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Full Length Article

Isolation, screening, and molecular characterization of indigenous *Trichoderma* isolates from West Java, Indonesia and their plant growth-promoting capability on rice plants (*Oryza sativa* L.)

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ABSTRACT

Trichoderma species are widely acknowledged as growth-promoting fungi that have been utilized to enhance the growth and yield of numerous crops. This research examined the capacity of 30 *Trichoderma* strains, isolated from two organic rice fields in West Java, Indonesia, to enhance the germination, growth, and physiological characteristics of rice plants (*Oryza sativa* L.). In general, *Trichoderma* strain TM10 demonstrated the greatest ability to increase seed germination (97.25 %), vigor index (3122.83), and germination speed (59.91 seeds/day). This strain also increased seedling root length and seedling height by 101.62 % and 112.20 %, respectively. Plants treated with TM10 exhibited a notable improvement in root length, plant height, fresh weight, and dry weight compared to control plants, demonstrating increases of 188.68 %, 69.90 %, 157.41 %, and 159.38 %, respectively. Furthermore, the total chlorophyll content and stomatal number in TM10-treated plants exhibited increments of 75.23 % and 75.53 %, respectively. Five selected isolates (TM7, TM10, SB2, SB8, and SB14) were evaluated for their potential to produce plant growth-promoting compounds, including phosphatase enzyme (ranging from 0.54 to 11.14 μg pNP $g^{-1}h^{-1}$), indole-3-acetic acid (IAA) (ranging from 28.96 to 63.91 μg/mL), ammonia (ranging from 1.96 to 5.79 μg/mL), and hydrogen cyanide (HCN) (ranging from 221.76 to 274.82 ppm). The best strain, namely TM10, was then molecularly identified as *Trichoderma yunnanense*. This investigation demonstrates that *Trichoderma* spp. isolated from organic rice fields could be used as a bioinoculant in sustainable rice production.

1. Introduction

Rice serves as a crucial staple food for the people of Indonesia, playing a central role in their daily diet and cultural traditions (Panuju et al., 2013). The demand for rice has surged in parallel with the escalating rates of economic and population growth in the country (Rozaki, 2021). From 2008 to 2020, there was a notable increase in rice demand, totaling 4.66 million tons, reflecting an average annual growth rate of 1.16 % (FAO, 2021). Rice production in Indonesia experienced fluctuations from 2018 to 2022, with corresponding figures of 59.20 million tons, 54.60 million tons, 54.64 million tons, 54.42 million tons, and 55.7 million tons (Statistics Indonesia, 2022). The challenge arises from domestic consumption outpacing production, leading the government to resort to rice imports to ensure ample supplies and prevent a spike in rice prices (Rifin, 2022). Accordingly, there is an immediate need to increase rice production.

Previously, the Indonesian government endeavored to uphold and enhance local rice output by employing methods such as reducing crop losses, improving irrigation systems, intensifying farming practices, maximizing land usage, and expanding cultivation areas (Rachman et al., 2022). However, efforts to increase rice production cause environmental pollution and harm due to the overutilization of chemical fertilizers and synthetic biocides (Huang et al., 2022). The excessive use of agrochemical products has a notable impact on diminishing soil

Abbreviations: PGPM, plant growth-promoting microorganisms; PGP, plant growth promoting; IAA, indole-3-acetic acid; HCN, hydrogen cyanide; PDA, potato dextrose agar; pNPP, p-nitrophenyl phosphate; PDB, potato dextrose broth; ITS, transcribed spacer region; BLAST, basic local alignment search tool; NCBI, National Center for Biotechnology Information; MEGA, molecular evolutionary genetics analysis; DNA, deoxyribonucleic acid; ACC, 1-aminocyclopropane-1-carboxylate.

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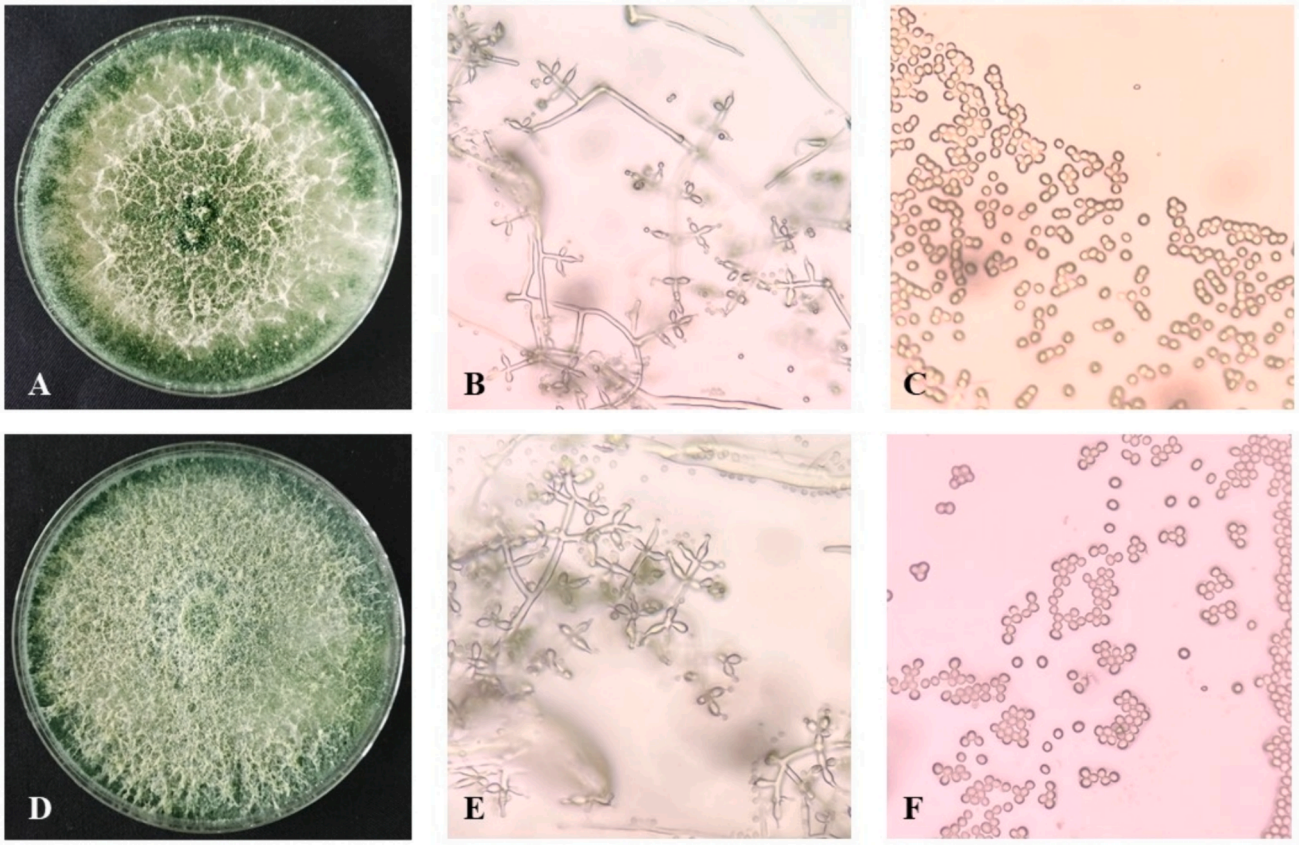

