applied sciences

MDPI

Article

Constant and Intermittent Contact with the Volatile Organic Compounds of *Serendipita indica* Alleviate Salt Stress In Vitro *Ocimum basilicum* L.

Hassiba Fraj * and Stefaan P. O. Werbrouck

Laboratory for Applied In Vitro Plant Biotechnology, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium
* Correspondence: hassibaing@live.fr or hassiba.fraj@ugent.be

Abstract: *Serendipita indica* is a plant growth-promoting fungus. It is a natural soil dweller that can colonize the roots of a wide range of plants, including cultivated crops. *S. indica* has been reported to improve plant nutrient uptake and increase stress tolerance when inoculated into the soil. The present study was undertaken to study the effect of volatile organic compounds (VOCs) of *S. indica* on salt-stressed *Ocimum basilicum* 'Fin vert' in vitro, either in a culture vessel with a semi-solid medium or via a modified temporary immersion bioreactor system (SETIS). For all salt concentrations, VOCs of *S. indica* significantly improved plant growth in both semi-solid medium and SETIS bioreactors. This resulted in heavier and taller plants, more shoots per plant, and longer roots. This was even observed for the control without salt. At 9 g/L NaCl, plants with Serendipita were able to give longer roots than those without (1.2 cm vs. 0.0 and 1.7 cm vs. 1.7 cm) in the semi-solid medium and SETIS, respectively. Nevertheless, the VOCs were not able to make the plant salt tolerant to this high concentration. The increase in total phenolic and flavonoid content and radical scavenging suggest that the antioxidant defense system is triggered by the *S. indica* VOCs. In the semi-solid system, without VOCs, 1 g/L NaCl led to an increase in total chlorophyll content (TCC) and a significant decrease in TCC was further measured only at 6 g/L NaCl or more. However, when VOCs were added, the bleaching effect of the salt was partially restored, even at 6 and 9 g/L NaCl. A significant decrease in TCC was also measured in the SETIS system at 6 g/L NaCl or more and treatment with VOC did not make any difference. An exception was 9 g/L, where the VOC-treated plants produced more than three times more chlorophyll than the non-treated plants. These findings will encourage the application of *Serendipita indica* for stress reduction. In addition, the proposed original adaptation of a temporary immersion system will be instrumental to investigate stress reduction associated with volatile compounds and better understand their mechanism of action.

Keywords: semi-solid medium; SETIS; NaCl; VOCs; basil

check for updates

Citation: Fraj, H.; Werbrouck, S.P.O. Constant and Intermittent Contact with the Volatile Organic Compounds of *Serendipita indica* Alleviate Salt Stress In Vitro *Ocimum basilicum* L. *Appl. Sci.* **2023**, *13*, 1776. https://doi.org/10.3390/app13031776

Academic Editor: Claudio De Pasquale

Received: 23 December 2022
Revised: 15 January 2023
Accepted: 27 January 2023
Published: 30 January 2023

1. Introduction

Beneficial rhizosphere microorganisms and their bioactive compounds have been widely studied as potential biofertilizers, biostimulants, and biocontrol agents to find sustainable alternatives to chemicals. In the rhizosphere, plants naturally coexist with various microorganisms. Sometimes they establish a symbiotic relationship with bacteria or fungi [1,2]. Microorganisms have not only diffusible metabolites to consider. The *volatile* organic compounds (VOCs) produced by microorganisms can modulate plant physiological processes, these metabolites, and their biological functions, which has gained interest [3]. The VOCs emitted by microorganisms are characterized by low molecular weight, high vapor pressure, low boiling point, and lipophilic [4]. Due to their small size, VOCs can spread through the atmosphere and soil. Some VOCs have neutral or inhibitory effects on the growth of plants, such as chlorosis and senescence [5,6]. However, VOCs that have positive effects on plant development could hold great promise for future applications as sustainable natural products. Indeed, some VOCs are capable of controlling plant pathogens, stimulating plant growth, and inducing systemic resistance to diseases [3,7–10]. This is reported for some bacteria such as *Bacillus* and *Burkholderia* [3,11,12] and some fungi like *Trichoderma* and the non-pathogenic *Fusarium oxysporum* MSA 35 [13,14].

The mutualistic root endosymbiont *Serendipita indica* colonizes a wide range of plant species and contributes to their biotic and abiotic stress tolerance under field conditions. *S. indica* increases salt stress tolerance in barley [15], rice [16], arabidopsis, alfalfa [17], tomato plants [18], and sweet basil [19]. It is involved in adjusting the osmotic balance [20] and modifying antioxidant enzyme levels, induces ROS capture systems, and regulates the K^+/Na^+ ratio of colonized plants [21]. However, its mode of action has not yet been definitively elucidated. Recently, attention has focused on the role of *S. indica* VOCs and their influence on plant growth. Some studies showed that the VOCs of *S. indica* and *S. williamsii* are able to increase the biomass of *Arabidopsis thaliana* in vitro seedlings [22].

In vitro or in fields, plants are facing different stress such as salinity, which is the main abiotic stress that threatens plant growth and thus agriculture, especially in arid and semi-arid areas. As a consequence, seed germination is inhibited, seedlings develop poorly, root development is restricted, oxidative stress is caused, and farmers suffer great losses. The problem has been addressed by conventional breeding and genetic modification, but mycorrhiza could also contribute to a sustainable strategy to increase crop yields under salt stress [23].

Ocimum basilicum, or sweet basil, is cultivated worldwide for the culinary use of its leaves and the pharmaceutical and cosmetic application of its essential oil [24]. Salinity negatively affects its germination, growth, flowering, and yield [25–27].

The objective of this research was to study the effect of the VOCs of *S. indica* on the salt stress tolerance the basil shoot, without direct contact of the fungus with the roots. Therefore, we made use of the controlled environment of in vitro culture systems. These included semi-solid systems and temporary immersion bioreactors (SETIS). We subjected the plants to salt stress and ensured that they came in contact with the fungus only through the volatiles in the headspaces. With this new approach, we were able to demonstrate for the first time that *Serendipita* can remotely influence salt stress resistance through its VOCs.

2. Materials and Methods

2.1. *Serendipita indica*

Serendipita (formerly *Piriformospora*) *indica* strain DSM 11,827 was obtained from Dr. Jolien Venneman [28]. It was stored at 4 °C in sterile potato dextrose agar (PDA) plates. Three days before the start of each experiment, *S. indica* was inoculated into the appropriate container on PDA and incubated at 29 °C.

2.2. Plant Material

Seeds of *Ocimum basilicum* 'Fin vert' were wrapped in filter paper, rinsed in 70% ethanol, and surface sterilized for 15 min in 0.8% NaOCl (10% commercial bleach) with 0.001% tween, and finally rinsed three times with sterile distilled water. They were individually placed in test tubes containing 20 mL of basal medium consisting of Murashige and Skoog (1962) macro- and microelements and vitamins (Duchefa, NL), 3% sucrose, and 0.7% plant agar (Duchefa NL). The pH of the medium was adjusted to 5.8 before autoclaving (120 °C, 15 min). Six weeks later, the seedlings were used as explants for micropropagation in 720 mL glass jars on the same basal medium.

