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## RESEARCH ARTICLE

# Organic hydroponics induces systemic resistance against the air-borne pathogen, *Botrytis cinerea* (gray mould)

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Here, we propose that organic hydroponics trigger induced systemic resistance (ISR) in lettuce against air-borne *Botrytis cinerea*, which causes gray mold. We compared effects of organic and chemical hydroponics, assessed presence of ISR elicitors in the hydroponic nutrient solution, and investigated molecular mechanism of ISR. Organic hydroponics significantly reduced gray mold lesions in lettuce (cultivated hydroponically) and cucumber (cultivated in soil and foliar sprayed with nutrient solution). The 1-aminocyclopropane-1-carboxylic acid synthase gene in lettuce and lipoxygenase and ethylene receptor-related gene in cucumber showed heightened expression, suggesting that the jasmonic acid/ethylene (JA/ET)-signaling pathway was involved in ISR for both crops. Low salicylic acid  $\beta$ -glucoside levels confirmed role of the ISR signaling pathway. ISR in both lettuce and cucumbers indicated that elicitors in organic hydroponics were nonhost-specific and that the JA/ET pathway was activated without microbe–root interaction. Thus, organic hydroponics can be an effective method for both soil-borne and air-borne disease control.

**Keywords:** Induced systemic resistance; organic hydroponics; *Botrytis cinerea*; elicitor

### 1. Introduction

Hydroponic cultivation systems have been developed to control soil-borne pathogens (Uyeda et al. 2011). Nevertheless, one of the most destructive air-borne pathogens, *Botrytis cinerea* (Williamson et al. 2007), is capable of causing severe disease in soilless cultivation systems. Infection by this pathogen has resulted in serious agronomic losses worldwide (van Kan 2005; Williamson et al. 2007), including in lettuce (Shim et al. 2014) and cucumber (Shishido 2011). *B. cinerea* is responsible for soft rot, a condition characterized by the collapse and water-soaking of parenchyma tissues followed by a rapid appearance of gray mold masses of conidia on leaves (Williamson et al. 2007). The pathogen favors cool and moist conditions, and is extremely difficult to control (van Kan 2005; Williamson et al. 2007; Shishido 2011; Shim et al. 2014). Organic hydroponics, which use corn steep liquor (CSL) as a nutrient source (Shinohara 2006; Shinohara et al. 2011), were developed to control bacterial (Fujiwara et al. 2012) and fungal soil-borne (Chinta et al. 2014) diseases. Thus, this specific type of hydroponic cultivation may become a potential disease management method for *B. cinerea* infection.

Previous research has shown that CSL promotes the growth of a beneficial microbial community (Shinohara 2006; Shinohara et al. 2011; Fujiwara et al. 2012; Chinta et al. 2014). These microbes rapidly colonize the nutrient solution and rhizosphere of the cultivated plants (Valance et al. 2010). In our earlier study, beneficial microorganisms were observed in the organic nutrient

solution through their antagonistic action against *Fusarium oxysporum* f.sp. *lactuca* (FOL), a causal agent of root rot disease in lettuce (Chinta et al. 2014). Similar to our results, Fujiwara et al. (2013) reported that rhizosphere microbes in organic hydroponics successfully suppressed FOL. Furthermore, the colonization of microbes on the plant root surface, also known as biofilm (Bogino et al. 2013; Ramey et al. 2014), has protective effects against pathogens (Ramamoorthy et al. 2001; Haggag 2010; Saharan & Nehra 2011).

The mode of disease suppression can be elucidated indirectly via stimulation of plant resistance (van Loon et al. 1998; Pieterse et al. 2003; Verhagen et al. 2006; Saharan & Nehra 2011), wherein the stimuli are called inducers or elicitors (Tamm et al. 2011; Thakur & Sohal 2013). Elicitors are divided into ‘general elicitors’ and ‘race specific elicitors’. Whereas general elicitors trigger defense responses in both host and nonhost plants, race specific elicitors only do so in specific host cultivars (Thakur & Sohal 2013). For instance, rhizosphere microbes (Ramamoorthy et al. 2001) and the biochemical they produce (Pal & Gardener 2006; Thakur & Sohal 2013) are possible elicitors that initiate responses on the root surface that activate signal transduction pathways and stimulate positive regulators of plant resistance (Thakur & Sohal 2013). The reduction of disease symptoms in above-ground plant parts due to the presence of rhizosphere microbes on the plant roots is known as induced systemic resistance (ISR) (van Loon et al. 1998; Ramamoorthy et al. 2001; Pieterse et al. 2003;

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van Loon 2007; Bogino et al. 2013). ISR was previously described by van Peer et al. (1991) in carnation and Wei et al. (1996) in cucumber. ISR may also occur against foliar pathogens, such as *B. cinerea*, in crops cultivated hydroponically.

