscopy, images obtained using equipment with optoelectric imaging detectors) have been of utmost significance.

Plants, similarly to animals, emit photons spontaneously without any external excitation by light or other chemical reagents as part of their vital activities (Abeles 1986). Biophoton generation is thought to represent spontaneous photon emission produced by way of chemical excitation arising from various metabolic processes, particularly from lipid peroxidation (Havaux 2003). These spontaneous ultra-weak bioluminescence signals can be strongly enhanced in plant tissue infected with a pathogen and provide an informative view of both oxidative stress signalling and oxidative stress damage (Arnhold 2001; Havaux et al. 2006). Because luminescence signals can be measured and visualised using a highly sensitive charge-coupled device (CCD) camera, this non-destructive technique could be a useful tool in phytopathological studies on plants.

The application of such devices to monitor plant defence responses at various stages of disease development by real-time imaging of free radical processes, measured by single-photon counting, has been presented and discussed here based on two plant-pathogen systems; i.e. potato (*Solanum tuberosum* L.) and a hemibiotrophic oomycete *Phytophthora infestans,* as well as ivy-leaved pelargonium (*Pelargonium peltatum* L.) and a necrotrophic pathogen *Botrytis cinerea*. Moreover, it has been demonstrated that imaging of temporal biophoton signals elicited by arachidonic acid might be a useful marker for visualizing oxidative changes leading to acquired resistance of potato leaves to *P. infestans.*

Materials and methods

Sterile potato plants (*Solanum tuberosum* L.) of cv. Bzura—highly resistant (HR-type) and of cv. Bintje—susceptible (lacking R genes) to *Phytophthora infestans*, derived from in vitro tissue culture were transferred to soil and they were grown in a phytochamber with 16 h of light (180 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 20±2°C and 60% humidity for 4 weeks.

The other experiments were conducted on ivy-leaved pelargonium (*Pelargonium peltatum* L.) cv. 'Cascade'®—moderately resistant to *Botrytis cinerea* Pers., bred by Fisher GmbH & Co. KG Company. Cultivar 'Cascade' originates from a very old cultivar 'Ville de Paris' (*P. hederaefolium*), is a diploid, propagated vegetatively and exhibits genetic stability. Plants were placed in a phytochamber with a constant air temperature of 21±2°C at light intensity of 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (fluorescent lamps, cool-white type—TLD 36/64 Philips) with a 12 h photoperiod. Analyses were performed on plants which developed 10 leaves.

Pathogen culture

Botrytis cinerea Pers Isolate 1072 was cultured in the dark at a temperature of 23±2°C, on a potato-agar medium with a 2% addition of glucose, pH 6.3. Cultures were restored monthly by mycelium passage onto fresh medium. Conidial spore suspensions of *B. cinerea* were produced as described by Floryszak-Wieczorek et al. (2007).

Phytophthora infestans An isolate with virulence of 1,3,4,7,10,11, was kindly supplied by Dr Renata Lebecka, Plant Breeding and Acclimatization Institute (IHAR), Research Division at Młochów,. The fungus was grown on a cereal-potato medium with an addition of dextrose. Zoospore suspensions of *P. infestans* were prepared exactly as described by Floryszak-Wieczorek (2000).

Method of inoculation

For *P. infestans* infections, detached compound leaves of both cultivars were inoculated by spraying with zoospores suspended in water (conc. 1.0×10^5 per ml) on the abaxial leaf surface and kept at 100% humidity in a growth chamber. To assess the influence of an SAR inducer on bioluminescence of potato leaves, a solution of arachidonic acid (1.5 mg per ml) was used, as prepared according to Cohen et al. (1991). The detached leaves were first placed on nutrient solidified medium in Petri dishes, and were then sprayed with arachidonic acid onto the adaxial side at 24 h before inoculation with *P. infestans* and finally put into the measuring chamber.

For *B. cinerea* inoculation, three drops (40 μl) of conidial suspension (conc. 7.5×10^5 per ml) were transferred onto the upper surface of detached pelargonium leaves, placed on solidified agar and subsequently put into the measuring chamber.

Equipment for imaging luminescence

A CCD "Night Owl" camera of a Molecular Light Imager LB 981 by EG & G Berthold (Germany) was used for imaging of enhanced luminescence without any external excitation by chemicals or light. The device consisted of a light-sensitive dark box measuring chamber, inside which a CCD slow scan camera was mounted. The size of the observed area containing a sample with ^{63}Ni-porcelain—radioluminescence standard was regulated by shifting the camera (up-down) by a camera lift. The applied camera consisted of an air cooler Peltier of a converter imaging CCD chip. The converter temperature was 200 K. Optical system retting was eliminated by the separation of the cold part from the part at room temperature by a vacuum–quartz gate. The image of the observed object was projected onto the CCD using a quartz lens. The camera was equipped with an automatic focus system. The image from the converter was directed by an imaging wire and an imaging interface to an IBM PC computer. The entire device was controlled by a camera stacking wire (RS 232 J). All required procedures of regulation, control, measurement and analysis of the obtained images were carried out by the "WinLight" software supplied by the producer.