Fig. 1. Colony of TM10 on PDA after 7 days (A), phialides of TM10 (B), conidia of TM10 (C), colony of SB8 on PDA after 7 days (D), phialides of SB8 (E), conidia of SB8 (F).

fertility, elevating rice yield losses attributable to pests and diseases, and diminishing beneficial soil microbial communities essential for soil biochemical processes (Meena et al., 2020). Consequently, the application of sustainable agricultural techniques is required to improve soil biological processes, reduce reliance on external inputs, and improve the structure and fertility of the soil (Abd-Alhamid et al., 2015).

Microorganisms perform an important function in environmental management and sustainable agriculture, and utilizing them in agroecosystems has achieved excellent results (Kaviyarasu et al., 2016; Magdalane et al., 2017; Valsalam et al., 2019). Utilizing microorganisms has several beneficial effects, including enhancing plant growth and productivity, enhancing plant resilience, and effectively managing diseases and pests (van Jaarsveld et al., 2021). Plant growth-promoting microorganisms (PGPM), including fungi, bacteria, cyanobacteria, and mycorrhiza, serve as environmentally friendly alternatives (Primavesi et al., 2023).

One of the PGPM members that can be used to promote the growth of rice plants is *Trichoderma* (Pang et al., 2017). *Trichoderma* species produce a variety of bioactive chemical compounds which enhance plant growth (phytohormones), control pathogens (e.g., cellulases, peptaibols, and hydrogen cyanide), and improve soil health (e.g., phosphate-solubilizing enzymes and ammonia) (Sharma et al., 2019). *Trichoderma* species are beneficial soil microorganisms and are widely studied as accessible and sustainable solutions for improving plant growth and development (Rawal et al., 2022). *Trichoderma* enhances plant growth through various mechanisms, encompassing both biochemical and molecular modulation of plants (Gajera et al., 2016). Studies have shown that the utilization of *Trichoderma* has the potential to enhance the growth and physiological characteristics of rice plants (Khadka and Uphoff, 2019).

The isolation of indigenous *Trichoderma* strains holds significant importance in agricultural practices, particularly in relation to the production of rice in an environmentally friendly way (Tegene et al., 2021). *Trichoderma* can enhance crop productivity and quality, reduce dependence on synthetic pesticides, and improve soil health (Abdullah et al., 2021; Akbari et al., 2024). It functions as a biofertilizer, biopesticide, and bioremediation agent, inhibiting harmful microorganisms and promoting beneficial ones (Sharma et al., 2019). In recent years, there has been a growing and intensive search for *Trichoderma* species with the potential to serve as bioinoculants in rice production (Abdullah et al., 2021). Therefore, this research aims to isolate the *Trichoderma* species from organic rice fields in West Java, Indonesia, and to elucidate the potential of *Trichoderma* strains in improving the growth and physiological characteristics of rice plants.

2. Materials and methods

2.1. *Trichoderma spp. isolation*

Trichoderma spp. were isolated from soil samples obtained from two organic rice fields in West Java, Indonesia: Nusantara Organic SRI Center in Sukabumi and SRI Mukti Sadaya Organic Rice Farm in Tasikmalaya. Purified isolates were transferred to fresh potato dextrose agar (PDA) medium. Morphological identification of the isolates was conducted through macroscopic and microscopic observations. Detailed procedures are provided in Supplementary Table S1.

2.2. *Evaluating the effect of Trichoderma on rice seedling growth*

The growth-promoting effect of 30 isolated *Trichoderma* strains was evaluated on IR64 rice variety seedlings in gnotobiotic conditions, tracking growth progress over 14 days, using a completely randomized

Table 1
The influence of *Trichoderma* inoculation on the growth of rice seedlings.

