

Transcriptomic Profiling of Rice Seedlings Inoculated with the Symbiotic Fungus *Trichoderma asperellum* SL2

by Fathurrahman Fathurrahman

Submission date: 22-Nov-2023 12:36PM (UTC+0700)

Submission ID: 2235921344

File name: 3.pdf (1.6M)

Word count: 6747

Character count: 38045

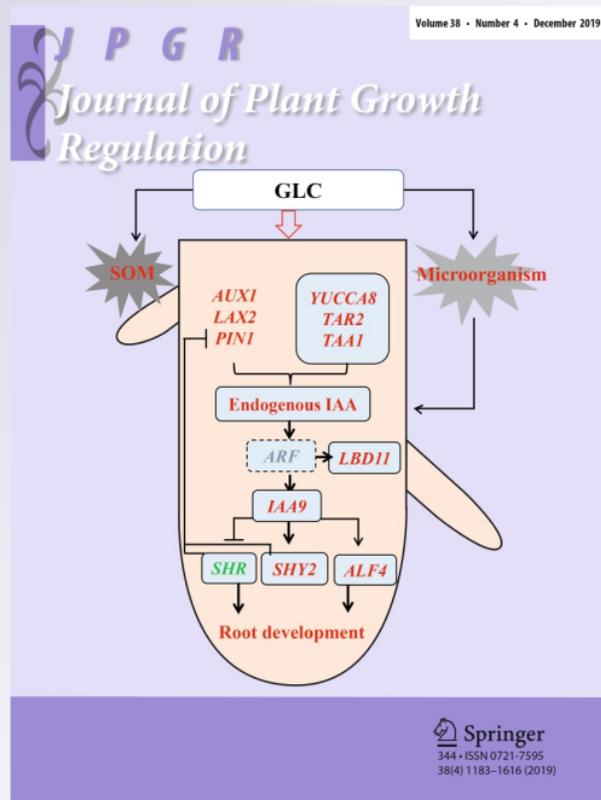
Transcriptomic Profiling of Rice Seedlings Inoculated with the Symbiotic Fungus *Trichoderma asperellum* SL2

Febri Doni, F. Fathurrahman, Muhamad Shakirin Mispan, Nurul Shamsinah Mohd Suhaimi, Wan Mohtar Wan Yusoff & Norman Uphoff

Journal of Plant Growth Regulation

ISSN 0721-7595
Volume 38
Number 4

J Plant Growth Regul (2019)
38:1507-1515
DOI 10.1007/s00344-019-09952-7



Your article is protected by copyright and all rights are held exclusively by Springer Science+Business Media, LLC, part of Springer Nature. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".



Transcriptomic Profiling of Rice Seedlings Inoculated with the Symbiotic Fungus *Trichoderma asperellum* SL2

Febri Doni¹ · F. Fathurrahman² · Muhamad Shakirin Mispan^{1,3} · Nurul Shamsinah Mohd Suhaimi¹ · Wan Mohtar Wan Yusoff⁴ · Norman Uphoff⁵

Received: 6 January 2019 / Accepted: 12 February 2019 / Published online: 20 March 2019
© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract

The fungal species *Trichoderma* is reported to have a significant impact on the growth and physiological performance of rice plants. However, the molecular mechanisms that induce these effects remain unspecified. Using next-generation sequencing technology, this study compared the differential expression of genes in rice seedlings that had been inoculated with *Trichoderma asperellum* SL2 with the gene expression in seedlings that had no such inoculation. The study showed that many genes related to plant growth enhancement and physiological functioning are differentially expressed in seedlings which have been symbiotically colonized by *T. asperellum* SL2. In these seedlings, specific genes related to photosynthesis, RNA activity, stomatal activity, and root development were found to be up-regulated as others were down-regulated. Although the exact causal mechanisms at the molecular level remain to be identified, the presence of *Trichoderma* versus its absence was associated with almost ten times more significant up-regulations than down-regulations for specific genes that have been identified from previous genomic mapping. Such analysis at the molecular level can help to explain observed phenotypic effects at the organismic level, and it begins to illuminate the observed beneficial relationships expressed phenotypically between crop plants and certain symbiotic microbes.

Keywords Gene expression · Rice · Transcriptomic analysis · *Trichoderma*

Introduction

Rice plant performance is shaped by environmental factors throughout the growth cycle. The influence of environmental factors must work through their effect on physiological

processes and molecular signaling inside rice plant cells (Nguyen et al. 1997; Colmer et al. 2014; Maruyama et al. 2014; Wu et al. 2018). Improving rice plant physiological characteristics and functioning is important for achieving higher yields (Haeefele et al. 2009; Mishra and Salokhe 2011; Alhasnawi et al. 2017).

Symbiotic fungi are reported to have significant impacts on rice plant growth and physiological performance (Redman et al. 2011; Doni et al. 2014; Contreras-Cornejo et al. 2014; Azad and Kaminskyj 2016; Zaidi et al. 2018). The mechanisms that enable symbiotic fungi to shape plant growth and performance include the production of diverse chemical substances affecting plant growth such as phytohormones; phosphate-solubilizing enzymes and siderophore production; and eliciting alterations in plant gene expression which affects among other things their metabolic and defense pathways (Rodriguez et al. 2008; Wani et al. 2015).

Trichoderma spp. is one of the prominent fungus species involved in these processes (Contreras-Cornejo et al. 2009, 2018). This species plays several roles in improving plant growth and engages in beneficial activities that

✉ Febri Doni
febridoni@um.edu.my

✉ Muhamad Shakirin Mispan
shakirin@um.edu.my

¹ Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

² Department of Agrotechnology, Faculty of Agriculture, Universitas Islam Riau, Pekanbaru 28284, Indonesia

³ Centre for Research in Biotechnology for Agriculture, University of Malaya, 50603 Kuala Lumpur, Malaysia

⁴ School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Selangor D.E, 43600 Bangi, Malaysia

⁵ SRI International Network and Resources Center, Cornell University, Ithaca, NY 14853, USA

include the degradation of toxins; increasing plant resistance to pathogens and environmental stress; solubilizing soil nutrients and enhancing nutrient uptake; and increasing root hair formation (Shoresh et al. 2010; Shukla et al. 2012; Nicolás et al. 2014; Guler et al. 2016).