The signal transduction pathway involved in plant responses to biotic and abiotic challenges depends on the action of the following plant hormones: salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) (Verhagen et al. 2006). In ISR, rhizosphere microbes trigger the JA/ET pathway by accumulating JA and ET. Moreover, pathogens and other challenges up-regulate SA, eventually leading to systemic acquired resistance, a defense response synonymous to ISR (Ramamoorthy et al. 2001; Pieterse et al. 2003; Verhagen et al. 2006; van Loon 2007; Haggag 2010; Saharan & Nehra 2011). SA also conjugates with  $\beta$ -glucoside to form salicylic acid  $\beta$ -glucoside (SAG), which accumulates around disease lesions as an early response to pathogen infection (Conrath et al. 1995). Therefore, plant resistance is often dependent on complex signaling interactions among JA, ET, and SA (Ramamoorthy et al. 2001; Verhagen et al. 2006).

Genes associated with plant health and resistance have been identified. For instance, a 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (*ACS*) gene promoted by ET was identified in melon (*ACSI*) (Gerchikov et al. 2008) and citrus (*CS-ACSI*) (Wong et al. 2011). In lettuce, *Ls-ACSI* is induced by ET and auxin (Takahashi et al. 2003). In addition, JA induces the lipoxygenase (*LOX*) gene in cucumber as a response to mechanical wounding (Matsui et al. 2006). Moreover, an ET receptor-related gene (*CTR*) was a candidate gene for plant tolerance to abiotic stimuli in cucumber (Xiao-Hua et al. 2012).

In the present study, we expanded on our previous findings, which demonstrated that organic hydroponics control soil-borne disease in lettuce (Chinta et al. 2014). We hypothesized that organic hydroponics should also induce systemic resistance in lettuce against the airborne causal disease, *B. cinerea*, and we aimed to clarify the underlying molecular mechanisms of this defense pathway. To verify that ISR is activated in lettuce, an inoculation test was conducted on leaves that were spatially separated from the lettuce biofilm. We also verified the existence and type of elicitors in the hydroponic nutrient solution through foliar treatments on cucumber, a nonhost for lettuce biofilm. Foliar sprays have been shown to successfully stimulate ISR (van Loon et al. 1998). Finally, to confirm the plant resistance signaling pathway, SA in lettuce and ISR marker genes in both lettuce and cucumber were examined.

## 2. Experimental

### 2.1. Hydroponic cultivation

The organic and chemical cultivation system used was as described in a previous study (Chinta et al. 2014). Treatments were divided into two categories: organic (CSL)

and chemical (Otsuka A; hereafter Ot A) hydroponics. Lettuce seeds (*Lactuca sativa* L. var. *crispa* cv. summer green) were grown on 23 × 23 × 28 mm (*L* × *D* × *H*) rock-wool cubes in growth chamber as an appropriate condition for preparing the lettuce seedlings. The prepared seedlings were transplanted in the hydroponic system for five weeks before the start of the experiments.

### 2.2. Nonhost plant preparation and foliar spraying technique

Cucumbers (*Cucumis sativus* L. cv. Shimoshirazujibai) were used as the nonhost plant. Seeds were grown in seed-sown soil (Supermix-A; Sakata Seed Corporation, Yokohama, Japan) at room temperature for two weeks or until the first leaf fully opened.

Using a 50 ml tube, organic and chemical nutrient solutions (Chinta et al. 2014) were collected from the hydroponic system with lettuce plants, which was built five weeks earlier. Each solution (1 ml per plant) was then sprayed on the upper side of cucumber leaves. Untreated cucumber plants (hereafter, 'No-treatment') were used as the control. Plants were incubated at room temperature for 24 h before the inoculation test. Eight to twelve plants were tested for each experiment.

### 2.3. Fungal preparation and inoculation method

*B. cinerea* was cultured on potato dextrose agar medium for a week in a 25°C incubator. The inoculum was prepared as 4-mm mycelial discs. Lettuce leaves and cucumber leaves sprayed with nutrient solution were detached from the plants and challenged with *B. cinerea* (Verhagen et al. 2010; Yoshino et al. 2011) by attaching mycelial discs to the lower side of the leaves. Leaf samples were incubated at 25°C for five days. On the final day of incubation, the diameter of the gray mold lesions was measured. Three lettuce plants in triplicate and three cucumber plants in four replications were prepared.

