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Research Article

Trichoderma spp. isolates stimulate rice seedling growth of Sertani 13 variety

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ABSTRACT

Trichoderma has become one of the most studied filamentous fungi to be used as a greener and more sustainable solution for improving the production and growth of numerous crops, due to its capability to form symbiotic associations with plants. This study aimed to evaluate the effect of Trichoderma isolates obtained from the rhizosphere of organic rice fields in Sukabumi, Indonesia, in enhancing rice germination and seedling growth. A laboratory experiment used a completely randomized design consisting of seed treatments of 21 Trichoderma isolates (T1-T21) and a control treatment without Trichoderma (C). The inoculation was employed to elucidate any potential effects of Trichoderma isolates. Results showed that five isolates, i.e., T5, T7, T9, T10, and T14 stimulated the highest seedling vigor index, root and shoot length, and fresh weight and dry weight. These findings exhibited the potential of these five isolates as plant growth-promoting fungi to improve rice seedling growth and contribute to our understanding of the role of symbiotic fungi in sustainable rice crop production.

Keywords: plant growth promoting fungi; seed treatment; plant-microbe interactions, symbiotic fungi

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INTRODUCTION

Rice (*Oryza sativa* L.) is an important cereal crop which serves as a staple food for over 50% of the global population, especially in Asia (Ng et al., 2015). However, there has been a lack of progress in the enhancement of rice productivity and expansion of cultivation land in the past twenty years (FAO, 2020). This is attributed to the growing scarcity of resources (land, water, and labor), as well as the ineffective utilization of agrochemical inputs, along with the escalating expenses associated with rice cultivation (Abdullah et al., 2021; Basu et al., 2021; Kumar et al., 2022; Prasad et al., 2017). Therefore, efforts must be made to increase rice production, and one innovative approach that should be considered due to its eco-friendly nature and low cost is the utilization of microbial-based inoculation (Akbari et al., 2023). Microbes can play crucial roles in enhancing rice growth by facilitating nutrient acquisition, influencing physiological processes and development, modulating gene expression, and suppressing phytopathogens without degrading the environment (Doni et al., 2022). Additionally, harnessing microbial-based inoculation could potentially lead to sustainable agricultural practices, ensuring food security for future generations while minimizing environmental impact (Wei et al., 2024).

For the past few decades, microbes have been scientifically examined and confirmed to play an important role in the preservation of soil health, as well as in the enhancement of rice productivity (Hanudin et al., 2018). Beneficial microorganisms have been used in sustainable agriculture for decades due to their potential to act as plant growth enhancers (Doni et al., 2014b; Yadav et al., 2017). The utilization of plant growth-promoting microorganisms has been empirically demonstrated to possess the capacity to enhance seed germination, enhance seed vigor, and promote seedling growth (Shahrajabian et al., 2021). *Trichoderma* is one of the plant growth-promoting microorganisms capable of enhancing the growth of rice plants (Doni et al., 2023).

Trichoderma (Hypocreales, Hypocreaceae; teleomorph Hypocrea) is a genus of mycotrophic filamentous ascomycete fungi that are widely isolated from rotting wood, bark, other fungi, construction materials, and mammals (Jaroszuk-ściseł et al., 2019). *Trichoderma* has been shown to be able to colonize plant roots in order to maintain symbiotic interactions with plants (Doni et al., 2017; Akbari et al., 2024). In exchange for providing *Trichoderma* with an appropriate environment and food, the host plant roots obtain favorable regulation of growth, yield, and stress tolerance (Alfiky & Weisskopf, 2021).

Trichoderma can be utilized as a bioinoculant for seed treatment in order to boost seedling growth (Pokhrel et al., 2022), or called plant growth-promoting fungi. Seed treatment emerges as a compelling method for applying antagonistic microorganisms, as other procedures require the utilization of larger quantities of propagules (Gava & Pinto, 2016). Seed treatment with beneficial microorganisms incurs relatively low application costs, does not induce alterations within the seed, and provides advantages to the crop during both germination and seedling growth phases (Cardarelli et al., 2022).

The efficacy of several *Trichoderma* isolates for enhancing rice seed germination has been documented in several studies. For instance, a study using a Malaysian rice variety MRQ74 demonstrated the positive effects of *Trichoderma* sp. SL2 on rice seedling growth and vigor, evidenced by notable increases in seedling shoot and root length (71% and 82%, respectively), shoot and root weight (47% and 153%, respectively), vigor index (91%), and germination speed (25%) compared to untreated control (Doni et al., 2014a). Furthermore, another study emphasized the efficacy of *Trichoderma* sp. 3 in enhancing the germination rate of the Junjuang rice variety by 21% and increasing the vigor index by 174% (Elita et al., 2023). Despite the documented benefits of *Trichoderma* in enhancing rice seed germination and seedling vigor, there is a notable gap in research regarding the influence of seed treatment with *Trichoderma* isolated from West Java, Indonesia on the growth of rice seedlings, especially in the context of Indonesian agricultural practices. Hence, the objective of this study was to assess the impact of seed treatment with 21 *Trichoderma* isolates, obtained from the rhizosphere of a rice field in Sukabumi, West Java, Indonesia, on rice seed germination and subsequent seedling growth, aiming to evaluate the promotion of rice seedling growth by *Trichoderma*.

