

Research Article

Morphoanatomical and Biochemical Changes in the Roots of Rice Plants Induced by Plant Growth-Promoting Microorganisms

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The goal of the present study was to characterize anatomical and biochemical changes in rice plant roots in response to seed treatment with rhizobacteria (*Burkholderia pyrrocinia* (R-46) + *Pseudomonas fluorescens* (R-55)) and *Trichoderma asperellum* (Ta: mixture of strains T-06, T-09, T-12, and T-52). The experimental design was completely randomized, with six treatments (R-46, R-55, R-46 + R-55, Ta + R-46 + R-55, Ta, and control) and ten replicates. Treatments Ta and R-46 + R-55 increased the root length and diameter as well as the cortex expansion and induced a 2% expansion of the aerenchymal space. Treatments Ta and R-46 increased the vascular cylinder diameter. The number of protoxylem poles and metaxylem vessel elements was increased by R-46 and R-55. The total phenol content increased with treatments Ta, R-46 + R-55, R-46, and R-55, and all the treatments increased the flavonoid content. The lignin content increased with the Ta and R-55 treatments. All the root architecture modifications resulting from the interaction between seedlings and bioagents (rhizobacteria and *Trichoderma* spp.) observed in the present study favored the root plasticity of rice seedlings.

1. Introduction

Upland rice is planted in few regions worldwide. However, it presents advantages compared to floodland rice due to its lower production costs and water consumption. The average productivity of upland rice has been under 3 ton·ha⁻¹, whereas the productivity potential of improved cultivars is greater than 5 ton·ha⁻¹. The low productivity is attributed to water stress, which causes low initial vigor of the seedling root, deficiency in the uptake of nitrogen in the form of nitrate (NO₃⁻) at early stages of rice plant development, lack of plant response to inputs under successive planting, and the occurrence of rice blast [1–3].

Biofertilizers are fertilizers composed of living microorganisms that promote plant growth when in contact with seeds or roots [4]. Plant growth-promoting rhizobacteria (PGPR) and the fungus *T. asperellum* are referred to in the literature as promoting plant growth through chemical signaling that elicits hormone and enzyme metabolic pathways related to oxidative stress [5], induction of structural changes in host plant cells, increases in the uptake of macronutrients such as P and N, and induction of systemic resistance (ISR) [6].

Inoculation with isolates of *Burkholderia pyrrocinia* (R-46) and *Pseudomonas fluorescens* (R-55) has been found to promote growth of rice plants and decrease the severity of

rice blast (*Magnaporthe oryzae*) through direct antibiosis and ISR [6], and isolates of *T. asperellum* (T.06, T.09, T.12, and T.52), applied to seeds and sprayed on leaves, decreased the severity of rice sheath blight and increased rice biomass and productivity both in the field and in greenhouse studies [7].

The characterization of histological and biochemical changes resulting from interactions between plant growth-promoting microorganisms (PGPMs) or bioagents and plant growth is important to understand the mechanisms involved in the growth process. This knowledge will be useful for the optimization of the current production system, making it more sustainable and environmentally friendly.

Bioagents constitute a sustainable option for plant production systems. In the present study, the hypothesis was advanced that bioagents R-46, R-55, and *T. asperellum* promote changes at the cell and biochemical levels in roots of rice plants. The goal of the present study was to identify and characterize anatomical and biochemical changes in rice roots treated with rhizobacteria (R-46 and R-55) and *T. asperellum*.

2. Materials and Methods

2.1. Plant Material and Microorganisms. The tested *P. fluorescens* (R-55) and *B. pyrrocinia* (R-46) isolates had been previously selected by [6], and the four *T. asperellum* isolates (T-06, T-09, T-12, and T-52) had been selected by [7]. All the isolates were stored at $\pm 4^{\circ}\text{C}$ at the microorganism bank of the Plant Protection Laboratory (Laboratório de Proteção de Plantas (LPP)) from the Rural Federal University of the Amazon (Universidade Federal Rural da Amazônia (UFRA)).

The treatments were composed of rice seeds (cultivar: Primavera Clearfield) microbiolized with T1: a mixture of four isolates of *T. asperellum* (T-06, T-09, T-12, and T-52); T2: two isolates of rhizobacteria, *P. fluorescens* (R-55) and *B. pyrrocinia* (R-46); T3: *P. fluorescens* (R-55); T4: *B. pyrrocinia* (R-46); T5: the microorganisms combined (all 4 isolates of *T. asperellum* + *P. fluorescens* + *B. pyrrocinia*); and T6: water-treated control.

The tested rhizobacteria (*B. pyrrocinia* (R.46) and *P. fluorescens* (R-55)) were separately inoculated in Petri dishes with 523 solid culture media [8] and incubated for 48 hours at 28°C . A bacterial suspension was subsequently prepared using sterile water and adjusted at 550 nm absorbance using a spectrophotometer ($A_{550} = 0.1$ corresponding to 10^8 CFU $\cdot\text{mL}^{-1}$).