### 2.4. Free SA and SAG quantification

Lettuce plants subjected to the CSL and OtA treatments (six for each condition) were prepared to quantify the amount of free SA and SAG. Quantification was performed following methods previously detailed by Widiastuti et al. (2013). Each leaf was cut and weighed (minimum weight 0.3 g). The leaves were frozen in liquid nitrogen and immediately ground using a pestle and mortar. A solution of 80% methanol (MeOH) was added to the ground leaves, and they were transferred to a 15 ml tube. MeOH was added until the volume was 10-fold. Samples were centrifuged at 4300 × *g* for 15 min. The supernatant was collected and 2 ml of the supernatant was applied to a solid-phase extraction cartridge (Cleanert C18; Bonna-agela Tech., DE, USA), which had been equilibrated with 80% MeOH. The cartridge was eluted twice with 1 ml of 80% MeOH. The volume of combined eluate was adjusted to 5 ml using 100% MeOH. An aliquot (1 ml) of the eluate was dried, and

the dried residue was dissolved in 1 ml of 20% MeOH, filtered through a Millipore 0.45 mm membrane filter (Merck Millipore, Billerica, MA, USA), and subjected to LC/MS/MS analysis. The standard methanolic solution was prepared by diluting SA (Kanto Chemical Co. Inc., Japan) and SAG provided by Dr Hasegawa from Ibaraki University. The concentrations of the standard solutions were 0.2, 2, 20, and 200 ppb. Analyses were conducted using a Shimadzu UFLC system (Shimadzu Corp., Japan) equipped with an SIL-20AC auto sampler and a Shim-pack XR-ODS (2.0 mm id × 30 mm) column (Shimadzu Corp., Japan). The column temperature was maintained at 40°C, and the injected volume was 20 µl. The elution gradient was obtained with a binary solvent system consisting of 0.1% CH<sub>3</sub>COOH in H<sub>2</sub>O (solvent A) and MeOH (solvent B) at a total flow rate of 0.2 ml/min. The gradient program was as follows: linear gradient of 5–100% B, 0–5 min; isocratic elution of 100% B, 5–8 min. MS/MS analysis was performed using a linear ion trap quadrupole LC/MS/MS spectrometer (3200 QTRAP; AB SCIEX, Framingham, MA, USA) controlled by Analyst 1.5.1 software. The ion source (Turbo V) was operated in the negative electrospray ionization mode. The source parameters were set as follows: curtain gas, 15 psi; temperature, 600°C; spray gas, 70 psi; dry gas, 80 psi; ion spray voltage, –4500 V; and declustering potential, –25 V. The mass spectrometer was operated in reaction-monitoring mode. SA and SAG levels were measured by monitoring the following transitions (in parentheses, entrance potential, EP; collision cell exit potential, CEP; collision energy, CE): SA: 136.9–93.0 (EP –2.5 V, CEP –16 V, CE –24 V); SAG: 299.0–136.9 (EP –2 V, CEP –20 V, CE –22 V). The amount of free SA and SAG was recorded as ng g<sup>-1</sup> fresh weight (FW) of lettuce leaves.

### 2.5. Gene expression analysis by quantitative real time-polymerase chain reaction (qRT-PCR)

Eleven lettuce plants and five cucumber seedlings were prepared for RNA extraction and first-strand cDNA synthesis, following methods described by Widiastuti et al. (2011, 2013). Subsequently, qRT-PCR was performed to evaluate the expression of defense-related genes (Table 1). The *Ls-ACSI* sequence was obtained from the National Center for Biotechnology Information

(NCBI, <http://www.ncbi.nlm.nih.gov/>). In addition, the cucumber *LOX* and *CTR* sequences were obtained from the DNA Data Bank of Japan (DDBJ, <http://www.ddbj.nig.ac.jp>). qRT-PCR was conducted on a Dice Real Time System Thermal Cycler (TP850; Takara Bio Inc., Otsu, Shiga, Japan). The thermal cycling conditions were as follows: incubation at 95°C for 10 min, and 45 cycles at 95°C for 15 s, 59°C for 30 s, and 72°C for 30 s. The dissociation step consisted of one cycle at 95°C for 15 s, 60°C for 30 s, and 95°C for 15 s. Actin expression of both lettuce and cucumber was used as a reference to normalize gene expression.

### 2.6. Statistical analyses

The lettuce data were analyzed using a two-tailed unpaired *t*-test. The cucumber data were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. Significance was defined as *P* < 0.05. All statistical analyses were performed in Excel Toukei 2012 software (SSRI Co., Ltd., Tokyo, Japan).