Treatments	Germination (%)	Vigor index	Speed of germination (seeds/day)	Root length (cm)	Shoot length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)
C	87.25 hij	1342.64 k	50.58 bcdefgh	6.16 g	9.26 h	0.09 d	0.01 c
TM1	92.25 cdef	2274.76 efghi	56.36 abc	8.02 bcdefg	16.66 cdef	0.13 abc	0.01 c
TM2	89.25 fghi	2410.22 defgh	47.81 cdefgh	8.81 bcdefg	18.21 abcde	0.13 abc	0.02 ab
TM3	89.25 fghi	2185.43 fghi	47.29 defgh	7.95 bcdefg	16.53 defg	0.12 abc	0.01 abc
TM4	90.25 efgh	2469.65 cdefg	48.47 cdefgh	9.02 bcdefg	18.34 abcde	0.14 abc	0.02 a
TM5	87.25 hij	2256.57 efghi	49.42 bcdefgh	8.47bcdefg	17.40 abcde	0.13 abc	0.02 abc
TM6	86.25 ijk	2085.11 hi	46.22 efgh	7.68 bcdefg	16.49 defg	0.12 abc	0.01 c
TM7	80.25 jk	2278.30 efghi	45.09 fgh	9.79 bc	18.63 abcd	0.15 ab	0.02 a
TM8	87.25 hij	2137.77 ghi	47.56 defgh	7.98 bcdefg	16.54 defg	0.12 abc	0.01 bc
TM9	89.25 fghi	2264.50 efghi	50.65 bcdefgh	8.20 bcdefg	17.19 bcde	0.13 abc	0.01 abc
TM10	97.25 a	3122.83 a	59.91 a	12.42 a	19.65 a	0.15 ab	0.02 a
TM11	85.25 jk	2294.11 defghi	49.61 bcdefgh	8.74 bcdefg	18.19 abcde	0.13 abc	0.02 ab
TM12	87.25 hij	2399.04 defgh	51.28 bcdefgh	9.16 bcdef	18.37 abcde	0.14 abc	0.02 ab
TM13	85.25 jk	2136.16 ghi	44.20 gh	8.11 bcdefg	16.92 bcde	0.13 abc	0.01 bc
TM14	87.25 hij	2331.16 defgh	49.86 bcdefgh	8.62 bcdefg	18.12 abcde	0.13 abc	0.02 ab
TM15	83.25 jk	1739.78 j	43.93 gh	6.52 fg	14.42 g	0.11 cd	0.01 c
SB1	88.25 ghij	2479.72 cdef	48.64 cdefgh	9.60 bcd	18.50 abcd	0.15 abc	0.02 a
SB2	94.25 abcd	2754.72 bc	53.72 abcde	10.28 abc	18.95 abc	0.15 a	0.02 a
SB3	89.25 fghi	2361.68 defgh	48.65 cdefgh	8.52 bcdefg	17.92 abcde	0.13 abc	0.02 ab
SB4	83.25 kl	2076.54 hi	43.17 h	6.73 defg	18.21 abcde	0.12 bcd	0.01 bc
SB5	94.25 abcd	1992.84 ij	54.72 abcde	6.67 efg	14.50 fg	0.12 cd	0.01 bc
SB6	96.25 ab	2532.35 cde	60.30 a	8.50 bcdefg	17.81 abcde	0.13 abc	0.02 ab
SB7	91.25 defg	2473.09 cdefg	52.88 abcdf	8.89 bcdefg	18.24 abcde	0.13 abc	0.02 abc
SB8	96.25 ab	2856.82 ab	57.39 ab	10.60 ab	19.09 ab	0.15 a	0.02 a
SB9	89.25 fghi	2498.89 cdef	51.16 bcdefgh	9.58 bcde	18.39 abcd	0.14 abc	0.02 a
SB10	88.25 ghij	2264.13 efghi	46.24 efgh	8.28 bcdefg	17.36 abcde	0.13 abc	0.02 abc
SB11	93.25 bcde	2565.58 bcde	50.42 bcdefgh	9.12 bcdef	18.35 abcde	0.14 abc	0.02 ab
SB12	93.25 bcde	2189.73 fghi	52.87 abcdf	7.53 cdefg	15.98 efg	0.12 abc	0.01 bc
SB13	94.25 abcd	2615.68 bcd	52.48 abcdfg	9.41 bcdef	18.38 abcd	0.14 abc	0.02 a
SB14	95.25 abc	2752.40 bc	55.26 abcd	10.19 abc	18.70 abcd	0.15 a	0.02 a
SB15	90.25 efgh	2277.26 efghi	50.97 bcdefgh	8.19 bcdefg	17.03 bcde	0.13 abc	0.01 bc

Note: Means with the same letter in the same column are not significantly different according to DMRT ($p \leq 0.05$).

design with 31 treatments (30 *Trichoderma* isolates and a control), and 10 replications were performed in a greenhouse at the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. Seeds were surface-sterilized and inoculated with *Trichoderma* spore suspension (10^7 spores/mL) for 24 h. Seeds were then assigned to each experimental condition—treatment groups (Tasikmalaya: TM and Sukabumi: SB, inoculated with *Trichoderma*) and the control group (C, not inoculated). Seedlings were grown in sterilized growth medium in a greenhouse with controlled conditions for 14 days. Root and shoot lengths, as well as fresh and dry weights, were measured. Furthermore, germination percentage, vigor index, and germination speed were then calculated. Detailed procedures are provided in Supplementary Table S1.

2.3. Evaluating the effect of Trichoderma on the growth and physiological characteristics of rice plants

After the initial 14-day germination period, seedlings were inoculated with *Trichoderma* spore suspension and transplanted into plastic pots under controlled greenhouse conditions for 60 days. This experiment utilized a completely randomized design with 31 treatments, consisting of 30 different isolates of *Trichoderma* and a control group without *Trichoderma* inoculation. The experiment was conducted with four replications. The planting medium composition was the same as in the previous experiment. The 2 kg of soil was put into a plastic pot with a diameter of 20 cm. Plant maintenance involved ensuring a water level 2 cm above the soil and aerating the soil surface physically every 10 days. Growth parameters such as plant height, root length, number of leaves, fresh weight, and dry weight were measured. Physiological characteristics included measuring chlorophyll content and stomatal density. Procedures are detailed in Supplementary Table S1.

2.4. Plant growth promoting traits and molecular identification of Trichoderma

The five best-performing *Trichoderma* isolates in enhancing rice germination, rice growth, and rice physiological traits will be tested for their plant growth promoting (PGP) abilities. The PGP observed is the activity of phosphatase and the production of indole-3-acetic acid (IAA), ammonia, and hydrogen cyanide (HCN). The molecular identification of two highest-performing isolates involved genomic DNA extraction, ITS region amplification, sequencing, and phylogenetic analysis using MEGA software. Detailed procedures are provided in Supplementary Table S1.

2.5. Statistical analysis

A statistical analysis of all the data was conducted using one-way analysis of variance (ANOVA). For significantly different parameters, mean separation was performed using Duncan's Multiple Range Test (DMRT) at a significance level of $p \leq 0.05$.

Table 2
The influence of *Trichoderma* inoculation on the growth of rice plants.