The four isolates of *T. asperellum* (T.06, T.09, T.12, and T.52) were individually grown in plastic bags, containing 50 g of sterilized rice grains, and incubated during 6 days at 25°C . From each plastic bag, a sample of 10 g of rice colonized with *T. asperellum* was blent and homogenized. Then a conidial suspension was prepared, which was adjusted to 10^8 CFU $\cdot\text{mL}^{-1}$ using a Neubauer chamber.

Rice seeds were sterilized with 70% ETOH and 2% NaClO, both for 1 minute, washed in sterile water for 1 minute, and placed on sterile filter paper, where they remained for 1 hour. The seeds were then steeped in the suspensions for each treatment for 24 hours at 28°C and at 115 rpm and were subsequently sown.

Two experiments were carried out: the first one in glass tube (60 mL) and the other in polyethylene plastic tube (180 cm³). The two experiments were completely randomized, with six treatments and ten replicates; the number of samples was 20 per treatment. The assay was performed in duplicate.

Experiment 1. After the microbiolization the seeds were sown in 60 mL tubes containing water-agar (1 L H₂O + 13.65 g of agar), where they remained for 21 days after sowing. Twenty plants of each treatment were collected for root anatomy description.

Experiment 2. After the microbiolization the seeds were sown in 180 cm³ tubes containing Gernino plant substrate (containing pine bark, peat, acidity corrective, triple superphosphate, and potassium nitrate). To quantify the dry mass (g) and length (cm) of leaves and roots, root/leaves ratio, and the lignin, lignin monomers, phenol, and flavonoid contents.

2.2. Anatomy Description and Morphometry. The plants were collected from the test tubes 21 days following sowing. The roots were carefully washed and taken to the Botany Laboratory of the Brazilian Enterprise for Agricultural Research (Embrapa) Eastern Amazon (Laboratório de Botânica da Embrapa Amazônia Oriental), fixed in FAA50 (50 mL of 37% formaldehyde, 50 mL of glacial acetic acid, and 900 mL of 50% ethanol), and subjected to vacuum in a desiccator for 24 hours. The fixative was then discarded, and the roots were stored in 70% ethanol until further processing.

Part of the samples was rehydrated in a graded ethanolic series to obtain sections [9] and analyzed by optical microscopy. Ten free-hand cross-sections were obtained from roots. The sections were cleared in sodium hypochlorite 2% NaClO for 1 minute, placed in 1% Astrablau for 30 seconds, and then placed in 1% Basic Fuchsin. Measurements were performed using a millimetric eyepiece on a Motic BA-400 microscope. In addition to the anatomical description, the root diameter, expansion of the cortex, thickness of the exodermis, and diameter of vascular cylinder were measured, and the numbers of protoxylem poles and metaxylem vessel elements were quantified.

Another part of the samples was dehydrated in a graded increasing ethanolic series, critical point-dried (CO₂), coated with a thin layer of gold, and observed using a scanning electron microscope (SEM). Cross-sections were obtained from the roots of three plants per treatment. The images obtained were used to determine the area occupied by the aerenchyma using the ANTI QUANT 2 software.

2.3. Dry Mass Gain and Length. Twenty-one days after growth, ten plants were collected from each treatment, and the following measurements were performed: leaf and root length, root/leaf ratio (cm), dry mass of leaves and roots, and root/leaf ratio (g). The dry mass was measured following drying of the plant material in a forced air circulation oven for 7 days at 65°C , using an analytical scale.

