Induction of Defense Responses in Cucumber Plants by Using the Cell-free Filtrate of the Plant Growth-Promoting Fungus *Penicillium simplicissimum* GP17-2

Kaori Shimizu¹, Mohamed Motaher Hossain¹, Kimihiko Kato², Mashaharu Kubota³ and Mitsuro Hyakumachi¹ *

Abstract: *Penicillium simplicissimum* GP17-2 is a plant growth-promoting fungus (PGPF) and an inducer of systemic defense responses. The mechanisms underlying the effect of GP17-2 on the reduction of cucumber leaf damage caused by the anthracnose pathogen *Colletotrichum orbiculare* were investigated. Cucumber leaves treated with the culture filtrate (CF) of GP17-2 exhibited a clear systemic resistance against subsequent infection with *C. orbiculare*. The number and size of lesions caused by the disease were reduced in CF-treated plants, in comparison with that in the control plants. The results showed that CF treatment could trigger a set of defense responses, including the production of hydrogen peroxide, formation of lignin, emission of ultra-weak photons, accumulation of salicylic acid, and increase in the transcription of the genes for the defense-related enzymes chitinase and peroxidase. Furthermore, subsequent inoculation of CF-pretreated plants with *C. orbiculare* resulted in higher systemic expression of the genes for chitinase, β-1,3-glucanase, and peroxidase relative to nontreated, inoculated plants; this indicated that CF mediates a potentiation state in the plant, enabling it to mount a rapid and effective response on infection by *C. orbiculare*. Our results indicate that the ability of CF of GP17-2 to stimulate active oxygen species, lignification, SA accumulation, and defense gene activation and potentiation in the host is the possible mode of action of the GP17-2 elicitor and inducer of induced systemic resistance against *C. orbiculare* infection in cucumber plants.

Key words: PGPF, *Colletotrichum orbiculare*, defense responses, potentiation

1 INTRODUCTION

Plant disease is one of the major factors detrimental to food production worldwide. Over the past few decades, producers have become more dependent on agrochemicals as a relatively reliable method for crop protection. However, because of the growing awareness of environmental damage caused by agrochemicals, their extensive use is intensively debated. Currently, extensive research is being conducted on the use of the genetic potential of plants to resist pathogens and the development of induced resistance as an environmentally safe means of disease control. Induced resistance involves the induction of plant defensive compounds after pathogen attacks, resulting in a marked decrease in the levels of susceptibility to diseases. Induced resistance can be triggered in plants by a previous infection with an avirulent, necrotizing pathogen (systemic acquired resistance [SAR]) or by previous colonization of the rhizosphere with beneficial microbes (induced systemic resistance [ISR]). In recent years, ISR in plants has involved the investigation of several selected strains of non-pathogenic plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF). PGPF and PGPR are rhizosphere fungi and bacteria, respectively, that, when reintroduced by inoculation into soil containing competitive microflora, exert stimulating effects on plant growth and manage soil and plant health. Both PGPF and PGPR can activate host defense mechanisms against subsequent infection by a pathogen through induced resistance. ISR mediated by...
PGPF and PGPR has been demonstrated in many plant species and is effective against a broad spectrum of pathogens\textsuperscript{6,7}. This ability of PGPF and PGPR to act as bioprotectants through ISR can be a significant component of future management practices.

Elicitation of various defense responses is the first step towards the elucidation of ISR that controls pathogen invasion. The early phase of plant response to elicitors is usually accompanied by the production hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}). H\textsubscript{2}O\textsubscript{2} can act as an antibiotic agent\textsuperscript{9}, and it contributes indirectly to a plant’s defense by causing cross-linking among hydroxyproline-rich glycoprotein monomers to form a network that provides anchorage for lignification\textsuperscript{10}. Induced lignification increases cell-wall endurance and arrests fungal penetration\textsuperscript{11}. Induced resistance also leads to the induction of local and systemic increases in the endogenous levels of various signaling compounds and the concomitant activation of large sets of genes related to the plant immune system, including those encoding pathogenesis-related (PR) proteins\textsuperscript{12-16}. PR proteins, such as β-1,3-glucanase (PR2) and chitinase (PR3), are known to disrupt the mycelial wall of a fungus and can be induced by salicylic acid (SA) and pathogenic attack\textsuperscript{17}. Peroxidases belong to the PR-protein 9 subfamily\textsuperscript{18} and are frequently responsive to SA, jasmonic acid (JA), or ethylene (ET)\textsuperscript{19}. They are known for their role in the formation of phytalexins and the formation of structural barriers\textsuperscript{20,21}. Despite this diversity in mechanisms, the induced defense is not always sufficient to protect the plant against damage by pathogens; therefore, plants have evolved an additional regulatory system that allows them to fine-tune their inducible defense system. When specific environmental cues are perceived, plants can develop an enhanced defensive capacity that enables a faster and stronger defense response after pathogen attack. This phenomenon is commonly referred to as “sensitization” or “priming”\textsuperscript{19,20}. Priming provides enhanced fitness under conditions and pressures associated with disease. Moreover, in recent years, a new type of defense-related phenomenon has been reported, in which plants generate relatively high levels of ultra-weak photon emissions in response to pathogen attack or elicitor treatment\textsuperscript{22-24}. This pathogen/elicitor-responsive photon emission, together with the generation of excited molecules involved in the defense response, could be used as a noninvasive indicator of physiological changes\textsuperscript{25}.