Despite the recognized potential of *Trichoderma* as a growth regulator in plants (Yedidia et al. 2001; Neumann and Laing 2006; Segarra et al. 2007; Contreras-Cornejo et al. 2015; Pascale et al. 2017), there are few studies of the exact mechanisms of *Trichoderma*–plant interaction. The influence of *Trichoderma* on plant gene expression can be assessed by doing transcriptomic studies of plants that have been inoculated with these microorganisms and comparing them with uninoculated plants.

There have been some transcriptomic studies previously conducted on gene expression associated with *Trichoderma* interactions with plants other than rice; for example, cacao (Bailey et al. 2006; Bae et al. 2009), tomatoes (Chacón et al. 2007), and cucumber (Samolski et al. 2012). These studies have shown that the inoculation of plants with *Trichoderma* up-regulates a number of functional genes which are related to plant physiological enhancement and to tolerance of abiotic stresses. Thus, transcriptomic analysis of the effects of *Trichoderma* inoculation in rice plants, while interesting because of this food crop's nutritional importance to half of the world's population, is not unique.

The molecular mechanisms associated with *Trichoderma* inoculation of rice plants, regarded as a model monocotyledon and one of the world's most important staple crops, are yet to be explored. In this study, the effects of a local plant growth-promoting fungus *T. asperellum* SL2 were evaluated under experimental conditions. Gene expression patterns in rice seedlings that had been inoculated with *Trichoderma* (or not inoculated as controls) were profiled using next-generation sequencing technology. This method allowed us to sequence entire plant genomes and to sample entire transcriptomes quite efficiently. This technology is also useful for undertaking rapid gene expression analysis, especially feasible for rice plants because the reference genome sequences for rice are readily available (Varshney et al. 2009).

This study thus explores mechanisms by which symbiotic *Trichoderma* can promote rice plant growth at the molecular level. The results of the study are expected to provide a better foundation for further work on plant–microbe interactions. The analysis shows many statistically significant associations between microbial inoculation and up-regulation or down-regulation of rice plant gene expression. Exactly how and why this influence is exerted at the molecular level remains to be established, but the patterns of association if reliable and coherent enough will advance appreciation of endophytic plant–microbial symbiosis.

Materials and Methods

Fungal Growth and Rice Seed Inoculation

T. asperellum SL2 (Public Accession Number: UPMC 1021) was propagated in potato dextrose agar and incubated for 7 days at 30 °C temperature. After incubation, *T. asperellum* SL2 spores were harvested from the plates by adding 10 ml of sterile water and were immediately transferred to an Erlenmeyer flask containing sterilized distilled water. The spore concentration was adjusted to 10^7 spores/ml.

Malaysian rice plants (*Oryza sativa* L.) of variety MRQ74 were used for this study. The seeds were surface-sterilized by soaking in 70% ethanol for 3 min, followed by soaking in 5% sodium hypochlorite for 3 min, and then washing them in sterilized distilled water. A total of 300 seeds that had been soaked for 24 h in a flask containing a suspension of 10^7 spores/ml were used as the treatment; 300 seeds that had been soaked for 24 h in sterilized distilled water without fungus spores served as the control.

Plant Material and Growth Conditions

The seeds for the treatment (T) and control (C) comparison were grown separately in trays for 10 days under greenhouse conditions (with 30 ± 4 °C temperature; 320 ± 3 μ mol light intensity; $80 \pm 3\%$ humidity; and 11 h 11 m 17 s \pm 9 s photoperiod). The 30 \times 50 cm seedling trays each contained 500 g of sterilized soil, 500 g sterilized sand, and 500 g sterilized compost as the growth medium. Water was supplied carefully to the trays by using a water sprayer to avoid trauma to the plants. The soil was kept moist, and no standing water was allowed.

Transcriptomic Analysis

Transcriptomic analysis was carried out based on rice seedlings at 10 days old. Two seedlings from the respective treatment and control populations which showed the best growth in terms of plant height and leaf area were selected for transcriptomic analysis. Seedling leaves were ground to a powdery form (for about 1–2 min) in a mortar with a pestle in the presence of liquid nitrogen, and then RNA extraction was done using a Sepasol-RNA I Super G kit (Nacalai Tesque, Inc., Japan). Extraction procedures followed the manufacturer's instructions. Total RNA went through the following four steps before library construction: (1) Agarose gel electrophoresis: testing RNA degradation and potential contamination; (2) Nanodrop: testing

RNA purity (OD260/OD280); (3) Qubit: quantifying the RNA and determining concentration; and (4) Agilent 2100: to check RNA integrity.

After the quality check procedures, two sets of RNA samples extracted from the two seedlings in each treatment group were pooled with a ratio 1:1. Further, mRNA was enriched using oligo(dT) beads. The mRNA was fragmented randomly in a fragmentation buffer followed by cDNA synthesis using random hexamers and reverse transcriptase. After first-strand synthesis, a custom second-strand synthesis buffer (Illumina) was added with dNTPs, RNase H, and *Escherichia coli* polymerase I to generate the second strand by nick-translation.

The final cDNA library was ready after a round of purification, terminal repair, A-tailing, ligation of sequencing adapters, size selection, and PCR enrichment. Library concentration was first quantified using a Qubit 2.0 fluorometer (Life Technologies), and then diluted to 1 ng/μl before checking insert size on an Agilent 2100 bioanalyzer and quantifying to greater accuracy by quantitative PCR (q-PCR) (library activity > 2 nM). Libraries were fed into HiSeq machines according to activity and expected data volume (Novogene 2017).