The 'Night Owl' camera was equipped with a slow scan light-sensitive CCD matrix, lighted from the back, with the resolving power of 512×512 points, sensitive to luminous radiation within the range of 200–1,100 nm. The obtained image was generated through the accumulation of a charge resulting from the action of photons with the matrix material. The method of image generation, adopted here, is called single photon counting imaging and makes it possible to record emissions of very low intensity (for methodology details see Górski et al. 2003).

Image acquisition and processing

The first stage of the applied procedure was to record images (photo) of a leaf under illumination in the growth chamber. Using this photograph, regions of interest (ROI) were defined, corresponding to individual leaves and a mask was generated, eliminating from the image the background area of the measuring chamber bottom. The second stage was to record a series of luminescent images after turning off the lights of the measuring chamber. The time of recording a single image was 30 min. The obtained images were undecipherable by the human eye, which does not distinguish between similar shades of black. A sufficient diversification of grey in the image was obtained by multiplying the luminescent image by 4000 (I*4000=>LUMI). Further improvement of image legibility was obtained with the application of the pseudocolour scale. Black (no emission) corresponds

to the left end of the applied scale. The right end corresponds to the lightest grey observed in the LUMI image (the highest emission). For the whole experiment one pseudocolour scale was adopted. For the purpose of presentation, on such colour images of the pseudocolour image (PCI) a mask was placed (PCI & MASK). In the next stage from the PCI & MASK images, an analyzed region (AR) was selected, corresponding to a single leaf, and the areas outside were eliminated (clear AR).

In order to read photometric data from luminescent images, another procedure was adopted. A MASK was placed on image (I) and regions of interest ROI. From such prepared images the clear AR was generated, omitting the stage of multiplication and pseudocolourization. Photometric data for leaves were referred to the emission of radioluminescence standard (RLS) and camera background (CBG).

Results

Mapping of biophoton emission from the plant-necrotroph system

Ultraweak bioluminescence triggered by *Botrytis cinerea*, (causative agent of grey mould), was measured after point-inoculation of leaves of pelargonium (*Pelargonium peltatum)* cv. 'Cascade'. Detached leaves were inoculated under green light and then put into dark conditions. Biophoton generation in leaves was measured from approx. 30 min after leaves were moved to the dark to allow for light-induced luminescence to cease. Mesophyll tissues containing chlorophyll emit luminescence for several minutes after moving from light to dark conditions. Leaves inoculated at three points with fungal spores caused an increase in photon emission due to the necrotrophic fungus (Fig. 1). The applied in vivo luminescence imaging technique made it possible to obtain the image of photon emission (2-D) of the leaf and an appropriately adjusted acquisition time image made it possible to create a library of images with examples presented in Fig. 1.

Around each of the three sites where fungal spores were placed on the leaf, a ring emitting photons was formed, starting from 3 to 4 days post inoculation (dpi). Luminescence surrounding the disease spots increased in size along with the extension of the area of grey mould spots (Fig. 1). The intensity of photon signals showed an upward trend until 5 dpi. Then the intensity of ultra-weak luminescence became weaker, which was connected with necrosis. Although initially an enhanced light-emitting ring of photons concomitant with disease development was observed, finally pelargonium leaves were able to display a general resistance response to *Botrytis* correlated with quenching of bioluminescence.

At the same time it was found that only the living inoculated leaf tissue exhibited an increased spontaneous luminescence emission. Neither the necrotic tissue of the host plant nor hyphae, sclerotia and spores of *B. cinerea* developing on various media showed such an enhanced ultra-weak photon production (data not shown).

Measurement and imaging of biophoton generation from a plant-hemibiotroph system

The single photon counting approach revealed pathogen-induced rapid biophoton responses, occurring approximately 30 min after potato leaves were challenged with *P. infestans*. Biophoton generation was much higher in a resistant potato cultivar than in a susceptible one, it even predominated over the Ni marker signal. It lasted for 10–12 h and then gradually decreased. In contrast to the transient light signal of the resistant cv., luminescence from susceptible potato leaves was at the beginning (0–10 h post inoculation, hpi; Fig. 2a) much weaker, but in the following hours and days (Fig. 2b), it increased consistently to a peak between 72 and 96 hpi. Imaging of biophoton emission using the CCD camera showed an enhanced transfer of light signal, where intensity was clearly associated with disease development and degradation of susceptible leaf tissue. Biophoton emission presented in Fig. 2b probably arose from metabolic disturbance, e.g. lipid peroxidation, a major consequence of oxidative stress and susceptible tissue damage caused by *P. infestans*. Utra-weak photon production from uninoculated leaves and subjected to senescence of both cultivars was very low and constant (data not shown).

