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# Rice Plants' Resistance to Sheath Blight Infection Is Increased by the Synergistic Effects of *Trichoderma* Inoculation with SRI Management

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**Abstract:** The capability of endophytic *Trichoderma* spp. to reduce sheath blight disease in rice caused by *Rhizoctonia solani* was assessed under the growth conditions established by practices of the System of Rice Intensification (SRI), compared to those of standard irrigated rice cultivation. Rice seeds inoculated with a local isolate of the fungus *Trichoderma asperellum* SL2 were grown under respective SRI and conventional conditions with the inoculated or uninoculated plants and then infected with the pathogen *R. solani*. It was seen that inoculation with this strain of *Trichoderma* protected rice plants against *R. solani* infection while enhancing plant growth, photosynthetic rate, and stomatal conductance. The biocontrol effectiveness of inoculation with a particular strain of *Trichoderma* was significantly greater under SRI management compared to conventional cultivation. This is the first report on how a crop management system, in this case, SRI, can influence the biocontrol effectiveness of *Trichoderma* spp.

**Keywords:** Biocontrol; *Trichoderma; Oryza sativa* L. 'MRQ74'; System of Rice Intensification; sheath blight disease; *Rhizoctonia solani;* symbiosis; *Trichoderma asperellum* SL2

# 1. Introduction

In terms of the disease damage caused to rice crops worldwide each year, sheath blight is second only to rice blast. It is estimated that, on average, sheath blight reduces rice production in Asia by about 6%, with localized losses of up to 50% [1–3]. Worldwide, sheath blight causes significant economic losses in rice yield [4–6].

The soil-borne pathogenic fungus that causes this disease, *Rhizoctonia solani*, is difficult to control due to its mechanisms of dispersal, propagation, and long-term survival, mediated through sclerotia, hardened masses of hyphae that contain reserves of food for the pathogen [7,8]. The infection spreads rapidly by means of hyphal runners that reach up from the soil to the upper plant parts, including leaf blades, and also to adjacent plants, especially at the early-heading and grain-filling stages of growth [9].

Disease development is most rapid under microclimatic conditions favorable to the pathogen, including low sunlight exposure, high temperature (28–32 °C), and humidity near 95% [9,10]. Close planting and high plant density create favorable conditions for its spread due to the crowding and shading of plants and the build-up of humidity within the canopy.

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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/license s/by/4.0/). One of the ways in which plant diseases can be controlled is by strengthening plants' immunity to pathogenic attack through specific plant–microbial interactions [11]. Plantbeneficial associations with soil-borne microbes, both bacteria and fungi, are commonplace in nature and are important for plant survival [12,13]. Certain fungi that have the capacity for biological control are able to suppress pathogenic activity and defend host plants against foliar pathogens and insect herbivores by priming above-ground plant parts against predation and infection [14].

Farming systems that are managed according to agroecological principles for achieving sustainable food production seek to utilize inter-species interactions that, among other functions, can control or inhibit crop disease [15,16]. The System of Rice Intensification (SRI) is one such methodology that promotes ecosystem diversity and beneficial plantmicrobial interactions [17–19]. SRI methods have been reported to affect agroecological dynamics within rice field ecosystems, for example, by increasing the diversity of insects [20–22] and microbes [23–26]. SRI-grown rice plants have also been reported to be more resistant to infestation by pests [27] and to plant diseases, including sheath blight [28].

The many species of the *Trichoderma* genus are ubiquitous in nature, being soil-borne ascomycetes that are highly interactive in soil, root, and foliar environments. Moreover, they are characterized by rapid growth [29,30]. Selected strains of *Trichoderma* spp. are increasingly used to enhance agricultural production as they can assist plants in resisting both biotic and abiotic stresses as well as to increase plants' capacity to produce certain growth-regulating compounds [31–34].

Being opportunistic plant symbionts and effective mycoparasites, numerous *Trichoderma* spp. have shown the potential to be used as commercial biofungicides that have no harmful effects on humans, wildlife, and other beneficial microorganisms [35]. Members of this genus can establish themselves within plants, contributing to the control of pathogens without harming their hosts [36].