Reference genome and gene-model annotation files were directly downloaded from a genome website (ftp://ftp.ensemblgenomes.org/pub/release-34/plants/gff3/oryza_sativa/). An index of the reference genome was built using Bowtie v2.2.3, and paired-end clean reads were then aligned to the reference genome using TopHat v2.0.12. TopHat was used as the mapping tool given that TopHat can generate a database of splice junctions based on the gene model annotation file, and thus it can give better mapping results than with other non-splice mapping tools (Novogene 2017).

HTSeq v0.6.1 was used to count the number of reads mapped to each gene, and then FPKM for each gene was calculated based on the length of the gene and the reads count mapped to this gene. FPKM—the expected number of fragments per kilobase of transcript sequence per million base pairs sequenced—considers the effect of sequencing depth and gene length for the reads count at the same time. It is currently the most commonly used method for estimating gene expression levels (Trapnell et al. 2010). Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the GOrse R package, in which gene length bias was corrected for. GO terms with corrected *p* values less than 0.05 were considered significantly enriched by differentially expressed genes (Novogene 2017).

Results

To parse the molecular changes in the rice seedling gene expression pattern that may be associated with *Trichoderma* colonization, transcriptome analysis was carried out

to identify the differentially expressed genes (DEGs) in the *Trichoderma*-inoculated seedlings vs. uninoculated seedlings (Fig. 1). Based on gene ontology (GO) analysis, it was determined that, overall, large numbers of genes related to various and specific biological processes were found to be more active in the *Trichoderma*-inoculated seedlings.

Biological Processes

The genes seen in this study to be differently expressed are linked to a great variety of known functions and processes: plant response to light; systemic acquired resistance; response to radiation; stomatal complex development; leaf development; root morphogenesis; carbon fixation; chlorophyll biosynthetic process; response to abiotic stimulus; auxin transport; response to symbiont; root development; defense response; reactive oxygen species metabolic processes; and photosynthesis. In Fig. 2, 301 of the 335 identified differentially affected genes were up-regulated in *Trichoderma*-inoculated rice seedlings, compared to only 34 genes that were down-regulated.

One of the most striking findings seen in the GO annotation chart is that with *Trichoderma* inoculation, more than 150 genes involved in the process of photosynthesis were up-regulated. Similarly, *Trichoderma* inoculation significantly increased the number of up-regulated genes associated with chlorophyll biosynthesis and with

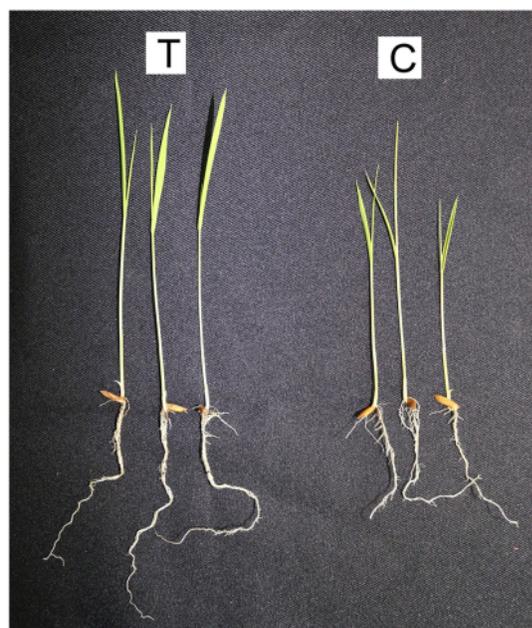


Fig. 1 *T. asperellum* SL2-inoculated seedlings, on left, have bigger leaves and roots compared to control plants

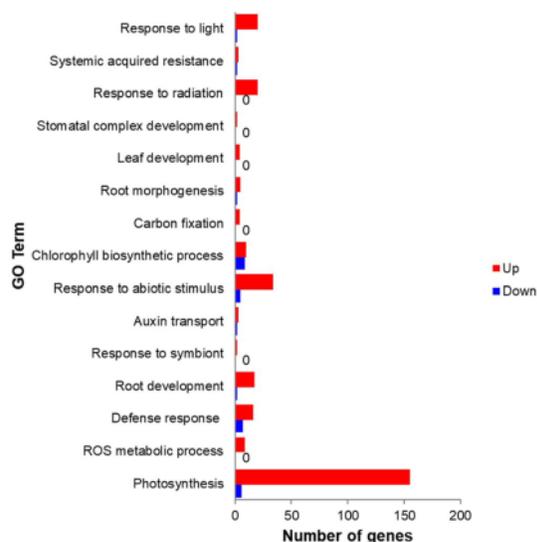


Fig. 2 Up- and down-regulated functional genes related to biological processes in rice seedlings inoculated with *T. asperellum* SL2 versus control seedlings, based on gene ontology (GO) annotation. This GO enrichment bar chart shows the differentially expressed genes (DEGs) and the counts of genes for each GO category. Many more genes related to biological processes were found to be up-regulated in *T. asperellum* SL2-inoculated seedlings compared to control seedlings

stomatal development. This clearly indicates that the presence of a symbiotic fungus such as *Trichoderma* can beneficially alter the physiological processes of the rice plant, resulting in enhanced plant growth.

Another noteworthy feature of *Trichoderma* inoculation was that many of the up-regulated genes are associated with the enhancement of root development. Our DEG analysis showed that more than 17 genes related to root development were found to be up-regulated in response of *Trichoderma* inoculation, whereas only two genes of this category were recorded as down-regulated. This was accompanied by the up-regulation of specific genes related to leaf development, carbon fixation, and auxin transport.