2.3. Semi-Solid Medium Bioassay

One hundred milliliters of basal medium containing 0, 1, 3, 6, or 9 g/L NaCl was added to 720 mL jars. The pH of the medium was adjusted to 5.8 before autoclaving (120 °C, 15 min). A sterile petri dish (ϕ 55 mm) was placed in each jar and then filled with 10 mL PDA. *S. indica* was inoculated into the petri dishes (Figure 1b VIII). After 3 days, nodal explants (0.5 cm) of basil from the previously mentioned in vitro stocks were transferred to the plant medium (Figure 1b VII). Three vessels were used per treatment and there were four biological replicates. Four weeks later, growth parameters (root length, shoot length, number of shoots, and fresh weight of the plant) were determined.

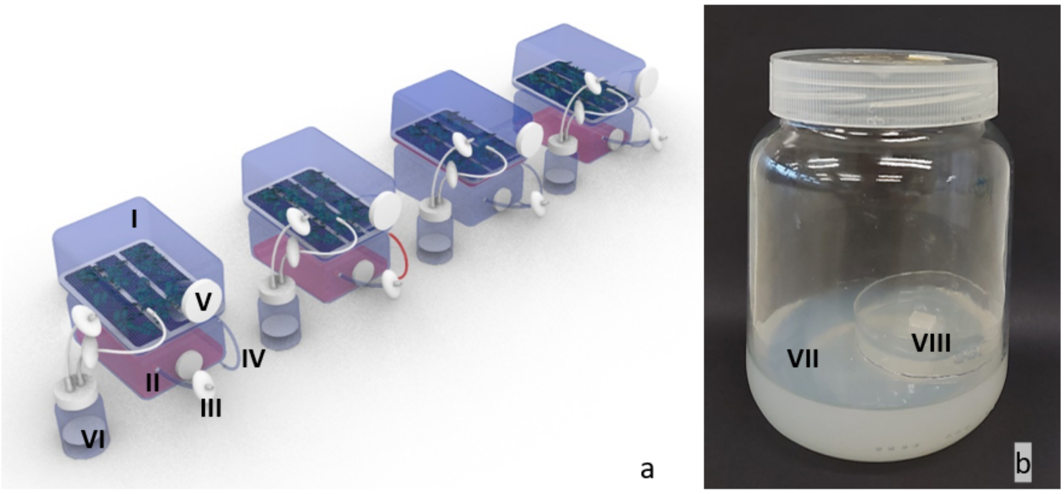

Figure 1. (**a**): The modified SETIS system. I: culture vessel; II: medium vessel; III: air filter; IV: silicone tube; V: screw caps; VI: Duran bottle containing PDA medium with *S. indica*. The four combinations show, from left to right, the resting phase, the pushing up of the liquid medium, the immersion phase, and the flowing back of the liquid medium. (**b**): The semi-solid system. VII: medium; VIII: Petri dish with *S. indica* growing on PDA.

2.4. SETIS Bioassay

SETIS™ (Vervit, Belgium) is a temporary immersion bioreactor (Figure 1a). Web-based software controls valves to supply compressed air that pushes liquid medium from a lower transparent reservoir (medium tank) (Figure 1a II), through a flexible silicone tube (Figure 1a IV), into an upper cube-like container that contains the explants (plant container) (Figure 1a I). The latter contains the explants, which are fully immersed in the medium. When the air pressure drops, the medium returns to the lower container by gravity. The main chamber is refreshed by separate flushes of compressed air. An additional 1000 mL Duran bottle containing 250 mL of PDA was added to this system (Figure 1a VI). *S. indica* was inoculated into this bottle 3 days before it was connected to the main aeration flow with silicone tubing. A 0.25 µm intermediate filter was used to prevent contamination (Figure 1a III). Every 8 h, a medium emersion phase of 60 s was programmed, as well as an air flux with or without VOCs of 60 s every hour (Figure 1 I).

The medium tank was filled with 1 L of liquid basal medium containing MS medium, 3% sucrose, and 0, 1, 3, 6, or 9 g/L NaCl. The pH of the medium was adjusted to 5.8 before autoclaving (120 °C, 15 min). After autoclaving the individual parts, the system was mounted in the laminar airflow. Then, 20 explants (0.5 cm) were transferred to each SETIS plant tank. Duran bottles containing the fungus were connected to half of the SETIS systems. Each experiment consisted of 10 SETIS systems and was repeated three times. In one repetition, PDA bottles without the fungus were also integrated.

2.5. Extraction

The basil leaves were collected after 4 weeks. They were freeze-dried, ground into a fine powder, and stored at –80 °C until use. Dried samples of 100 mg were extracted with 10 mL of methanol (80%). After 10 min of sonication, the samples were allowed to rest for 30 min. They were then centrifuged 10 times at 5500 rpm. The supernatant was collected and used for the determination of phytochemicals, TPC, TFC, and antioxidant activity.

2.6. Total Phenolic Content (TPC)

The determination of TPC was performed according to the following procedure [29]. Briefly, 50 µL of the sample was pipetted into a test tube. To this, 125 µL of 10% Folin-Ciocalteu solution was added and vortexed. After 5 min, 400 µL of 7.5% Na2 CO3 solution and 425 µL milliQ water were added, followed by 2 h incubation in the dark. The absorbance was measured at a wavelength of 760 nm with a UV-Vis spectrometer. The TPC of 1 g of dry extract was related to gallic acid as a standard (mg GAE/g).

2.7. Total Flavonoid Content (TFC)

The TFC was calculated according to the following method [30]. Five hundred microliters of the extract was mixed with 500 µL of a reagent (20 µL of 10% AlCl3, 20 µL of 1 M sodium acetate, 300 µL methanol, and 560 µL MilliQ water) and then shaken vigorously. The absorbance was measured at a wavelength of 415 nm with the UV-Vis spectrometer. The TFC was determined using a standard curve made with quercetin and the results were expressed as mg QE per mg dry extract.

2.8. DPPH Free Radical Scavenging Activity

The DPPH method was performed as follows [31]. The reaction mixture contained 0.1 mL of methanolic extract of basil, 0.4 mL of 0.1 M Tris-HCl (pH 7.4), and 0.5 mL of 0.3 mM DPPH. This was shaken vigorously and incubated for 20 min in the dark at room temperature. Free radical scavenging activity was measured spectrophotometrically at 517 nm and calculated using the following formula:

$$\text{Percentage inhibition\%} = [(A0 - A1)/A0] \times 100 \quad (1)$$

where A0 was the absorbance of the control (blank, without extract) and A1 the absorbance in the presence of the extract. The results were reported as IC50 value (µg/mL), where a lower IC50 value represents a stronger DPPH scavenging capacity.

2.9. Total Chlorophyll Content

The total chlorophyll of the leaves was extracted with 80% acetone [32]. One hundred milligrams of fresh basil leaf was cut into small pieces and ground for 5 min in 10 mL of 80% acetone in a mortar and pestle. The homogenate was put into a 15 mL Falcon tube and centrifuged at 3000× g for 15 min. The supernatant was moved into a new 15 mL Falcon tube. The optical density (OD) of the extract was measured at both 663 and 644 nm using spectrophotometer. The concentration of total chlorophyll, in milligrams per gram of FW tissue, was calculated using the following formula:

$$\text{Chl (total)} = 17.76 \times OD_{644} + 7.34 \times OD_{663} \quad (2)$$

2.10. Cultivation Conditions

All cultures were incubated at 25 ± 2 °C under a 16 h/8 h light/dark photoperiod and a light intensity of 45 µmol m^{-2} s^{-1} fluorescent light (FL) provided by PHILIPS master TLD 36 W 830 Refex ECO. After 4 weeks of culture, the proliferation rate was calculated as the number of shoots per explant. Shoot length, root length, and weight were measured.