Treatments	Root length (cm)	Plant height (cm)	Total fresh weight (g)	Total dry weight (g)	Leaf number (per plants)
C	4.24 o	35.05 n	2.16 q	0.32 q	5
TM1	7.41 ijkl	40.95 ij	3.26 jkl	0.49 jkl	5
TM2	8.74 defghi	45.55 efg	3.66 ghijk	0.55 ghijk	5
TM3	6.65 klm	40.05 jk	3.06 lmn	0.46 lmn	4.33
TM4	8.94 defgh	46.05 ef	3.86 fghi	0.58 fghi	5
TM5	8.19 fghij	42.05 i	3.38 ijkl	0.51 jkl	4.33
TM6	6.65 klm	39.15 kl	2.76 mno	0.41 mno	5
TM7	9.74 cde	49.05 cd	4.56 bcd	0.68 bcd	5
TM8	6.9 1jklm	40.55 ijk	3.16 klm	0.47 klm	5
TM9	7.74 ghijk	41.25 ij	3.36 jkl	0.50 jkl	5
TM10	12.24 a	59.55 a	5.56 a	0.83 a	5
TM11	8.61 efghi	45.05 fg	3.59 ghijk	0.54 ghijk	5
TM12	9.04defgh	46.55 ef	4.06 efg	0.61 efg	5
TM13	7.41 ijkl	41.175 ij	3.33 jkl	0.5 jkl	4.33
TM14	8.41 efghi	44.15 gh	3.56 hijk	0.53 hijkl	5
TM15	4.24 o	35.55 n	2.26 pq	0.34 pq	5
SB1	9.65 cdef	48.55 d	4.46 cde	0.67 cde	5
SB2	10.65 bc	52.05 b	4.76 bc	0.71 bc	5
SB3	8.26 efghij	43.55 h	3.46 ijkl	0.52 ijkl	5
SB4	5.61 mn	37.55 m	2.56 opq	0.38 opq	5
SB5	5.15 no	36.05 n	2.46 opq	0.37 opq	4.33
SB6	8.25 efghij	42.05 i	3.43 ijkl	0.51 ijkl	7
SB7	8.81 defghi	45.55 efg	3.76 fghij	0.56 fghij	4.33
SB8	11.24 ab	52.55 b	4.96 b	0.74 b	5
SB9	9.24 defg	47.05 e	4.16 def	0.62 def	5
SB10	8.11 ghij	41.55 ij	3.36 jkl	0.50 jkl	5
SB11	8.94 defgh	46.05 ef	3.96 fgh	0.59 fgh	5
SB12	6.24 lmn	38.55 lm	2.66 nop	0.40 nop	5
SB13	9.15 defgh	47.00 e	4.06 efg	0.61 efg	5
SB14	10.15 bcd	50.05 c	4.66 bc	0.70 bc	4.33
SB15	7.65 hijkl	41.25 ij	3.35 jkl	0.50 jkl	5

Note: Means with the same letter in the same column are not significantly different according to DMRT ($p \leq 0.05$).

3. Results

3.1. Isolation and morphological identification of Trichoderma

Trichoderma spp. were successfully isolated from the soil of organic rice fields in Tasikmalaya (TM) and Sukabumi (SB) with 15 isolates, respectively. The isolated *Trichoderma* strains exhibited similar macroscopic and microscopic characteristics (Supplementary Table S2). Fig. 1 shows the morphological appearance of TM10 and SB8 isolates.

3.2. Rice seed germination and vigor

The germination capacity of rice seeds varied, with the lowest percentage shown by the TM7 treatment (80.25 %) and the highest percentage shown by the TM10 (97.25 %). TM10 treatment increased seed vigor significantly, as indicated by a high seed vigor index (3122.83). Likewise, in the germination speed parameter, the highest average number of seeds capable of germinating in a day was shown by the TM10 treatment (59.91 seeds/day). The findings summarized in Table 1 demonstrate that *Trichoderma* inoculation improved seed germination and seedling early growth.

3.3. Rice seedling growth

The *Trichoderma* treatment resulted in significant improvements in root length, seedling height, and the fresh and dry weight of seedlings, as shown in Table 1. The TM10 treatment yielded the greatest results in root length, measuring 12.42 cm, which represents a 101.62 % increase. Seedling height increased significantly, up to 112.20 %, with the TM10 treatment. In addition, TM7, TM10, SB2, SB8, and SB14 treatments increased fresh weight and dry weight significantly compared to the control.

Table 3
The influence of *Trichoderma* inoculation on the chlorophyll content and stomatal density of rice plants.

Treatments	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Total chlorophyll (mg/g)	Stomata/ mm^2
C	1.25 e	1.17 g	2.41 h	213.84 r
TM1	1.71 abcde	1.30 bcdefg	3.01 bcdefg	251.57 klmno
TM2	1.86 abcd	1.34 bcdefg	3.20 bcdef	276.73 efghi
TM3	1.59 bcde	1.28 cdefg	2.87 defgh	246.86 mnop
TM4	1.92 abc	1.35 bcdefg	3.27 bcde	289.31 defg
TM5	1.81 abcd	1.31 bcdefg	3.12 bcdef	264.15 hijklm
TM6	1.54 cde	1.27 defg	2.80 efgh	245.28 mnop
TM7	2.03 abc	1.44 bcd	3.46 abc	336.48 b
TM8	1.60 bcde	1.29 bcdefg	2.90 cdefgh	248.43 lmnop
TM9	1.80 abcd	1.31 bcdefg	3.11 bcdef	257.86 ijklmn
TM10	2.22 a	1.67 a	3.89 a	371.07 a
TM11	1.85 abcd	1.33 bcdefg	3.18 bcdef	276.73 efghi
TM12	1.94 abc	1.37 bcdef	3.31 bcde	295.60 cde
TM13	1.74 abcde	1.31 bcdefg	3.04 bcdefg	254.72 jklmn
TM14	1.83 abcd	1.33 bcdefg	3.16 bcdef	273.58 fghij
TM15	1.29 e	1.19 fg	2.48 gh	224.84 qr
SB1	2.03 abc	1.41 bcde	3.43 abcd	312.89 c
SB2	2.08 ab	1.47 bc	3.55 ab	342.77 b
SB3	1.82 abcd	1.32 bcdefg	3.15 bcdef	272.01 fghijk
SB4	1.53 cde	1.26 defg	2.78 efgh	232.70 opqr
SB5	1.40 de	1.22 efg	2.62 fgh	227.99 pqr
SB6	1.82 abcd	1.32 bcdefg	3.14 bcdef	268.87 ghijkl
SB7	1.91 abc	1.35 bcdefg	3.25 bcde	279.87 efgh
SB8	2.09 ab	1.49 b	3.58 ab	352.20 b
SB9	2.01 abc	1.40 bcdef	3.40 abcd	308.18 cd
SB10	1.81 abcd	1.31 bcdefg	3.12 bcdef	261.01 hijklm
SB11	1.92 abc	1.36 bcdefg	3.28 bcde	292.45 def
SB12	1.54 cde	1.26 defg	2.80 efgh	238.99 nopq
SB13	1.95 abc	1.39 bcdef	3.34 abcde	303.46 cd
SB14	2.06 ab	1.45 bcd	3.51 ab	339.62 b
SB15	1.79 abcd	1.31 bcdefg	3.10 bcdef	256.29 ijklmn

Note: Means with the same letter in the same column are not significantly different according to DMRT ($p \leq 0.05$).