\textit{Penicillium simplicissimum} GP17-2 (GP17-2) is a PGPF collected from the rhizosphere of the zoysiagrass \textit{Zoysia tenuifolia}. The barley grain inoculum and cell-free filtrate (CF) of GP17-2 has been shown to protect plants through induced resistance\textsuperscript{24}. The CFs of other PGPFs have also been shown to initiate a host defense\textsuperscript{6,25-28}; therefore, there is evidence for the presence of one or more elicitors in the CF. The purpose of this study was to investigate the effect of treatment with CF of GP17-2 on host defense responses to pathogen infection. Using cucumber and \textit{Colletotrichum orbiculare} as a model system, we examined the possibility that CF of GP17-2, when applied to the second true leaves of cucumber plants, results in ISR to \textit{C. orbiculare} in the upper (top one-third) non-treated leaves of the plants. Furthermore, we investigated an array of defense responses in cucumber plants after treatment with CF of GP17-2 to determine the mode of action of the GP17-2 elicitor.

2 MATERIALS AND METHODS

2.1 PGPF, pathogen, and host

The PGPF used in this experiment was an isolate of GP17-2, obtained from the Laboratory of Plant Pathology, Gifu University, Gifu, Japan. Anthracnose pathogen \textit{C. orbiculare} 104T maintained on 3% potato-dextrose agar (PDA) slants (Laboratory of Plant Pathology, Gifu University, Gifu, Japan) was used for this study. The cucumber plant \textit{Cucumis sativus} L. \textit{‘Jibai’} was used as the host.

2.2 Preparation of CF of GP17-2 and inocula of the pathogen

The CF of GP17-2 was prepared according to the procedure described by Hossain et al. (2007)\textsuperscript{24}. The pathogen \textit{C. orbiculare} 104T was transferred to PDA (3% agar) plates and incubated for 7-10 days at 25°C for maximum sporulation. Spores of \textit{C. orbiculare} were scraped from the culture by using a sterile scalpel, and a spore suspension was collected in sterile water and filtered through 8 layers of cheesecloth. The spore concentration was adjusted to 105 spores mL\textsuperscript{-1} by using a hemacytometer.

2.3 ISR assays in cucumber plants

Cucumber plants were grown in the commercial Star Bed potting medium (soilless, peat-based potting medium, Kyodohiyro Co., Ltd, Aichi, Japan), containing humus, peat, rock phosphate, and composted plant materials. Surface-sterilized (2% NaOCl for 25 min) cucumber seeds were germinated on autoclaved filter papers soaked with sterile distilled water. After incubating for 48 h at 25°C, 1 pre-germinated cucumber seed was planted per sterilized plastic pot (6 × 7.5 cm). Plants were grown at 25°C in growth chambers with a 12 h/12 h light/dark period. The second true leaf of a 3-week-old plant was dipped into the CF of GP17-2 for 1 min. In the control plants, the leaves were treated with sterilized distilled water. Pathogen inoculation was performed on the third true leaf 1 day after induction treatment. Twenty drops (each 10 μL) of the spore suspension (5 × 105 spores mL\textsuperscript{-1}) were placed at 20 different positions on the third leaf of each plant. A disk of lens paper (diameter, 5 mm) was placed on each drop to prevent runoff and to maintain
uniform distribution of the spores. The inoculated plants were incubated at 25°C for 36 h in a dark, humid chamber (85-90% relative humidity (RH)) and transferred back to the same growth chamber for another 6 days to ensure better disease development. The total number of lesions and the area of each lesion (mm²) developed by C. orbiculare were measured. On the basis of the total lesion area (TLA), the level of protection induced by each treatment was calculated as follows: Protection % = (1 - TLA in induced plants/TLA in control plants) × 10,029.