Imaging of spontaneous photon emission induced by arachidonic acid

Treatment of susceptible potato leaves with arachidonic acid—an effective inducer of SAR, showed a transient

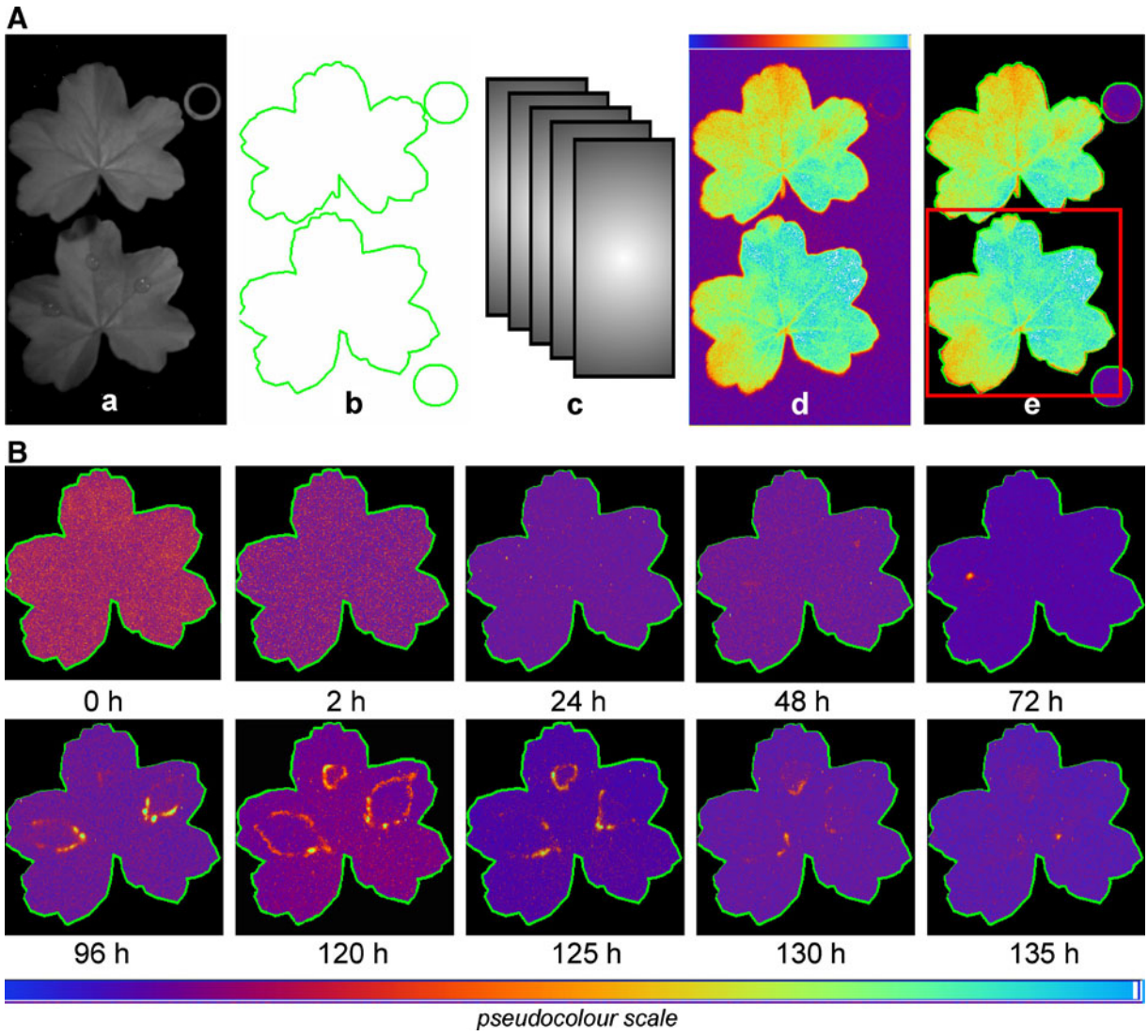
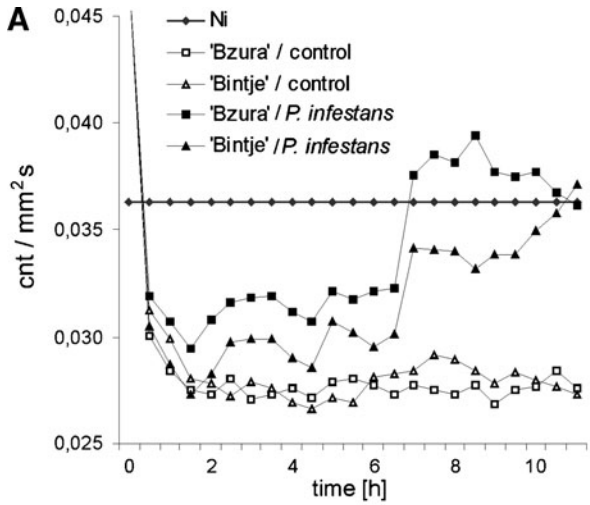