3.4. Rice plant growth

The findings presented in Table 2 indicate that the use of *Trichoderma* led to improved root length, plant height, fresh weight, and dry weight in comparison to the uninoculated plants. Meanwhile, the *Trichoderma* treatment had no noticeable effect on the observed leaf number.

3.5. Chlorophyll content

The chlorophyll content exhibited a notable increase in the *Trichoderma*-treated plants, as shown in Table 3. The highest chlorophyll *a* content was shown in the TM10 treatment (2.22 mg/g), with an increase of 77.60 % when compared to the control. The highest chlorophyll *b* content was shown in the TM10 treatment (1.67 mg/g), followed by SB8

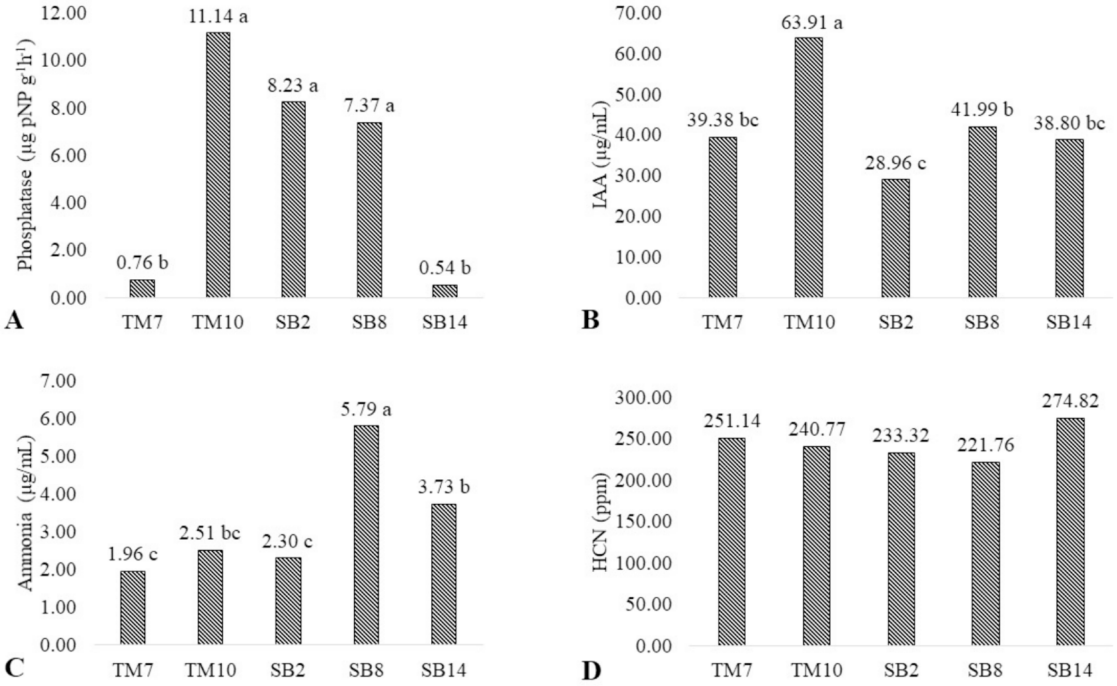

Fig. 2. Plant growth promoting traits of five *Trichoderma* strains isolated from West Java, Indonesia. Phosphatase activity (A), IAA production (B), ammonia production (C), HCN production (D).

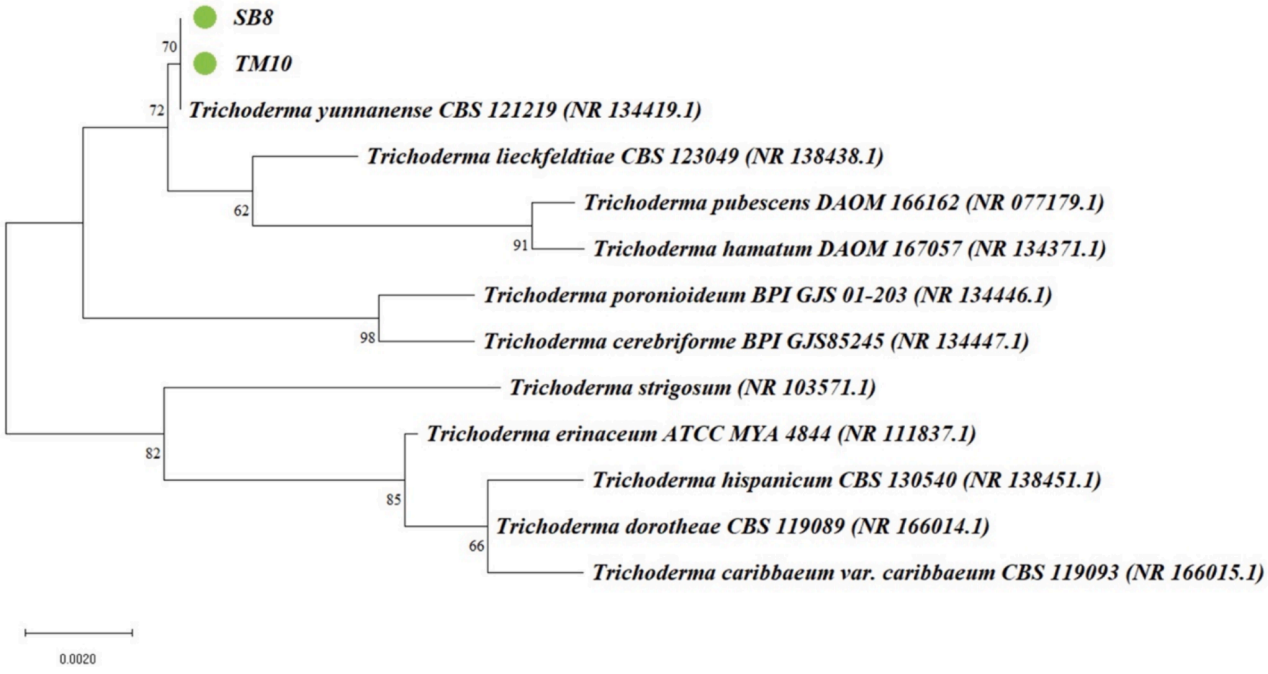

Fig. 3. The phylogenetic tree uses DNA sequences from the isolated *Trichoderma* spp. (SB8 and TM10, green circle) used for the recent study which are constructed based on the neighbor-joining method and the p-distance genetic distance model with the MEGA11 application. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(1.49 mg/g), with an increase of 42.73 % and 27.35 %.