2.4 H2O2 generation assay using diaminobenzidine tetra-hydrochloride staining

Diaminobenzidine tetrahydrochloride (DAB) (Nacalai Tesque, Kyoto, Japan) staining was used to evaluate H2O2 generation. The second true leaves of 21-day-old plants grown as previously described were treated with CF of GP17-2 and incubated for 24 h. After incubation, the third leaves were inoculated with 20 drops (each 10 μL) of the spore suspension (5 × 10⁵ spores • mL⁻¹). Leaf discs were punched out at inoculation points by using a sterile cork borer (diameter, 5 mm) at 12, 24, 36, and 48 h post-inoculation. For detecting H2O2 generation in the leaf samples, a modified Thordal-Christensen et al. method (1997) was used. Leaf samples were incubated with DAB solution (1 µg • mL⁻¹) for 8 h at room temperature in the dark and then fixed in a mixture of ethanol and acetic acid (96:4, v/v) overnight. The DAB-stained leaf discs were then observed under a microscope for the occurrence of DAB polymerization indicated by the absence or presence of a dark reddish-brown color in the disc.

2.5 Ultra-weak photon emission assays

Cucumber seeds were sown in moist sterilized filter paper and incubated for 4 days in the dark at 25°C. The seedlings were then transferred to petri dishes. To suppress photon emissions reported to occur from injured or activated tissues, the cucumber seedlings were incubated at 25°C for 3 h in the dark. After incubation, the cucumber seedlings were treated with CF of GP17-2. The time-dependent intensity variation in the photon emissions was assessed using a C1230 photon counter (Hamamatsu Photonics K.K., Shizuoka, Japan) equipped with an R208 photomultiplier tube, which provides a spectral response from 185 to 650 nm. A special dark-box system with a rotating tray containing space for 16 samples was used to measure multiple samples under the same conditions. Each sample was measured every 42.4 s at 20°C for 24 h. Photons were counted at 1-s intervals.

2.6 Testing for lignin formation

Cucumber seeds were sown in moist sterilized filter paper and incubated for 7 days in the dark at 25°C. The roots of each seedling were individually dipped in 5 mL of CF of GP17-2, contained in glass tubes (2.2 × 12 cm) and incubated for 24 h at 25°C. Sterilized distilled water was used instead of CF of GP17-2 for the control. The incubated roots were washed in distilled water, and the seedlings were placed on moistened sterile blotters. The hypocotyls were then inoculated by placing 5 drops of 5-μL spore suspension (5 × 10⁵ spores • mL⁻¹). A disc of lens paper (diameter, 5 mm) was placed on each drop to prevent runoff and maintain a uniform spore distribution. The inoculated plants were then incubated at 25°C for 24 h. The epidermal strips from the hypocotyls of the seedlings were peeled off using a fine-point tweezers and stained with toluidine blue in 0.1 M phosphate buffer (pH 6.5) for 5 min or soaked in a solution of 2% phenolglucin in 95% ethanol for 1.2 h. The epidermal strips were placed on a glass slide and one drop of 35% hydrochloric acid was added on it. The glass slide was then heated over a low flame until the veins of the epidermal strips turned reddish-purple. These samples were observed for the degree of lignin formation under the microscope (80×), which was evaluated by determining the percentage of germinated spores (>90%) together with the number of appressoria around which lignin depositions were induced. For each treatment, 100 germinated spores were evaluated.

2.7 Extraction and analysis of endogenous SA

Cucumber plants were grown in pots for 21 days, and the second true leaves were treated with CF of GP17-2 or sterilized distilled water for 1 min in the same manner as described for the ISR assays. One day after induction treatment, second and third leaves were collected (1.2 g) and boiled for 10 min in a 2% (v/v) acetic acid (pH 2.7) solution (10 × volume of leaf weight). Boiling under acidic conditions hydrolyzes the conjugated SA into free SA and glucose; therefore, the amount of SA detected using high-performance liquid chromatography (HPLC) in this study indicates the total amount of free and conjugated SA in cucumber tissue.