We have reported previously on the plant growth-promotion potentials of *Trichoderma asperellum* SL2 and its ability to enhance rice plants' height, tillering, biomass, photosynthesis efficiency, chlorophyll content, and stomatal density [37–39]. However, the ability of this strain to control phytopathogenic fungi in rice has not been documented, especially its capacity to control plant disease in combination with SRI management practices. These agronomic practices create environmental conditions that are different from standard rice paddy management. By greatly reducing plant density m<sup>2</sup>, they permit more airflow through the canopy, and this lowers humidity within that microclimate. By stopping the continuous flooding of paddies and by introducing soil-aerating mechanical weeding, paddy soils under SRI management are aerated both passively and actively. This supports root growth and aerobic soil organisms, which also benefit from increased organic matter in the soil, with more substrate for the soil biota ranging from microbes to earthworms.

Previous reports from field evaluations have indicated that SRI methods have an inhibiting effect on sheath blight in rice crops. In 2004, researchers from the China National Rice Research Institute in Hangzhou reported that farmers in Tian Tai County of Zhejiang province were observing 70% less sheath blight in their SRI fields compared to in fields cultivated with conventional methods [40]. The next year in Vietnam, the National IPM Program of the Ministry of Agriculture and Rural Development conducted on-farm trials in 8 districts to evaluate SRI's effect on major rice pests and diseases in that country. These trials showed the prevalence of sheath blight on SRI plots to be 63% and 74% less in the spring and summer seasons, respectively, compared to adjoining plots that were cultivated with standard practices [28].

The study reported here was designed to determine the effects of planting methods (SRI vs. conventional) when rice plants were enhanced (or not) by inoculation with a selected *Trichoderma* strain and whether there were synergistic effects between SRI practices and *Trichoderma* for jointly controlling sheath blight infection. Changes in plants' growth and in their physiological processes were monitored, as were associated disease manifestations.

#### 2. Materials and Methods

# 2.1. Design of the Study and Crop Management

This study was designed to assess the respective and interactive effects of the planting methods (SRI vs. conventional) and the application of *T. asperellum* SL2, as well as any synergistic effects that there might be between SRI management and *T. asperellum* SL2 inoculation for reducing infection by the pathogenic fungus *R. solani*. A completely randomized design with six treatments and seven replications was used for the study (Table 1). As only this particular strain of *Trichoderma* was used in the treatments, in the rest of this paper, we will usually refer to it simply as *Trichoderma*.

Treatments	Label	<b>Rice Crop</b>	Trichoderma	Rhizoctania
		Management	Inoculation	Inoculation
1	SRI+T+RS	SRI	+	+
2	CONV+T+RS	Conventional	+	+
3	SRI+RS	SRI	-	+
4	CONV+RS	Conventional	-	+
5	SRI only	SRI	-	-
6	CONV only	Conventional	-	-

Table 1. Experimental design.

All plants were cultivated under greenhouse conditions with the following microclimatic characteristics: temperature  $30^\circ \pm 4$  °C; light intensity  $320 \pm 3 \mu$ mol;  $80 \pm 3\%$  humidity; and photoperiod 11 h 11 m 17 s  $\pm 9$  s for 65 days prior to the *R. solani* inoculation. The trials were all conducted under gnotobiotic conditions, as described below. Details of the differences between the SRI and conventional crop management methods employed are given in Table 2.

Table 2. Crop management in this study: SRI compared with conventional methods.

Practices	SRI Treatment *	<b>Conventional Treatment</b>
Seedling transplant age	5-day-old seedlings	20-day-old seedlings
Weed control	Soil was plowed manually using a rake (a 30 cm long wood han- dle attached to a 5 cm × 5 cm square wooden block) at inter- vals of 10, 20, 30, and 40 days af- ter transplanting, thereby elimi- nating weeds as well as enhanc- ing the soil's aeration	Hand weeding at 10, 20, 30, and 40 days after trans- planting
Water management	Non-flooded, slightly aerobic soil conditions but enough soil hu- midity to sustain plant growth. This enables better aeration and growth of <i>Trichoderma</i> . Before weeding, 2 cm water height from soil surface was applied, and af- ter weeding, the water was drained out immediately from the pots	A 5–6 cm water level was