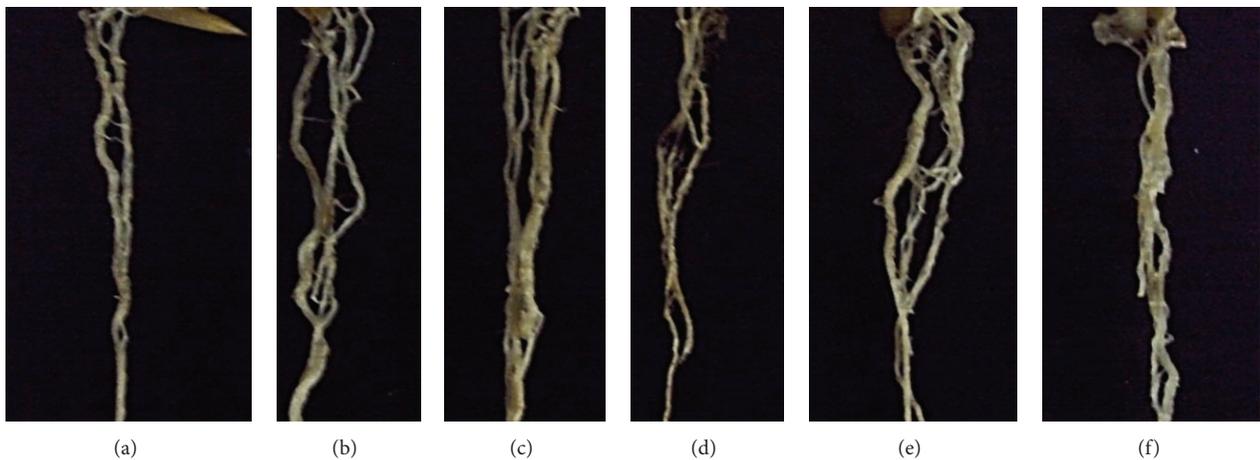


FIGURE 1: Adventitious roots of rice plants originating from microbiolized seeds. (a) Control plant (nonmicrobiolized seeds). (b–f) Roots of plants from seeds microbiolized with (b) *T. asperellum* (T-06, T-09, T-12, and T-52) + *B. pyrrocinia* + *P. fluorescens*, (c) *T. asperellum* (T-06, T-09, T-12, and T-52), (d) *B. pyrrocinia* + *P. fluorescens*, (e) *B. pyrrocinia*, and (f) *P. fluorescens* (f). Evaluation was performed 9 days after sowing.

2.4. Determination of Phenols, Flavonoids, Lignin, and Lignin Monomers. Ten plants of each treatment were collected, and three replicates were performed, for each evaluation. Roots were separated from the shoot, washed, and placed in a forced air circulation oven at 65°C for 7 days, until a constant mass was reached. The roots were then ground using liquid nitrogen and stored at $\pm 4^{\circ}\text{C}$.

Total lignin and lignin monomers: samples were homogenized in 50 mM sodium phosphate buffer, pH 7.0, purified in 1% Triton X-100, 1 M NaCl, and acetone, according to [10], and centrifuged for 15 minutes. The final pellet was dried and considered to be the protein freed from cell walls. Lignin was quantified using the thioglycolic acid method [10], and lignin monomers were quantified using alkaline nitrobenzene peroxidation [11].

Total phenols and flavonoids: one gram of root was extracted in 20 mL methanol (80 : 20, v/v) for 15 minutes, and the extract was resuspended in 20 mL of 1% Triton X-100. The extract was used for the determination of the total phenol content using a spectrophotometer (absorbance 735 nm). A standard curve was obtained using gallic acid. Flavonoid contents were determined in extracts in aluminum chloride by a colorimetric assay (735 nm) [12].

2.5. Statistical Analysis. An ANOVA was performed, followed by a Duncan test ($P < 0.05$ and $P < 0.1$), and the standard error was calculated ($P \leq 0.05$). The SPSS 17.0 software was used for all the analyses.

3. Results

3.1. Dry Mass and Length Gain. The root length and dry mass of plants treated with biopromoters were higher than control treatment. Significant differences in root length and root/leaf ratio were observed among the treatments with seeds microbiolized with the four *T. asperellum* isolates and

the combination of the two rhizobacteria (*B. pyrrocinia* + *P. fluorescens*) (Table 1).

Plants treated with the mix of the four *T. asperellum* isolates presented higher leaf and root dry mass, when compared to the other treatments. The root/leaf dry mass was higher for all biopromoter treatments (Table 1).

3.2. Anatomy Description and Morphometry. The root diameter increased in all the treatments with PGPMs. This change was especially pronounced for the treatments with *B. pyrrocinia* and with *T. asperellum* + *B. pyrrocinia* + *P. fluorescens*, for which statistically significant differences from the control were observed (Table 2).

Plants originating from seeds microbiolized with PGPMs presented more adventitious roots, based on visual assessments, compared to control plants (Figure 1, Table 1). In addition, SEM observations (Figure 2) and morphometry revealed that some of the anatomical characteristics of the roots changed, such as vascular cylinder diameter, cortex and exodermis thickness, and number of protoxylem poles and metaxylem vessel elements (Figure 2).

The cortex and exodermis thickness and number of protoxylem poles were significantly higher in the roots of all the PGPM treatments. The treatments with *B. pyrrocinia* and with *B. pyrrocinia* + *P. fluorescens* statistically presented the largest increase in cortex thickness and in the number of poles of protoxylem, respectively (Table 2).

The lacunae of aerenchyma area and the number of metaxylem vessel elements increased in all the treatments with PGPMs, except for the treatment with the rhizobacteria (R-55 and R-46) and *T. asperellum* mix (Table 2), which were not significantly different from the control roots (Table 2).

3.3. Determination of Phenols, Flavonoids, Lignin, and Lignin Monomers. The total phenol and flavonoid contents increased in all the plants treated with bioagents (Table 3), except for the treatment with *B. pyrrocinia* + *P. fluorescens*,

TABLE I: Length (cm) and dry mass (g) of leaves, root, and root/leaf ratio of rice plants obtained from seeds microbiolized with PGPMs, 21 days following sowing in tubes (180 cm³) containing Gernino plant substrate.