The concentration of SA in the extract was measured using HPLC. The extract was injected into a C-18 column (Shim-Pack CLC-ODS; Shimadzu, Kyoto, Japan) running with 20% methanol in 20 mM sodium acetate buffer at a flow rate of 1 mL•min⁻¹. SA was detected with a fluorescence detector at 290-nm excitation and 402-nm emission, as described by Ishikawa et al. (2005).

2.8 RNA extraction and northern blot analysis

Seed sowing, induction treatment, and pathogen inoculation were performed using the same techniques as described during the test for lignin formation. The hypocotyls were harvested several times before and after inoculation. Total RNA was extracted from the sampled hypocotyls by using the acid guanidinium thiocyanate-phenol-chloroform extraction method and further purified by ultracentrifugation.
Ten micrograms of total RNA from hypocotyls was fractionated using electrophoresis in a 1.2% (w/v) agarose-formaldehyde gel. The RNA was then transferred onto a Hybond-N nylon membrane (Amersham Biosciences Corp, NJ, USA). Hybridization was performed at 68°C by using digoxigenin (DIG)-UTP-labeled RNA probes (Boehringer Mannheim, Mannheim, Germany). After hybridization, the membranes were washed twice (10 min per wash) with 0.1× SSC (0.165 M Na₃C₆H₅O₇, 1.65 M NaCl, pH 7.0) and 0.1% SDS, and twice (30 min per wash) with 0.1× SSC and 0.1% SDS at 60°C. DIG was detected using an enzyme-linked immunoassay with an anti-DIG antibody conjugated to alkaline phosphatase and CDP-Star (Roche, Germany), an ultrasensitive chemiluminescent substrate for alkaline phosphatase.

### Statistical analysis

The experimental design was completely randomized, consisting of 3 replications for each treatment. The experiment was repeated at least twice, and treatment means were compared using a paired t-test at $p \leq 0.05$.

### 3 RESULTS

#### 3.1 ISR in cucumber against *C. orbiculare* by CF of GP17-2

Application of CF of GP17-2 as leaf treatment resulted in ISR against the anthracnose pathogen *C. orbiculare* in 3-week-old cucumber plants. ISR significantly reduced total lesion numbers and TLA per infected leaf, as determined on the third true leaf 8 days after inoculation. The level of protection from *P. simplicissimum* GP17-2 was 79% higher than that in the control plants (Table 1).

#### 3.2 H₂O₂ detection in the leaves of cucumber plants in response to *C. orbiculare* by using the DAB-uptake method

The DAB-uptake method was used to study H₂O₂ production in cucumber leaves in response to treatment with CF of GP17-2 and inoculation with *C. orbiculare*. DAB staining of leaf discs was performed at different time points post inoculation (12, 24, 36 and 48 h).

#### 3.3 Ultra-weak photon emissions from cucumber seedlings treated with CF of GP17-2

Consecutive spectral analyses of ultra-weak photon emissions from cucumber seedlings that showed a defense

---

**Table 1** Effect of cell free filtrate of *Penicillium simplicissimum* GP17-2 on the total lesion number and lesion area on leaves of cucumber plants challenge inoculated with *Colletotrichum orbiculare*, and the level of protection.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TLN</th>
<th>TLA (mm²)</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. simplicissimum</em> GP17-2</td>
<td>17.00*</td>
<td>82.72*</td>
<td>79%</td>
</tr>
<tr>
<td>Control</td>
<td>20.00</td>
<td>393.28*</td>
<td>–</td>
</tr>
</tbody>
</table>

TLN, Total lesion number; TLA, total lesion area  
* Significant difference by t-test ($p \leq 0.05$). Values are means of three replications, each consisting of 4 plants.  
* Protection = $[1 - (\text{TLA in treated plants/TLA in control plants})] \times 100$, where TLA = sum of the area (mm²) of lesions caused by *C. orbiculare* on leaves of cucumber plants.
response were conducted to observe the process of physiological transition. The spectrum showed a drastic transition where the emission intensity increased to a very high level shortly after CF treatment at over 750000 counts $\cdot$ s$^{-1}$ $\cdot$ cm$^{-2}$ (Fig. 2); however, the emissions rapidly decreased after the first high surge and the spectrum became stable after 10 to 14 h of treatment.