## 3. Results

### 3.1. Induction of lettuce resistance against gray mold disease

The diameter of gray mold lesions on lettuce cultivated under CSL and Ot A treatments is shown in Figure 1. At five days after inoculation, slight symptoms of gray mold disease were observed on lettuce leaves subjected to CSL treatment (Figure 1a). In contrast, lettuce leaves subjected to Ot A treatment were severely susceptible to *B. cinerea* and exhibited serious soft rot (Figure 1b). The CSL treatment significantly reduced the disease symptoms by 64% compared to the Ot A treatment (Figure 2).

### 3.2. Expression of ISR-related genes in lettuce

Figure 3 shows the lettuce gene expression in response to different environmental conditions. Cultivation of lettuce under the CSL treatment increased expression of *Ls-ACSI* in comparison to lettuce in the Ot A treatment, and this difference was statistically significant.

Table 1. Primers pairs used for qRT-PCR analysis.

Gene name	GenBank accession number	Forward/reverse primers
<i>Lactuca sativa</i> L. actin ( <i>Ls-Act</i> )	AY260165	F 5'-tggtgtgatgtaggtatggccag-3' R 5'-agggtgctcttcaggagcaacacg-3'
<i>Lactuca sativa</i> L. ACC synthase gene ( <i>Ls-ACSI</i> )	AF380836	F 5'-ggtttgaccctaattcgattgtg-3' R 5'-gtggcaccacaagaaggcttcac-3'
Cucumber actin ( <i>Actin</i> )	AB698859	F 5'-tctgtccctctacgtagtgac-3' R 5'-tccaaacggagaatggcatgagg-3'
Lipoxygenase gene ( <i>LOX</i> )	AJ271161	F 5'-cgctgtgaacttcgggcaatac-3' R 5'-tgtccctggtccggcatgaa-3'
Ethylene receptor-related gene ( <i>CTR</i> )	AB026498	F 5'-acgagtgtctcccaggaaca-3' R 5'-agcccatccatgccagctctga-3'

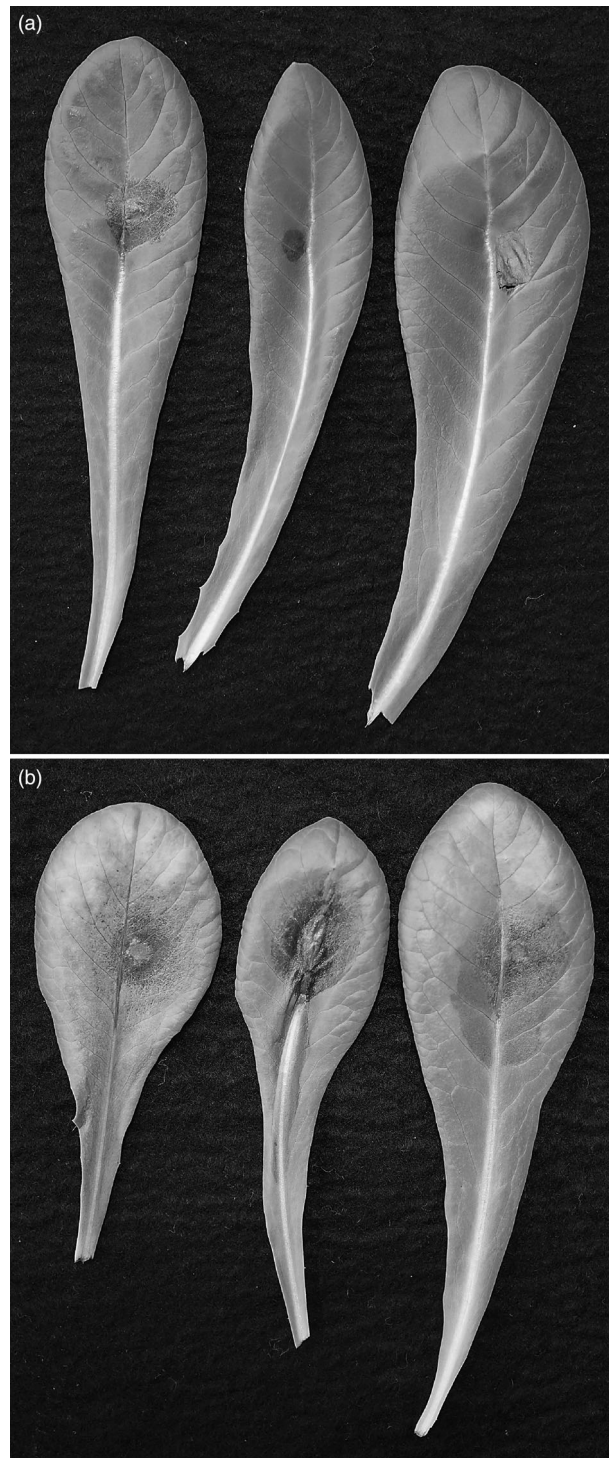


Figure 1. Comparison of lettuce (*Lactuca sativa* L.) resistance against *Botrytis cinerea* at 5 days post-inoculation on lettuce cultivated in (a) organic hydroponics and (b) chemical hydroponics.