MATERIALS AND METHODS

Research site and experimental design

The research was carried out at the Applied Microbiology and Plant Pathology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor. The research was conducted from December 2021 – February 2022.

In this experiment, a completely randomized design was employed, consisting of 22 treatments and three replications. The treatments were 21 isolates of *Trichoderma* (T) and one control (C). The seed treatments evaluated for this study are rice seeds soaked in distilled water as the control group (C) and rice seeds soaked in spore suspension of *Trichoderma* isolated from Nusantara Organic SRI Center (NOSC), Sukabumi, West Java (T). For each treatment, the seeds were soaked for 24 hours. The data were measured quantitatively. Rice germination parameters were measured including germination percentage and vigor index. Rice seedling growth parameters consisted of root and shoot length, fresh weight, and dry weight.

Fungal cultures and preparation of inocula

Trichoderma spp. was isolated from soil samples collected from organic rice fields in Sukabumi using a multilevel dilution technique in the Applied Microbiology and Plant

Pathology Laboratory, Universitas Padjadjaran. The process involved serial dilution of a 10 g soil sample with 100 mL distilled water, creating homogenized suspensions. Before the soil became a precipitate, a sterile pipette was used to collect 1 mL of the suspension, which was then diluted into 9 mL of distilled water. The dilution process was performed three times, resulting in a dilution rate of 10^{-1} to 10^{-5} g of soil per mL. A volume of 100 µL of the solution was pipetted out and transferred using the pour plate technique onto the prepared potato dextrose agar (PDA) medium. The agar was then incubated at 26 °C until fungal colonies formed. Subsequently, the isolates underwent purification and were moved to a new petri dish containing fresh PDA medium (Mishra et al., 2019).

After a seven-day incubation at 26°C, twenty-one purified isolates of *Trichoderma* (T1-T21) were used to make a spore suspension. The spores were obtained from plates with the addition of 10 mL of sterile water. Subsequently, the spores were directly moved to an erlenmeyer flask filled with sterilized distilled water. The spore concentration was adjusted to 10^7 spores mL^{-1} based on measurements obtained using a hemocytometer. The following formula was used to calculate spore density (Anhar et al., 2018):

$$\text{Spore density} = (\text{number of conidia} \times 5 \times \text{dilution factor})/(\text{haemocytometer volume})$$

Rice seed inoculation and seedling preparation

In this research, the Indonesian Sertani 13 rice (*Oryza sativa* L.) cultivar was used. The seeds were sterilized by soaking them in 70% ethanol for 30 minutes and then rinsing them with sterile distilled water. For the control treatment, 300 seeds were soaked for 24 hours in sterile, distilled water.

Trichoderma-treated seeds (300 seeds per treatment) were soaked for 24 hours in a spore suspension. The seedlings were then grown for a duration of five days within sterile Petri dishes, wherein the growth media consisted of filter paper moistened with distilled water (Doni et al., 2017).

Observations

The germination percentage refers to the mean proportion of seeds that undergo germination within five days. The seeds were cultivated on filter paper in petri dishes under controlled conditions at a temperature of 26°C. The total amount of normal seedlings was collected, which indicated by rice seedlings with essential structures (seminal roots, mesocotyl, coleoptile, cotyledon, and primary leaf). The germination percentage was determined using the subsequent formula:

$$\text{Germination (\%)} = (\text{number of normally germinated seeds})/(\text{number of seeds}) \times 100$$

The calculation of a seed vigor index (SVI) was performed using the formula provided by Abdul-Baki and Anderson (1973):

$$\text{Seed vigor index} = \text{germination (\%)} \times \text{seedling length (shoot length + root length) (cm)}$$

The lengths of the roots and shoots were assessed on the fifth day after the radicle had emerged. A total of 20 seedlings were randomly chosen from each treatment, and their lengths were measured using a ruler. The measurement of root length was conducted by determining the distance between the apex of the primary root and the base of the hypocotyl. The measurement of shoot length was conducted by determining the distance between the base of the primary leaf and the base of the hypocotyl. The fresh weight and dry weight (in milligrams) of seedlings were measured using a digital scale. The seedlings were subjected to drying in an oven at 65°C for 48 hours, following which their dry weight was measured (Mishra et al., 2019).

Statistical analyses

All data were statistically examined using one-way analysis of variance (ANOVA). The Duncan Multiple Range Test (DMRT) at $p<0.05$ was used to separate mean values for variables with significant effect.

