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Increased Expression of the Tomato *SlSWEET15* Gene During Grey Mold Infection and the Possible Involvement of the Sugar Efflux to Apoplasm in the Disease Susceptibility

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Abstract

Host plant susceptibility genes, which facilitate pathogen growth during plant infection, are attractive targets for disease-resistance breeding. To explore candidate susceptibility genes in tomatoes during *Botrytis cinerea* infection, the fungal infection-responsive *SWEET* genes were screened for out of all 31 tomato *SlSWEET* genes. The expression of only one gene, *SlSWEET15*, was induced by *B. cinerea* at the pre-necrotic stage (16 h post inoculation), whereas most of the other *SWEET* genes were downregulated. The expression of the *SlSWEET15* transiently increased by 16 h post inoculation, then reduced to basal levels by 24 h post inoculation. We measured the glucose and sucrose contents of apoplasmic fluid of infected cotyledons at the pre-necrotic stage (20 h post inoculation). The sugar contents of the apoplasmic fluids were significantly higher in the infected cotyledons compared to 0 h. Furthermore, glucose and sucrose can promote growth and invasion of *B. cinerea* both *in vitro* and *in vivo*. SWEET proteins in clade III, including the deduced SlSWEET15, are well-known sugar efflux transporters. These results suggest that *SlSWEET15* is induced by *B. cinerea* and that this is exploited by the fungus, which may provide sugars to promote hyphal growth in the pre-necrotic stage of infection in tomato.

Keywords: Tomato; *Botrytis cinerea*; SWEET sugar transporters; Susceptibility genes

Introduction

Necrotrophic fungi are the largest class of fungal phytopathogens and cause serious crop losses worldwide [1]. *Botrytis cinerea*, a typical necrotrophic fungus, is an important plant pathogen with a wide range of host plants, causing gray mold disease in over 200 plant species, including most vegetable and fruit crops, trees and flowers [2]. *B. cinerea* causes massive losses in some field and greenhouse-grown horticultural crops, including tomatoes [3]. The damage to tomato yields caused by *B. cinerea* is enormous, and the fungus is difficult to control because it has a variety of pathogenic mechanisms, that can use diverse hosts as inoculum sources, and can survive as mycelia and/or conidia or for extended periods as sclerotia in crop debris [3]. Furthermore, promising genetic resources concerning grey mold resistance have not been found in the cultivated tomato (*Solanum lycopersicum* L.), despite ongoing efforts to identify genetic loci for resistance [4].

One of the alternative strategy for the breeding of disease-resistant crops is the utilization of a reverse genetics approach. Along with suppressing or evading plant immunity, most pathogens, especially biotrophs, require the cooperation of the host to establish a compatible interaction [5]. Host plant genes that facilitate infection and support compatibility are considered to be susceptibility genes. If a defective mutation occurs in a gene essential for pathogen susceptibility, the plant acquires resistance to that pathogen. A recessive resistance gene to powdery mildew, *mlo* (*mildew resistance locus O*) is the best example [6,7]. Powdery mildew resistant gene *mlo* was originally discovered in barley and characterized as a membrane-anchored protein [6]. The role of *MLO* in powdery mildew susceptibility has been confirmed in *Arabidopsis*, pea, tomato, pepper, wheat, and strawberry [5]. *MLO* seems to be required for susceptibility to adapted pathogens, and *mlo* mutants display a loss of susceptibility resembling that described for non-host resistance [7].

Recently, it has been revealed that several sugar efflux transporter encoding genes, *SWEETs*, are important susceptibility factors, therefore, could be candidate recessive disease resistance genes. Plant pathogens, especially biotrophs and hemi-biotrophs, depend on nutrient uptake from host plants. Thus, a reasonable hypothesis is that plant pathogens hijack the host nutrient transport system. In rice, two recessive genes that confer resistance to the hemi-biographic bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), *xa13* and *xa25* loci, encode SWEET proteins [8,9]. Additionally, the sugar transporter genes *OsSWEET11*, *12* and *14* are confirmed targets of *Xoo* effectors [10-12]. Furthermore, the expression of *Arabidopsis SWEET* genes is upregulated by several pathogens, including *B. cinerea* [9]. Interestingly, in addition to *Arabidopsis*, it was recently reported that the *VvSWEET4* gene, encoding a member of the grapevine SWEET sugar transporter family, is also induced during *B. cinerea* infection [13]. These findings support the hypothesis that necrotrophs, which had been thought to only take up nutrients from dead host cells, may be able to take up sugar from live host cells. Therefore, identifying pathogen-responsive *SWEET* genes will be an effective way of finding essential susceptibility factors, which may be ideal targets for disease-resistance breeding.

Here, we explored *SWEET* genes responsive to *B. cinerea* infection from all 31 *SlSWEET genes* in tomato and found that the fungus only induces expression of the *SlSWEET15* gene, which is a clade III SWEET sugar efflux transporter and transports sucrose and glucose [14]. The

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identification of possible candidate susceptibility factors for *B. cinerea* may lead to a deeper understanding of the complex process of *B. cinerea* infection and may provide attractive targets for breeding grey mold resistance in tomato. In the present study, we discuss the characteristics of *SlSWEET15* gene induction during *B. cinerea* infection and the possible utilization of the sugar transporter by the fungus.