Fig. 1 Biophoton emission following challenge of pelargonium leaves with *Botrytis cinerea*. **a** The first panel provides a conventional photo of two pelargonium leaves; *a*, upper line shows a uninoculated detached leaf, lower line shows a *B. cinerea* point-inoculated leaf—3 drops (40 μl) of fungal suspension approx. $7.5 \cdot 10^5$ spores per ml on each leaf; *b–e*, using this photograph of infected leaf a mask was generated and a region of interest was defined (for more details see "Materials and Methods"). **b** The following sequence illustrates changes in biophoton emission intensity from analysed regions of infected leaves from 0 to 135 h post-inoculation (hpi). It presents only selected examples. A series of 300 images reflecting luminescence changes of a single leaf were recorded during over 5 days (135 hpi). Acquisition time of a single image was 5 min. during the first day, and 30 min. at later dates after inoculation

burst of light in response to *P. infestans* (Fig. 3). This single elevation of photon emission began around 30 h after inoculation, lasted for a subsequent 15 h and finally disappeared after 45 hpi. The infected leaves that were not induced with arachidonic acid, produced a slightly higher baseline light emission compared to both of the uninoculated leaves.

Discussion

Each living cell of a plant or an animal body constantly produces photons, similarly to the plasmalemma producing a potential gradient on its surface. Biophotons are generated by a live cell when its molecules in an electronically excited state decay to a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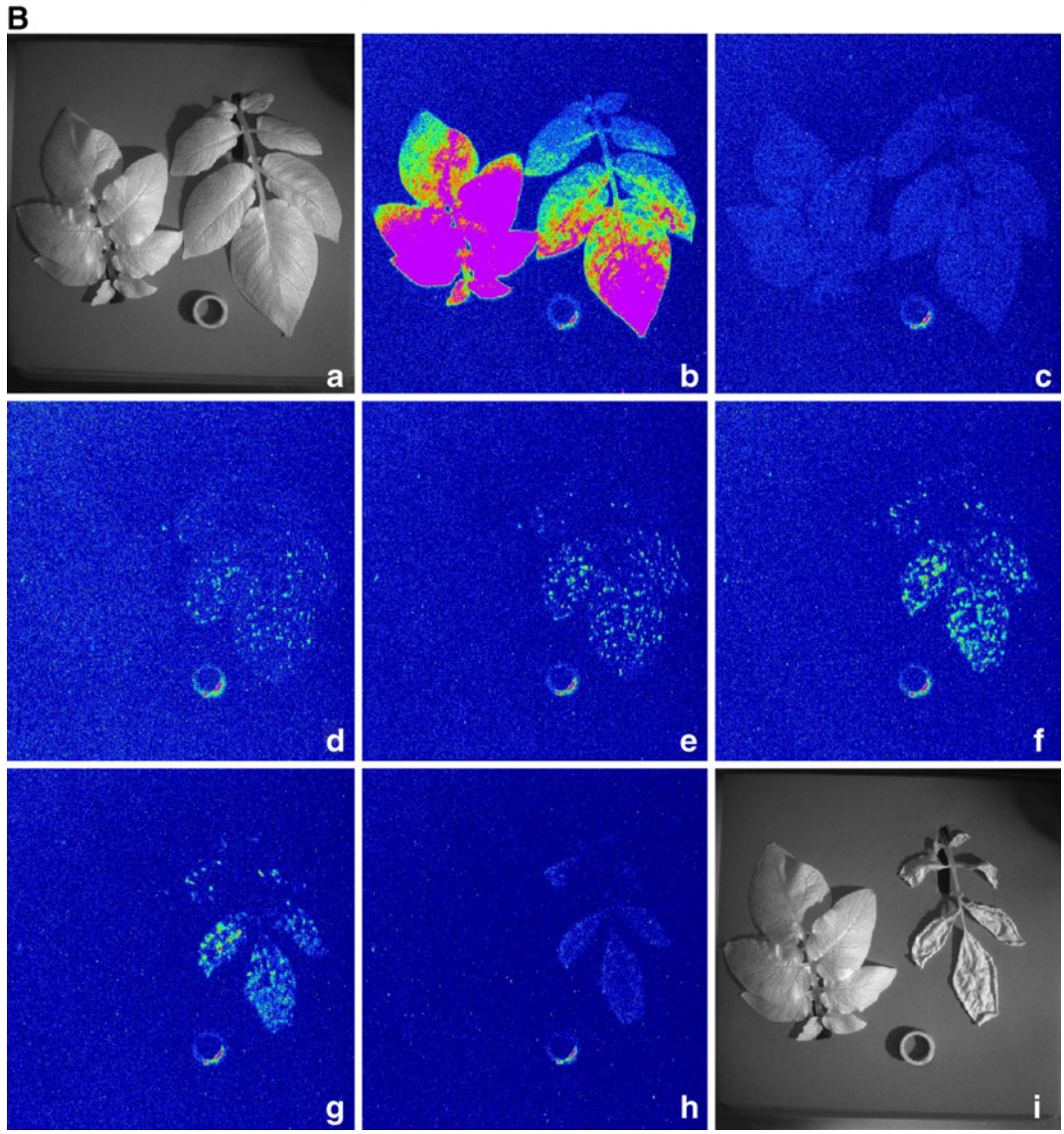
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◀ **Fig. 2** **a** Biophoton emission from potato leaves (resistant and susceptible) early after challenge with *Phytophthora infestans*. Single photons were counted with a photon counter. Data are expressed as changes in biophoton emission from the area of inoculated leaves (cnt ·mm^{-2}·s). Values represent the average of triplicate experiments. **b** Imaging of biophoton generation from potato leaves during late blight development. *a* The first panel provides a starting photo of inoculated potato leaves—*P. infestans* resistant cv. Bzura (*left*) and susceptible cv. Bintje (*right*); *b* The second panel shows quenching of leaf autofluorescence; *c–h* The sequence of images presents a pattern of biophoton generation in the dark for 1, 24, 48, 72, 96 and 120 h after inoculation; *i* The last panel provides a photo of infected leaves of both cultivars at 120 h after *P. infestans* inoculation