3.6. Stomatal density

The highest stomatal density was shown in the TM10 treatment (371.07 stomata/mm^2), followed by SB8 (352.20 stomata/mm^2) with an increase of 73.53 % and 64.70 %, respectively. The results summarized in Table 3 show that *Trichoderma* treatment increases the stomatal density of rice plants.

3.7. Plant growth-promoting traits of Trichoderma

Our study recorded the phosphatase activity, IAA production, ammonia production, and HCN production abilities of five *Trichoderma*

isolates, revealing significant differences among treatments (Fig. 2).

3.8. Molecular identification and phylogenetic analysis

According to the results of BLAST analysis, both TM10 and SB8 were identified as belonging to the *T. yunnanense* species (Fig. 3). The sequences of TM10 and SB8 were then deposited in GenBank with accession numbers OR915448 and OR915449, respectively.

4. Discussion

Trichoderma-inoculated rice seeds in this study exhibited enhanced germination and vigor index, consistent with prior research (Doni et al., 2014). Phenolics, secondary metabolites, and phytohormones released by *Trichoderma* contribute to improved seed vigor (Swain et al., 2018), influenced by the strain and environmental factors (Nieto-Jacobo et al., 2017). However, some *Trichoderma* isolates in this study did not enhance germination percentage, aligning with observations of inhibitory effects reported by Santos et al. (2020).

Trichoderma has been discovered to synthesize secondary metabolites, such as harzianolide, that affect initial phases of seedling growth by increasing root and root tip length, thereby regulating general root development (Cai et al., 2013). These combined effects support the notable increase observed in root and shoot growth, as well as fresh and dry weight, in *Trichoderma*-inoculated seedlings. Such findings underscore the potential of *Trichoderma*-based bioinoculants as an environmentally friendly strategy for promoting rice seedling growth.

The enhanced growth in rice inoculated with *Trichoderma* during the late vegetative phase aligns with previous findings (Doni et al., 2017). *Trichoderma* strain TM10 exhibited high IAA production, correlating with increased root and shoot growth. The capability of *Trichoderma* to stimulate plant root growth is linked to IAA synthesis (Nieto-Jacobo et al., 2017). In seed treatments, *Trichoderma* can enhance the plants' endogenous IAA production (Backer et al., 2018). IAA released by *Trichoderma* can directly enhance root growth through cell elongation or division and may have an indirect effect on ethylene (Contreras-Cornejo et al., 2016).

Efficient phosphorus absorption facilitated by phosphate-solubilizing microorganisms is crucial for chlorophyll biosynthesis, contributing to the increased chlorophyll observed in this study, consistent with a previous study by Swain et al. (2018). The five tested isolates exhibited acid phosphatase activity, essential for converting organic P into soluble inorganic forms, similar to that reported by Gaind (2016). The phosphatase production process by *Trichoderma* is influenced by various factors, with inorganic phosphate availability playing a pivotal role (Bononi et al., 2020).

Our findings align with earlier studies that revealed ammonia production by *Trichoderma* increased fresh weight, dry weight, plant height, and root length (Wang et al., 2021). In addition, the ample supply of nitrogen and phosphorus in the media can be utilized by plants for efficient photosynthesis, ultimately generating energy for growth (Singh et al., 2017). The five isolates tested for ammonia production align with several studies that reported ammonia production by *Trichoderma* strains (Gateta et al., 2023; Thakkar and Saraf, 2015).

An increase in stomatal density was observed in rice plants subjected to *Trichoderma* inoculation. These results are in agreement with the findings of Doni et al. (2017), who reported an increase in stomatal density (69 %) in rice plants following *Trichoderma* inoculation as opposed to those that were not inoculated. The improved stomatal density observed in this study holds potential implications for regulating water loss and augmenting CO_2 absorption, which are essential for the growth of inoculated plants (Paradiso et al., 2017).

In addition, *Trichoderma*, as a biocontrol agent, demonstrates another plant growth-promoting property through the production of HCN, a gaseous volatile substance that hampers electron transport, leading to energy supply disruption and eventual cell death in targeted phytopathogens (Kolandasamy et al., 2023). Our findings showed the high production of HCN, which ranged from 221.76 ppm to 274.82 ppm, surpassing those reported by Vijayan et al. (2015), indicating that HCN production from various *Trichoderma* spp. isolates from India ranged from 0.8 ppm to 180.6 ppm. This holds significant implications for agricultural practices, offering a promising avenue for improving crop productivity and sustainability.

5. Conclusions

Trichoderma isolates significantly enhance rice growth and germination through mechanisms like IAA production, phosphate solubilization, ammonia production, and HCN regulation. The TM10 strain, in particular, showed superior results in improving rice growth and physiological traits of rice plants. For future studies, it is essential to investigate and compare different *Trichoderma* isolates, with a specific focus on discovering and characterizing secondary metabolites and proteins that may act as stimuli for plant defense systems. This comparative investigation of *Trichoderma* isolates will provide a deeper understanding of their potential use.

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CRediT authorship contribution statement

Sulistya Ika Akbari: Formal analysis, Methodology, Writing – original draft. **Dedat Prismantoro:** Data curation, Writing – review & editing. **Joko Kusmoro:** Supervision, Writing – review & editing. **Rusdi Hasan:** Supervision, Writing – review & editing. **Mohamad Nurzaman:** Supervision, Writing – review & editing. **Nia Rossiana:** Supervision, Validation, Writing – review & editing. **Febri Doni:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2024.103378.

References

Abd-Alhamid, Hassan, H.S.A., Haggag, L.F., Hassan, A.M., 2015. Effect of mineral and bio-fertilization on vegetative growth, leaf mineral contents and flowering of manzanillo olive trees. Int. J. Chem. Technol. Res. 8, 51–61.