Materials and Methods

Plant materials and growth conditions

The tomato (*Solanum lycopersicum* L.) cultivar Ponderosa was primarily used in the present study, and other cultivars, including strawberry, Micro-Tom, Soprano and Misora, were also used for *SWEET* gene expression analysis. Tomato seeds were germinated and grown on wet filter paper for 3 d before being transferred to soil. The seedlings were then incubated in a growth chamber under 16-h-light at 25°C/8-h-dark conditions at 19°C.

Fungal strains and growth conditions

Botrytis cinerea highly pathogenic strains MB1209 were used for inoculation assays. The fungus was maintained on potato dextrose agar (PDA; 1.5% agar was added to potato dextrose broth, BD Difco, Sparks, MD, USA) at 25°C. For sporulation, 5 mm × 5 mm mycelial mats were transferred to PDA plates and incubated for 8 d under BLB light at 25°C.

Inoculation methods and assays for fungal invasion

Tomato (cv. Ponderosa) cotyledons were harvested from 3- to 4-weeks-old plants and placed in glass Petri dishes moistened with a wet filter paper. Conidia of *B. cinerea* were collected from 2-week-old cultures and suspended in 2-fold diluted PDB potato dextrose broth (1/2 PDB) at 1×10^6 conidia/ml. A small piece of filter paper (3 mm × 3 mm) was put on the center of the tomato cotyledon, and then a droplet of the fungal spore suspension (5 µl) was deposited beneath the filter paper. As mock treatment, 5 µl of 1/2 PDB was applied on the filter paper. The cotyledons were then incubated on water-moistened filter paper for the appropriate incubation time at 22°C. The biomass of *B. cinerea* was measured by quantitative PCR (qPCR) using *B. cinerea*-specific ITS primers (Table S1). qPCR was performed with the StepOne Plus system (Life Technologies, Foster City, CA, USA) and the Fast SYBR Green Master Mix kit (Life Technologies), using a program of 20 sec at 95°C, followed by 40 cycles of 95°C for 3 sec, annealing for 30 sec at 60°C, and melt curve stage at 95°C for 15 sec, 60°C for 1 min and 95°C for 15 sec. The average threshold cycle (Ct) was used to determine the fungal biomass.

All experiments were performed in biological triplicate, and statistical significance was determined using analysis of variance (ANOVA) with MS Excel software (Microsoft, Redmond, WA, USA).

Gene expression analyses

The tomato cotyledons inoculated with fungal spores were used for total RNA extraction at the 0, 8, 16 and 24 h post inoculation. RNA was extracted using the RNA Sui-Sui P kit (Rizo, Tsukuba, Japan) following the manufacturer's instruction. RNA concentrations and 260/280 nm ratio were measured using a spectrophotometer (Du650, Beckman Coulter, Fullerton, CA, USA). Total RNA from healthy tissues was extracted by the same method. For quantitative reverse transcription PCR (qRT-PCR), total RNA from each sample was treated with DNase I (DNase I recombinant, RNase-free; Roche Diagnostics, Mannheim, Germany), followed by cDNA synthesis using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies). cDNA derived from 25 ng of total RNA was used for each qRT-PCR reaction with gene-specific primer sets (Table S1). The tomato SAND family protein-encoded gene (*SlSAND*) was used as an internal control [15]. Additionally, expression of the *PR1a1* gene was measured using a *PR1a1* specific primer set to monitor fungal invasion (Table S1). Primer sets for each *SWEET* gene were designed using Primer Express 3 software (Life Technologies). The names of tomato *SWEET* genes are written according to the nomenclature of Lin et al. [16]. Real-time qRT-PCR was performed with the StepOne plus system as described in the methods for qPCR, using a program of 20 sec at 95°C, followed by 40 cycles of 95°C for 3 sec, annealing for 30 sec at 60°C, and melt curve stage at 95°C for 15 sec, 60°C for 1 min and 95°C for 15 sec. The average threshold cycle (Ct) was used to determine the fold change of gene expression.

All experiments were performed in biological triplicate and statistically analyzed by ANOVA.

Observation of hypersensitive cell death in tomato cotyledons

The tomato cotyledons were inoculated with spores of *B. cinerea* and incubated as described above. Cotyledons were collected at 0 h, 20 h and 48 h post inoculation, followed by staining-clearing treatment described by Bruzzese and Hasan [17]. Whole leaves were fixed and stained in a staining solution (95% ethanol 300 ml; chloroform 150 ml; 90% lactic acid 125 ml; phenol 150 g; chloral hydrate 450 g; aniline blue 0.6 g) for 48 h and then treated with concentrated chloral hydrate (2.5 mg/ml in distilled water) for 24 h. After rinsing by water, samples were examined with a light microscope (Axioskop2 equipped with a digital camera AxioCam HRc, Zeiss, Hallbergmoos, Germany).

Extraction of apoplasmic fluids from tomato cotyledon and measurements of sugar concentration

The tomato cotyledons were inoculated with spores of *B. cinerea* and incubated as described above. Cotyledons were collected at 0 h and 20 h post inoculation. Apoplasmic fluid was collected and measured sugar concentrations, according to a method described by Zhou et al. [12]. In brief, distilled water was infiltrated into tomato cotyledons with 2.5 ml plastic syringes. The cotyledons were centrifuged for 10 min at 1,000 × g in a swinging bucket rotor at 4°C. Apoplasmic fluids were move into new sample tubes. The concentrations of glucose and sucrose were measured by the Sucrose/D-Glucose assay kit (Roche/R-Biopharm, Darmstadt, Germany).