Nutrient management	<ul> <li>2.5 g of sterilized compost (total nitrogen (N) 16.2%; phosphorus (P) 6.7%; potassium (K) 11.4%) was applied per plastic pot 10 days after transplanting. This is equivalent to an application rate of ~5 tons ha<sup>-1</sup></li> </ul>	425 mg urea, 325 mg P <sub>2</sub> O <sub>5</sub> (phosphorus pentoxide), and 300 mg K <sub>2</sub> O (potas- sium oxide) were applied per plastic pot 10 days af- ter transplanting
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\* These methods are described as 'simulated' because when rice plants are grown in pots, there is no opportunity for unconstrained root growth, and the surface soil aeration of mechanical weeding cannot be duplicated in pots. Both root growth and soil aeration are important components of SRI methodology [38].

#### 2.2. Fungal Cultures and Preparation of Inocula

An endophytic isolate of *T. asperellum* SL2 (public accession number: UPMC 1021) was used in this study [38]. An isolate of *R. solani* 1802/KB (public accession number: KF312465, previously used in Hossain et al. [5] and Kalaivani et al. [41]) was obtained from the Plant Molecular Biology Laboratory, Faculty of Science and Technology, Universiti Kebangsaan Malaysia.

For multiplication, each isolate was cultured on potato dextrose agar and incubated at 30 °C in an incubator. The *Trichoderma* inoculum used in this experiment was in the form of a fungal spore suspension. The spores were harvested from a seven-day-old fungal culture by adding 10 mL of sterile water to the *Trichoderma*-containing petri dish, and the spores were transferred immediately to an Erlenmeyer flask containing sterilized distilled water. Spore concentration was adjusted to 10<sup>7</sup> spores/mL based on hemocytometer counts [42]. The *R. solani* inocula used were in the form of mycelial plugs taken from a three-day-old fungal culture.

## 2.3. Soil Preparation

Homogeneous sandy clay loam soil (pH 6.9; EC; 2.1 dS/m; total N 0.8%; total P 0.52%; total K 0.78%) was used for this experiment. The soil was sterilized in an autoclave prior to use, then was immediately placed in plastic pots,  $25 \times 16$  cm. In both treatments, the amount of soil used per plastic pot was 3 kg.

## 2.4. Rice Plant Preparation and Inoculation with T. asperellum SL2

In this experiment, a Malaysian rice cultivar moderately susceptible to sheath blight disease, *Oryza sativa* L. cultivar MRQ74, was used [43]. Seeds of the 'MRQ74' cultivar were surface-sterilized by soaking them in 70% ethanol for 30 min, followed by soaking in 5% NaOCl for 30 min and then washing them with sterilized distilled water. For their treatment with *Trichoderma*, the seeds were soaked for 24 h in a flask containing 10<sup>7</sup> spores/mL. For the control plants, seeds were soaked the same way for 24 h but in sterilized distilled water.

The *Trichoderma*-inoculated seeds and the untreated seeds were grown separately at the same time under the greenhouse with microclimatic conditions described above. They were cultivated in seedling trays 30 × 50 cm containing a mixture of 500 g sterilized soil, 500 g sterilized sand, and 500 g sterilized compost as the growth medium. For the SRI-method trials, seedlings were transplanted into their respective plastic pots 5 days after sowing, while seedlings for the conventional-method trials were maintained in their seedling tray until reaching an age of 20 days, when they were transplanted. Seedlings for both the SRI and conventional trials were transplanted singly per plastic pot.

# 2.5. Inoculation with R. solani

Rice plants at 65 days old, i.e., in their late-tillering stage, were inoculated with *R*. *solani* by placing mycelial plugs beneath the leaf sheath [44]. The inoculated sheath was

covered immediately with aluminum foil. When typical lesions appeared after three days, the aluminum foil was removed, and the infected plants were left in a humidity chamber  $(4 \times 3 \times 2 \text{ m})$ . Optimum humidity  $(95 \pm 3\%)$  and temperature  $(30^\circ \pm 4 \text{ °C})$  were maintained using a 3–5 cm water level placed inside the humidity chamber. Plants were left in the humidity chamber for three weeks to allow for normal disease development [5,44].