Treatment	Length (cm)		Dry mass (g)		Root/leaf ratio	Root/leaf ratio
	Leaves	Root	Leaves	Root		
<i>Trichoderma asperellum</i> ⁽¹⁾	33.5 ± 1.5 ⁽²⁾ a ⁽³⁾	24.3 ± 1.9 ab	2.527 × 10 ⁻² ± 2.0 × 10 ⁻³ a	1.079 × 10 ⁻² ± 0.2 × 10 ⁻² a	0.73 ± 0.23 a	0.429 ± 0.32 a
<i>Burkholderia pyrrocinia</i> + <i>Pseudomonas fluorescens</i>	30.3 ± 1.5 a	22.6 ± 1.8 ab	1.645 × 10 ⁻² ± 7.0 × 10 ⁻³ c	0.733 × 10 ⁻² ± 0.4 × 10 ⁻² c	0.75 ± 0.17 ab	0.464 ± 0.86 a
<i>B. pyrrocinia</i>	31.1 ± 1.6 a	17.5 ± 2.1 bc	2.120 × 10 ⁻² ± 1.0 × 10 ⁻³ b	0.912 × 10 ⁻² ± 0.7 × 10 ⁻² b	0.63 ± 0.26 bc	0.412 ± 0.24 a
<i>P. fluorescens</i>	33.1 ± 1.5 a	16.3 ± 1.6 c	1.500 × 10 ⁻² ± 3.8 × 10 ⁻³ c	0.680 × 10 ⁻² ± 0.4 × 10 ⁻² d	0.49 ± 0.16 c	0.450 ± 5.33 a
<i>T. asperellum</i> + <i>B. pyrrocinia</i> + <i>P. fluorescens</i>	29.7 ± 1.3 a	14.3 ± 1.8 c	1.530 × 10 ⁻² ± 2.0 × 10 ⁻³ c	0.615 × 10 ⁻² ± 0.2 × 10 ⁻² d	0.48 ± 0.25 c	0.402 ± 0.15 b
Control (water)	28.2 ± 1.8 a	14.9 ± 2.5 c	1.182 × 10 ⁻² ± 2.0 × 10 ⁻³ d	0.464 × 10 ⁻² ± 0.3 × 10 ⁻² e	0.53 ± 0.28 c	0.379 ± 0.19 b

⁽¹⁾ *T. asperellum* (mix of four isolates: T-06, T-09, T-12, and T-52). ⁽²⁾ Standard error ($P \leq 0.05$). ⁽³⁾ Averages followed by the same letter within the same column were not significantly different according to Duncan's test ($P \leq 0.1$). Number of samples = 20.

TABLE 2: Root diameter, cortex and exodermis thickness, lacunae aerenchyma area (μm^2), vascular cylinder diameter (μm), and number of protoxylem poles and metaxylem vessel elements of rice roots obtained from seeds microbiolized with PGPMs, 21 days following sowing.

Treatment	Root diameter (μm)	Cortex thickness (μm)	Exodermis thickness (μm)	Aerenchyma lacunae (μm^2)	Vascular cylinder diameter (μm)	Number of protoxylem poles	Number of metaxylem vessel elements
<i>TrichodermaAsperillum</i> ⁽¹⁾	375.05 ± 59.60 ⁽²⁾ c ⁽³⁾	159.16 ± 17.03 b	12.1 ± 1.07 a	9.77 × 10 ⁶ ± 18.99 × 10 ³ a	229.6 ± 26.97 a	18.66 ± 6.11 a	3.66 ± 1.10 a
<i>Burkholderia pyrrocinia</i> + <i>Pseudomonas fluorescens</i>	373.94 ± 70.98 c	161.13 ± 14.09 b	13.28 ± 0.48 a	9.76 × 10 ⁶ ± 0.55 × 10 ³ a	221.4 ± 80.92 ab	20.00 ± 1.90 a	4.00 ± 1.90 a
<i>B. pyrrocinia</i>	456.63 ± 13.37 a	197.28 ± 11.10 a	12.3 ± 0.80 a	9.70 × 10 ⁶ ± 18.93 × 10 ³ ab	229.6 ± 26.97 a	19.00 ± 1.90 a	4.33 ± 1.10 a
<i>P. fluorescens</i>	369.37 ± 49.53 c	155.48 ± 3.43 b	11.8 ± 1.07 a	9.72 × 10 ⁶ ± 3.80 × 10 ³ ab	213.0 ± 53.95 ab	18.00 ± 3.80 a	4.66 ± 1.10 a
<i>T. asperillum</i> + <i>B. pyrrocinia</i> + <i>P. fluorescens</i>	411.11 ± 63.51 b	174.32 ± 15.58 b	12.79 ± 0.80 a	9.68 × 10 ⁶ ± 0.38 × 10 ³ bc	205.0 ± 26.97 ab	17.33 ± 2.90 a	2.33 ± 1.10 b
Control (water)	303.81 ± 46.22 d	130.13 ± 12.32 c	7.38 ± 0.96 b	9.61 × 10 ⁶ ± 0.76 × 10 ³ c	180.4 ± 26.97 b	13.00 ± 7.60 b	2.00 ± 1.90 b