stable ground state (Abeles 1986). A spontaneous emission of ultra-weak luminescence is associated with biochemical changes, most frequently with peroxidation of poly-unsaturated fatty acids and endoperoxides, which generate light–emitting molecules such as triplet carbonyls and singlet oxygen (Voeikov 2000). So far, basic molecular mechanisms underlying biophoton emissions are unknown.

In order to understand processes occurring in plants subjected to stress conditions, including labile oxidative changes, attempts should be made to preserve intracellular parameters instead of referring to drastic and invasive methods (Havaux et al. 2006). On the other hand, the diversity of experimental data obtained forces researchers to search for convenient methods for their collection and presentation. As stated in the introduction, a human being is physiologically prepared for the perception and processing of information contained in an image, thus techniques using these forms of data transmission are of great interest. Therefore, digital image processing techniques are employed in order to achieve complete photometrical and mathematical analysis of the investigated objects underlying light-generation processes. Functional imaging of ultra-weak photon emission used in this study makes it possible to evaluate visually in real time the degree of intracellular oxidative homeostasis misbalance in a selected fragment of an organ or the whole plant, and thus conclude

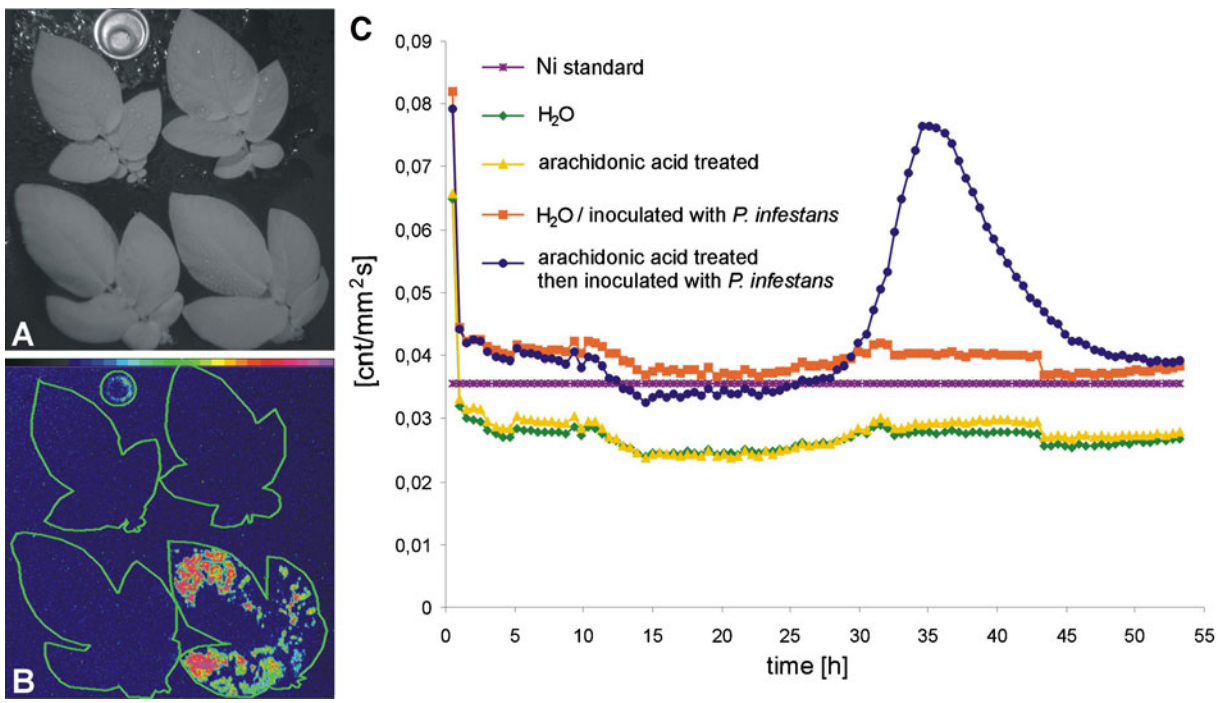

Fig. 3 The effect of *Phytophthora infestans* on biophoton emission of susceptible potato elicited by a SAR inducer—arachidonic acid. **a** photo; **b** bioluminescence image. Leaf position—control treated with water (*upper range, from left*); inoculated with *P. infestans* (*upper range, from right*); arachidonic acid treated (*lower range, from left*); arachidonic acid treated and then inoculated with *P. infestans* (*lower range, from right*). Ring in the middle of upper line—radioluminescence standard, **c** Time curve of biophoton intensity generation from potato leaves

on the level of plant resistance or susceptibility to biotic stress.

The results presented here have shown that a resistant potato variety challenged with *P.infestans* emitted biophotons immediately after inoculation. Recorded bioluminescence lasted for approximately 12 h, with maximum intensity observed from 8 to 10 h post inoculation. At the same time, photon emissions associated with a compatible interaction were weaker than the signal generated by an incompatible one. However, beginning from 2 dpi, susceptible leaves produced more photons than resistant ones, which reflected oxidative damage, being an outcome of disease progress (Floryszak-Wieczorek 2000).