# 2.6. Disease Evaluation

To assess the early stages of disease development after *R. solani* inoculation, the lengths of lesions on the sheath of the inoculated rice plants were measured (in cm) 7 days after inoculation. To evaluate sheath blight resistance/susceptibility, lesion lengths and the degree of disease severity in each sheath of the inoculated plants were recorded. Both total lesion length and disease severity were calculated at 3 weeks after the plants were inoculated with *R. solani*.

Total lesion length was the sum of all the lesion lengths on the leaves in a particular culm. The degree of disease severity was scored as follows: 0 = no lesions; 1 = appearance of water-soaked lesions; 2 = appearance of necrotic lesions; 3 = less than 50% necrosis on the leaf cross-section; 4 = more than 50% necrosis on the leaf cross-section; 5 = necrosis across the entire leaf section resulting in leaf death.

The index of disease susceptibility was calculated as follows: susceptibility index =  $(5n_5 + 4n_4 + 3n_3 + 2n_2 + 1n_1 + 0n_0)/5N \times 100$ ; where  $n_{0-5}$  is the number of leaves observed in each degree of necrosis (0 to 5), and N is the number of total leaves investigated in a culm. All lesions from a culm were added together to arrive at a total lesion length (in cm) for each culm [44].

#### 2.7. Measurement of Rice Growth and Physiological Components

Rice physiological and growth components were measured at 3 weeks after *R. solani* inoculation, i.e., in plants 86 days old. Measurements for the physiological traits were made on the flag leaves of the rice plants. Measurements of photosynthesis rate ( $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>), leaf stomatal conductance (mmol m<sup>-2</sup>s<sup>-1</sup>), internal carbon dioxide concentration (ppm), and transpiration rate (mmol m<sup>-2</sup>s<sup>-1</sup>) were monitored using a LICOR 6400 (Lincoln, Nebraska, USA) and an infrared gas analyzer (IRGA), as previously described in Doni et al. [38].

Plant height (in cm) was measured from ground level to the tip of the longest leaf. The tiller number was counted for each plant. For measurements of canopy fresh weight (g) and root fresh weight (g), the rice plants were separated carefully from the soil. Root fresh weight and canopy fresh weight were measured using digital scales. For the measurement of canopy dry weight and root dry weight (in g), all plants were dried in an oven for 7 days at 65 °C; after drying, each was weighed on a digital scale with all of the root and canopy dry weights recorded [37].

#### 2.8. Statistical Analysis

All data were analyzed statistically using one-way analysis of variance (ANOVA) methods. Mean separation was carried out for significantly different parameters using Duncan's Multiple Range Test (DMRT) at p < 0.05.

#### 3. Results

## 3.1. Rice Growth Responses to R. solani Inoculation

The height of SRI+T+RS plants was significantly greater than SRI+RS plants but not significantly more than CONV+T+RS plants. *R. solani* did not have any adverse effect on the SRI-grown plants that had been inoculated with *Trichoderma* in terms of the plantheight parameter. Moreover, the plant height of SRI+RS plants was seen to be significantly higher than that of CONV+RS plants (Table 3).

Treatments	Plant Height (cm)	Tiller Number	Root Fresh Weight (g)	Canopy Fresh Weight (g)	Root Dry Weight (g)	Canopy Dry Weight (g)
SRI+T+RS	71.71 a	6.57 a	40.05 a	20.90 ab	20.48 a	6.73 b
CONV+T+RS	68.12 a	5.28 bc	30.38 b	20.02 bc	16.63 b	4.73 c
SRI+RS	55.71 b	5.28 bc	22.61 c	16.98 d	13.91 c	4.33 c
CONV+RS	48.22 c	4.85 c	12.74 d	11.84 e	7.78 d	2.45 d
SRI only	70.92 a	6.14 ab	42.35 a	21.95 a	20.50 a	9.07 a
CONV only	69.48 a	6.57 a	30.59 b	19.13 c	17.08 b	6.65 b

Table 3. Rice plant growth responses to *R. solani* inoculation.

Means with the same letters within the column do not differ significantly according to DMRT (p < 0.05).