⁽¹⁾ *T. asperillum* (mix of four isolates: T-06, T-09, T-12, and T-52). ⁽²⁾ Standard error ($P \leq 0.05$). ⁽³⁾ Averages followed by the same letter within the same column were not significantly different according to Duncan's test ($P \leq 0.05$) at 15 and 21 days following sowing. Number of samples = 20. Measurements were performed using a millimetric eyepiece in a MOTIC BA-400 light microscope.

TABLE 3. Phenols, flavonoids, lignin, and lignin monomer contents of roots of rice plants obtained from seeds microbiolized with bioagents, 21 days following sowing.

Treatment	Phenols			
	Total phenols	Flavonoids	Lignin	Lignin monomers
<i>T. asperellum</i> ⁽¹⁾	$64 \times 10^{-2} \pm 0.15 \times 10^{-2(2)}$ a ⁽³⁾	$10.93 \times 10^{-2} \pm 0.67 \times 10^{-2}$ a	$2.93 \times 10^{-2} \pm 1.05 \times 10^{-2}$ a	$1.44 \times 10^{-2} \pm 1.37 \times 10^{-2}$ c
<i>B. pyrrocinia</i> + <i>P. fluorescens</i>	$8.15 \times 10^{-2} \pm 1.21 \times 10^{-2}$ ab	$8.05 \times 10^{-2} \pm 0.62 \times 10^{-2}$ b	$0.40 \times 10^{-2} \pm 0.10 \times 10^{-2}$ b	$0.49 \times 10^{-2} \pm 0.30 \times 10^{-2}$ c
<i>B. pyrrocinia</i>	$9.11 \times 10^{-2} \pm 1.47 \times 10^{-2}$ a	$8.16 \times 10^{-2} \pm 0.34 \times 10^{-2}$ b	$0.42 \times 10^{-2} \pm 1.82 \times 10^{-2}$ b	$2.47 \times 10^{-2} \pm 1.83 \times 10^{-2}$ b
<i>P. fluorescens</i>	$8.92 \times 10^{-2} \pm 2.27 \times 10^{-2}$ a	$8.06 \times 10^{-2} \pm 0.74 \times 10^{-2}$ b	$2.18 \times 10^{-2} \pm 1.82 \times 10^{-2}$ a	$4.05 \times 10^{-2} \pm 0.56 \times 10^{-2}$ a
<i>T. asperellum</i> ⁽¹⁾ + <i>B. pyrrocinia</i> + <i>P. fluorescens</i>	$9.48 \times 10^{-2} \pm 1.96 \times 10^{-2}$ a	$7.54 \times 10^{-2} \pm 0.37 \times 10^{-2}$ c	$0.90 \times 10^{-2} \pm 1.45 \times 10^{-2}$ b	$0.38 \times 10^{-2} \pm 0.73 \times 10^{-2}$ c
Control	$6.80 \times 10^{-2} \pm 0.77 \times 10^{-2}$ b	$6.79 \times 10^{-2} \pm 0.19 \times 10^{-2}$ d	$0.74 \times 10^{-2} \pm 0.30 \times 10^{-2}$ b	$0.47 \times 10^{-2} \pm 8.00 \times 10^{-2}$ c

All the results are presented in mg per g dry mass. ⁽¹⁾ *T. asperellum* (mix of four *T. asperellum* isolates: T-06, T-09, T-12, and T-52). ⁽²⁾ Standard error ($P \leq 0.05$). ⁽³⁾ Averages followed by the same letter within the same column were not significantly different according to Duncan's test ($P \leq 0.05$). Number of samples = 20.