It has also been detected that *Trichoderma* can trigger defensive responses of rice seedlings against phytopathogenic infection. Sixteen genes that are related to the plant defense response were significantly up-regulated in the rice seedlings that had symbiotic *Trichoderma*. Specifically, another two genes associated with systemic acquired resistance (SAR) were up-regulated with *Trichoderma* inoculation, whereas one such gene was down-regulated.

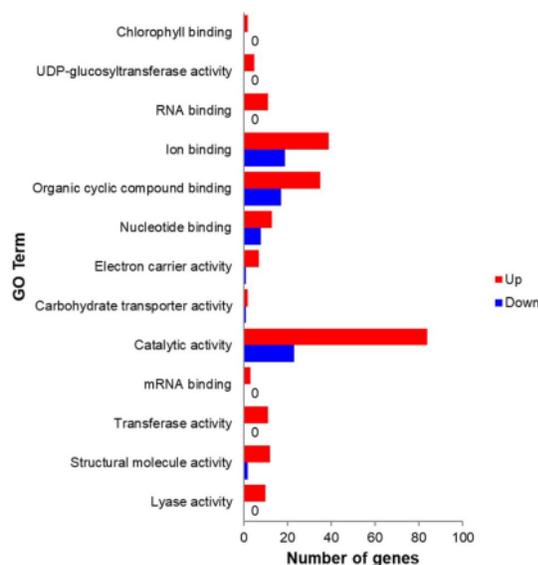


Fig. 3 Up- and down-regulated functional genes related to molecular functions in rice seedlings inoculated with *T. asperellum* SL2 versus control seedlings, based on gene ontology (GO) annotation. This GO enrichment bar chart shows the differentially expressed genes (DEGs) and the counts of genes for each GO category. Many more genes related to molecular functions were found to be up-regulated in *T. asperellum* SL2-inoculated seedlings compared to control seedlings

Molecular Functions

Most of the genes involved in molecular functioning and metabolism were also up-regulated by the *Trichoderma* inoculation of rice seedlings. According to our DEG analysis, 84 genes involved in catalytic activity and 39 genes involved in ion-binding activity were up-regulated with rice plant–*Trichoderma* interaction, compared to, respectively, 23 and 19 genes that were down-regulated (Fig. 3). Following the same pattern, 35 genes involved in organic cyclic compound binding and another 13 genes involved in nucleotide binding were observed to be up-regulated. These numbers were roughly double the number of down-regulations: 17 genes involved in organic cyclic compound binding, and eight genes involved in nucleotide binding.

Interestingly, from examining the GO charts, we found that six categories of molecular-function characteristics were over-presented in the rice seedlings with *Trichoderma* presence: chlorophyll binding, UDP-glucosyltransferase activity, RNA binding, mRNA binding, transferase activity, and lyase activity. All of the DEGs in these categories were observed to be up-regulated, whereas none were down-regulated. These data are consistent with the observed up-regulation of seven genes involved in electron carrier activity. Only one gene in this GO category was seen to be down-regulated.

Cellular Components

In term of genes known to affect cellular components, it was seen that especially the cell organs associated with photosynthesis were influenced by *Trichoderma* inoculation. A total of 238 genes related to the thylakoid membranes, the crucial components within the chloroplasts that carry out the light reactions of photosynthesis, were observed to be up-regulated with *Trichoderma* inoculation. Only four genes related to these membranes were down-regulated (Fig. 4).

Another 192 genes related to the chloroplast, one of the most important organelles in plant cells, were observed to be up-regulated, compared to just ten genes that were down-regulated. Regarding the organelles and cytosol of the cytoplasm, 76 genes were up-regulated in rice seedlings as a result of *Trichoderma*–rice plant interaction, whereas 12 genes were down-regulated.

With respect to water membrane transport, one gene was up-regulated with *Trichoderma* inoculation, whereas none were observed to be down-regulated. In relation to the respiration process, 18 genes bearing on photosystem components and one gene linked to the mitochondria were up-regulated with *Trichoderma* inoculation, whereas two genes for photosystem components were down-regulated. Likewise, multiple genes affecting plastids and the cell wall (> 80)

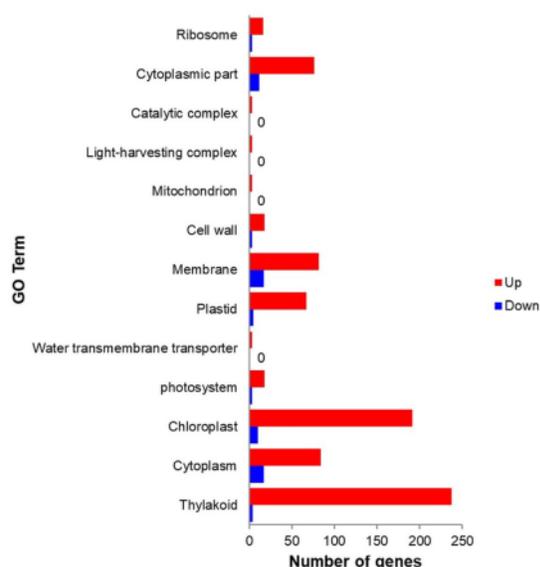


Fig. 4 Up- and down-regulated functional genes related to cellular components in rice seedlings inoculated with *T. asperellum* SL2 vs. control seedlings, based on gene ontology (GO) annotation. This GO enrichment bar chart shows the differentially expressed genes (DEGs) and the counts of genes for each GO category. Many more genes related to cellular components were found to be up-regulated in *T. asperellum* SL2-inoculated seedlings compared to control seedlings

were identified as up-regulated, whereas only six genes were down-regulated.