There were no significant differences in tiller number among SRI+T+RS plants, SRI plants, and CONV plants (Table 3). However, inoculation with *Trichoderma* significantly increased the tiller number on SRI+T+RS plants compared to CONV+T+RS and SRI+RS plants. These data indicate that the combined treatment of *Trichoderma* and SRI methods (young seedlings with aerobic soil conditions and increased soil organic matter) protected rice plants better against the negative effects of *R. solani*. The tiller number for SRI+RS matched that for CONV+T+RS, indicating that SRI management methods by themselves were conferring as much protection as when conventional methods were enhanced by *Trichoderma* inoculation.

Similarly, SRI+T+RS plants and SRI plants had the highest values for root fresh weight and dry weight (Table 3). Inoculating SRI-grown plants with both *Trichoderma* and *R. solani* (SRI+T+RS) did not reduce plant root weight. Further, when comparing results from SRI+T+RS and CONV+T+RS plants, it was seen that the *Trichoderma* by itself did not protect rice plants grown under conventional methods from *R. solani* infection as well as it protected SRI-grown plants. Significant differences were observed between SRI+RS plants and CONV+RS plants, with SRI+RS plants having greater fresh weight and dry weight of roots compared to CONV+RS plants.

SRI+T+RS plants also had higher fresh weight and dry weight of their canopies compared to SRI+RS and CONV+RS, with the differences being statistically significant. The results also showed that inoculation with *R. solani* significantly and adversely affected canopy fresh weight and dry weight, especially for plants grown with conventional methods since both CONV+T+RS and CONV+RS plants exhibited lower canopy weights when compared to SRI+T+RS plants. Generally, there was decreased weight of rice plants infected by *R. solani*, with the combined effects of SRI methods and *Trichoderma* inoculation giving rice plants the most protection from *R. solani* infection.

#### 3.2. Rice Plant Physiological Responses to R. solani Inoculation

The photosynthesis rate measured in SRI+T+RS plants (7.08  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>) was significantly greater than that in CONV+T+RS plants (6.14  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>), SRI+RS plants (2.36  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>), and CONV+RS plants (1.66  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>). The sheath blight disease caused by *R. solani* infection affected the physiological processes, especially in rice plants that were grown under conventional methods (Table 4). SRI-grown plants recorded the highest rate of photosynthesis compared to other treatments in all three pairings.

Treatments	Photosynthetic Rate (µmol m <sup>-2</sup> s <sup>-1</sup> )	Stomatal Conductance (mmol m <sup>-2</sup> s <sup>-1</sup> )	Internal Car- bon Dioxide Concentration (ppm)	Transpiration Rate (mmol m <sup>-2</sup> s <sup>-1</sup> )
SRI+T+RS	7.08 c	564.50 c	356.54 d	6.41 d
CONV+T+RS	6.14 d	351.67 d	383.18 c	7.27 с
SRI+RS	2.36 e	131.56 e	443.19 b	0.89 f
CONV+RS	1.66 f	85.08 f	490.72 a	1.25 e
SRI only	9.20 a	935.92 a	291.52 e	8.26 b
CONV only	8.19 b	850.88 b	354.27 d	8.96 a

Table 4. Rice plant physiological responses to R. solani infection.

Means with the same letters within the column do not differ significantly according to DMRT (p < 0.05).

Our physiological evaluation found that SRI+T+RS plants exhibited the highest stomatal conductance among the *R. solani*-inoculated treatments, while *Trichoderma*-inoculated plants generally showed a consistent ability to enhance plants' stomatal conductance. *Trichoderma* appeared to be less able to regulate stomatal conductance in plants that had been grown with conventional methods, as we found stomatal conductance to be moderately affected by *R. solani* infection in the CONV+T+RS plants. Rice plants that had been grown with conventional methods and were not inoculated with *Trichoderma* definitely experienced the most reduction in stomatal conductance. CONV+RS plants had the lowest value for stomatal conductance (85.08 mmol m<sup>-2</sup>s<sup>-1</sup>) compared to the values with other treatments.

Reduction of plants' physiological capability was also observed in the marked differences in their transpiration rates according to the treatment received. A lower transpiration rate is generally understood to represent high water use efficiency. The low transpiration rates in SRI+RS and CONV+RS plants reflect that the *R. solani* infection was affecting both the plants' water transport and their physiological processes.