2.11. Statistical Analysis

A completely randomized design was used for all experiments. By SPSS statistics 28, a three-way ANOVA was performed, which was followed by a one-way ANOVA to separate the means of the 5 × 2 combinations of salt and VOCs treatment, for each system, using the least significant difference test ($p < 0.05$).

3. Results

3.1. Effect of Volatile Substances on Growth Parameters

The results are summarized in Figure 2. Without *S. indica* volatiles, NaCl was found to have a negative effect on total plant growth, which was estimated from the average biomass per shoot. Increasing the NaCl concentration from 0 to 9 g/L gradually decreased the biomass from 1.3 g to 0.2 g in semi-solid media and from 1.5 g to 0.04 g in SETIS. The longest plants and roots were obtained on salt-free medium. With increasing NaCl concentration, both parameters decreased significantly in both systems as visually shown in Figure 3.

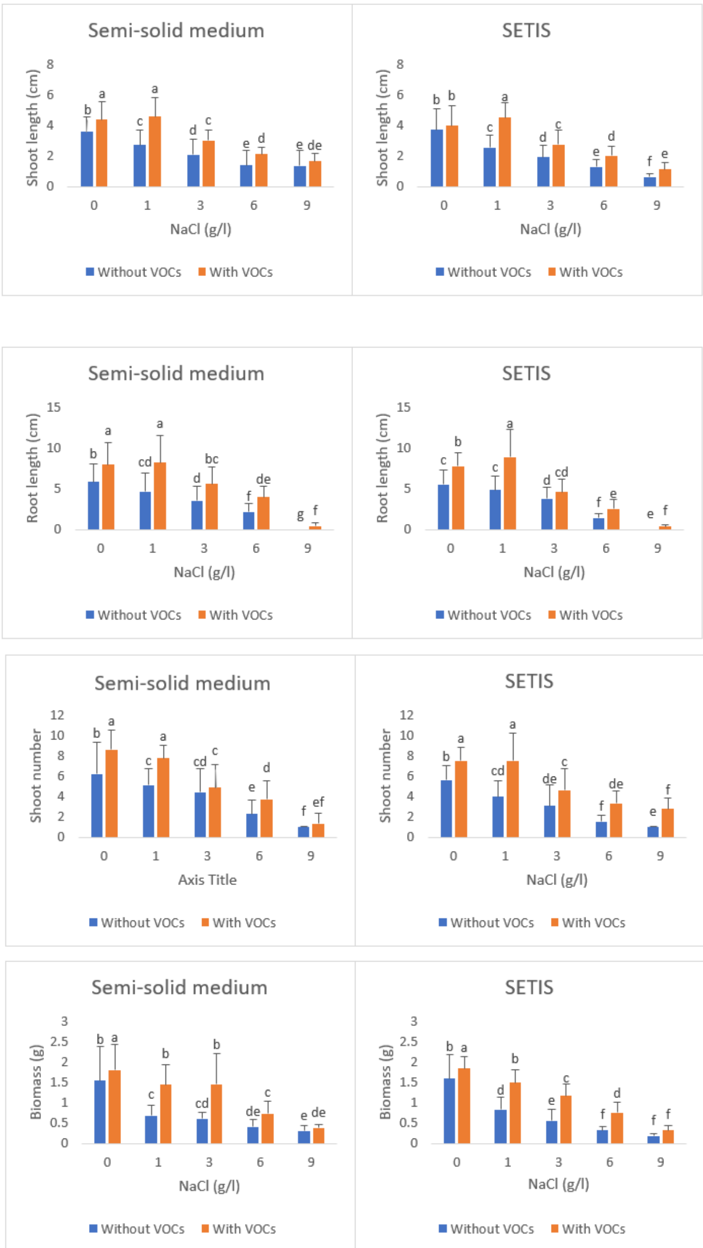

Figure 2. Effect of increasing salt concentration with or without VOCs emitted by *Serendipita indica* on shoot and root length, number of shoots, and biomass of *Ocimum basilicum* grown in semi-solid medium and SETIS (means of 10 combinations of salt and VOCs treatment, for each system, were analyzed by one way ANOVA: for each graph, bar plots of the means overlaid by standard error bars. Lower letters above the columns indicate significant differences between values (p-value < 0.05).

(**a**)

(**b**)

Figure 3. Morphology of *Ocimum basilicum* plants treated with volatiles emitted by *S. indica*: (**a**) in SETIS; (**b**) in semi-solid medium.

For all salt concentrations, VOCs of *S. indica* significantly improved plant growth in both semi-solid medium and SETIS bioreactors. This resulted in heavier and taller plants, more shoots per plant, and longer roots. This was even observed for the control without salt. At 9 g/L NaCl, plants with *Serendipita* were able to give longer roots than without (1.2 cm vs. 0.0 and 1.7 cm vs. 1.7 cm) in the semi-solid medium and SETIS, respectively. Nevertheless, the VOCs were not able to make the plant salt tolerant to this high concentration.

3.2. Effect of VOCs on Chlorophyll Content

In the semi-solid system, without VOCs, 1 g/L NaCl led to an increase in TCC, and a significant decrease in TCC was further measured only at 6 g/L NaCl or more (Table 1). However, when VOCs were added, the bleaching effect of the salt was partially restored at 6 and 9 g/L NaCl. A significant decrease in TCC was also measured in the SETIS system at 6 g/L NaCl or more. VOC-treated plants were similar to non-treated plants experiencing the same salt stress. An exception was 9 g/L, where the VOC-treated plant produced more than three times more chlorophyll than the non-treated plants.

Table 1. Effect of *Serendipita indica* volatiles on total chlorophyll content of *Ocimum basilicum* grown on semi-solid medium and SETIS under salt stress conditions.

	Total Chlorophyll Content mg/g FW			
	Semi-Solid Medium		**SETIS**	
Salt Treatment	**without VOCs**	**with VOCs**	**without VOCs**	**with VOCs**
Control (0 g/L NaCl)	20.8 ± 2.5 ^{b}	29.8 ± 6.0 ^{a}	18.6 ± 1.5 ^{b}	26.0 ± 3.5 ^{a}
1 g/L NaCl	25.4 ± 3.4 ^{a}	30.0 ± 3.7 ^{a}	20.0 ± 3.1 ^{ab}	26.0 ± 2.8 ^{a}
3 g/L NaCl	$22.\ 0 \pm 2.3$ ^{ab}	26.7 ± 4.6 ^{a}	16.6 ± 0.5 ^{bc}	18.8 ± 4.6 ^{b}
6 g/L NaCl	14.8 ± 1.5 ^{c}	17.6 ± 1.3 ^{b}	12.0 ± 2.0 ^{cd}	13.4 ± 1.3 ^{cd}
9 g/L NaCl	4.2 ± 1.4 ^{d}	13.5 ± 1.9 ^{cd}	3.8 ± 1.5 ^{e}	12.8 ± 1.9 ^{d}

For each system, values are means ± SE. Different lowercase letters indicate significant difference ($p < 0.05$), between the 10 combinations of salt x VOCs, based on one-way ANOVA.