All experiments were performed in biological triplicate and statistically analyzed by ANOVA.

Assay for the effects of sugar on invasion of tomato cotyledons and fungal spore growth

To mimic sugar exudation from mesophyll cells to the plant-fungus interface, tomato cotyledons were inoculated with fungal spores that were suspended in 20 mM or 40 mM glucose or sucrose instead of 1/2 PDB. The lesion size and fungal biomass were measured as described in the methods above.

To investigate the direct effects of sugars on fungal spore growth, spores were suspended in sugar solutions, and then droplets of the fungal spore suspension (5 µl) were deposited on glass slides. The spore suspension was incubated in a moist chamber at 22°C, followed by observation under Zeiss Axioskop2 microscope. The length of the germ tube was measured on digital images using ImageJ software [18] 8 h after incubation.

All experiments were performed in biological triplicate and statistically analyzed by ANOVA.

Results

Changes in the expression of tomato *SWEET* genes during *B. cinerea* infection

Plant genomes typically contain approximately 20 *SWEET* paralogs, which are differentially expressed [19]. According to Lin et al. [16], 30 *SWEET* genes were identified in the tomato genome. Cell death in infected cotyledons was observed under the microscope appeared 48 h post inoculation, whereas no cell death had formed by 20 h post inoculation (see Figure 3a). We focused on the pre-necrotic stage of *B. cinerea* infection (until 20 h post inoculation), because it is presumed that no sugar leakage from dead cells occurred in this stage. Primer sets for all 31 tomato *SWEET* (*SlSWEET*) genes were designed, and the expression of each gene was investigated by qRT-PCR at 0 h and 16 h post inoculation with *B. cinerea*. According to phylogenetic analysis based on the deduced amino acid sequence of *SWEET* gene products, *Arabidopsis* [19] and rice [11] *SWEET* genes are subdivided into four clades, clades I to IV. Thirty *SlSWEET* genes also fall into these four clades (Figure S1). The changes in gene expression of the 31 *SlSWEET* at an early stage of *B. cinerea* infection were investigated by qRT-PCR (Figure 1). Expression of all *SlSWEET* genes was detected, except for *SlSWEET8a*. The expression of *SlSWEET8a* was never detected at all, even though two different primer sets were used for qRT-PCR (Table