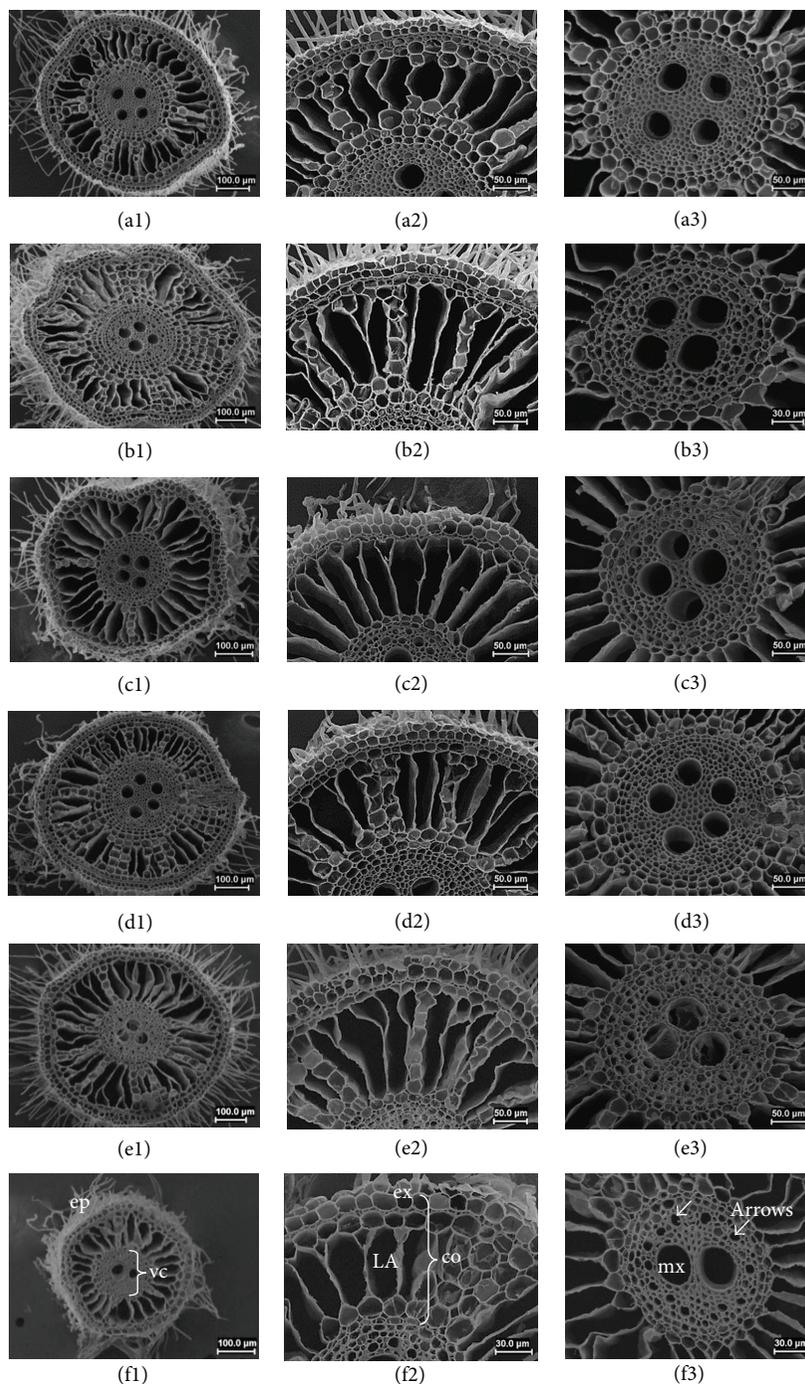


FIGURE 2: Electromicrographs of adventitious roots of rice obtained from seeds treated with PGPMs, 21 days following sowing. (a1–a3) *T. asperellum* (T-06, T-09, T-12, and T-52), (b1–b3) *B. pyrrocinia* + *P. fluorescens*, (c1–c3) *B. pyrrocinia*, (d1–d3) *P. fluorescens*, (e1–e3) *T. asperellum* (T-06, T-09, T-12, and T-52) + *B. pyrrocinia* + *P. fluorescens*, and (f1–f3) control. Epidermis with root hairs (ep), cortex (co), aerenchyma lacunae (LA), exodermis (ex), vascular cylinder (vc), protoxylem poles (arrows), and metaxylem vessel elements (mx).

which presented a phenol content similar to that of the control. However, the lignin content only increased in the roots of the plants treated with *T. asperellum* and *P. fluorescens* isolates. Microbiolization of seeds with *P. fluorescens* resulted in the higher expression of lignin monomers, followed by the seeds treated with *B. pyrrocinia*. The remaining treatments were not significantly different from the control (Table 3).

4. Discussion

Treatment with *T. asperellum* (mix of four *T. asperellum* isolates: T-06, T-09, T-12, and T-52) and the combination of rhizobacteria (*B. pyrrocinia* + *P. fluorescens*) resulted in 58 and 43% gain in root length of rice seedlings, respectively, and *T. asperellum* resulted in 46% increase in root/leaves ratio

(Table 1). These results are, probably, explained by the fact that *T. asperellum* isolates T-06, T-09, T-12, and T-52 were reported to produce phosphate-solubilizing enzymes, and among them T-12 and T-52 were identified as auxins producers. The same isolates also decrease rice sheath blight (*Rhizoctonia solani*) severity by 43%, a sclerotide viability up to 50%, and the area under the disease progress curve by 45% (Silva et al., 2012) [7]. It was earlier observed that isolates R-55 and R-46 were siderophores producers, besides β -1,3-glucanase during rice blast severity suppression [6].