### 3.3. Quantity of SA in CSL and Ot A treatments

Free SA and SAG quantities under the two treatments are illustrated in Figure 4. CSL and Ot A treatments caused no significant differences in the amount of free SA per FW of plant. Conversely, a significant difference was found in SAG quantities between the two treatments. The SAG levels of lettuce cultivated in Ot A and CSL treatments were 79.45 and 15.13 ng g<sup>-1</sup> FW, respectively.

### 3.4. Existence of elicitors

Foliar spraying of organic nutrient solution onto cucumber leaves clearly reduced the gray mold lesion caused by *B. cinerea* (Figure 5b). In contrast, similarly severe soft lesions occurred in the No-treatment (Figure 5a) and Ot A treatment (Figure 5c). Figure 6 shows that CSL treatment significantly decreased the diameter of soft rot lesions on the leaves, compared to the No-treatment condition and Ot A nutrient solution treatment.

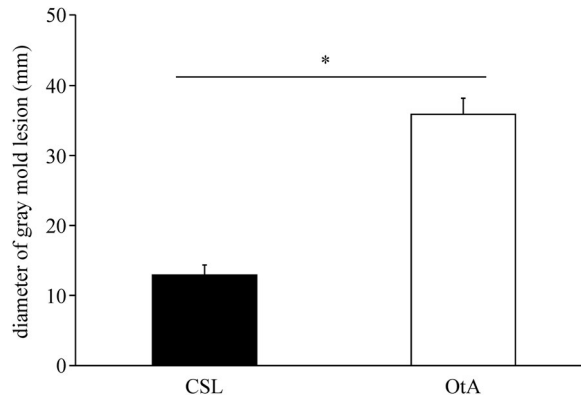


Figure 2. Induction of lettuce resistance against gray mold disease (*Botrytis cinerea*) in organic hydroponics. The asterisk indicates a significant difference according to a two-tailed unpaired *t*-test ( $n = 3$ ;  $P < 0.05$ ). Vertical bars show the standard error.

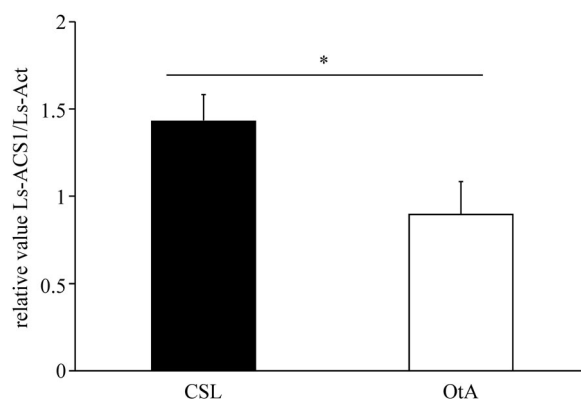


Figure 3. Expression of ACC synthase gene in lettuce (*Ls-ACSI*) on lettuce plants cultivated under different hydroponic systems. Relative values of gene expression are compared to actin gene expression for each treatment. The asterisk indicates a significant difference according to a two-tailed unpaired *t*-test ( $n = 11$ ;  $P < 0.05$ ). Vertical bars show the standard error.

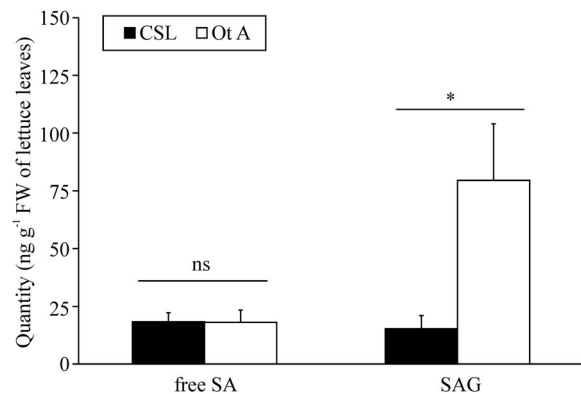


Figure 4. Quantities of free SA and SAG in lettuce leaves of different hydroponic systems. SA and SAG amounts were measured as  $\text{ng g}^{-1}$  FW of five-week old lettuce leaves. The asterisk indicates a significant difference and ns indicates non-significance, according to a two-tailed unpaired *t*-test ( $n = 3$ ;  $P < 0.05$ ). Vertical bars show the standard error.