### 3.4 Lignification in cucumber tissues by using CF of GP17-2

A quantitative assay was performed to determine the intensity of CF-induced lignification in cucumber hypocotyls. The epidermal tissues of cucumber hypocotyls were stained and scanned to determine the percentage of germinating pathogen spores with appressoria around which lignin depositions were induced. Lignin deposition was significantly enhanced in CF-treated plants after inoculation with *C. orbiculare*. One day after inoculation with *C. orbiculare*, 63.8% of germinating spores showed lignin deposition at the point of attempted penetration in the hypocotyls of CF-treated plant, while in the control plants, only 12% of the germinating spores showed lignin deposition at the penetration point (Fig. 3). These results suggest that CF-treated plants are predisposed to mounting a set of defense responses, whereas plants not pretreated with CF display a minimal response to resisting *C. orbiculare* infection.

### 3.5 SA accumulation in cucumber leaves treated with CF of GP17-2

An investigation was conducted to determine the role of SA in ISR elicited by CF of GP17-2. The second true leaf of cucumber plants was treated with CF, and both the treated and the upper, non-treated leaves (the third true leaf) of the plants were collected separately at 0, 2, 4, 6, 8, and 10 days after treatment for quantification of SA accumulation.

![Fig. 2](image_url) Increases in elicitor-responsive photon emissions treated from cucumber seedlings treated with CF of GP17-2. Ultraweak photon emissions were measured after 0, 2, 4, 6, 8, 10 and 12 h of CF treatment.

![Fig. 3](image_url) Lignifications of hypocotyls of cucumber seedlings induced by CF of GP17-2 following challenge inoculation with *C. orbiculare*. *Mean of one trial each with eight replicates. Means carrying with an asterisk are significantly different ($p = 0.05$) according to student’s t-test.

![Fig. 4](image_url) Accumulation of total salicylic acid (SA) in second (A) and third true leaves (B) of cucumber plants treated with CF of GP17-2 or SDW (control). The experiments were repeated three times with similar results. *Mean of one trial each with eight replicates. Means carrying with an asterisk are significantly different ($p = 0.05$) according to student’s t-test.

Increased quantities of SA were detected in both second and third true leaves of the CF-treated plants (Figs. 4A and 4B). The highest increase in SA accumulation was observed in both leaves at 2 days after CF treatment; however, second true leaf contained about twice as much salicylic acid as third true leaf of the same plants at this point in time. After that, both leaves of the CF-treated plants showed a more or less gradual decrease in SA accumulation over time.

3.6 Expression of genes for peroxidase, chitinase, and β-1,3-glucanase in CF-treated plants

CF of GP17-2 was tested to determine whether it was capable of stimulating the expression of several genes for defense-related enzymes such as peroxidase, chitinase, and β-1,3-glucanase in cucumber plants. Expression of these genes was assayed systematically at 1, 2, 3, 4, 5, 6, 12, and 24 h after CF treatment. Increased expression of peroxidase and chitinase genes was observed in CF-treated plants, beginning at 2 h and reaching a maximum at 18 h after CF application (Fig. 5); however, no detectable transcript accumulation of the β-1,3-glucanase gene was evident in either treated or untreated plants, even 24 h after CF application.

To investigate whether priming for faster or stronger defense-gene activation also occurs in CF-induced ISR, we examined the expression of the defense genes in C. orbiculare-inoculated plants at 1, 2, 3, 4, 5, 6, 12, 18, and 24 h post-inoculation. Figure 5 shows that inoculation of plants pretreated with CF of GP17-2 further enhanced expression of the peroxidase and chitinase genes in hypocotyls of cucumber plants, beginning at 1 h after inoculation. Similarly, although no transcript accumulation of the β-1,3-glucanase gene was observed before pathogen inoculations, the gene was classically induced after pathogen inoculation. Greater expression of this gene was observed at 1 h post-inoculation in CF-treated plants, compared to at 12 h post-inoculation in the control plants. This result suggests that a systemically resistant plant induced by CF of GP17-2 is primed for potentiated gene activation against pathogens.

4 DISCUSSION

A previous study demonstrated that CF of GP17-2 is as effective as living inocula in suppressing diseases in Arabidopsis^24^. CF of GP17-2 was also observed to exhibit similar suppression of anthracnose disease in cucumber, reducing total lesion numbers and TLA per infected leaf. This suggests that CF of GP17-2 induces defense against a wide range of pathogens in a variety of systems. Suppression of the disease appeared to be systemic, as indicated when second leaves were treated with CF of GP17-2 while the pathogen was inoculated on third leaves.