3.3. Effect of Volatile Substances on Antioxidant Parameters

3.3.1. Total Phenol Content

TPC content (Tables 2 and 3) was measured in each extract using the Folin-Ciocalteu reagent. Results were derived from a standard curve (y = 0.002x + 0.0268; R^2 = 0.9963) of gallic acid (0–500 µg/mL) and expressed as gallic acid equivalents (GAE) per gram of dry weight. In semi-solid medium, starting from 6 g/L NaCl, the content of TPC in the plants decreased significantly. In the presence of the VOCs, the phenolic compounds were almost doubled in all treatments. In SETIS, salt did not affect TPC content, but VOCs strongly stimulated TPC at 0 and 1 g/L NaCl. Although not at 3 and 6 g/L salt, at 9 g/L salt, there was a positive effect on TPC content.

Table 2. Effect *of Serendipita indica* volatiles on total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (DPPH) of *Ocimum basilicum* in semi-solid medium under stress condition.

Semi-Solid Medium	**TPC (mg GAE/g)**		**TFC (mg QE/g)**		**DPPH (IC_{50}) (µg/mL)**	
Salt Treatment	**without VOCs**	**with VOCs**	**without VOCs**	**with VOCs**	**without VOCs**	**with VOCs**
Control (0 g/L NaCl)	59.9 ± 5.2 ^{cd}	107.8 ± 1 ^{a}	45.6 ± 4.2 ^{c}	86.1 ± 0.8 ^{a}	45.4 ± 10.8 ^{b}	53.6 ± 7.5 ^{b}
1 g/L NaCl	58.8 ± 7.4 ^{cd}	105.3 ± 11.5 ^{a}	42.7 ± 4.5 ^{cd}	61.6 ± 12.1 ^{b}	49.0 ± 6.8 ^{b}	72.6 ± 6.5 ^{a}
3 g/L NaCl	65.5 ± 7.0 ^{c}	86.1 ± 6.7 ^{b}	45.5 ± 8.7 ^{c}	64.2 ± 1.0 ^{b}	37.0 ± 12.0 ^{c}	60.2 ± 9.1 ^{a}
6 g/L NaCl	18.9 ± 2.3 ^{f}	36.0 ± 0.7 ^{e}	31.2 ± 2.4 ^{e}	36.0 ^{de} ± 0.7	47.9 ± 9.3 ^{b}	76.7 ± 8.4 ^{a}
9 g/L NaCl	18.5 ± 5.8 ^{f}	38.3 ± 3.1 ^{e}	18.5 ± 3.5 ^{f}	38.3 ± 3.1 ^{cd}	64.0 ± 5.8 ^{a}	97.0 ± 22.0 ^{a}

For each parameter, values are means ± SE. Different lowercase letters indicate significant difference ($p < 0.05$), between the 10 combinations of salt x VOCs, based on one-way ANOVA.

Table 3. Effect *of Serendipita indica* volatiles on total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (DPPH) of *Ocimum basilicum* in SETIS under stress condition.

SETIS Salt Treatment	TPC (mg GAE/g) without VOCs	TPC (mg GAE/g) with VOCs	TFC (mg QE/g) without VOCs	TFC (mg QE/g) with VOCs	DPPH (IC_{50}) (μg/mL) without VOCs	DPPH (IC_{50}) (μg/mL) with VOCs
Control (0 g/L NaCl)	49.0 ± 7.8 ^{d}	128.2 ± 29 ^{a}	50.8 ± 7.8 ^{a}	56.7 ±14.1 ^{a}	27.3 ±6.8 ^{de}	44.5 ± 11.0 ^{bc}
1 g/L NaCl	60.3 ± 12.6 ^{cd}	108.4 ± 22.6 ^{b}	33.4 ± 5.3 ^{cd}	56.7 ± 14.1 ^{a}	19.2 ± 6.2 ^{e}	29.0 ± 5.2 ^{d}
3 g/L NaCl	55.5 ± 11.2 ^{d}	71.1 ± 13.4 ^{cd}	30.1 ± 6.6 ^{de}	44.4 ± 7.8 ^{b}	43.6 ± 10.2 ^{bc}	75.2 ± 10.2 ^{a}
6 g/L NaCl	45.5 ± 13.2 ^{d}	53.1.3 ± 10 ^{d}	28.0 ±5.5 ^{de}	36.5 ± 6.1 ^{c}	21.6 ±10.2 ^{e}	53.2 ± 7 ^{b}
9 g/L NaCl	55.8 ±15.5 ^{d}	85.4 ±20.1 ^{bc}	24.3 ± 5.2 ^{e}	26.1 ± 4.6 ^{e}	12.8 ± 1.9 ^{e}	32.4 ± 4.5 ^{d}

For each parameter, values are means ± SE. Different lowercase letters indicate significant difference ($p < 0.05$), between the 10 combinations of salt x VOCs, based on one-way ANOVA.

3.3.2. Total Flavonoid Content

TFC was measured with aluminum chloride in a colorimetric method. The results were subtracted from the calibration curve ($y = 0.0689x - 0.0113$; $R^2 = 0.9994$) of quercetin (0–12.5 μg/mL) and expressed as quercetin equivalents (QE) per gram of dry extract weight (Tables 2 and 3). In semi-solid medium, starting from 6 g/L NaCl, the content of TFC in the plants decreased significantly. VOCs increased TFC, but from 6 g/L, there was no difference anymore with the plants without VOC. The highest amount of TFC (86.1 mg QE/g) was found in the control plant in semi-solid medium treated with VOCs. The smallest amount (18.5 mg QE/g) of flavonoids was found in the semi-solid medium when the plants were exposed to 9 g/L NaCl. In the SETIS system, TFC decreased steadily as the salt concentration increased. VOCs were able to partially inhibit this, but no longer did so at 9 g/L NaCl in the medium.

3.3.3. Radical Scavenging Capacity

The antioxidant capacity of the methanolic extract of the leaves of *Ociumum basilicum* was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. In semi-solid medium, when the salt concentration in the medium increased, DDPH fluctuated. It was found that the DPPH was always higher when VOCs came into play. In SETIS, without VOCs, DPPH did not change with increasing salt concentration except at 3 g/L NaCl. VOCs always increased DPPH, and again there was a strong DPPH increase at 3 g/L.

3.3.4. Comparison between Experimental Systems

Although the effects of the VOC were fairly analogous in both systems, the modified temporary immersion system is preferable. Indeed, in the semi-solid system, it occasionally happened that the fungus escaped from its recipient and colonized the medium, requiring extra replication.