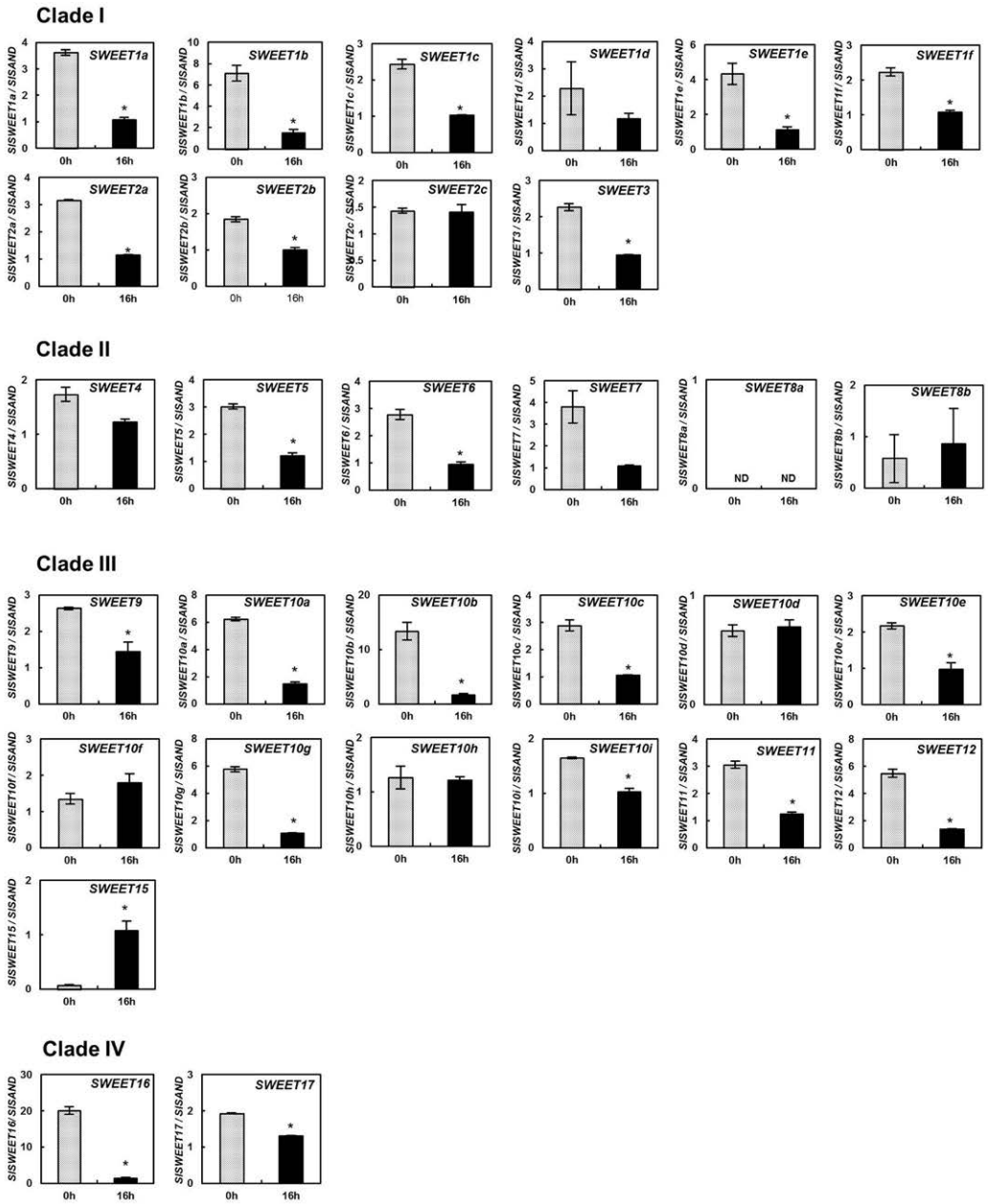

Figure 1: Expression of tomato *SlSWEET* genes in the cotyledon at the early stage of *B. cinerea* infection. Expression of each *SlSWEET* gene was measured by qRT-PCR 0 h and 16 h post inoculation. Transcript levels were normalized to the *SlSAND* gene. Values represent the mean and standard error of triplicate results. ND means 'not detected'. Asterisks indicate values that are statistically significantly different from the 0 h control using ANOVA ($P<0.05$).

S1). Also a large deletion (24 amino acids) was found in the functional domain (two triple-helix-bundle repeats) of the deduced gene product (Figure S2). Therefore, *SlSWEET8a* gene is likely to be a pseudogene. Twenty-one of the 30 expressing *SlSWEET* genes were significantly downregulated in infected tomato cotyledons by 16 h post inoculation relative to expression at the time of infection. Also *SlSWEET1d* and *7* were obviously downregulated by the fungal infection, although no significant differences were detected between 0 h and 16 h post inoculation because of relatively large fluctuations. All of these genes belong to one of the four *SlSWEET* gene clades. Thus, most of the *SlSWEET* genes were downregulated by *B. cinerea* inoculation at the early stage of infection. The expression of 6 *SlSWEET* genes, *SlSWEET2c* (clade I), *SlSWEET4* and *8b* (clade II), *SlSWEET10d*, *10f* and *10h* (clade III), was not significantly changed. Only *SlSWEET15*, which belongs to clade III, was significantly upregulated, and the expression of this gene was increased more than ten times 16 h post inoculation compared with the 0 h control (Figure 1).

Expression patterns of *SlSWEET15* gene in infected tomatoes

When the cotyledon was inoculated with *B. cinerea* spores, the expression of *SlSWEET15* was increased 3.4 to 8.1 times 16 h post inoculation, relative to the 0 h control, regardless of differences in tomato cultivars (Figure 2a). When the tomato cotyledon (cultivar Ponderosa) was inoculated with *B. cinerea*, expression of the *SlSWEET15* gene remained at basal levels until 8 h post inoculation, then transiently increased to 5.4 times at 16 h post inoculation compared with levels at 0 h. The transiently induced expression later decreased back to basal levels by 24 h post inoculation. No obvious changes in *SlSWEET15* gene expression were detected in the mock control over the course of the infection (Figure 2b). The expression of a well-known defense marker gene, *PR1a1*, was slightly upregulated at 16 h post inoculation and tended to continue increasing until at least 24 h post inoculation, showing the same tendency as the increase in fungal biomass (Figure 2c, 2d). These results suggest that *SlSWEET15* is a relatively early responsive and transiently expressed gene during *B. cinerea* infection in tomato.

Changes of sugar contents in apoplasm of *B. cinerea* infected cotyledons

Induced expression of sugar efflux transporter gene *SlSWEET15*, in the pre-necrotic stage of infection may trigger sugar leakage from live cotyledon cells to apoplasm. Therefore, we measured glucose and sucrose contents of apoplasmic fluids collected from infected cotyledons at the pre-necrotic stage. We preferred 20 h post inoculation as a time point for measurements of sugar contents, because no cell death was observed in cotyledons by 20 h post inoculation, while prominent cell death was induced in cotyledon tissue by 48 h post inoculation (Figure 3a). Infected and mock treated cotyledons were infiltrated with distilled water 20 h post inoculation, and the cotyledons were subjected to centrifugation to recover apoplasmic fluids. The glucose and sucrose content of the supernatant was 3.4 and 2.4-fold higher in apoplasmic fluids from infected cotyledons compared to 0 h control, respectively,