The rapid release of H$_2$O$_2$ is generally assumed to be a key event in the orchestration of various cellular defense responses^35^, and it is a well-described response of plant cells to molecules with eliciting activities. The cucumber

---

**Fig. 5** Northern blot analysis of the transcription of defense-related genes in cucumber hypocotyls in response to treatment with CF of GP17-2 or SDW (control). For each treatment total RNA was extracted from a pool of hypocotyls collected from different plants. Transcripts levels of peroxidase, chitinase and β-1,3-glucanase were analysed by northern blot.
leaves at different times of pathogen infection were stained with DAB to determine the generation of active oxygen species during the interaction of C. orbiculare with the host plant. H$_2$O$_2$ generation was detected as a response to pathogen infection of CF-treated plants. Similarly, in chemiluminescence (CL) assays, a massive accumulation of H$_2$O$_2$ was also triggered by CF treatment. Given that H$_2$O$_2$ is a critical element in a plant’s defense against pathogens, the ability of CF to stimulate host active oxygen species suggests the possible mode of action of the CF elicitor and inducer of ISR.

Our studies demonstrate that lignin is produced in the epidermal tissues of CF-treated cucumber plants surrounding spores or appressoria of inoculated fungi, thus inhibiting fungal penetration in the systemically protected plant parts. Similar observations were also made by Koike et al. (2001) with 12000 Da fractions and lipid fractions of CF of GP17-2. Several other researchers also reported a possible relationship of induced lignification to an induced systemic protection mechanism in cucumber anthracnose; therefore, lignification appears to explain, at least in part, the CF of GP17-2-mediated resistance of cucumber to C. orbiculare.

Cucumber seedlings treated with CF of GP17-2 transiently generated relatively high levels of elicitor responsive photon emissions. A similar result was also obtained by Iyozumi et al. (2005) in rice cells in response to the PGPF elicitor CF of GP17-2. These data indicate that, as with other defense responses, elicitor-responsive photon emissions are enhanced in cells showing induced resistance and can be used as an indicator of plant defense activation.

Cucumber plants induced for systemic resistance by treatment with the CF of GP17-2 accumulated SA in treated and non-treated leaves. This suggests that SA might play a role in a CF-mediated plant defense against pathogens. By analyzing a set of transgenic and mutant Arabidopsis, Hossain et al. (2007) suggested that SA is partially required for the establishment of CF of GP17-2-mediated resistance in Arabidopsis. The link between SA production and systemic resistance has also been well established in many other plant-elicitor interactions. In contrast, Trichoderma asperellum T203-mediated ISR in cucumber plants requires a JA/ethylene-dependent pathway. This indicates that the systemic resistance induced by GP17-2 seems to be distinct from that mediated by T203.

To understand the involvement of defensive compounds in plant reactions to CF treatment, we studied the expression pattern of several defense-related genes. CF treatment induced a marked increase in the expression of the genes for chitinase and peroxidase within 2 h of treatment, suggesting that the protection afforded by CF of GP17-2 was associated with the accumulation of mRNA of the defense genes for chitinase and peroxidase; however, the increased expression of these genes in the CF-pre-elicited plants was further elevated by inoculation with C. orbiculare. In addition, a significant elevation of mRNA levels for the β-1,3-glucanase gene was manifested in CF-pretreated plants subsequently inoculated with C. orbiculare. A similar phenomenon was described for the same set of genes in the Trichoderma-mediated ISR against Pseudomonas syringae pv. Lachrymans, indicating that different elicitor-mediated resistance in cucumber genotypes might share a common set of defense mechanisms to respond to pathogen infection. The phenomenon of potentiation or conditioning has also been found in other studies; therefore, potentiation of defense-related genes could have a wider spectrum and significantly contribute to the plant’s resistance to pathogen infection.

In conclusion, the results indicate that CF of GP17-2 is an effective inducer of ISR and pathogen defense responses in cucumber plants. The induction effect by CF of GP17-2 was associated with the production of H$_2$O$_2$, formation of lignin, emissions of ultra-weak biophotons, accumulation of SA, and activation of genes for defense-related enzymes such as chitinase, peroxidase, and β-1,3-glucanase, all of which might affect the growth and development of C. orbiculare.

REFERENCES
30) Thordal-Christensen, H.; Zhang, Z.; Wei, Y.; Collinge, D. B. Subcellular localization of $H_2O_2$ in plants. H$_2$O$_2$ accumulation in papillae and hypersensitive response during the barley- powdery mildew interaction. Plant J., 11, 1187-1194 (1997).
Induction of defense responses by GP17-2