RESULTS AND DISCUSSION

Seed germination and vigor

The germination percentage and seedlings vigor differed significantly among treatments ($p \leq 0.05$) (Table 1). The germination percentage of rice seeds in this study ranged from 66% to 94%. While the seed treatment with *Trichoderma* isolates did not exhibit a significantly higher germination percentage compared to the control, certain *Trichoderma* isolates showed a tendency to enhance vigor index values. Seed treatment with T1 showed a notable seed germination percentage (94%); however, this result did not demonstrate a significant difference compared to C, T2, T4, T8, T12, T14, T17, T18, T19, and T20 treatments, indicating comparable effects across these *Trichoderma* isolates and control. Meanwhile, T10 exhibited the lowest germination percentage among the treatments. Among *Trichoderma* isolates, T14 demonstrated the highest seed vigor index (1337.70), in comparison to the other treatments. Furthermore, findings suggest that isolates T4, T5, T7, T9, T13, T15, T16, T17, and T20 also displayed higher vigor index values compared to the control treatment, underscoring their potential as promising isolates for improving rice seedling vigor. Conversely, the control treatment (C) exhibited the lowest vigor index (759.29).

Table 1. Effects of *Trichoderma* on germination percentage and seedling vigor index of rice seedlings.

Trichoderma isolates	Germination (%)	Vigor index
Control (C)	84.67abcd	759.29gh
T1	94.00a	942.54cdefg
T2	88.00ab	852.08efgh
T3	79.00bcde	936.66cdefg
T4	87.33abc	1160.57b
T5	72.00de	1027.73bcde
T6	73.00de	812.50fgh
T7	80.00bcd	1123.54bc
T8	82.33abcd	881.11defg
T9	78.33bcde	1061.20bcde
T10	65.67e	904.31defg
T11	79.00bcde	887.10defg
T12	81.00abcd	909.13defg
T13	79.00bcde	972.11bcdef
T14	89.33ab	1337.70a
T15	78.67bcde	1018.62bcdef
T16	78.33bcde	1028.09bcde
T17	84.00abcd	1062.90bcd
T18	82.33abcd	899.19defg
T19	84.00abcd	920.24cdefg
T20	83.33abcd	964.72bcdefg
T21	73.67cde	863.15defgh

Note: The treatments T1-T21 (inoculated with native *Trichoderma* isolates). Values followed by the same letters in the same column are not significantly different according to DMRT ($p<0.05$).

The presence of high seed vigor is correlated with the capacity to enhance growth and productivity within the realm of agricultural production (Han et al., 2014). The findings of our study align with those of a prior investigation conducted by Doni et al. (2014b), who observed an enhancement of germination percentage and seedling vigor of rice with the application of seven *Trichoderma* isolates. In another study, the inoculation of *T. harzianum* and *T. minutisporum* increased the germination percentage, vigor index, and germination speed of local Indonesian rice varieties (AA75, Mikonga, Batang Sungkai, Saganggam) (Anhar et al., 2021). The enhanced germination and seed vigor seen in this study may be attributed to the presence of several phytohormones like auxin, cytokinin, zeatin, and gibberellin, which are released by *Trichoderma* isolates (Osiewacz, 2002;

Swain et al., 2018). Furthermore, seed treatment with the promising *Trichoderma* isolates can stimulate the production of gibberellin which activates germination by promoting enzyme activity, such as amylase, which facilitates starch metabolism in rice seedlings (Piri et al., 2019). In addition, the type of *Trichoderma* strain and numerous external stimuli influence the production of growth regulators (Nieto-Jacobo et al., 2017). However, in this study, not all *Trichoderma* isolates are able to enhance germination. Santos et al. (2020) reported a comparable opposing outcome, wherein *Trichoderma* was discovered to have a suppressive impact on the germination percentage of *Handroanthus serratifolius*. The variation in germination outcomes can be attributed to the isolate-specific effect, as certain isolates have the potential to inhibit germination (Machado et al., 2015). This inhibition may result from the presence of phytotoxic secondary metabolites, such as trichothecenes produced by *Trichoderma* at specific concentrations (Yin et al., 2020).

Seedling growth

Trichoderma application had a significant impact on seedling growth (Table 2). Overall *Trichoderma* treatment had a significant and positive effect on both the root and shoot length. Root lengths among the treatments were notably greater for T14 (7.25 cm), T5 (6.77 cm), T7 (6.61 cm), T10 (6.35 cm), T9 (6.21 cm), T4 (6.08 cm), T16 (6.06 cm), and T15 (5.93 cm), suggesting similar effects across these *Trichoderma* isolates. Similarly, the greater shoot lengths were achieved by T14 (7.73 cm), T5 (7.52 cm), T7 (7.44 cm), T10 (7.37 cm), T4 (7.24 cm), T9 (7.35 cm), T16 (7.08 cm), T15 (7.02 cm), and T13 (6.94 cm).