Discussion

Trichoderma has been recognized as a symbiotic fungus that can interact with plants by colonizing their roots, establishing chemical communication within the plant, and then systematically altering the expression of numerous plant functional genes (Harman 2011). In this study, inoculating rice seedlings with *Trichoderma* was associated with the induced expression of hundreds of genes that are involved in energy metabolism, plant growth, water transport, and other metabolic pathways within the plant.

This research showed that the presence (vs. absence) of *Trichoderma* was correlated with the up-regulation of many genes which have been identified to be involved in photosynthesis and chlorophyll biosynthesis. This suggests an overall increase of the rice plants' capacity for photosynthesis as a result of *Trichoderma* inoculation. In addition, the up-regulation of many genes in the chloroplast (the organelle in which photosynthesis occurs) would also be contributing to enhanced photosynthesis.

Furthermore, the up-regulation of genes related to CO₂ fixation, response to light, and stomatal complex development indicated an enhancement of the plant's efficiency in photosynthesis. The findings from this study are consistent with those of Shores and Harman (2008a) who showed the ability of *Trichoderma* to induce significant changes in the proteome of the shoots of maize (*Zea mays*) seedlings, changes that were mostly related to carbohydrate metabolic and photosynthetic processes.

Our previous studies on the effects of *T. asperellum* SL2 on rice plants showed that this microbe's presence (vs. absence) was correlated with the enhancement of rice seedling growth, vigor, and chlorophyll content. The effects of *T. asperellum* SL2 inoculation on the rice plants during their crop cycle led to significant increases in photosynthetic rate, stomatal density, and other physiological traits (summarized in Table 1). The relationship between the System of Rice Intensification (SRI) management and *T. asperellum* SL2 inoculation has been examined in parallel research (Doni et al. 2017, 2018; Doni 2018). This documented synergistic effect on rice plant growth and resistance to sheath blight when inoculation was accompanied by SRI changes in rice cultivation methods that enhanced root growth and the soil biota. The effects that those studies documented at the organismic level are seen here to be paralleled at the molecular level.

In our trials, inoculation of rice seedlings with *Trichoderma* had a significant influence on the up-regulation of many genes related to root development, root

Table 1 Agronomic and physiological enhancement in rice plants upon *T. asperellum* SL2 inoculation

Plant growth stage	Agronomic advantages	Physiological advantages	References
Seedling stage	Higher vigor index Better shoot and root growth Higher germination rate	Higher leaf chlorophyll content	Doni et al. (2017)
Vegetative stage	More tillers More leaf numbers Better shoot and root growth	Higher leaf chlorophyll content Higher NPK uptake Higher photosynthetic rate More stomatal number Higher stomatal conductance Lower transpiration rate Higher water use efficiency More resistance to <i>Rhizoctonia solani</i>	Doni et al. (2014), Doni et al. (2017), Doni et al. (2018), Doni (2018)
Ripening stage	More panicles More leaf numbers	Higher leaf chlorophyll content Higher photosynthetic rate	Doni et al. (2014), Doni et al. (2017), Doni et al. (2018)
Harvest stage	More grains per panicle More filled grains Heavier grains		Doni et al. (2017), Doni et al. (2018)

morphogenesis, and leaf development. A robust root system can improve water and nutrient uptake, and subsequently can promote plant growth and assist them in tolerating stress conditions (Gunapati et al. 2016). A previous study by Viterbo et al. (2010) revealed the capacity of *Trichoderma* to alter a gene that encodes an ACC deaminase in *Brassica napus*, a gene that is involved in root elongation. Additionally, the up-regulation of genes involved in auxin transport implies that the colonization of *Trichoderma* in rice roots enhances plant phytohormone dynamics.

In response to abiotic and biotic stresses, several genes that affect the plant response to these stresses were up-regulated in plant interactions with symbiotic *Trichoderma*. Numerous genes that are involved in the plant defense response, in their response to abiotic stimuli, and in reactive oxygen species (ROS) metabolism were up-regulated with *Trichoderma* inoculation. An abundance of up-regulated genes that relate to plant defense mechanisms can be considered as an indication of better plant fitness and greater plant ability to resist infection. This finding is in agreement with earlier findings by Shores et al. (2005) that demonstrated the up-regulation of defense-related genes such as Lox1, Pal1, ETR1, and CTR1 in cucumber plants following *Trichoderma* colonization.

In this study, many genes related to the cell wall were also observed to be up-regulated after *Trichoderma* inoculation. As a physical barrier against pathogen invasion, the cell wall is critical for the plant defense system because it has numerous functions such as producing antimicrobial enzymes and secondary metabolites (Underwood 2012; Malinovsky et al. 2014). A study by Nawrocka and Malolepsza (2013) has verified the capacity of *Trichoderma* to release elicitors that may induce certain signals transmitted within the plant such as salicylic acid (SA), jasmonic acid (JA), and ROS.

The gene expression alterations in ROS metabolism-related genes in this research indicated the capacity of *Trichoderma* to modulate ROS systems in rice plants. In plant systems, ROS have both negative and positive roles as reactive oxygen species are, on the one hand, toxic byproducts of aerobic metabolism, and at the same time, key regulators for growth, development, and defense pathways, on the other (Mittler et al. 2004). When plants are exposed to abiotic stresses, ROS are overproduced within plants. As these molecules are highly reactive and toxic, they cause damage to proteins, lipids, carbohydrates, and DNA, eventually resulting in oxidative stress (Gill and Tuteja 2010). Thus, balancing the generation and elimination of ROS becomes a crucial process for maintaining plant health and growth.