### 3.5. Elicitor-induced expression of ISR-related genes in cucumber

Figure 7 shows the relative values of *LOX* and *CTR* gene expression in cucumber after the application of CSL and Ot A nutrient solutions. Expression of both ISR-related genes significantly increased after foliar spraying of the organic nutrient solution. In contrast, expression of *LOX* and *CTR* was not significantly different from that under either the Ot A treatment or the No-treatment.

## 4. Discussion

Organic hydroponics was demonstrated to protect crops against the air-borne pathogen *B. cinerea* in this study. At five days after *B. cinerea* challenge, lettuce grown using organic hydroponics showed a significant suppression of gray mold symptoms, whereas lettuce grown in chemical hydroponics exhibited large disease lesions. Hammerschmidt et al. (2001) suggested that the suppression of disease incidence indicates an induction of plant

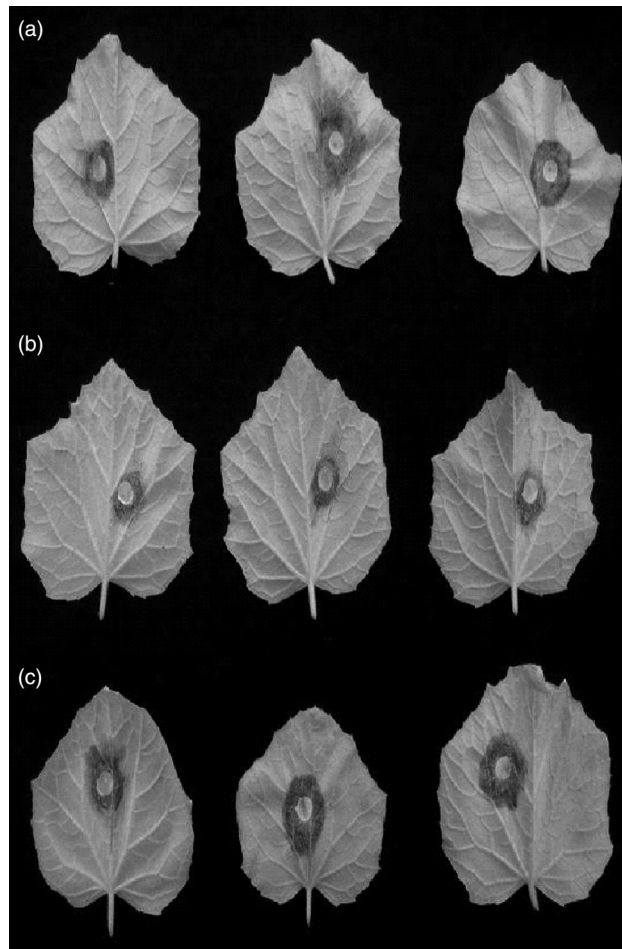


Figure 5. Gray mold (*Botrytis cinerea*) symptoms on nonhost cucumber (*Cucumis sativus* L. cv. Shimoshirazujibai) seedlings after foliar spraying of organic and chemical nutrient solutions at 5 days after inoculation. (a) No-treatment; (b) organic nutrient solution; and (c) chemical nutrient solution.

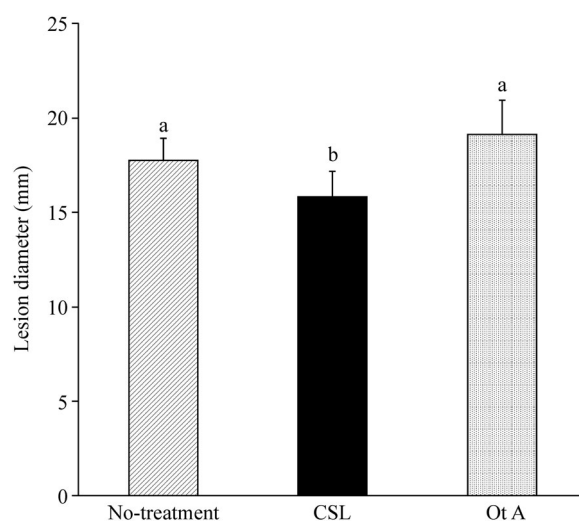


Figure 6. Effects of different nutrient solutions sprayed on nonhost cucumber (*Cucumis sativus* L. cv. Shimoshirazujibai) seedlings against gray mold disease (*Botrytis cinerea*). The asterisk indicates a significant difference according to Tukey's multiple comparison test ( $n = 4$ ;  $P < 0.05$ ). Vertical bars show the standard error.

resistance. Therefore, our finding suggests that resistance was induced in lettuce plants cultivated in organic hydroponics, but not in lettuce cultivated in chemical hydroponics.