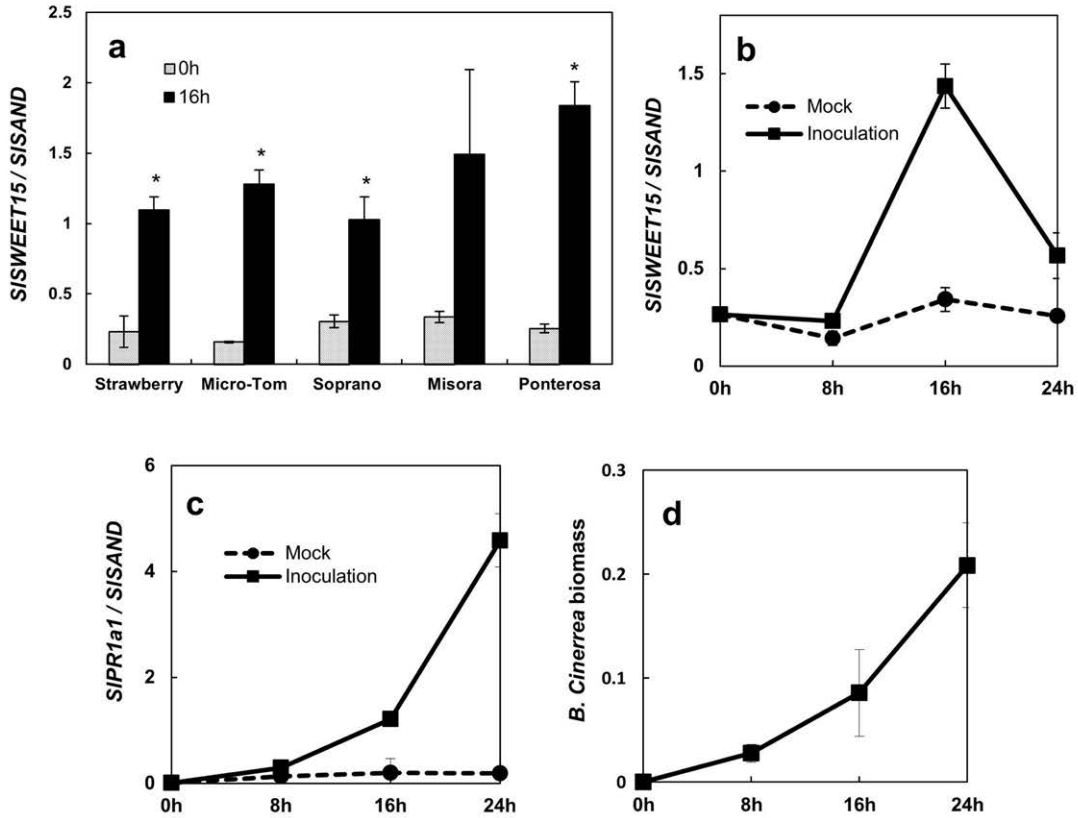

Figure 2: Expression of the *SlSWEET15* gene in different tomato varieties and time course of *SlSWEET15* gene expression. (a) Expression of the *SlSWEET15* gene was measured by qRT-PCR at 0 h and 16 h post inoculation in 5 different tomato cultivars. Asterisks indicate values that are statistically significantly different from the 0 h control using ANOVA ($P<0.05$). Expression of *SlSWEET15* (b) and *SlPR1a1* (c) genes were measured by qRT-PCR at 0, 8, 16 and 24 h post inoculation. In mock inoculations, cotyledons were treated with 1/2 PDB. Transcript levels were normalized to the *SlSAND* gene. (d) Biomass of *B. cinerea* was measured with qPCR at the same time points. Values represent the mean and standard error of triplicate results.

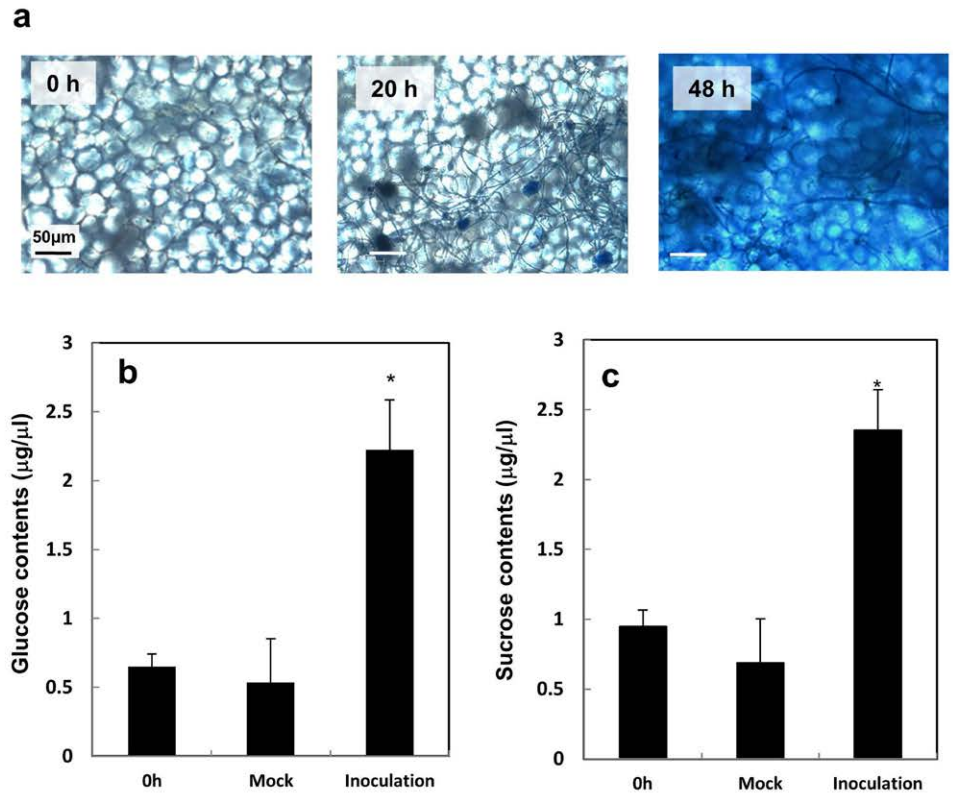

Figure 3: Sugar contents in apoplasmic fluids of *B. cinerea* infected cotyledons. (a) Light microscopies of aniline blue stained cotyledons inoculated with *B. cinerea* spores at 0, 20 and 48 h post inoculation. Note that no cell death was observed by 20 h post inoculation. Glucose (b) and sucrose (c) contents in apoplasmic fluids collected from 0 h, 20 h after mock treated and 20 h post inoculated cotyledons. Values represent the mean and standard error of triplicate results. Asterisks indicate values that are statistically significantly different from the 0 h control using ANOVA ($P<0.05$).