Transpiration rate activity is closely related to plant leaves' stomatal conductance. For example, the transpiration rate of CONV+RS plants was measured as 1.25 mmol m<sup>-2</sup>s<sup>-1</sup>, which represents rather inefficient physiological functioning because these plants had very low stomatal conductance while transpiring much water. On the other hand, the transpiration rate in plants that had been inoculated with *Trichoderma* was not much affected by *R. solani* infection. SRI+T+RS plants had a more desirable, i.e., lower, transpiration rate than did the CONV+T+RS plants: 6.4 mmol m<sup>-2</sup>s<sup>-1</sup> vs. 7.3 mmol m<sup>-2</sup>s<sup>-1</sup>.

SRI+T+RS plants, with their higher stomatal conductance compared to CONV+T+RS plants, also transpired less water than did the CONV+T+RS plants. This means that SRI+T+RS plants were metabolically more efficient in their use of water compared to CONV+T+RS plants. This difference was seen in the SRI plants generally. SRI plants, while having a stomatal conductance rate of 935.9 mmol m<sup>-2</sup>s<sup>-1</sup>, transpired only 8.3 mmol m<sup>-2</sup>s<sup>-1</sup> water. This was significantly lower than the 8.9 mmol m<sup>-2</sup>s<sup>-1</sup> water transpired by CONV plants (Table 4).

In converting carbon dioxide into carbohydrates, the SRI+T+RS plants were the most efficient among those treated with *R. solani*. These plants also exhibited the lowest internal concentrations of carbon dioxide compared to rice plants that received other treatments (Table 4). Severe infection caused by *R. solani* significantly increased the internal carbon dioxide concentration in CONV+RS plants (to 490.7 ppm). On the other hand, comparing the internal carbon dioxide concentration in CONV+T+RS and SRI+T+RS plants indicated that conventional methods of rice cultivation were reducing the ability of *Trichoderma* to improve rice plants' efficiency in converting carbon dioxide into carbohydrates, presumably because of the hypoxic soil conditions.

#### 3.3. Disease Evaluation Screening

SRI+T+RS plants recorded the lowest lesion length (0.3 cm), total lesion length (17.4 cm), and susceptibility index (21%), contrary to CONV+RS plants. The latter had an average lesion length of 6.1 cm, a total lesion length of 56.8 cm, and a susceptibility index score of 64%. Moreover, SRI+T+RS plants had lower lesion length, less total lesion length, and lower susceptibility index score compared to CONV+T+RS plants (Table 5). In general, the SRI plants were less susceptible compared to CONV plants. This indicated that alternative crop management methods resulted in respectively different manifestations of disease.

**Table 5.** Effects of *T. asperellum* SL2 and SRI methods for protecting rice plants from *R. solani* infection.

Treatments	Lesion Length (cm)	Total Lesion Length Susceptibility Index		
Treatments		(cm)	(%)	
SRI+T+RS	0.31 d	17.42 d	20.7 с	
CONV+T+RS	1.31 c	22.27 с	30.6 b	
SRI+RS	3.21 b	42.88 b	57.8 a	
CONV+RS	6.12 a	56.84 a	63.7 a	

Means with the same letters within the column do not differ significantly according to DMRT (p < 0.05).

The synergistic effects of *Trichoderma* combined with SRI methods had the most impact on rice plants' tolerance of *R. solani* infections as manifested in physical assessments of the plants. There was lower lesion length and susceptibility in SRI+T+RS plants compared to CONV+T+RS plants and lower lesion length and susceptibility in SRI+RS plants compared to CONV+RS plants, suggesting a synergistic interaction between *Trichoderma* inoculation and SRI cultivation methods (Table 5).

The positive interaction between *Trichoderma* and SRI management enhanced rice plants' fitness, which led to greater protection against the effects of sheath blight. The results also indicated that conventional methods diminished the potential of *Trichoderma* as a biocontrol agent that could counteract the negative effects of *R. solani* in rice plants.