These root modifications promoted by PGPMs result in positive physiological responses of plants. This response was observed in *Carthamus tinctorius* L. treated with *Azotobacter vinelandii* and *Azospirillum brasilense*, which presented up to 76% gain in root diameter, resulting in greater biomass [13]. When the PGPMs were combined with chemical fertilizers applied every three months, a maximum 64% gain in root diameter was observed compared to the control [14]. These modifications of the root system in response to the PGPMs may be associated with changes in signaling in phytohormone pathways, such as indol-3-acetic acid (IAA), resulting in increases of the surface area and the number of roots and root hairs. Parallel to the increase in root system induced by PGPRs and *T. asperellum*, an increase in mineral ion translocation via stimulation of ATPase proton pumps has been suggested [15]. The root gains induced by the PGPMs tested in the present study, *T. asperellum* and PGPRs, are very important for upland rice production systems. In this planting system, rice is limited due to the low vigor of the plant during the first 21 days following sowing. This limitation is a consequence of the low efficiency in N uptake due to the higher levels of N available in the form of NO_3^- [1] and to the fact that rice plants take up ammonium (NH_4^+) with higher efficiency [3]. Nitrogen influences the formation of roots at the meristematic tissues of the root-elongation region [16].

All the tested PGPMs resulted in an expansion of the root cortex up to 30% and of the exodermis up to 68% compared to the control, 21 days following sowing. This change was more pronounced for *B. pyrrocinia* and the combination of the two rhizobacteria (*B. pyrrocinia* + *P. fluorescens*), for which differences were observed starting 15 days following sowing. The exodermis constitutes barriers to the radial movement of ions and water at the roots [17]. A greater expansion of those layers induced by PGPMs may confer to the rice plants adequate levels of nutrients, especially K^+ , Ca^{2+} , and Mg^{2+} , lower loss of water, resulting in better maintenance of physiological and metabolic activities of plants, and greater resistance to water and salt stress [18, 19]. The aerenchyma lacunae increased up to 2% in plants treated with *T. asperellum* and *B. pyrrocinia* + *P. fluorescens*. Increasing the aerenchymal lacunae is very important for rice root in upland conditions, probably for helping adaptation during drought periods [20]. The aerenchyma expansion results in greater gas exchange for the irrigated rice plants and is mediated by the formation of reactive oxygen species (ROS). ROS have been identified as a signal required for cell death to occur at the cortex, which is induced by ethylene [21–23].

Ethylene was identified as the hormonal signal mediating formation of aerenchyma in corn and rice [24, 25]. However, in corn, root cortical aerenchyma reduces root respiration by converting living cortical tissue to air volume and increases drought tolerance by reducing root metabolic costs, permitting greater root growth and water acquisition from drying soil [26]. Our data makes us believe that in rice the same results could be found.

The vascular cylinder diameter increased 27% in the treatments with *T. asperellum* (mix of four isolates: T-06, T-09, T-12, and T-52) and *B. pyrrocinia*. All the tested PGPMs increased the number of protoxylem poles (43% in average). The number of metaxylem vessel elements increased 108% in the plants treated with *T. asperellum* (mix of four isolates: T-06, T-09, T-12, and T-52), *B. pyrrocinia* + *P. fluorescens*, *B. pyrrocinia*, and *P. fluorescens* (Table 2). Rice genotypes with more xylem vessels present better water conduction and will be more tolerant to water stress under Indian summer conditions. Some studies showed that increasing the number and the diameter of xylem vessels promotes drought resistance in legumes such as chickpeas (*Cicer arietinum* L.), peanuts (*Arachis hypogaea* L.), pigeon pea (*Cajanus cajan* (L.) Millsp.), cowpea (*Vigna unguiculata* (L.) Walp.), soybean (*Glycine max* (L.) Merr.), common bean (*Phaseolus vulgaris* L.), and millet (*Pennisetum glaucum* (L.) R. Br.) [27, 28]. This tolerance may result in higher productivity compared to plants not treated with PGPMs in upland systems [29, 30]. The occurrence of Indian summers in the savanna results in plant water stress, which may be minimized by anatomical adaptations. Studies have shown that some anatomical root characteristics, such as the metaxylem vessel elements diameter, are related to the maintenance of water conduction [31].