To investigate the molecular mechanism underlying the induction of lettuce resistance, the expression of a plant health-related gene was examined. *Ls-ACSI* gene expression increased significantly under the CSL

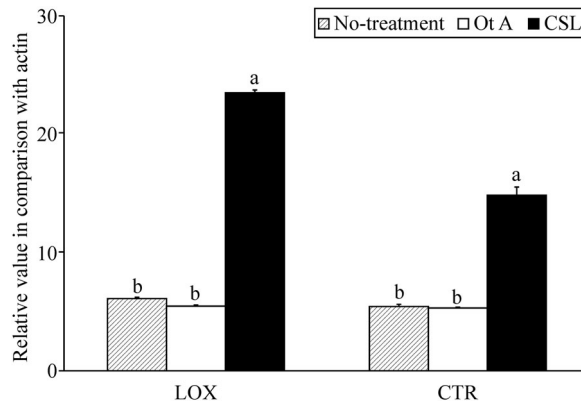


Figure 7. Expression pattern of JA/ET-response genes in nonhost cucumber (*Cucumis sativus* L. cv. Shimoshirazujibai) seedlings in response to foliar spraying with different nutrient solutions. Defense-related genes were lipoxygenases (*LOX*) and ethylene receptor related (*CTR*). The asterisk indicates a significant difference between treatments for each defense-related gene according to the Tukey's multiple comparison test ( $n = 5$ ;  $P < 0.05$ ). Vertical bars show the standard error.

treatment compared to the Ot A treatment, implying that resistance was triggered at a molecular level after cultivation in organic hydroponics. Conversely, the chemical hydroponic cultivation system did not molecularly induce systemic resistance in lettuce plants.

Regulation of the marker gene *Ls-ACSI* in lettuce correlates with root hair development and is promoted by auxin and ET (Takahashi et al. 2003). Lettuce roots grew well and reached the nutrient solution rapidly in organic hydroponics, which also yielded luxuriant lettuce root hair, similar to previous studies by another group (Shinohara et al. 2011) and our group (Chinta et al. 2014). This root hair may be critical for the formation of biofilm by beneficial microorganisms. We had previously suggested that production of a natural auxin involved in root growth, indole-3-acetic acid, may be triggered by the association between lettuce roots and beneficial microorganisms (Chinta et al. 2014). Furthermore, increasing levels of ACC, the direct precursor of ET, in the plant biosynthesis pathway also increases root growth (van Loon et al. 2006). Our current results imply that CSL treatment in organic hydroponics stimulated the production of auxin and ET, which then induced high expression of *Ls-ACSI*. Thus, auxin and ET triggered root hair development and facilitated biofilm formation. In contrast, chemical hydroponics resulted in very little root hair development and a lack of biofilm, which is consistent with the low expression of *Ls-ACSI* in this treatment.

The beneficial microorganisms that form biofilm may trigger ISR by altering biochemical reactions in the host (Haggag 2010). We suggest that microbial wounding, one of the mechanisms causing ISR, likely occurred during the interaction between beneficial microorganisms and lettuce roots to induce biofilm formation. This physical interaction then increased ET production in CSL-cultivated lettuce plants. Multiple lines of research have indicated that high amounts of ET produced around microbial wounding sites trigger the ET-signaling pathway to activate ISR (van Loon et al. 2006). Therefore, the present results clearly demonstrate that organic hydroponics induces plant-mediated systemic resistance,

wherein beneficial microorganisms initiate plant resistance in lettuce roots through the ET-signaling defense pathway. The resultant cascade then suppresses disease incidence in lettuce leaves. Furthermore, the variation in *Ls-ACSI* expression in this study suggests that *Ls-ACSI* has the potential to be a marker gene for plant defense, which should contribute to the currently limited research on molecular mechanisms of induced resistance in lettuce.