In the case of a strong stress exceeding the adaptive and tolerance abilities of an organism, when an irreversible disruption of metabolism and peroxidative damage to lipid structures occur, a rapid increase in luminescence is observed, called death radiation (Sławiński 2003). However, it is known from reports by other authors that ultra-weak biophoton emission generated from sweet potato was related to defence responses, e.g. phytoalexin accumulation, to non-pathogenic *Fusarium oxysporum* (Makino et al. 1996; Hiramatsu 1998; Iyozumi et al. 2002). According to Bennett et al. (2005) and Mansfield (2005) biophoton generation is associated with hypersensitive resistance reaction in *Arabidopsis*, bean and tomato. Tissues undergoing the HR were recognized by a biophoton flare or "biophoton fingerprint" that occurred several hours before cell death.

In our study, early biophoton emission in a resistant potato variety preceded the timing of leaf collapse, observed as the TUNEL positive reaction by several hours as well (Floryszak-Wieczorek et al. unpublished).

In response to *Botrytis cinerea*, the point inoculated pelargonium leaves showed local photon generation localized first at the site of the attempted penetration, then from 3 to 5 dpi in the region adjacent to progressing disease spots, between dying and living cell layers directly surrounding expanding primary necrotic lesions. Biophotons were not emitted from dry necrotic spots.

The presented results supply a valuable supplement and confirmation of our previously published data (Floryszak-Wieczorek et al. 2007). This reported a strong NO burst, recorded electrochemically with a microsensor, and was followed by a wave of secondary NO generation, shown by bio-imaging with a NO-specific fluorescent dye (DAF-2DA) in pelargonium cells challenged with *B. cinerea*. The enhanced NO synthesis correlated with hydrogen peroxide (H_2O_2) accumulation located in targeted cells, which exhibited an active cell death (the TUNEL-positive reaction). The induced NO generation initially expanded and then gradually disappeared on successive days, provoking non-cell-death resistance with an enhanced pool of antioxidants, which finally favoured the maintenance of homeostasis in surrounding cells.