Moreover, both fresh weight and dry weight significantly increased with *Trichoderma* seed treatment compared to the control (Table 3). Higher fresh weights were observed in T14 (110.67 mg), T5 (105.33 mg), T7 (101.33 mg), T10 (99.33 mg), and T9 (96.67 mg) treatments. Similarly, greater dry weights were observed in T14 (82.40 mg), T5 (77.60 mg), T7 (74.13 mg), T10 (72.67 mg), and T9 (71.60 mg) treatments.

Table 2. Root length and shoot length of rice seedlings with *Trichoderma* inoculation.

Trichoderma isolates	Root length (cm)	Shoot length (cm)
Control (C)	3.44h	5.51ij
T1	3.84fgh	6.21ghi
T2	3.56gh	6.15hi
T3	5.25bcdef	6.77bcdefgh
T4	6.08abcd	7.24abcdef
T5	6.77ab	7.52ab
T6	4.62defgh	6.51defgh
T7	6.61ab	7.44abc
T8	4.44efgh	6.26ghi
T9	6.21abc	7.35abcde
T10	6.35abc	7.37abcd
T11	4.63defgh	6.57cdefgh
T12	4.66defgh	6.64bcdefgh
T13	5.37bcdef	6.94abcdefgh
T14	7.25a	7.73a
T15	5.93abcde	7.02abcdefgh
T16	6.05abcd	7.08abcdefg
T17	5.62bcde	7.02abcdefgh
T18	4.46efgh	6.45fgh
T19	4.49efgh	6.47efgh
T20	4.94cdefgh	6.65bcdefgh
T21	5.01cdefg	6.73bcdefgh

Note: The treatments T1-T21 (inoculated with native *Trichoderma* isolates). Values followed by the same letters in the same column are not significantly different according to DMRT ($p<0.05$).

Table 3. Fresh weight and dry weight of rice seedlings with *Trichoderma* inoculation.

Trichoderma isolates	Fresh weight (mg)	Dry weight (mg)
Control (C)	56.00jk	42.67jk
T1	68.00hij	52.53hij
T2	65.33ij	47.73ij
T3	93.33bcdef	68.27bcdef
T4	94.67bcde	69.60bcde
T5	105.33ab	77.60ab
T6	78.00fghi	57.20fghi
T7	101.33abc	74.13abc
T8	72.00ghi	50.67ghi
T9	96.67abcde	71.60abcde
T10	99.33abcd	72.67abcd
T11	82.67efgh	61.87efgh
T12	84.00defgh	61.07defgh
T13	89.33bcdef	65.87bcdef
T14	110.67a	82.40a
T15	90.67bcdef	72.00bcdef
T16	92.67bcdef	68.13bcdef
T17	89.33bcdef	68.00bcdef
T18	72.00ghi	50.67ghi
T19	77.33fghi	58.67fghi
T20	84.00defgh	63.20defgh
T21	86.00cdefg	67.87cdefg

Note: The treatments T1-T21 (inoculated with native *Trichoderma* isolates). Values followed by the same letters in the same column are not significantly different according to DMRT ($p<0.05$).

The observed effects on shoot and main root length in rice seedlings induced by *Trichoderma* isolates mainly may be attributed to the synthesis of growth-stimulating hormones and secondary metabolites. The observed enhancements in shoot and main root length align with findings from previous research, establishing a consistent pattern across diverse crops such as wheat (Saadaoui et al., 2023), Mongolian pine (Halifu et al., 2019), and tomato (Singh et al., 2014).

Beneficial root-colonizing microorganisms like *Trichoderma* that release IAA can potentially influence the plant's endogenous IAA when employed in seed treatment (Backer et al., 2018). IAA is essential for root and shoot growth in plants (Halifu et al., 2019). Apart from plant hormones, *Trichoderma* was discovered to produce harzianolide, a secondary metabolite capable of influencing the early phases of seedling growth by increasing root length and root tips and regulating overall root development (Cai et al., 2013). These findings not only contribute to the growing body of knowledge on seed treatment with *Trichoderma* but also suggest its potential application as a sustainable strategy for enhancing seedling growth in rice and other crops.

CONCLUSIONS

Five out of 21 *Trichoderma* isolates, i.e., T5, T7, T9, T10, and T14 were promising bioinoculants as shown by positive effects on higher root, shoot length, fresh and dry weight of rice seedlings. The germination percentage of *Trichoderma*-treated seeds was not significantly different from the control treatment, therefore, evaluation of seedling growth variable is important in the future. Further research is needed to investigate the performance of *Trichoderma*-treated seeds under actual field conditions, considering factors such as soil types, climatic conditions, and agronomic practices.

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