One of the ways that adverse effects of ROS in plants can be modulated is by inoculating plants with symbiotic fungi (Rodriguez and Redman 2005). Mastouri et al. (2010) earlier pointed out the capacity of *Trichoderma* to reduce damage from the accumulation of ROS in tomato plants, by inducing physiological protection against oxidative damage. Further, Mastouri et al. (2012) have reported on the ability of *Trichoderma* to remove damaging ROS in tomato seedlings. The ability of seedlings to protect themselves from oxidative damage was accompanied by the up-regulation of certain genes for enzymes that reduce glutathione and ascorbate synthesis; by an increase in the activity of these enzymes; as well as by a shift in the redox state of glutathione and ascorbate pools towards a reduced state.

Recently, Pandey et al. (2016) revealed that the inoculation of rice plants with *Trichoderma* improved drought tolerance by modulating transcript levels for proline, superoxide dismutase, lipid peroxidation product, and dehydrin/aquaporin. The large-scale up-regulation of many genes

that affect ribosomes, thylakoid membranes, water transmembrane transporters, the cytoplasm, and plastids in rice seedlings provide further evidence that *Trichoderma* inoculation triggered large-scale changes in plant cell gene expression and activates numerous plant metabolic pathways.

The present work had thus shown promising features of *Trichoderma* as a soil inoculant. Moreover, an abundance of rice root exudates may attract *Trichoderma* to colonize rice roots and penetrate root tissues. Agronomic methods that enhance plant root growth such as SRI could create a positive feedback loop where larger root systems attract more *Trichoderma*, and these endophytes in turn support more plant root growth.

Vargas et al. (2011) have reported a plant-derived sucrose which is an important resource as a signaling molecule that attracts *T. virens*. Our study revealed a particular change in the rice seedlings inoculated with *Trichoderma* that may contribute to better crosstalk between this symbiont and rice plants because our analysis showed up-regulation of two genes that are involved in the plant response towards symbionts. This warrants further study.

The molecular functions of rice seedlings were very evidently enhanced in response to *Trichoderma* inoculation. Many genes with specific functions in molecular signaling were up-regulated in interaction with *Trichoderma*: UDP-glucosyltransferase activity, RNA binding, mRNA binding, transferase activity, catalytic activity, and lyase activity. These multiple changes indicated that there may be some direct connection between the capacity of *Trichoderma* to induce plant molecular signaling and its ability to promote plant growth. These results support the findings of Shores and Harman (2008b) who showed a large portion of the up-regulated proteins in maize (*Z. mays*) seedlings to be involved in amino acid metabolism, cell wall metabolism, and genetic information processing.

A large majority of the genes that were affected by *Trichoderma* inoculation were up-regulated. However, a minority were down-regulated. For example, genes involved in photosynthesis were found to be 155 up-regulated and six down-regulated. This is due to the multiplicity of genes involved in a specific biochemical pathway. Genes were up- or down-regulated at certain times depending on their function. Some genes were also functioning as negative regulators in a specific pathway (Zipfel and Oldroyd 2017). Bharti et al. (2016) reported that the expression levels of stress-related genes in control and *Dietzia natronolimnaea*-inoculated wheat plants showed major differences when under saline conditions, but minor differences under non-saline conditions. These differences are because salinity stress tolerance mechanisms are complex phenomena and involve a cumulation of changes in various genes guiding plant pathways. Previously, Srivastava et al. (2012) revealed that genes involved in particular

biological processes were 21% down-regulated in *Arabidopsis* plants inoculated with *Pseudomonas putida*.

Microbe-mediated plant growth is a multigenic process that is likely to be specific to the participating microbes and plant species (Bharti et al. 2016). An earlier study that showed a lipid-transfer protein gene was up-regulated in rice roots in response to colonization by *Glomus mosseae* (Blilou et al. 2000). In another study, some genes in *P. stutzeri* bacteria were switched on during rice root colonization but were switched off when grown on a synthetic medium (Rediers et al. 2003). These indicate that plant–microbial interactions are unique processes that involve two-way communication between microbes and host. Beneficial outcomes of these interactions include increasing the host's fitness and growth while enabling microbes to successfully colonize the roots or to take up and utilize more sugars and amino acids exuded by the plants (Rodriguez et al. 2009).

In conclusion, as a symbiotic microbe, *Trichoderma* was able to alter gene expression in rice seedlings on a large scale under controlled, experimental conditions. Extensive up-regulation of genes with relatively little down-regulation contributed to better energy metabolism and plant growth and facilitated water transport, photosynthesis, and other metabolic pathways in the rice seedlings. These abilities are of great agriculture importance for supporting more plentiful and sustainable rice production.

Acknowledgements We express appreciation for helpful feedback and suggestions from Dr. Gary Harman, Professor Emeritus, Cornell University, Geneva, NY, USA.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest.

References

- Alhasnawi AN, Radziah CC, Kadhim AA, Isahak A, Mohamad A, Yusoff WM (2017) Relationship observed between salinity-tolerant callus cell lines and anatomical structure of Line 2 (*Oryza sativa* L.) indica under salinity stress. *Biocatal Agric Biotechnol* 10:367–378
- Azad K, Kaminskyj S (2016) A fungal endophyte strategy for mitigating the effect of salt and drought stress on plant growth. *Symbiosis* 68:73–78
- Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, Melnick RL, Bailey BA (2009) The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. *J Exp Bot* 60:3279–3295
- Bailey BA, Bae H, Strem MD, Roberts DP, Thomas SE, Crozier J, Samuels GJ, Choi IY, Holmes KA (2006) Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. *Planta* 224:1449–1464
- Bharti N, Pandey SS, Barnawal D, Patel VK, Kalra A (2016) Plant growth promoting rhizobacteria *Dietzia natronolimnaea*