4. Discussion

Glycophytes, such as *Ocimum*, suffer from salt stress and grow slower, branch less, and show wilting symptoms. This is caused by two successive processes. Firstly, salt stress reduces water uptake due to osmotic stress. Secondly, excess salts in the transpiration stream damages plant cells by inhibiting photosynthesis, affecting ion homeostasis, and peroxidizing membrane lipids [33]. Not surprisingly, we observed in both in vitro systems that plant growth was strongly affected by salt stress. In the semi-solid medium, the number of shoots, root length, shoot length, and biomass decreased at salt levels ranging from 0 g/L (control) to 9 g/L NaCl. Similar studies in basil, both in pots and with nutrient solutions, confirmed the negative effects of salt. When basil was grown in soil containing 3 g/L NaCl, leaf area and the fresh and dry weight of leaves and stems decreased to 77% compared to the control [34]. Even 1.5 g/L NaCl reduced the total fresh weight and dry weight of basil roots [35]. Plant height, stem diameter, leaf number, and dry mass of basil plants were significantly reduced when salt concentration was increased from 0 to 4.80 g/L NaCl [36]. In SETIS, the addition of 1 g/L NaCl seemed to be beneficial for shoot and root length, but at 3 g/L NaCl, there were already negative effects. The stimulatory effect of temporary immersion in 1 g/L NaCl suggests that *O. basilicum* prefers mild salinity. Some authors demonstrated this positive effect in other species. For example, the addition of 1.5 or 3 g/L NaCl to the culture medium had a positive impact on the growth of *Solanum lycopersicum seedlings* [37].

Another negative effect of salinity demonstrated in this experiment was the reduced chlorophyll content in both systems. The reduction of chlorophyll under salt stress has been demonstrated in basil [36], and in many other species, such as sugarcane [38], wheat and barley [39], tomato [40], *Prosopis alba* [41] and maize [42]. The reduction in chlorophyll content can be explained by the accumulation of intracellular Na^+ that blocks the uptake of K^+ from the medium. Therefore, the plants suffer from deficiency of K^+, leading to leaf chlorosis [43].

Phenolic compounds, including flavonoids, are the most widely distributed secondary metabolites present in the plant kingdom. These compounds play numerous biochemical and molecular roles in plants, such as signaling molecules, plant defense, mediating auxin transport, antioxidant activity, and free radical scavenging [44]. Phenolic compounds are highly produced by the Lamiaceae family [45], particularly in basil. Our results showed that in SSM, the TPC and TFC decreased when the salt concentration was 6 g/L or more, but DPPH increased. In contrast, in SETIS, salt concentration did not affect the TPC, but decreased the TFC and DPPH. The latter showed a remarkable increase at 3 g/L NaCl. This trend was also reported in pot experiments. Salt stress decreased the TPC and TFC for *Schizonepeta tenuifolia,* except at the lowest concentration (1.5 g/L NaCl) where the TPC and TFC increased [46]. Salt stress not only affected the TPC, but also the profile to phenolic compounds in basil leaves [47].

In both the SSM and the SETIS bioreactor, VOCs of *S. indica* significantly improved plant growth under salt stress, expressed as an increase in shoot length and number and biomass. There was also an overall positive effect on root length, even without salt stress. The growth-promoting effects of VOCs produced by *S. indica were* only recently discovered. The shoot biomass of in vitro seedlings of *Arabidopsis* increased up to nine-fold in the presence of VOCs from *S. indica* compared to control [22]. The most abundant volatile compound produced by *S. indica* was methyl benzoate, but its single application did not affect plant growth [22]. The beneficial effect of this fungus on seed germination, growth, and biomass was further reported only under field conditions in numerous horticultural and medical plant species [48–55]. A positive effect of *S. indica* was also reported on the growth of *Ocimum sanctum* [56]. Root colonization by *S. indica* has also been found to improve crop tolerance to a number of abiotic stressors, including low temperatures, heavy metals, and salt [52,57–60].

Regarding the positive effect of VOCs, reference should also be made to *Trichoderma* and other fungi. In arabidopsis, beneficial effects of *Trichoderma* VOCs on growth were reported [61,62]. Physically separated *Trichoderma* spp. and *A. thaliana* enhanced both shoot and root biomass, root production, and chlorophyll content [63]. Stimulation of salt stress tolerance by *Trichoderma* VOCs was likewise reported [64]. VOCs emitted by *T. viride* showed a growth promotion of tomato plants [61]. *Streptomyces* VOCs were found to promote growth of tomato seedlings irrigated with 12 g/L NaCl, with an increase in fresh weight, shoot length, and number of fibrous roots [65]. The volatiles emitted by *Cladosporium cladosporioides* promote tobacco growth [66]. In addition, VOCs from rhizosphere bacteria were reported to promote plant growth [11,67].

In both examined systems, salt stress led to chlorosis. But the *S. indica* VOCs counteracted this effect, which was reflected in a significant increase in chlorophyll content. In a pot experiment, *S. indica* was found to significantly increase the chlorophyll content of rice plants under salt conditions compared to non-inoculated plants [16], which helped the plants to survive the stress conditions. Several *Trichoderma* strains also significantly increased the chlorophyll content of *A. thaliana*, in control plants or plants with salt stress [64]. Similarly, volatiles emitted by *Fusarium oxysporum* and *Verticillium dahliae were reported to* affect *the* response of *A. thaliana* to salt stress by promoting growth and increasing chlorophyll content [68].

Salt stress is known to cause oxidative damage by stimulating the production of ROS that, in turn, damages proteins, lipids, DNA, and carbohydrates. To capture and detoxify the ROS, the biosynthesis of antioxidants (phenols and flavonoids) is stimulated. In our study, VOCs produced by *S. indica* significantly increased TPC, TFC, and antioxidant activity in all salt treatments, suggesting that these extra phenolics, including flavonoids and other antioxidants, help the plants to survive under saline conditions [69]. When treated with *S. indica*, *Bacopa monniera* plants showed a higher level of antioxidant activity comparing to the control plants [70]. The fungus *S. indica* was also found to increase the production of osmoprotectants (proline and glycein) in salt stressed barely plants [15]. The level of phenolic compounds may be directly related to their antioxidant capacity because of their ability to scavenge free radicals [37,71].

Volatiles released by microbes have been shown to be important drivers of plant growth [13,22,61]. The mechanisms of this growth promotion are complex. The single application 6-pentyl-2H-pyran-2-one (6-PP), a major VOC of *Trichoderma*, affected root morphogenesis [13]. However, VOCs can also work in a mixture. Methyl benzoate was found to be the dominant molecule produced by *Serendipita*, but its single application did not affect plant growth [22]. Some authors suggest that it is actually CO_2 emitted by fungi that is responsible for the increased plant growth; however, this hypothesis was rejected by CO_2 capture [22,71]. The profile of fungal volatiles changes as the fungi grow and mature. It has been suggested that VOCs emitted by microorganisms mimic plant metabolites, providing signals to plants that ultimately generate growth changes [61]. There are not a lot of studies on the effect of *Serendipita* metabolites. For this reason, strong evidence for a correlation between growth promotion and the production of specific compounds is lacking, suggesting complex molecular interactions are required. Future analytical research on the composition of fungal volatiles at different stages, and molecular research on the specific genes and pathways, may shed light on how fungi can also remotely affect plant growth and their defense mechanisms against stress.