These findings corroborate a model for cellular responses of *Arabidopsis* to *Botrytis cinerea* described by van Baarlen et al. (2007). Efficient resistance against *B. cinerea* infection was found to depend on two major cellular processes in distinct zones surrounding the primary infection site. According to the cited authors, the first zone is characterized by fast death and desiccation of cells in the sites of invading hyphae, while the second zone with progressive cell death contains both living and dead cells. Communication between these zones and the resulting local balance of death and survival factors defines the diameters of each zone and promotes resistance or susceptibility to the necrotroph.

Functional imaging of ultra-weak photon emission presented here makes it possible to evaluate visually in real time this balance of dead and living tissue of pelargonium leaves. Initially intensification of light as 'dead radiation' leads to a shift towards tissue death at sites of pathogen invasion, then gradually quenching and finally suppressed biophoton production conducive to a shift towards survival and establishment of plant resistance.

Increased luminescence, observed in our study and preceding or concomitant with cell death, might be not only a mechanism of radiation deactivation of electron excited states, but also it constitutes an additional biological signal of electromagnetic nature, similar to the SOS signal when the organism faces a threat. However, the possible informative-regulatory meaning of ultra-weak light is less known than the energetic role (Arnhold 2001). In confirmation, spraying susceptible potato with arachidonic acid—an effective inducer of SAR and then challenging it with *P. infestans* resulted in a transient burst of light lasting for several hours. Kageyama et al. (2006) documented thoroughly that elicitor-responsive photon emission from rice cells,

induced by N-acetylchitooligosaccharide, occurred in a close relation with ROS generation. Putative signal transduction leading to elicitor-induced biophoton response was found to operate through phospholipid signalling. Similarly, Park et al. (1998) demonstrated induction of a sub-systemic oxidative burst by an elicitor-stimulated local oxidative burst, which was monitored as luminol-mediated chemiluminescence in potato plants. The authors suggested that an elicitor derived from *Phytophthora infestans* might cause a local oxidative burst in potato tuber slices and compound leaves that provides a mobile signal for the induction of a systemic oxidative burst, responsible for the induction of SAR.

The results reported here have shown that imaging of biophoton emission might be used to monitor pathogen-induced changes associated with processes of auto-oxidation and bio-communication occurring between cells of the host body. Various pathogens resulted in a modulation of the redox potential of targeted plant cells, which activate signalling pathways and induce plant defence responses according to the genetic make up of the plant. Since the understanding of the mechanism of intracellular redox potential regulation requires simultaneous monitoring of many important signals, the technique of chemical imaging, linking luminescence and spectroscopy seems to be a promising innovation in this field (Boppart 2008).

References

Abeles, F. B. (1986). Plant chemiluminescence. *Annual Review of Plant Physiology, 37*, 49–72.

Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology, 55*, 373–399.

Arasimowicz, M., & Floryszak-Wieczorek, J. (2007). Nitric oxide as a bioactive signalling in plant stress responses. *Plant Science, 172*, 876–887.

Arnhold, J. (2001). Application of chemiluminescence methods in the investigation of redox regulation in cells. In S. Albrecht, T. Zimmermann, & H. Brandl (Eds.), *Chemiluminescence at the turn of the millennium* (pp. 85–94). Dresden: Schweda-Werbendruck GmbH.

Bennett, M., Mehta, M., & Grant, M. (2005). Biophoton imaging: a nondestructive method for assaying R gene responses. *Molecular Plant-Microbe Interactions, 18*, 95–102.

Boppart, S. A. (2008). Optical coherence tomography with applications in cancer imaging. In L. Pavesi & P. M. Fauchet (Eds.), *Biophotonics* (pp. 127–149). Berlin: Springer-Verlag.

Cohen, Y., Gisi, U., & Mosinger, E. (1991). Systemic resistance of potato plants against *Phytophthora infestans* induced by unsaturated fatty acids. *Physiological and Molecular Plant Pathology, 38*, 255–263.

Floryszak-Wieczorek, J. (2000). Effect of *Phytophthora infestans* on the activity of oxygen scavenging enzymes in leaves of different potato genotypes. *Phytopathologia Polonica, 19*, 147–155.

Floryszak-Wieczorek, J., Arasimowicz, M., Milczarek, G., Jeleń, H., & Jackowiak, H. (2007). Only an early nitric oxide burst and the following wave of secondary nitric oxide generation enhanced effective defence responses of pelargonium to a necrotrophic pathogen. *The New Phytologist, 175*, 718–730.

Garcia-Brugger, A., Lamotte, O., Vandelle, E., Bourque, S., Lecourieux, D., Poinssot, B., et al. (2006). Early signalling events induced by elicitors of plant defenses. *Molecular Plant-Microbe Interactions, 19*, 711–724.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology, 43*, 205–207.