The plants treated with *B. pyrrocinia* presented a thicker pericycle than the remaining treatments (Figures 2(a3)–2(f3)). The pericycle is related to the formation of lateral roots (Figure 1), which play an important role in plant development, facilitating oxygen diffusion from the base to the apex as well as water, ion, and nutrient uptake [32, 33], allowing the construction of ramified root systems. The process of lateral root formation consists of two main stages: reactivation of the pericycle cell cycle and establishment of a new meristem [34]. In *Arabidopsis*, lateral roots are initiated by the local activation of pericycle cells and of the protoxylem poles [35]. In the present study, the tested PGPMs stimulated all these cells, pericycle and protoxylem, which may result in increased lateral root formation and consequently more vigorous establishment of rice plants.

All the tested PGPMs increased the levels of at least two phenols in roots. The maximum total phenol content was 42% in the plants treated with *T. asperellum*, and the average increase in flavonoids for all the treatments was 26% (Table 3). The increase in total phenols and flavonoids in plants treated with *Trichoderma* starts with the host plant root penetration and colonization by *Trichoderma*. At that moment, the plant increases the production of phenols to restrict the *Trichoderma* spp. colonization of the intercellular spaces at the epidermis, cortex, and vascular bundles. However, some isolates of this fungus can tolerate the increase in concentration of antimicrobial compounds inside the cell and

keep growing intra- and intercellularly at the roots of treated plants. This tolerance has been associated with the presence of ion-transport systems, which are key factors of the multiple effects of *Trichoderma* species as biocontrol and growth-promoting agents [36, 37]. Biotic and abiotic factors are known to induce systemic resistance (ISR) through structural changes. The physical and mechanical strength of the cell wall is induced by several factors and is generally expressed first as a hypersensitivity reaction (HR) to the penetration of phytopathogen cells. This reaction leads to histological changes, such as formation of papillae, lignification, deposition of callose, and thickening of the cell wall due to deposition of phenols and substances similar to hydrogen peroxide [38].

The increase in lignin content was approximately 300% in the plants treated with *T. asperellum* (mix of four isolates: T-06, T-09, T-12, and T-52) and 200% in the plants treated with *P. fluorescens*. The highest accumulation of lignin monomers was observed in the plants treated with *P. fluorescens* (762%) (Table 3). Lignocellulosic biomass is composed of cellulose, hemicellulose, and lignin, and lignin is the most recalcitrant to biodegradation [39]. The increase of these polymers may confer better organization of microfibrils, which mediate the structural stability of the root cell wall, to rice roots treated with *T. asperellum* and *P. fluorescens* [40].

The rhizobacteria *P. fluorescens* presented biofilm formation, which helps the bacteria attach to roots of rice seedlings. This benefit of biofilm formation was shown in roots of *Arabidopsis thaliana*, where the inhibition of biofilm formation at the root surface resulted in decreased adhesion of *Bacillus subtilis* to the substrate surface and could lead to the produced catechol (phenol), which can generate reactive oxygen species [41]. Studies with *Arabidopsis* and rice showed that catechol induces higher levels of superoxide H_2O_2 [42, 43].

5. Conclusions

All the root architecture modifications resulting from the interaction between seedlings and bioagents (rhizobacteria and *Trichoderma* spp.) observed in the present study favored the root plasticity of rice seedlings, resulting in greater plant growth due to a better water uptake, resistance to water stress, and mechanical impedance. The tested bioagents were shown to be potential biofertilizer to be inserted in upland rice management, with the goal of minimizing the disadvantages of this system, besides increasing defense response and increasing the productivity levels of genetically improved rice cultivars, without increasing the application of chemicals for fertilization and plant protection.

Abbreviations

Embrapa:	Brazilian Enterprise for Agricultural Research
HR:	Hypersensitivity reaction
IAA:	Indol-3-acetic acid
ISR:	Induced systemic resistance
PGPMs:	Plant growth-promoting microorganisms
PGPR:	Plant growth-promoting rhizobacteria
ROS:	Reactive oxygen species
SEM:	Scanning electron microscopy.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

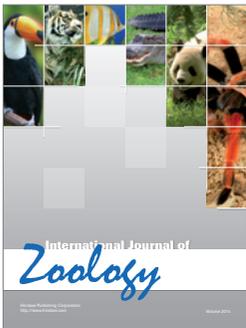
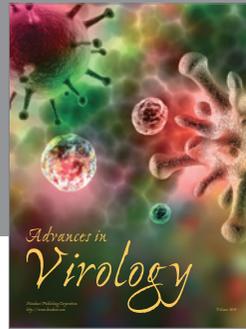
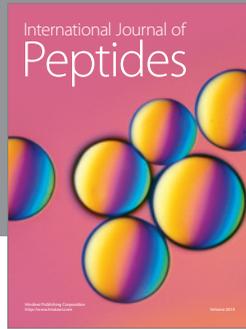
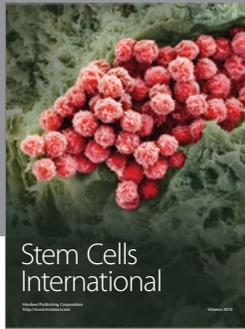
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