5. Conclusions

For the first time, a modified SETIS system was used to regularly flush the headspace of in vitro plants with fresh VOCs from a microorganism, in this case, *S. indica*. In addition, in a semi-solid culture system, it was possible to spatially separate this endophytic fungus from the in vitro plants, but colonization of the plant medium by the fungus could not always be avoided. Both systems were suitable to demonstrate that the volatiles of *S. indica* can partially alleviate the negative effect of increasing NaCl concentration on root and shoot development and chlorophyll content. These VOCs probably triggered the antioxidant defense system. Currently, the effects of the volatiles of *S. indica* on tolerance to other abiotic stressors are being studied.

Author Contributions: Conceptualization, H.F. and S.P.O.W.; methodology, data acquisition, data analyses and original draft preparation, H.F.; visualization, H.F. and S.P.O.W.; manuscript revision, S.P.O.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available on request from the corresponding author.

Acknowledgments: The authors wish to acknowledge Jeroen Werbrouck for the 3D-drawings of SETIS system.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Smith, S.E.; Read, D. *Mycorrhizal Symbiosis*, 3rd ed.; Academic Press: London, UK, 2008; pp. 1–9.
2. Bulgarelli, D.; Schlaeppi, K.; Spaepen, S.; van Themaat, E.V.L.; Schulze-Lefert, P. Structure and Functions of the Bacterial Microbiota of Plants. *Annu. Rev. Plant Biol.* **2013**, *64*, 807–838. [CrossRef] [PubMed]
3. Ryu, C.-M.; Farag, M.A.; Hu, C.-H.; Reddy, M.S.; Wei, H.-X.; Pare, P.W.; Kloepper, J.W. Bacterial volatiles promote growth in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4927–4932. [CrossRef] [PubMed]
4. Hung, R.; Lee, S.; Bennett, J.W. Fungal volatile organic compounds and their role in ecosystems. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 3395–3405. [CrossRef] [PubMed]
5. Lee, S.; Behringer, G.; Hung, R.; Bennett, J. Effects of fungal volatile organic compounds on Arabidopsis thaliana growth and gene expression. *Fungal Ecol.* **2018**, *37*, 1–9. [CrossRef]
6. Garbeva, P.; Weisskopf, L. Airborne medicine: Bacterial volatiles and their influence on plant health. *New Phytol.* **2020**, *226*, 32–43. [CrossRef] [PubMed]
7. Lee, B.; Farag, M.A.; Park, H.B.; Kloepper, W.J.; Lee, S.H.; Ryu, C.-M. Induced Resistance by a Long-Chain Bacterial Volatile: Elicitation of Plant Systemic Defense by a C13 Volatile Produced by Paenibacillus polymyxa. *PLoS ONE* **2012**, *7*, e48744. [CrossRef]
8. Park, Y.S.; Dutta, S.; Ann, M.; Raaijmakers, J.M.; Park, K. Promotion of plant growth by Pseudomonas fluorescens strain SS101 via novel volatile organic compounds. *Biochem. Biophys. Res. Commun.* **2015**, *461*, 361–365. [CrossRef]
9. Raza, W.; Yousaf, S.; Rajer, F.U. Plant growth promoting activity of volatile organic compounds produced by biocontrol strains. *Sci. Lett.* **2016**, *4*, 40–43.
10. Tahir, H.A.S.; Gu, Q.; Wu, H.; Niu, Y.; Huo, R.; Gao, X. Bacillus volatiles adversely affect the physiology and ultra-structure of Ralstonia solanacearum and induce systemic resistance in tobacco against bacterial wilt. *Sci. Rep.* **2017**, *7*, 40481. [CrossRef] [PubMed]
11. Blom, D.; Fabbri, C.; Connor, E.C.; Schiestl, F.P.; Klauser, D.R.; Boller, T.; Eberl, L.; Weisskopf, L. Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. *Environ. Microbiol.* **2011**, *13*, 3047–3058. [CrossRef]
12. Groenhagen, U.; Baumgartner, R.; Bailly, A.; Gardiner, A.; Eberl, L.; Schulz, S.; Weisskopf, L. Production of Bioactive Volatiles by Different Burkholderia ambifaria Strains. *J. Chem. Ecol.* **2013**, *39*, 892–906. [CrossRef]
13. Garnica-Vergara, A.; Barrera-Ortiz, S.; Muñoz-Parra, E.; Raya-González, J.; Méndez-Bravo, A.; Macías-Rodríguez, L.; Ruiz-Herrera, L.F.; López-Bucio, J. The volatile 6-pentyl-2*H*-pyran-2-one from *Trichoderma atroviride* regulates *Arabidopsis thaliana* root morphogenesis via auxin signaling and ethylene insensitive 2 functioning. *New Phytol.* **2015**, *209*, 1496–1512. [CrossRef] [PubMed]
14. Minerdi, D.; Bossi, S.; Maffei, M.E.; Gullino, M.L.; Garibaldi, A. Fusarium oxysporum and its bacterial consortium promote lettuce growth and expansin A5 gene expression through microbial volatile organic compounds (MVOC) emission. *FEMS Microbiol. Ecol.* **2011**, *76*, 342–351. [CrossRef]
15. Waller, F.; Achatz, B.; Baltruschat, H.; Fodor, J.; Becker, K.; Fischer, M.; Heier, T.; Hückelhoven, R.; Neumann, C.; von Wettstein, D.; et al. The endophytic fungus Piriformospora indica reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc. Natl. Acad. Sci. USA* **2013**, *102*, 13386–13391. [CrossRef] [PubMed]
16. Jogawat, A.; Saha, S.; Bakshi, M.; Dayaman, V.; Kumar, M.; Dua, M.; Varma, A.; Oelmüller, R.; Tuteja, N.; Johri, A.K. *Piriformospora indica* rescues growth diminution of rice seedlings during high salt stress. *Plant Signal. Behav.* **2013**, *8*, e26891. [CrossRef]
17. Abdelaziz, M.E.; Kim, D.; Ali, S.; Fedoroff, N.V.; Al-Babili, S. The endophytic fungus Piriformospora indica enhances Arabidopsis thaliana growth and modulates Na^+/K^+ homeostasis under salt stress conditions. *Plant Sci.* **2017**, *263*, 107–115. [CrossRef] [PubMed]
18. Li, L.; Wang, X.; Zhu, P.; Wu, H.; Qi, S. Plant growth-promoting endophyte Piriformospora indica alleviates salinity stress in Medicago truncatula. *Plant Physiol. Biochem.* **2017**, *119*, 211–223. [CrossRef]
19. Ghorbani, A.; Razavi, S.M.; Ghasemi Omran, V.O.; Pirdashti, H. *Piriformospora indica* inoculation alleviates the adverse effect of NaCl stress on growth, gas exchange and chlorophyll fluorescence in tomato (*Solanum lycopersicum* L.). *Plant Biol. (Stuttg.)* **2018**, *20*, 729–736. [CrossRef] [PubMed]
20. Jogawat, A.; Vadassery, J.; Verma, N.; Oelmüller, R.; Dua, M.; Nevo, E.; Johri, A.K. PiHOG1, a stress regulator MAP kinase from the root endophyte fungus Piriformospora indica, confers salinity stress tolerance in rice plants. *Sci. Rep.* **2016**, *6*, 36765. [CrossRef] [PubMed]
21. Baltruschat, H.; Fodor, J.; Harrach, B.D.; Niemczyk, E.; Barna, B.; Gullner, G.; Janeczko, A.; Kogel, K.H.; Schäfer, P.; Schwarczinger, I.; et al. Salt tolerance of barley induced by the root endophyte Piriformospora indica is associated with a strong increase in antioxidants. *New Phytol.* **2008**, *180*, 501–510. [CrossRef] [PubMed]
22. Venneman, J.; Vandermeersch, L.; Walgraeve, C.; Audenaert, K.; Ameye, M.; Verwaeren, J.; Steppe, K.; Van Langenhove, H.; Haesaert, G.; Vereecke, D. Respiratory CO2 combinedwith a blend of volatiles emitted by endophytic Serendipita strains strongly stimulate growth of Arabidopsis implicating auxin and cytokinin signaling. *Front. Plant Sci.* **2020**, *11*, 544435. [CrossRef] [PubMed]
23. Rodriguez, R.J.; Henson, J.; Van Volkenburgh, E.; Hoy, M.; Wright, L.; Beckwith, F.; Kim, Y.-O.; Redman, R.S. Stress tolerance in plants via habitat-adapted symbiosis. *ISME J.* **2008**, *2*, 404–416. [CrossRef]