while no changes were observed in the mock treated cotyledons (Figure 3b,3c).

Effects of glucose and sucrose on fungal spore growth and invasion into plant tissue

The results above led us to hypothesize that sugar exudation to the plant-fungus interface may play an important role in plant permissiveness to fungal infection. This is supported by the fact that SWEET proteins are well-known hexose and sucrose efflux transporters [9,16], especially clade III SWEET proteins, which transport glucose and sucrose [14]. To test this hypothesis, spores were suspended in a glucose or sucrose solution to mimic the exudation of sugar to the plant-fungus interface, and tomato cotyledons were inoculated with the suspensions. When cotyledons were inoculated with spores suspended with distilled water, necrotic lesions never formed on tomato cotyledon 48 h post inoculation. However, necrotic lesions appeared on cotyledons inoculated with spores suspended in sugar solutions (Figure 4a). The lesions expanded in a sugar concentration-dependent manner in both sugar treatments, and lesion sizes tend to be larger in the case of sucrose treatment compared to glucose treatment (Figure 4b). A similar tendency was observed in the biomass of *B. cinerea*. The fungal biomass was more prominently and significantly increased 48 h post inoculation when spores were suspended in sucrose (Figure 4c).

To determine the direct effects on the fungal growth, spores were suspended in distilled water (control), glucose or sucrose, and then germ tubes elongation on a glass slides was measured 8 h after incubation (Figure 5). When spores were suspended with 20 mM and 40 mM glucose, germ tubes of *B. cinerea* elongated to approximately 50 µm, whereas only short germ tubes (27.7 µm in average) were formed in distilled water (Figure 5b). More prominently, germ tubes from spores suspended in 20 mM and 40 mM sucrose solutions were nearly twice as long (108 and 139 µm, respectively) as those suspended in glucose solution (Figure 5b).

These results suggest that fungal growth in the early stage of infection can be promoted by plant exudation of sugar, especially of sucrose, to the plant-fungus interface.

Discussion

Since 2010, when actual sugar efflux transporter activity was shown [9], knowledge of important roles of SWEET sugar transporters has been accumulated from a number of research groups over the last few years. The redundancy of SWEET genes (approximately 20 to 30 copies in the plant genome) implicates them as fundamental sugar transporters. Indeed, a wide variety of physiological roles, including pollen development [20], nectar secretion [16], phloem loading [14], seed development [21], salt stress [22] and freezing tolerance [23], have been described in *Arabidopsis*.

Simultaneously, it has become increasingly clear that SWEET transporters were hijacked by plant pathogens and used as a source of nutrients. Biotrophic pathogens must acquire sugars through their host plasma membrane [24,25] thus, the exploitation of host sugar efflux systems by pathogens is a reasonable strategy. Chen et al. [9] reported that the powdery mildew pathogen *Golovinomyces cichoracearum*