# 4. Discussion

The ability of *T. asperellum* SL2 to increase rice plant growth has been found previously to be different between rice plants grown under SRI conditions and those with conventional cultivation [38]. Plants grown with SRI management and inoculated with *Trichoderma* exhibited better growth performance in all of the parameters measured compared to rice plants that were grown conventionally. A previous study by Daryaei et al. [45] reported that the biological activity of *Trichoderma* spp. against *R. solani* is heavily influenced by abiotic factors.

Lower soil saturation and high soil pH lead to more rapid germination and greater biological control activity of *Trichoderma* spp. This is in agreement with an earlier study by Neuman and Laing [31], which found that the plant growth-enhancement effects of *Trichoderma* are greater in an environment that is conducive to plant health and with conditions favorable for the growth and functioning of plant root systems. With SRI, soils are kept mostly aerobic, with oxygen in the soil enhanced during mechanical weeding operations in addition to having water on the field intermittently rather than continuously.

Furthermore, with SRI, there is less or no use of synthetic fertilizers and little use of pest-control compounds that can unbalance or inhibit the growth of beneficial microorganisms [15,46]. An increase in the number of beneficial microbes in soils under SRI management has been previously reported by other authors [25,26,47,48].

Under SRI growing conditions, even the rice plants inoculated with *R. solani* but not with *Trichoderma* were observed to fare better than their counterparts cultivated under standard flooded-rice management. Rice plants under SRI field conditions have been

reported to benefit from specific changes in their morphologic characteristics and physiological performance, as summarized in Thakur et al. [18]. It has been shown, for example, that simply transplanting seedlings at a young age can minimize root trauma and increase root growth and plant tillering [49]. Aeration of the soil during weeding enhances root growth and root health as the unsaturated soil conditions give plant roots more oxygen to take in. These effects of SRI cultivation practices would, respectively, enhance rice plants' ability to tolerate *R. solani*.

In the present study, *T. asperellum* SL2 protected rice plants from *R. solani* infection, at least in part, by increasing or protecting the plants' physiological capacities. This in itself would render the plants better able to withstand the effects of sheath blight disease. SRI+T+RS plants, for example, exhibited physiological activity that was more efficient, with the highest photosynthetic rates and a low rate of transpiration which indicates higher water use efficiency.

Thakur et al. [50] reported that SRI rice plant phenotypes have more than twice as much water use efficiency in their production of photosynthate, fixing 3.6  $\mu$ mols of carbon dioxide per mmol of water transpired, compared to 1.6  $\mu$ mols of carbon dioxide that conventionally-grown plants of the same cultivar converted into photosynthate per mmol of water transpired. This means that the SRI plants studied were producing 'more crop per drop', something that will become ever more important as farmers around the world have to cope with less irrigation water supply or more poorly-distributed rainfall.

At the same stage of development, SRI-grown plants with *Trichoderma* inoculation also showed the lowest levels of internal carbon dioxide among the treatments receiving *R. solani* inoculation. This indicates that there was more activity of carboxylation in carbon dioxide fixation when producing glucose for carbohydrate metabolism in rice plants. This result also agrees with the earlier findings of Thakur et al. [50,51].

*Trichoderma* spp. have also been reported to be able to reprogram plants' gene expression to evoke induced systemic resistance (ISR) within plants [13,34]. This genetic reprogramming induces mechanisms in plants that alleviate physiological and abiotic stresses and improve their N-use efficiency [29,52]. These effects have already been seen with SRI-grown plants, and they could be heightened by endophytic *Trichoderma* [53–55].

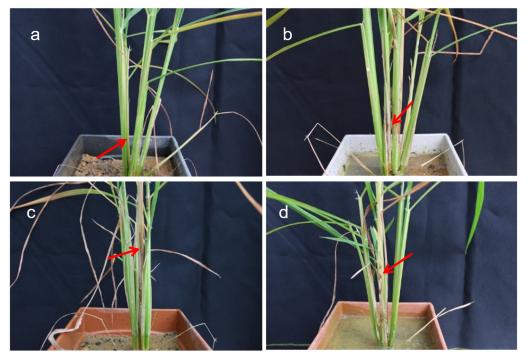
It was interesting that the SRI+RS plants demonstrated higher physiological efficiency than the CONV+RS plants, as the latter exhibited the greatest reduction in physiological parameters among all of the plants that were infected with *R. solani*. Although this finding cannot be directly extrapolated from greenhouse to field conditions, it does indicate that the negative effects of *R. solani* can be reduced by providing rice plants with more favorable environments above-ground and healthier soil conditions in which to grow.