Gòmez-Lim, M. A. (2002). Genes involved in plant defense. In V. Valpuesta (Ed.), *Fruit and vegetable biotechnology* (pp. 114–135). Cambridge: Woodhead Publishing Ltd., CRC.

Górski, Z., Bembnista, T., Floryszak-Wieczorek, J., Domański, M. & Sławiński, J. (2003). Computer-aided photometric analysis of dynamic digital bioluminescent images. In M. Pluta, M. Szyjer & E. Powichrowska (Eds.), *Metrology and testing techniques using light* (pp. 1–10), Proceedings of SPIE, 5064, 1–10. Bellingham, Washington USA.

Grant, J. J., & Loake, G. J. (2000). Role of reactive oxygen intermediates and cognate redox signalling in disease resistance. *Plant Physiology, 124*, 21–29.

Havaux, M. (2003). Spontaneous and thermoinduced photon emission: new methods to detect and quantify oxidative stress in plants. *Trends in Plant Science, 8*, 409–413.

Havaux, M., Triantaphylides, C., & Genty, B. (2006). Autoluminescence imaging: a non-invasive tool for mapping oxidative stress. *Trends in Plant Science, 11*, 480–484.

Hiramatsu, M. (1998). Biophotons and defense response in plants. In J. J. Chang, J. Fisher, & F.-A. Popp (Eds.), *Biophotons* (pp. 45–55). London: Kluwer Academic.

Iyozumi, H., Kato, T., & Makino, T. (2002). Spectral shift of ultaweak photon emission from sweet potato during a defense response. *Photochemistry and Photobiology, 75*, 322–325.

Kageyama, C., Kato, K., Iyozumi, H., Inagaki, H., Yamaguchi, A., Furuse, K., et al. (2006). Photon emissions from rice cells elicited by N-acetylchitooligosaccharide are generated through phospholipid signalling in close association with the production of reactive oxygen species. *Plant Physiology and Biochemistry, 44*, 901–909.

Makino, T., Kato, K., Iyozumi, H., Honzawa, H., Tachiiri, Y., & Hiramatsu, M. (1996). Ultraweak luminescence generated by sweet potato and *Fusarium oxysporum* interactions associated with a defense response. *Photochemistry and Photobiology, 64*, 953–956.

Mansfield, J. W. (2005). Biophoton distress flares signal the onset of the hypersensitive reaction. *Trends in Plant Science, 10*, 307–309.

Miller, G., Coutu, J., Shulaev, V., & Mittler, R. (2008). Reactive oxygen signalling in plants. *Annual Plant Reviews, 33*, 189–201.

Mur, L. A., Carver, T., & Prats, E. (2006). NO way to live; the various roles of nitric oxide in plant-pathogen interactions. *Journal of Experimental Botany, 57*, 489–505.

Overmyer, K., Brosché, M., & Kangasjärvi, J. (2003). Reactive oxygen species and hormonal control of cell death. *Trends in Plant Science, 8*, 335–342.

Park, H. J., Doke, N., Miura, Y., Kawakita, K., Noritake, T., & Komatsubara, H. (1998). Induction of a sub-systemic oxidative burst by elicitor-stimulated local oxidative burst in potato plant tissues: a possible systemic signalling in systemic acquired resistance. *Plant Science, 138*, 197–208.

Schulze-Lefert, P. (2004). Knocking on the heaven's wall: pathogenesis of and resistance to biotrophic fungi at the cell wall. *Current Option in Plant Biology, 7*, 377–383.

Sławiński, J. (2003). Biophotons from stressed and dying organisms: toxicological aspects. *Indian Journal of Experimental Botany, 41*, 483–493.

Spoel, S. H., Johnson, J. J., & Dong, X. (2007). Regulation of tradeoff between plant defenses against pathogens with different life styles. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 18842–18847.

van Baarlen, P., Woltering, E. J., Staats, M., & van Kan, J. A. L. (2007). Histochemical and genetic analysis of host and non-host interactions of *Arabidopsis* with *Botrytis species*: an important role for cell death control. *Molecular Plant Pathology, 8*, 41–54.

Voeikov, V. L. (2000). Processes involving reactive oxygen species are the major source of structured energy for organismal biophotonic field pumping. In L. Beloussov, F.-A. Popp, V. Voeikov & R. van Wijk (Eds.), *Biophotonics and coherent systems* (pp. 203–228). Moscow University Press.

Zago, E., Morsa, S., Dat, F., Alard, P., Ferrarini, A., Inze, D., et al. (2006). Nitric oxide- and hydrogen peroxide-responsive gene regulation during cell death induction in tobacco. *Plant Physiology, 141*, 404–411.

Zhao, J., Davis, L. C., & Verpoorte, R. (2005). Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnology Advances, 23*, 283–333.