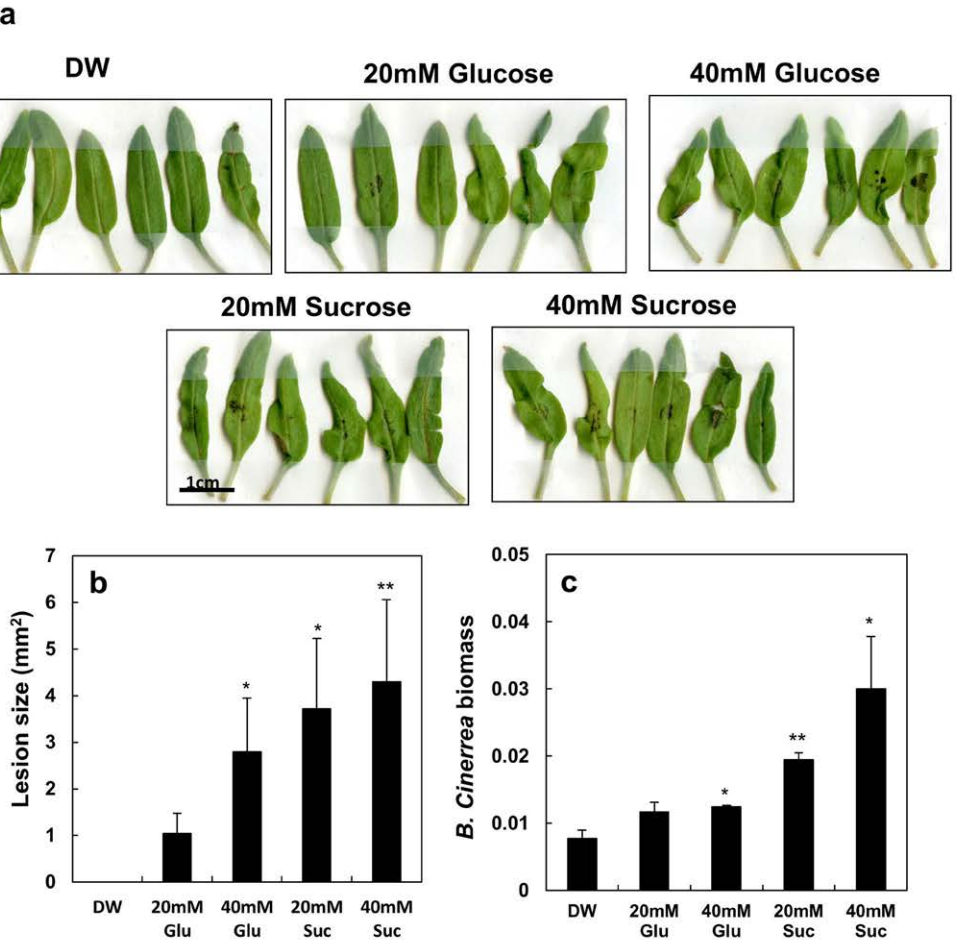

Figure 4: Effects of glucose and sucrose on lesion expansion and fungal growth of *B. cinerea*. To mimic sugar exudation from mesophyll cells, the fungal spores were suspended in 20 mM or 40 mM of glucose (Glu) or sucrose (Suc) solutions. (a) Images of tomato cotyledons infected with *B. cinerea* spores suspended in 20 mM and 40 mM glucose and sucrose solutions 48 h post inoculation. DW, cotyledons inoculated with distilled water-suspended spores. (b) Images of cotyledons were digitally scanned and the lesion areas were measured. (c) The biomass of *B. cinerea* was measured using qPCR 48 h post inoculation. Values represent the mean and standard error of triplicate results. Asterisks indicate values that are statistically significantly different from the DW control using ANOVA (*, P<0.05; **, P<0.01).

induced the accumulation of *AtSWEET* mRNAs, most prominently those of *AtSWEET12*. Furthermore, the hemi-biotrophic bacterial blight pathogen of rice, *Xoo*, induces rice clade III *OsSWEET* genes [10-12]. Direct evidence for the role of SWEET transporters on hemi-biotrophic fungus has not been shown yet. It is, however, strongly suggested that limitation of the availability of sucrose in the rice leaf apoplasm to prevent hemi-biotrophic fungus, *Magnaporthe oryzae*, infection [26]. Infection of rice by *Xoo* strain PXO99A requires the bacterial type III effector gene *pthXo1*, encoding a transcriptional activator-like (TAL) effector PthXo1, which directly interacts with the *OsSWEET11* promoter [9].

Two of the *OsSWEETs* were identified as recessive blight resistance loci, named *xa13* [8,27] and *xa25* [28], corresponding to *OsSWEET11* and *OsSWEET13*, respectively, are now known to be functional sugar transporters. *OsSWEET11* and *OsSWEET13* belong to clade III SWEETs (Figure S3). Moreover, only 5 out of the 21 *OsSWEET* genes, all of which belong to clade III (*OsSWEET11*to *15*), have been shown to support pathogen growth [11]. Recently, *SWEETs* have been shown to be induced by TAL effectors from the Cassava blight pathogen *X. axonopodis* pv. *manihotis*. In this case, two clade III genes, *MeSWEET10* and *15*, were induced by the pathogen [29]. Interestingly, in the present study, only one tomato *SWEET* gene out of 31 *SlSWEETs*, *SlSWEET15*, which is also a member of clade III, was specifically induced by *B. cinerea* infection (Figure 1). Clades I, II, and IV appear to be predominantly hexose transporters, whereas clade III SWEETs transport predominantly sucrose, and also can transport glucose [14]. Thus, clade III SWEET proteins may provide some specific advantage to pathogens by providing sucrose and glucose.

SlSWEET15 was the only *SWEET* gene activated in response to *B. cinerea* infection in tomato cotyledons. It was reported that *B. cinerea* induced expression of *SWEET* genes in *Arabidopsis* [9] and grapevine [13], although the details have not yet been clarified. In *Arabidopsis*, *B. cinerea* induced expression of *AtSWEET4*, *AtSWEET15* and *AtSWEET17* 48 h post inoculation, although no induction of gene expression was observed at the earlier stage of infection, 18 h post inoculation [30]. Similarly with *Arabidopsis*, *VvSWEET4*, which was the only SWEET gene induced by *B. cinerea* infection in grapevine, was not induced until 72 h post inoculation, the stage when visible necrotic lesions expand. Compared with *VvSWEET4*, two typical markers of *B. cinerea infection* in grapevine, *VvSTS*, a key enzyme in the synthesis of stilbene phytoalexins, and *VvHSR1*, a cell death marker, were induced at a much earlier stage, 24 h and 48 h post inoculation, respectively