The combined positive effects of *Trichoderma* inoculation and SRI management are seen to reduce the susceptibility of rice plants to sheath blight disease and its adverse effects. These results are consistent with the findings of Adak et al. [56] and Shivay et al. [57], who reported that under SRI management, plant growth-promoting microorganisms (PGPM) increased the activity of defense- and pathogenesis-related enzymes compared to those in rice plants grown under conventional, continuously-flooded conditions.

Previous studies have shown that *Trichoderma* spp. can induce host resistance to sheath blight disease by mechanisms such as the induction of pathogenesis-related proteins and the increasing activity of enzymes such as chitinase,  $\beta$ -1,3-glucanase, cellulose, and peroxidase [58], as well as increasing the synthesis of terpenoids in host plants [59]. In maize, proteomic analysis has shown that inoculation with *T. harzianum* T22 can upregulate many proteins related to plants' defense mechanisms [60,61]. Research by Chen et al. [62] showed that when the populations of *T. asperellum* T12 on the surface of rice leaf sheaths were increased, the populations of *R. solani* decreased.

Antagonistic activity against *R. solani* has been well-documented in previous studies as most *Trichoderma* spp. have been shown to produce toxic volatile metabolites such as  $\beta$ -1,3-glucanase, chitinase, acid phosphatase, acid proteases, and alginate lyase. These metabolites have significant effects on the growth and development of *R. solani* [63,64], producing cell wall-degrading enzymes [65] and antifungal compounds such as 6-pentyl pyrone [66] and cremenolide [67].

The influence of planting and cultural methods on plant growth and health was seen in our trials when rice plants grown with SRI methods were exposed to *R. solani*. These plants exhibited lower lesion length, total lesion length, and susceptibility compared to rice plants that were growing under conventional management (Figure 1). This corresponded with the more favorable physiological traits exhibited by these plants compared with other plants of the same cultivar grown with conventional methods (Table 4).



**Figure 1.** Disease manifestations in different treatments: (**a**) *T. asperellum* SL2-inoculated rice plants grown under SRI condition exhibited the lowest lesion length compared to (**b**) *T. asperellum* SL2-inoculated plants grown under conventional condition, (**c**) rice plants without *T. asperellum* SL2 inoculation grown under SRI condition, and (**d**) rice plants without *T. asperellum* SL2 inoculation grown under conventional condition.

Previous research has reported that SRI plants have superior physiological and morphological characteristics, e.g., more tillers produced, more panicle formation, deeper and better-distributed root systems, higher rates of xylem exudation, more open plant architecture with more erect and larger leaves that have a higher leaf area index (LAI) which leads to greater light interception, higher leaf chlorophyll content and a higher rate of photosynthesis, lower transpiration of water and higher water-use efficiency, and greater fluorescence efficiency [18,50,51,68].

## 5. Conclusions

This research has shown that inoculation of rice plants with the symbiotic fungus *T. asperellum* SL2 was effective in reducing the incidence and severity of sheath blight disease caused by the pathogenic fungus *R. solani* while also improving desirable physiological characteristics of rice plants and promoting their growth. Thus, *Trichoderma*, as well as other soil microbes which warrant similar study, can likely play important roles in maintaining sustainable rice production in cost-effective and environmentally-friendly ways.

We conclude that *Trichoderma* can probably assist in providing optimal ecosystem services in combination with SRI management methods that maintain moist but aerobic soil conditions, reduce or avoid agrochemical use, apply compost to build up soil organic matter, and oxygenate surface soil during mechanical weeding. These practices provide favorable conditions for *Trichoderma* to thrive around, on, and inside the rice plants. However, the efficacy of the proposed approach should be further evaluated on a larger scale under field conditions with farmers.

Much more remains to be known scientifically about this particular plant-microbial association and similar endophytic relationships. The inter-relationships measured and reported here may well be indicative of a broader set of beneficial plant-microbial associations not limited to this particular microorganism.

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