24. Lawrence, B.M. Labiatae oils-Mother Nature's chemical factory. In *Essential Oils*; Allured Publishing: Carol Stream, IL, USA; pp. 188–206.
25. Heidari, M. Effects of salinity stress on growth, chlorophyll content and osmotic components of two basil (*Ocimum basilicum* L.) genotypes. *Afr. J. Biotechnol.* **2012**, *11*, 379–384. [CrossRef]
26. Zahedi, S.M.; Nabipour, M.; Azizi, M.; Gheisar, H.; Jalali, M.; Amimi, Z. Effect of kinds of salt and its different levels on seed germination and growth of basil plant. *World Appl. Sci. J.* **2010**, *15*, 1039–1045.
27. Mousavi, S.G.; Jouyban, Z. Effect of salinity stress on germination and growth parametrs of seedlings of basil (*Ocimum basilicum* L.). *J. Cent. Eur. Agric.* **2021**, *22*, 546–556.
28. Venneman, J.; De Tender, C.; Debode, J.; Audenaert, K.; Baert, G.; Vermeir, P.; Cremelie, P.; Bekaert, B.; Landschoot, S.; Thienpondt, B.; et al. Sebacinoids within rhizospheric fungal communities associated with subsistence farming in the Congo Basin: A needle in each haystack. *FEMS Microbiol. Ecol.* **2019**, *95*, fiz101. [CrossRef]
29. Pham, H.N.T.; Tang Nguyen, V.; Van Vuong, Q.; Bowyer, M.C.; Scarlett, C.J. Bioactive compound yield and antioxidant capacity of Helicteres hirsuta lour. Stem as affected by various solvents and drying methods. *J. Food Process. Preserv.* **2017**, *41*, e12879. [CrossRef]
30. Mahboubi, M.; Kazempour, N.; Boland Nazar, A.R. Total phenolic, total flavonoids, antioxidant and antimicrobial activities of Scrophularia striata boiss extracts. *Jundishapur. J. Nat. Pharm. Prod.* **2013**, *8*, 15–19. [CrossRef]
31. Yamaguchi, T.; Takamura, H.; Matoba, T.; Terao, J. HPLC Method for Evaluation of the Free Radical-scavenging Activity of Foods by Using 1,1-Diphenyl-2-picrylhydrazyl. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1201–1204. [CrossRef]
32. Arnon, D.I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **1949**, *24*, 1–15. [CrossRef]
33. Hao, S.; Wang, Y.; Yan, Y.; Liu, Y.; Wang, J.; Chen, S. A Review on Plant Responses to Salt Stress and Their Mechanisms of Salt Resistance. *Horticulturae* **2021**, *7*, 132. [CrossRef]
34. Tarchoune, I.; Degl'Innocenti, E.; Kaddour, R.; Guidi, L.; Lachaâl, M.; Navari-Izzo, F.; Ouerghi, Z. Effects of NaCl or Na_2SO_4 salinity on plant growth, ion content and photosynthetic activity in *Ocimum basilicum* L. *Acta Physiol. Plant.* **2012**, *34*, 607–615. [CrossRef]
35. Saia, S.; Corrado, G.; Vitaglione, P.; Colla, G.; Bonini, P.; Giordano, M.; Rouphael, Y. An endophytic fungi-based biostimulant modulates volatile and non-volatile secondary metabolites and yield of greenhouse basil (*Ocimum basilicum* L.) through variable mechanisms dependent on salinity stress level. *Pathogens* **2021**, *10*, 797. [CrossRef]
36. Rivera, P.; Moya, C.; O'Brien, J.A. Low Salt Treatment Results in Plant Growth Enhancement in Tomato Seedlings. *Plants* **2022**, *11*, 807. [CrossRef]
37. Elawad, S.H.; Gascho, G.J.; Street, J.J. Response of sugarcane to silicate source and rate. I: Growth and yield. *Agron. J.* **1982**, *74*, 781–783. [CrossRef]
38. Munns, R.; Schachtman, D.; Condon, A. The Significance of a Two-Phase Growth Response to Salinity in Wheat and Barley. *Funct. Plant Biol.* **1995**, *22*, 561–569. [CrossRef]
39. Dasgan, H.; Aktas, H.; Abak, K.; Cakmak, I. Determination of screening techniques to salinity tolerance in tomatoes and investigation of genotype responses. *Plant Sci.* **2002**, *163*, 695–703. [CrossRef]
40. Meloni, D.A.; Gulotta, M.R.; Martínez, C.A.; Oliva, M.A. The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in Prosopis alba. *Braz. J. Plant Physiol.* **2004**, *16*, 39–46. [CrossRef]
41. Mansour, M.M.F.; Salama, K.H.A.; Ali, F.M.; Abou Hadid, A.F. Cell and plant responses to NaCl in Zea mays L. cultivars differing in salt tolerance. *Gen. Appl. Plant Physiol.* **2005**, *31*, 29–41.
42. Gopal, R.; Dube, B.K. Influence of variable potassium on barley metabolism. *Ann. Agric. Res.* **2003**, *24*, 73–77.
43. Tohidi, B.; Rahimmalek, M.; Arzani, A. Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of *Thymus* species collected from different regions of Iran. *Food Chem.* **2017**, *220*, 153–161. [CrossRef] [PubMed]
44. Valifard, M.; Mohsenzadeh, S.; Kholdebarin, B. Salinity Effects on Phenolic Content and Antioxidant Activity of Salvia macrosiphon. *Iran. J. Sci. Technol. Trans. Sci.* **2016**, *41*, 295–300. [CrossRef]
45. Zhou, Y.; Tang, N.; Huang, L.; Zhao, Y.; Tang, X.; Wang, K. Effects of Salt Stress on Plant Growth, Antioxidant Capacity, Glandular Trichome Density, and Volatile Exudates of Schizonepeta tenuifolia Briq. *Int. J. Mol. Sci.* **2018**, *19*, 252. [CrossRef]
46. Stagnari, F.; Galieni, A.; D'Egidio, S.; Falcinelli, B.; Pagnani, G.; Pace, R.; Pisante, M.; Benincasa, P. Effects of sprouting and salt stress on polyphenol composition and antiradical activity of einkorn, emmer and durum wheat. *Ital. J. Agron.* **2017**, *12*, 293–301. [CrossRef]
47. Varma, A.; Verma, S.; Sahay, N.; Bütehorn, B.; Franken, P. Piriformospora indica, a cultivable plant-growth-promoting root endophyte. *Appl. Environ. Microbiol.* **1999**, *65*, 2741–2744. [CrossRef]
48. Varma, A.; Singh, A.; Sudha, M.; Sahay, N.S.; Sharma, J.; Roy, A.; Kumari, M.; Rana, D.; Thakran, S.; Deka, D.; et al. Piriformospora indica: A cultivable mycorrhiza-like endosymbiotic fungus. In *The Mycota*, 9th ed.; Hock, B., Ed.; Springer: Berlin, Germany, 2001; pp. 125–150.
49. Rai, M.K.; Varma, A.; Pandey, A.K. Antifungal potential of Spilanthes calva after inoculation of Piriformospora indica. *Mycoses* **2004**, *47*, 479–481. [CrossRef] [PubMed]
50. Achatz, B.; Franken, P. Untersuchungen zum Einfluss des Wurzelendophyten Piriformospora indica auf das Wachstum von Hordeum vulgare, die Resistenz gegen Blumeria graminis f.sp. hordei und die Genexpression in den Blättern. Ph.D. Thesis, Philipps-Universität Marburg, Marburg, Germany, 2006. [CrossRef]