- modulates the expression of stress responsive genes providing protection of wheat from salinity stress. *Sci Rep* 6:34768
- Bllilou I, Ocampo JA, García-Garrido JM (2000) Induction of Ltp (lipid transfer protein) and Pal (phenylalanine ammonia-lyase) gene expression in rice roots colonized by the arbuscular mycorrhizal fungus *Glomus mosseae*. *J Exp Bot* 51:1969–1977
- Chacón MR, Rodríguez Galán O, Benítez Fernández CT, Sousa S, Rey M, Llobell González A, Delgado Jarana J (2007) Microscopic and transcriptome analyses of early colonization of tomato roots by *Trichoderma harzianum*. *Int Microbiol* 10(1):19–27
- Colmer TD, Armstrong W, Greenway H, Ismail AM, Kirk GJD, Atwell BJ (2014) Physiological mechanisms of flooding tolerance in rice: transient complete submergence and prolonged standing water. In: Lutttge U, Beyschlag W, Cushman J (eds) *Progress in botany*, vol 75. Springer, Berlin, pp. 255–307
- Contreras-Cornejo HA, Macías-Rodríguez L, Cortés-Penagos C, López-Bucio J (2009) *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. *Plant Physiol* 149:1579–1592
- Contreras-Cornejo HA, Macías-Rodríguez L, Alfaro-Cuevas R, Lopez-Bucio J (2014) *Trichoderma* spp. improve growth of Arabidopsis seedlings under salt stress through enhanced root development, osmolyte production, and Na⁺ elimination through root exudates. *Mol Plant Microbe Interact* 27(6):503–514
- Contreras-Cornejo HA, Macías-Rodríguez L, Vergara AG, López-Bucio J (2015) *Trichoderma* modulates stomatal aperture and leaf transpiration through an abscisic acid-dependent mechanism in Arabidopsis. *J Plant Growth Regul* 34:425–432
- Contreras-Cornejo HA, Macías-Rodríguez L, del-Val E, Larsen J (2018) The root endophytic fungus *Trichoderma atroviride* induces foliar herbivory resistance in maize plants. *Appl Soil Ecol* 124:45–53
- Doni F (2018) *Trichoderma asperellum* SL2 for improving growth, gene expression pattern, physiological traits, yield and disease resistance of rice plants under System of Rice Intensification (SRI) management system. PhD thesis, The National University of Malaysia, Bangi, Malaysia
- Doni F, Anizan I, Che Radziah CMZ, Wan Mohtar WY (2014) Physiological and growth response of rice (*Oryza sativa* L.) plants to *Trichoderma* spp. inoculants. *AMB Express* 4:45
- Doni F, Che Radziah CMZ, Anizan I, Norela S, Fathurrahman F, Uphoff N, Wan Mohtar WY (2017) Relationships observed between *Trichoderma* inoculation and characteristics of rice grown under System of Rice Intensification (SRI) vs. conventional methods of cultivation. *Symbiosis* 72:45–59
- Doni F, Zain CR, Isahak A, Fathurrahman F, Anhar A, Mohamad WN, Yusoff WM, Uphoff N (2018) A simple, efficient, and farmer-friendly *Trichoderma*-based biofertilizer evaluated with the SRI Rice Management System. *Org Agric* 8:207–223
- Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* 48:909–930
- Guler NS, Pehlivan N, Karaoglu SA, Guzel S, Bozdeveci A (2016) *Trichoderma atroviride* ID20G inoculation ameliorates drought stress-induced damages by improving antioxidant defence in maize seedlings. *Acta Physiol Plant*. <https://doi.org/10.1007/s11738-016-2153-3>
- Gunapati S, Naresh R, Ranjan S, Nigam D, Hans A, Verma PC, Gadre R, Pathre UV, Sane AP, Sane VA (2016) Expression of GhNAC2 from *G. herbaceum*, improves root growth and imparts tolerance to drought in transgenic cotton and Arabidopsis. *Sci Rep* 6:24978
- Haefele SM, Siopongco JD, Boling AA, Bouman BA, Tuong TP (2009) Transpiration efficiency of rice (*Oryza sativa* L.). *Field Crops Res* 111(1):1–10
- Harman GE (2011) Multifunctional fungal plant symbionts: New tools to enhance plant growth and productivity. *New Phytol* 189:647–649
- Malinovsky FG, Fangel JU, Willats WG (2014) The role of the cell wall in plant immunity. *Front Plant Sci*. <https://doi.org/10.3389/fpls.2014.00178>
- Maruyama K, Urano K, Yoshiwara K, Morishita Y, Sakurai N, Suzuki H, Kojima M, Sakakibara H, Shibata D, Saito K, Shinozaki K (2014) Integrated analysis of the effects of cold and dehydration on rice metabolites, phytohormones, and gene transcripts. *Plant Physiol* 164:1759–1771
- Mastouri F, Björkman T, Harman GE (2010) Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. *Phytopathol* 100:1213–1221
- Mastouri F, Björkman T, Harman GE (2012) *Trichoderma harzianum* enhances antioxidant defense of tomato seedlings and resistance to water deficit. *Mol Plant Microbe Interact* 25:1264–1271
- Mishra A, Salokhe VM (2011) Rice root growth and physiological responses to SRI water management and implications for crop productivity. *Paddy Water Environ* 9:41–52
- Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. *Trend Plant Sci* 9:490–498
- Nawrocka J, Malolepsza U (2013) Diversity in plant systemic resistance induced by *Trichoderma*. *Biol Control* 67:149–156
- Neumann B, Laing M (2006) *Trichoderma*: an ally in the quest for soil system sustainability. In: Uphoff N, Fernandes E, Herren H, Husson O, Laing M, Palm C, Pretty J, Sanchez P, Sanginga N, Thies J (eds) *Biological approaches to sustainable soil systems*. CRC Press, Boca Raton, pp 491–500
- Nguyen HT, Babu RC, Blum A (1997) Breeding for drought resistance in rice: Physiology and molecular genetics considerations. *Crop Sci* 37(5):1426–1434
- Nicolás C, Hermosa R, Rubio B, Mukherjee PK, Monte E (2014) *Trichoderma* genes in plants for stress tolerance-status and prospects. *Plant Sci* 228:71–78
- Novogene (2017) Whole genome sequencing. <https://en.novogene.com/next-generation-sequencing-services/>
- Pandey V, Ansari MW, Tula S, Yadav S, Sahoo RK, Shukla N, Bains G, Badal S, Chandra S, Gaur AK, Kumar A (2016) Dose-dependent response of *Trichoderma harzianum* in improving drought tolerance in rice genotypes. *Planta* 243:1251–1264
- Pascale A, Vinale F, Manganiello G, Nigro M, Lanzuise S, Ruocco M, Marra R, Lombardi N, Woo SL, Lorito M (2017) *Trichoderma* and its secondary metabolites improve yield and quality of grapes. *Crop Prot* 92:176–181
- Rediers H, Bonnacerrere V, Rainey PB, Hamonts K, Vanderleyden J, De Mot R (2003) Development and application of a dapB-based in vivo expression technology system to study colonization of rice by the endophytic nitrogen-fixing bacterium *Pseudomonas stutzeri* A15. *Appl Environ Microbiol* 69:6864–6874
- Redman RS, Kim YO, Woodward CJ, Greer C, Espino L, Doty SL, Rodriguez RJ (2011) Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change. *PLoS ONE* 6(7):e14823
- Rodriguez R, Redman R (2005) Balancing the generation and elimination of reactive oxygen species. *Proc Natl Acad Sci USA* 102:3175–3176
- Rodriguez RJ, White JF Jr, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. *New Phytol* 182:314–330
- Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim YO, Redman RS (2008) Stress tolerance in plants via habitat-adapted symbiosis. *ISME J* 2(4):404–416
- Samolski I, Rincón AM, Pinzón LM, Viterbo A, Monte E (2012) The qid74 gene from *Trichoderma harzianum* has a role in