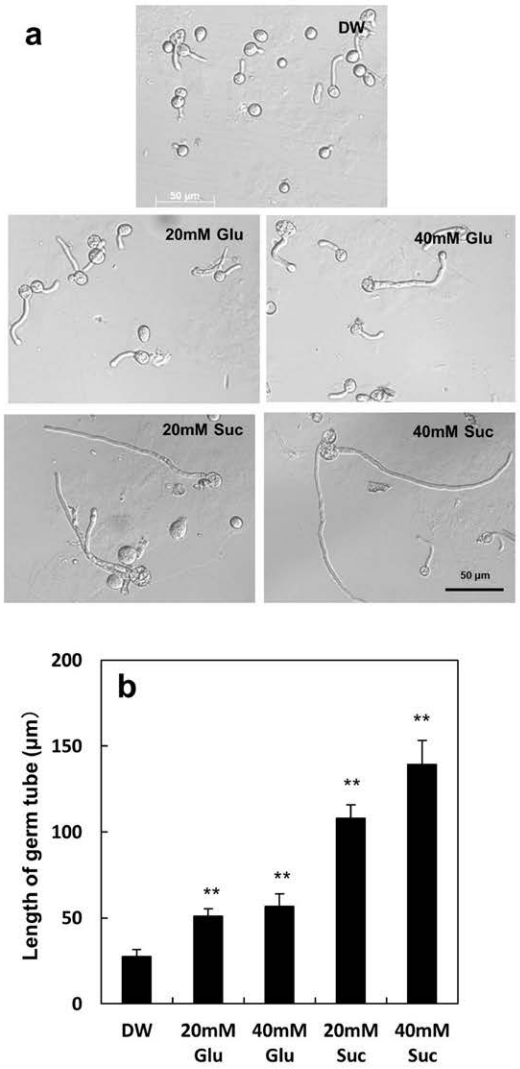

Figure 5: Effects of glucose and sucrose on growth of *B. cinerea* germ tubes. To investigate the direct effects of sugars on fungal spore growth, spores were suspended into sugar solutions, germ tube lengths were measured on digital images 8 h after incubation. (a) Light microscopies of germ tubes, suspended in distilled water (DW), glucose (Glu) or sucrose (Suc) 8 h after incubation. (b) Germ tube length. Values represent the mean and standard error of triplicate results. Asterisks indicate values that are statistically significantly different from the DW control using ANOVA (**, P<0.01).

[13]. In contrast, *B. cinerea* infection in tomato transiently induced expression of *SlSWEET15* at 16 h post inoculation, the early infection stage when no cell death observed (Figure 3a). With regards to the late induction of *VvSWEET4*, Chong et al. [13] proposed a possible role for VvSWEET4 in plant cell death because *B. cinerea*, a typical necrotrophic pathogen, is promoted by and requires the active cell death of the host in order to feed on dead macerated tissues [31]. However, our present results strongly suggest that *SlSWEET15* has no involvement in necrotic lesion formation or cell death in *B. cinerea*-infected tomatoes because the expression pattern was different from a typical defense-related gene, *PR1a1*(Figure 2b,2c). Expression of *SlSWEET15* was transiently induced at the pre-necrotic stage of infection, considerably earlier than the formation of cell death (Figures 2 and 3a). Actually, contents of glucose and sucrose in the apoplasm of infected cotyledons were significantly increased in the stage when no cell death was observed (Figure 3). Furthermore, glucose and sucrose can promote growth and invasion of *B. cinerea* both *in vitro* and *in vivo* (Figures 4 and 5). These results support a model where the necrotrophic pathogen *B. cinerea* may need to obtain sugars, glucose and sucrose, from live tomato cells by hijacking *SlSWEET15* at the pre-necrotic stage of infection.

Unexpectedly, most of tomato *SWEET* genes were downregulated at the pre-necrotic stage of infection (16 h post inoculation) (Figures 1 and 2). This suggests that most *SWEET* genes, whose original function is inter- and intracellular sugar transport in plant cells, are positively involved in defense reactions by reallocating and retaining carbohydrates [32]. Therefore, the downregulation of *SlSWEET* genes may be caused by *B. cinerea*, which transfers small RNA effectors into host plant cells to suppress host immunity and achieve infection [33].

To improve plant resistance against pathogens, dominant resistance genes have typically been used. However, because resistance is based on the recognition of a single pathogen-derived molecular pattern, altering a plant gene that critically facilitates compatibility (susceptibility genes) could provide a more broad-spectrum and durable type of resistance [5]. Indeed, *SWEET*-derived recessive *Xoo* resistance genes from rice have been obtained not only from natural mutations of some rice clade III *SWEET* genes [26,27] but also from an artificially generated target mutation of the *SWEET* gene via genome editing using CRISPR/Cas9 technology [12]. Also, Li et al. [34] produced *Xoo* resistant rice by knockdown of clade III *OsSWEET11* with an artificial microRNA technology. These facts indicate that knockout or knockdown of an appropriate plant *SWEET* gene, which functions as a susceptibility gene of a pathogen, may present a promising strategy for the generation of resistant plants to the pathogen. Recent protein-base plant genome editing technology [35] may enhance practical utility of the strategy by developing non-transgenic recessive alleles at the targeted susceptibility genes.

The results of this study strongly suggest that *SlSWEET15* is induced and exploited by *B. cinerea* and may provide sucrose to promote hyphal growth in the early stages of infection in tomato, although direct evidence has not been shown in the present study. An understanding of the detailed mechanisms will help in the development of grey mold-resistant tomato plants. Future studies aimed at generating a *SlSWEET15* transgenic tomato will provide a better understanding of the role of this candidate tomato susceptibility gene in *B. cinerea* infection.

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