Abdullah, N.S., Doni, F., Mispan, M.S., Saiman, M.Z., Yusuf, Y.M., Oke, M.A., Mohd Suhaimi, N.S., 2021. Harnessing *Trichoderma* in agriculture for productivity and sustainability. Agronomy 11, 1–17. https://doi.org/10.3390/agronomy11122559.

Akbari, S.I., Prismantoro, D., Permadi, N., Rossiana, N., Miranti, M., Mispan, M.S., Mohamed, Z., Doni, F., 2024. Bioprospecting the roles of *Trichoderma* in alleviating plants' drought tolerance: Principles, mechanisms of action, and prospects. Microbiol. Res. 283, 127665 https://doi.org/10.1016/j.micres.2024.127665.

Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., Smith, D.L., 2018. Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for

sustainable agriculture. Front. Plant Sci. 871, 1–17. https://doi.org/10.3389/fpls.2018.01473.
Bononi, L., Chiaramonte, J.B., Pansa, C.C., Moitinho, M.A., Melo, I.S., 2020. Phosphorus-solubilizing *Trichoderma* spp. from Amazon soils improve soybean plant growth. Sci. Rep. 10, 1–14. https://doi.org/10.1038/s41598-020-59793-8.
Cai, F., Yu, G., Wang, P., Wei, Z., Fu, L., Shen, Q., Chen, W., 2013. Harzianolide, a novel plant growth regulator and systemic resistance elicitor from *Trichoderma harzianum*. Plant Physiol. Biochem. 73, 1–8. https://doi.org/10.1016/j.plaphy.2013.08.011.
Contreras-Cornejo, H.A., Macías-Rodríguez, L., Del-Val, E., Larsen, J., 2016. Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: Interactions with plants. FEMS Microbiol. Ecol. 92, 1–17. https://doi.org/10.1093/femsec/fiw036.
Doni, F., Isahak, A., Che Mohd Zain, C.R., Mohd Ariffin, S., Wan Mohamad, W.N., Wan Yusoff, W.M., 2014. Formulation of *Trichoderma* sp. SL2 inoculants using different carriers for soil treatment in rice seedling growth. Springerplus 3, 1–5. https://doi.org/10.1186/2193-1801-3-532.
Doni, F., Zain, C.R.C.M., Isahak, A., Fathurrahman, F., Sulaiman, N., Uphoff, N., Yusoff, W.M.W., 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. https://doi.org/10.1007/s13199-016-0438-3.
FAO, 2021. In: World Food and Agriculture, Statistical Yearbook 2021. FAO, Rome. https://doi.org/10.1016/S0140-6736(59)91820-3.
Gaind, S., 2016. Phosphate dissolving fungi: Mechanism and application in alleviation of salt stress in wheat. Microbiol. Res. 193, 94–102. https://doi.org/10.1016/j.micres.2016.09.005.
Gajera, H.P., Hirpara, D.G., Katakpara, Z.A., Patel, S.V., Golakiya, B.A., 2016. Molecular evolution and phylogenetic analysis of biocontrol genes acquired from SCoT polymorphism of mycoparasitic *Trichoderma koningii* inhibiting phytopathogen *Rhizoctonia solani* Kuhn. Infect. Genet. Evol. 45, 383–392. https://doi.org/10.1016/J.MEEGID.2016.09.026.
Gateta, T., Nacoon, S., Seemakram, W., Ekprasert, J., Theerakulpisut, P., Sanitchon, J., Suwannarach, N., Boonlue, S., 2023. The potential of endophytic fungi for enhancing the growth and accumulation of phenolic compounds and anthocyanin in maled phai rice (*Oryza sativa* L.). J. Fungi 9, 1–19. https://doi.org/10.3390/jof9090937.
Huang, M., Zeng, L., Liu, C., Li, X., Wang, H., 2022. Research on the eco-efficiency of rice production and its improvement path: A case study from China. Int. J. Environ. Res. Public Health 19, 1–20. https://doi.org/10.3390/IJERPH19148645.
Kaviyarasu, K., Manikandan, E., Kennedy, J., Jayachandran, M., Maaza, M., 2016. Rice husks as a sustainable source of high quality nanostructured silica for high performance Li-ion battery requital by sol-gel method - a review. Adv. Mater. Lett. 7, 684–696. https://doi.org/10.5185/amlett.2016.6192.
Khadka, R.B., Uphoff, N., 2019. Effects of *Trichoderma* seedling treatment with system of rice intensification management and with conventional management of transplanted rice. PeerJ 2019, 1–22. https://doi.org/10.7717/peerj.5877.
Kolandasamy, M., Mandal, A.K.A., Balasubramanian, M.G., Ponnusamy, P., 2023. Multifaceted plant growth-promoting traits of indigenous rhizospheric microbes against *Phomopsis theae*, a causal agent of stem canker in tea plants. World J. Microbiol. Biotechnol. 39, 1–20. https://doi.org/10.1007/s11274-023-03688-z.
Magdalane, C.M., Kaviyarasu, K., Judith Vijaya, J., Jayakumar, C., Maaza, M., Jeyaraj, B., 2017. Photocatalytic degradation effect of malachite green and catalytic hydrogenation by UV–illuminated CeO_2/CdO multilayered nanoplatelet arrays: Investigation of antifungal and antimicrobial activities. J. Photochem. Photobiol. B Biol. 169, 110–123. https://doi.org/10.1016/j.jphotobiol.2017.03.008.
Meena, R.S., Kumar, S., Datta, R., Lal, R., Vijayakumar, V., Brtnicky, M., Sharma, M.P., Yadav, G.S., Jhariya, M.K., Jangir, C.K., Pathan, S.I., Dokulilova, T., Pecina, V., Marfo, T.D., 2020. Impact of agrochemicals on soil microbiota and management: A review. Land 9, 1–21. https://doi.org/10.3390/LAND9020034.
Nieto-Jacobo, M.F., Steyaert, J.M., Salazar-Badillo, F.B., Vi Nguyen, D., Rostás, M., Braithwaite, M., De Souza, J.T., Jimenez-Bremont, J.F., Ohkura, M., Stewart, A., Mendoza-Mendoza, A., 2017. Environmental growth conditions of *Trichoderma* spp. affects indole acetic acid derivatives, volatile organic compounds, and plant growth promotion. Front. Plant Sci. 8, 1–18. https://doi.org/10.3389/FPLS.2017.00102.