- root architecture and plant biofertilization. *Microbiology* 158(1):129–138
- Segarra G, Casanova E, Bellido D, Odena MA, Oliveira E, Trillas I (2007) Proteome, salicylic acid, and jasmonic acid changes in cucumber plants inoculated with *Trichoderma asperellum* strain T34. *Proteomics* 7:3943–3952
- Shoresh M, Harman GE (2008a) The relationship between increased growth and resistance induced in plants by root colonizing microbes. *Plant Signal Behav* 3:737–739
- Shoresh M, Harman GE (2008b) The molecular basis of shoot responses of maize seedlings to *Trichoderma harzianum* T22 inoculation of the root: a proteomic approach. *Plant Physiol* 147:2147–2163
- Shoresh M, Yedidia I, Chet I (2005) Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203. *Phytopathol* 95:76–84
- Shoresh M, Harman GE, Mastouri F (2010) Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu Rev of Phytopathol* 48:21–43
- Shukla N, Awasthi RP, Rawat L, Kumar J (2012) Biochemical and physiological responses of rice (*Oryza sativa* L.) as influenced by *Trichoderma harzianum* under drought stress. *Plant Physiol Biochem* 54:78–88
- Srivastava S, Chaudhry V, Mishra A, Chauhan PS, Rehman A, Yadav A, Tuteja N, Nautiyal CS (2012) Gene expression profiling through microarray analysis in *Arabidopsis thaliana* colonized by *Pseudomonas putida* MTCC5279, a plant growth promoting rhizobacterium. *Plant Signal Behav* 7:235–245
- Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ, Pachter L (2010) Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat Biotechnol* 28:511–515
- Underwood D (2012) The plant cell wall: a dynamic barrier against pathogen invasion. *Front Plant Sci* 3:1–6
- Vargas WA, Crutcher FK, Kenerley CM (2011) Functional characterization of a plant-like sucrose transporter from the beneficial fungus *Trichoderma virens*: Regulation of the symbiotic association with plants by sucrose metabolism inside the fungal cells. *New Phytol* 189:777–789
- Varshney RK, Nayak SN, May GD, Jackson SA (2009) Next-generation sequencing technologies and their implications for crop genetics and breeding. *Trends Biotechnol* 27:522–530
- Viterbo A, Landau U, Kim S, Chernin L, Chet I (2010) Characterization of ACC deaminase from the biocontrol and plant growth-promoting agent *Trichoderma asperellum* T203. *FEMS Microbiol Lett* 305:42–48
- Wani ZA, Ashraf N, Mohiuddin T, Riyaz-Ul-Hassan S (2015) Plant-endophyte symbiosis, an ecological perspective. *Appl Microbiol Biotechnol* 99:2955–2965
- Wu Q, Peng X, Yang M, Zhang W, Dazzo FB, Uphoff N, Jing Y, Shen S (2018) Rhizobia promote the growth of rice shoots by targeting cell signaling, division and expansion. *Plant Mol Biol* 97:507–523
- Yedidia I, Srivastava AK, Kapulnik Y, Chet I (2001) Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. *Plant Soil* 235:235–242
- Zaidi NW, Singh M, Kumar S, Sangle UR, Singh R, Prasad R, Singh SS, Singh S, Yadav AK, Singh A, Waza SA (2018) *Trichoderma harzianum* improves the performance of stress-tolerant rice varieties in rainfed ecologies of Bihar, India. *Field Crops Res* 220:97–104
- Zipfel C, Oldroyd GE (2017) Plant signaling in symbiosis and immunity. *Nature* 543:328

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Transcriptomic Profiling of Rice Seedlings Inoculated with the Symbiotic Fungus *Trichoderma asperellum* SL2

ORIGINALITY REPORT

22%

SIMILARITY INDEX

12%

INTERNET SOURCES

%

PUBLICATIONS

17%

STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

1%

★ Submitted to Royal Botanic Garden Edinburgh

Student Paper

Exclude quotes Off

Exclude matches Off

Exclude bibliography Off