In addition to ET, SA is also an important signal in induced resistance (Conrath et al. 1995; Verhagen et al. 2006). To determine whether SA played a role in ISR in lettuce, free SA and SAG quantity was analyzed. The amount of free SA in lettuce leaves was not significantly different between organic and chemical hydroponics. Conversely, Ot A treatment resulted in significantly higher SAG quantity than CSL treatment. Despite this result, lettuce plants cultivated in chemical hydroponics were susceptible to gray mold disease. Conrath et al. (1995) reported that a threshold level of SA is required for enhanced plant resistance; thus, the SA concentration in Ot A-treated lettuce leaves may have been inadequate to induce defense responses against infection by *B. cinerea*. In comparison, SAG levels were reduced in organic hydroponics. Several studies have shown that SA is not present in rhizobacteria-mediated ISR (Pieterse et al. 2003; Shores et al. 2005; Verhagen et al. 2006). Therefore, we conclude that in contrast to ET, SA was not induced by microbe–root interactions and played no role in ISR induced by organic hydroponics.

Resistance of lettuce to gray mold was stimulated by biotic elicitors in the form of biofilm. Furthermore, cucumber resistance against *B. cinerea* also increased significantly after spraying with organic nutrient solution. This outcome on a nonhost plant indicates the existence of elicitors that are independent from direct interactions with beneficial microorganisms. These independent elicitors could be substances produced by microbes and substances contained in the organic nutrient solution. Microorganisms generate many substances that can trigger plant resistance, such as carbohydrates, proteins, lipids, glycoproteins, and volatile compounds (Thakur



& Sohal 2013). Specifically, *Bacillus subtilis* and its bio-products, polysaccharides (Beauregard et al. 2013) and siderophores (Saharan & Nehra 2011), have been reported to induce resistance in a number of plants. We suggest that *Bacillus* sp. bacteria, identified in organic hydroponics (Fujiwara et al. 2013), also produced polysaccharides and siderophores as elicitors in this study. Moreover, CSL also consists of carbohydrates and volatile compounds that possibly act as antimicrobial substances (Chinta et al. 2014) and are likely to contribute as elicitors in the organic nutrient solution. Therefore, the organic nutrient solution was likely rich in both nutrition and elicitors.

Spraying of the chemical nutrient solution resulted in disease symptoms as severe as those in the untreated leaves, which suggests that the chemical nutrient solution lacked elicitors and was unable to induce resistance against *B. cinerea* infection in cucumber. The organic nutrient solution was sprayed on the upper side of leaves, whereas the pathogen was inoculated on the lower side of the leaves. The difference in the location of application implies that an indirect interaction between the elicitors and *B. cinerea* may have occurred. Consequently, cucumber resistance was assumed to be a plant-mediated induced resistance, similar to ISR in lettuce. In addition, organic hydroponics induced resistance in both lettuce and cucumber, implying that the elicitors in the nutrient solution are general elicitors.

The gene expression analyses in cucumber corroborated the observed *Ls-ACSI* expression in lettuce. Specifically, the organic nutrient solution increased the gene expression of both *LOX* and *CTR*. *LOX* is regulated by JA (Matsui et al. 2006) and *CTR* is controlled by ET (Xiao-Hua et al. 2012). Thus, we suggest that the general elicitors in the organic nutrient solution were recognized by cucumber cells, which then activated JA and ET to regulate the over-expression of *LOX* and *CTR*, respectively. These defense mechanisms in CSL-treated cucumbers confirmed that JA- and ET-response pathways are essential for ISR in the cucumber. In contrast, the presumed absence of elicitors in the chemical nutrient solution caused *LOX* and *CTR* gene expression in Ot A-treated cucumbers to be as low as that in No-treatment cucumbers. In summary, the pattern of ISR-related gene expression in lettuce and cucumber plants indicates that both crops responded to general elicitors and that plant resistance was activated via the JA/ET-signaling pathway.

In conclusion, organic hydroponics can trigger ISR in lettuce through biofilm formed from the interaction between beneficial microorganisms and lettuce roots. Moreover, foliar spraying of the organic nutrient solution conferred the same beneficial effects on cucumber, a nonhost for lettuce biofilm. This result indicates that general elicitors are present in the organic nutrient solution. Additionally, both organic hydroponics and foliar sprays are potentially valuable methods to protect crops against plant disease. In particular, organic hydroponics successfully suppressed *B. cinerea*, demonstrating that CSL-enriched hydroponics is effective

against soil-borne and air-borne pathogens. Finally, our study contributes to an increased understanding of the mechanisms underlying elicitor, host, and pathogen interactions, which should provide facilitate future research on the molecular basis of plant responses to environmental stress.

### Disclosure statement

The authors declare that they have no competing interests.

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