Pang, G., Cai, F., Li, R., Zhao, Z., Li, R., Gu, X., Shen, Q., Chen, W., 2017. *Trichoderma*-enriched organic fertilizer can mitigate microbiome degeneration of monocropped soil to maintain better plant growth. Plant Soil 416, 181–192. https://doi.org/10.1007/s11104-017-3178-0.
Panuju, D.R., Mizuno, K., Trisasongko, B.H., 2013. The dynamics of rice production in Indonesia 1961–2009. J. Saudi Soc. Agric. Sci. 12, 27–37. https://doi.org/10.1016/J.JSSAS.2012.05.002.
Paradiso, R., Arena, C., De Micco, V., Giordano, M., Aronne, G., De Pascale, S., 2017. Changes in leaf anatomical traits enhanced photosynthetic activity of soybean grown in hydroponics with plant growth-promoting microorganisms. Front. Plant Sci. 8, 1–13. https://doi.org/10.3389/fpls.2017.00674.
Primavesi, O., Harman, G.E., Uphoff, N., Doni, F., 2023. The plant–soil microbiome: an overview. In: Uphoff, N., Thies, J. (Eds.), Biological Approaches to Regenerative Soil Systems. CRC Press, Boca Raton, Florida, USA, pp. 99–108.
Rachman, B., Ariningsih, E., Sudaryanto, T., Ariani, M., Septanti, K.S., Adawiyah, C.R., Ashari, Agustian, A., Saliem, H.P., Tarigan, H., Syahyuti, Yuniarti, E., 2022. Sustainability status, sensitive and key factors for increasing rice production: A case study in West Java, Indonesia. PLoS One 17, 1–19. https://doi.org/10.1371/JOURNAL.PONE.0274689.
Rawal, R., Scheerens, J.C., Fenstemaker, S.M., Francis, D.M., Miller, S.A., Benitez, M.S., 2022. Novel *Trichoderma* isolates alleviate water deficit stress in susceptible tomato genotypes. Front. Plant Sci. 13, 1–16. https://doi.org/10.3389/fpls.2022.869090.
Rifin, A., 2022. Marketed surplus of Indonesian rice production. Cogent Econ. Financ. 10, 1–14. https://doi.org/10.1080/23322039.2022.2119694.
Rozaki, Z., 2021. Food security challenges and opportunities in indonesia post COVID-19, pp. 119–168. In: Cohen, M.J. (Ed.), Advances in Food Security and Sustainability. Elsevier Inc., Washington DC https://doi.org/10.1016/bs.af2s.2021.07.002.
Santos, M.F.D., Santos, L.E.D., da Costa, D.L., Vieira, T.A., Lustosa, D.C., 2020. *Trichoderma* spp. on treatment of *Handroanthus serratifolius* seeds: Effect on seedling germination and development. Heliyon 6, 1–8. https://doi.org/10.1016/j.heliyon.2020.e04044.
Sharma, S., Kour, D., Rana, K.L., Dhiman, A., Thakur, Shiwani, Thakur, P., Thakur, Sapna, Thakur, N., Sudheer, S., Yadav, N., Yadav, A.N., Rastegari, A.A., Singh, K., 2019. *Trichoderma*: Biodiversity, Ecological Significances, and Industrial Applications. In: Recent Advancement in White Biotechnology Through Fungi. Switzerland: Springer Nature, pp. 85–120. doi: 10.1007/978-3-030-10480-1.
Singh, D., Dubey, A.K., Kumar, Y., Rajput, P.S., Chandraker, S.K., Verma, A.K., 2017. Effect of npk on plant growth, yield and quality of capsicum (*Capsicum annum* L.) c.v. Swarna under shade net condition. Int. J. Curr. Microbiol. Appl. Sci. 6, 1007–1013. https://doi.org/10.20546/ijcmas.2017.604.125.
Statistics Indonesia, 2022. Harvest Area and Rice Production in Indonesia, 2022. Official Statistics News, Statistics Indonesia, Jakarta.
Swain, H., Adak, T., Mukherjee, A.K., Mukherjee, P.K., Bhattacharyya, P., Behera, S., Bagchi, T.B., Patro, R., Shasmita, Khandual, A., Bag, M.K., Dangar, T.K., Lenka, S., Jena, M., 2018. Novel *Trichoderma* strains. isolated from tree barks as potential biocontrol agents and biofertilizers for direct seeded rice. Microbiol. Res. 214, 83–90. https://doi.org/10.1016/j.micres.2018.05.015.
Tegene, S., Dejene, M., Terefe, H., Tegegn, G., Tena, E., Ayalew, A., 2021. Evaluation of native *Trichoderma* isolates for the management of sugarcane smut (*Ustilago scitaminea*) in sugar plantations of Ethiopia. Cogent Food Agric. 7, 1–21. https://doi.org/10.1080/23311932.2021.1872853.
Thakkar, A., Saraf, M., 2015. Development of microbial consortia as a biocontrol agent for effective management of fungal diseases in *Glycine max* L. Arch. Phytopathol. Plant Prot. 48, 459–474. https://doi.org/10.1080/03235408.2014.893638.
Valsalam, S., Agastian, P., Arasu, M.V., Al-Dhabi, N.A., Ghilan, A.K.M., Kaviyarasu, K., Ravindran, B., Chang, S.W., Arokiyaraj, S., 2019. Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of *Tropaeolum majus* L. and its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties. J. Photochem. Photobiol. B Biol. 191, 65–74. https://doi.org/10.1016/j.jphotobiol.2018.12.010.
van Jaarsveld, W.J., Halleen, F., Bester, M.C., Pierron, R.J.G., Stempien, E., Mostert, L., 2021. Investigation of *Trichoderma* species colonization of nursery grapevines for improved management of black foot disease. Pest Manag. Sci. 77, 397–405. https://doi.org/10.1002/PS.6030.
Vijayan, A.K., Nair, R.B., Rajendran, R., Francis, S.M., Nair, A.S., Thomas, J., 2015. Evaluation of native *Trichoderma* spp. against pathogens infecting small cardamom. J. Plant. Crop. 43, 35–39.
Wang, H., Zhang, R., Duan, Y., Jiang, W., Chen, X., Shen, X., Yin, C., Mao, Z., 2021. The endophytic strain *Trichoderma asperellum* 6s–2: An efficient biocontrol agent against apple replant disease in china and a potential plant-growth-promoting fungus. J. Fungi 7, 1–27. https://doi.org/10.3390/jof7121050.