51. Sun, C.; Johnson, J.M.; Cai, D.; Sherameti, I.; Oelmüller, R.; Lou, B. Piriformospora indica confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. *J. Plant Physiol.* **2010**, *167*, 1009–1017. [CrossRef]
52. Satheesan, J.; Narayanan, A.K.; Sakunthala, M. Induction of root colonization by Piriformospora indica leads to enhanced asiaticoside production in Centella asiatica. *Mycorrhiza* **2011**, *22*, 195–202. [CrossRef]
53. Smriti, S.; Ajit, V. From Piriformospora indica to Rootonic: A review. *Afr. J. Microbiol. Res.* **2014**, *8*, 2984–2992. [CrossRef]
54. Harrach, B.D.; Baltruschat, H.; Barna, B.; Fodor, J.K.; Ogel, K.H. The Mutualistic Fungus *Piriformospora indica* Protects Barley Roots from a Loss of Antioxidant Capacity Caused by the Necrotrophic Pathogen *Fusarium culmorum*. *Mol. Plant Microbe. Interact.* **2013**, *26*, 599–605. [CrossRef] [PubMed]
55. Das, A.; Kamal, S.; Shakil Najam, A.; Sherameti, I.; Oelmuller, R.; Dua, M.; Tuteja, N.; Johri, A.K.; Varma, A. The root endophyte fungus *Piriformospora indica* leads to early flowering, higher biomass and altered secondary metabolites of the medicinal plant, *Coleus forskohlii*. *Plant Signal. Behav.* **2012**, *7*, 103–112. [CrossRef] [PubMed]
56. Ansari, M.W.; Bains, G.; Shukla, A.; Pant, R.C.; Tuteja, N. Low temperature stress ethylene and not Fusarium, might be responsible for mango malformation. *Plant Physiol. Biochem.* **2013**, *69*, 34–38. [CrossRef] [PubMed]
57. Unnikumar, K.R.; Sowjanya, S.K.; Varma, A. Piriformospora indica: A versatile root endophytic symbiont. *Symbiosis* **2013**, *60*, 107–113. [CrossRef]
58. Zarea, M.J.; Hajinia, S.; Karimi, N.; Goltapeh, E.M.; Rejali, F.; Varma, A. Effect of Piriformospora indica and Azospirillum strains from saline or non-saline soil on mitigation of the effects of NaCl. *Soil Biol. Biochem.* **2012**, *45*, 139–146. [CrossRef]
59. Zarea, M.J.; Chordia, P.; Varma, A. Piriformospora indica Versus Salt Stress. *Soil Biol.* **2013**, *33*, 263–281. [CrossRef]
60. Lee, S.; Yap, M.G.; Hung, R.; Bennett, J.W. Volatile organic compounds emitted by Trichoderma species mediate plant growth. *Fungal Biol. Biotechnol.* **2016**, *3*, 1–14. [CrossRef]
61. Hung, R.; Lee, S.; Bennett, J.W. Arabidopsis thaliana as a model system for testing the effect of Trichoderma volatile organic compounds. *Fungal Ecol.* **2015**, *6*, 19–26. [CrossRef]
62. Nieto-Jacobo, M.F.; Steyaert, J.M.; Salazar-Badillo, F.B.; Nguyen, D.V.; Rostás, M.; Braithwaite, M.; De Souza, J.T.; Jimenez-Bremont, J.F.; Ohkura, M.; Stewart, A.; et al. Environmental Growth Conditions of Trichoderma spp. Affects Indole Acetic Acid Derivatives, Volatile Organic Compounds, and Plant Growth Promotion. *Front. Plant Sci.* **2017**, *8*, 102. [CrossRef]
63. Jalali, F.; Zafari, D.; Salari, H. Volatile organic compounds of some Trichoderma spp. increase growth and induce salt tolerance in Arabidopsis thaliana. *Fungal Ecol.* **2017**, *29*, 67–75. [CrossRef]
64. Gong, Y.; Chen, L.-J.; Pan, S.-Y.; Li, X.-W.; Xu, M.-J.; Zhang, C.M.; Qin, S. Antifungal potential evaluation and alleviation of salt stress in tomato seedlings by a halotolerant plant growth-promoting actinomycete Streptomyces sp. KLBMP5084. *Rhizosphere* **2020**, *16*, 100262. [CrossRef]
65. Paul, D.; Park, K.S. Identification of Volatiles Produced by Cladosporium cladosporioides CL-1, a Fungal Biocontrol Agent That Promotes Plant Growth. *Sensors* **2013**, *13*, 13969–13977. [CrossRef]
66. Lugtenberg, B.; Kamilova, F. Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.* **2009**, *63*, 541–556. [CrossRef]
67. Li, N.; Kang, S. Do volatile compounds produced by *Fusarium oxysporum* and *Verticillium dahliae* affect stress tolerance in plants? *Mycology* **2018**, *9*, 166–175. [CrossRef]
68. Abogadallah, G.M. Insights into the significance of antioxidative defense under salt stress. *Plant Signal. Behav.* **2010**, *5*, 369–374. [CrossRef] [PubMed]
69. Prasad, R.; Kamal, S.; Sharma, P.K.; Oelmueller, R.; Varma, A. Root endophyte Piriformospora indica DSM 11827 alters plants morphology, enhances biomass and antioxidant activity of medicinal plant Bacopa monniera. *J. Basic Microbiol.* **2006**, *53*, 1016–1024. [CrossRef]
70. Huang, Y.C.; Chang, Y.H.; Shao, Y.Y. Effects of genotype and treatment on the antioxidant activity of sweet potato in Taiwan. *Food Chem.* **2006**, *98*, 529–538. [CrossRef]
71. Kai, M.; Piechulla, B. Plant growth promotion due to rhizobacterial volatiles—An effect of CO_2? *FEBS Lett.* **2009**, *583*, 3473–3477. [CrossRef] [PubMed]