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Research Article

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Posted Date: August 19th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10346068/v1

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Additional Declarations: No competing interests reported.

Diversity, distribution, and abundance of native *Trichoderma* species in selected irrigated lowland rice agroecosystems of Tanzania

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Abstract

Aim: The genus *Trichoderma* is recognized for its potential biocontrol, plant biostimulant, and biofertilizer abilities. This study assessed the diversity and ecological distribution of native *Trichoderma* species isolated from lowland irrigated rice agroecosystems within six agroecological zones of Tanzania. **Methodology and Results**: Isolates were obtained from rhizospheric soils using a serial dilution technique. The isolates were assessed for colony color, growth pattern, conidiophore characteristics, phialide, and conidial morphology. DNA was extracted using the CTAB method, and PCR amplification was performed using ITS1F (5'-TCCGTAGGTGAACCTGCGG-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′). The sequences were compared in the BLASTn program in the National Center for Biotechnology Information (NCBI). Phylogenetic trees provided species resolution, with four main clades: *Harzianum* (*T. harzianum and T. pleuroti*); *Longibrachiatum* (*T. longibrachiatum*); *Virens* (*T. virens*); and *Viridae* (*T. atroviride, T. asperellum, T. erinaceum, T. koningiopsis,* and *T. paraviridescens*). The coastal zone had the highest species diversity and evenness (H'=1.21, J=0.88), while the Southern and western highlands zone was completely dominated by a single species (H'=0, J=0). High genetic similarity of 100% was in the Southern and western highlands zone,

and the greatest genetic differentiation between the semi-arid and Central plateaux zones was 93%. Soil texture (Clay, Sandy clay to Sandy clay loam), pH (3.36 to 7.31), organic matter (1.07 to 3.18 %), and electrical conductivity (2.5 to 4.58 ds/m) influenced the assemblage of the *Trichoderma* community within the zones. **Conclusion, significance and impact of study:** The findings highlight the importance of local environmental conditions in shaping microbial communities and suggest that native *Trichoderma* isolates could be exploited as biocontrol agents.

Keywords: rice agroecosystems**,** *Trichoderma*, diversity, distribution, characterization

1.0 INTRODUCTION

Trichoderma is a fungus belonging to the family Hypocreaceae within the Ascomycete division. The members of this genus are rhizospheric filamentous fungi that dominate various temperate and tropical soil ecosystems (Rebolledo-Prudencio *et al.,* 2020). Their rapid growth, high stress tolerance capacities, diverse metabolic capabilities, and highly competitive nature make them successful colonizers of various habitats, including wide range soils, decayed wood, and aquatic habitats (Rebolledo-Prudencio *et al.,* 2020). They are also known to be aggressive against several phytopathogens, thus used as biocontrol agents (Hashim, 2020; Saharan *et al.,* 2023).

Their effectiveness is due to the production of several metabolites with antimicrobial properties (cell wall degrading enzymes, antibiotics, volatile, and non-volatile compounds) and biostimulating properties (phytohormones, phytoregulators) (Misra and Ansari, 2021)

Trichoderma species produce bioactive substances that have antagonistic effects on plant-pathogenic fungi, bacteria and plant-pathogenic nematodes (Druzhinina et al*.,* 2018). Species such as *T. harzianum, T. hamatum, T. longibrachiatum, T. koningii, T. viride, T. polysporum,* and *T. asperellum* have been widely used as biological control agents of several diseases, including cotton verticillium wilt, crop

gray mold, tomato gray mold, melon wilt, potato dry rot, tobacco root rot, and others (Alfiky and Weisskopf, 2021; Lazazzara *et al.,* 2021; Pollard-Flamand *et al.,* 2022; Rees *et al.,* 2022).

The ability of *Trichoderma* to produce metabolites and biostimulating compounds enables them to utilize different mechanisms for phytopathogens' growth inhibition. These include, competition for nutrients and space, plant resistance, antibiosis, and parasitism (Druzhinina *et al.,* 2018; Villar *et al*., 2022; Walia *et al*., 2021). Antibiosis and mycoparasitism normally occur in synergy, whereby the target fungus secretes a chemical stimulus (Oligochottin) that attracts an antagonist fungus to recognize the host cell surface (Villar *et al*., 2022).

After attacking, *Trichoderma* uses hydrophobin-like proteins to produce haustoria that penetrate the pathogen cell surface, grow its hyphae along the host's hyphae, and finally release specific lytic enzymes (β-glucanase, chitinase, and protease), leading to degradation of the cell membrane or cell wall. Upon entering the pathogen cell, *Trichoderma* utilizes nutrients from the host fungi and produces antibiotics that affect the host’s hyphae and inhibit resynthesize of the cell wall, leading to death (Druzhinina *et al.,* 2018; Villar *et al*., 2022).

As biostimulants and biofertilizers, *Trichoderma* promote plant growth and induce tolerance to biotic and abiotic stresses by producing growth hormones, enzymes, and metabolites that improve nutrient availability and uptake (Nofal *et al.,* 2021). Phytohormones such as indole-3-acetic acid (IAA), gibberellins, auxin, and cytokinins regulate plant growth and development in plants. Enzymes such as phosphatases and phytases solubilize iron, phosphorus, and other nutrients, making them more accessible to plant roots and increasing nutrient assimilation, especially under low-fertility conditions (Hernández-Huerta *et al*., 2025). Secondary Metabolites, such as harzianolide, and antioxidants have been found to induce systemic plant resistance and reduce oxidative stress (Villar *et al.,* 2022).

Trichoderma is diverse, with over 340 known species, and a few species and strains are effective in agricultural production (Peixoto *et al.,* 2024). Understanding this diversity supports accurate identification and selection of species and/or strains for controlling plant pathogens and promoting plant growth. This is because species and strains vary widely in their antagonistic capacities (metabolic profiles, modes of action) and in their ability to adapt to local environmental conditions.

Studies on *Trichoderma* species diversity have mainly been conducted in Asia, Europe, and America (Peixoto et al., 2024). In Africa, particularly in Tanzania, information on the availability, ecological abundance, and diversity of native *Trichoderma* species is limited. Thus, the objective of the present study was to evaluate the taxonomic diversity and population dynamics of native isolates of *Trichoderma* species recovered from irrigated lowland rice agroecosystems using morphological and molecular tools.

2.0 MATERIALS AND METHODS

2.1 SELECTION OF SAMPLING SITES

Soil samples were obtained from six agroecological zones of Tanzania between July and December 2023; each zone was represented by one irrigated lowland rice agroecosystem. A purposive sampling method was used to select the sampling sites based on their potential for rice production and a long history (minimum of 10 years) of rice cultivation. A total of six lowland rice irrigation scheme were selected: Kitere irrigation scheme in the Coastal zone, Bahi irrigation scheme in the Central plateaux zone; Kahangara irrigation scheme in the Semi-arid zone; Makwale irrigation scheme in the Southern and western highlands zone; Lower Moshi irrigation scheme in the Northern highlands zone; and Msolwa irrigation scheme in the Alluvial zone

(**Figure1**).

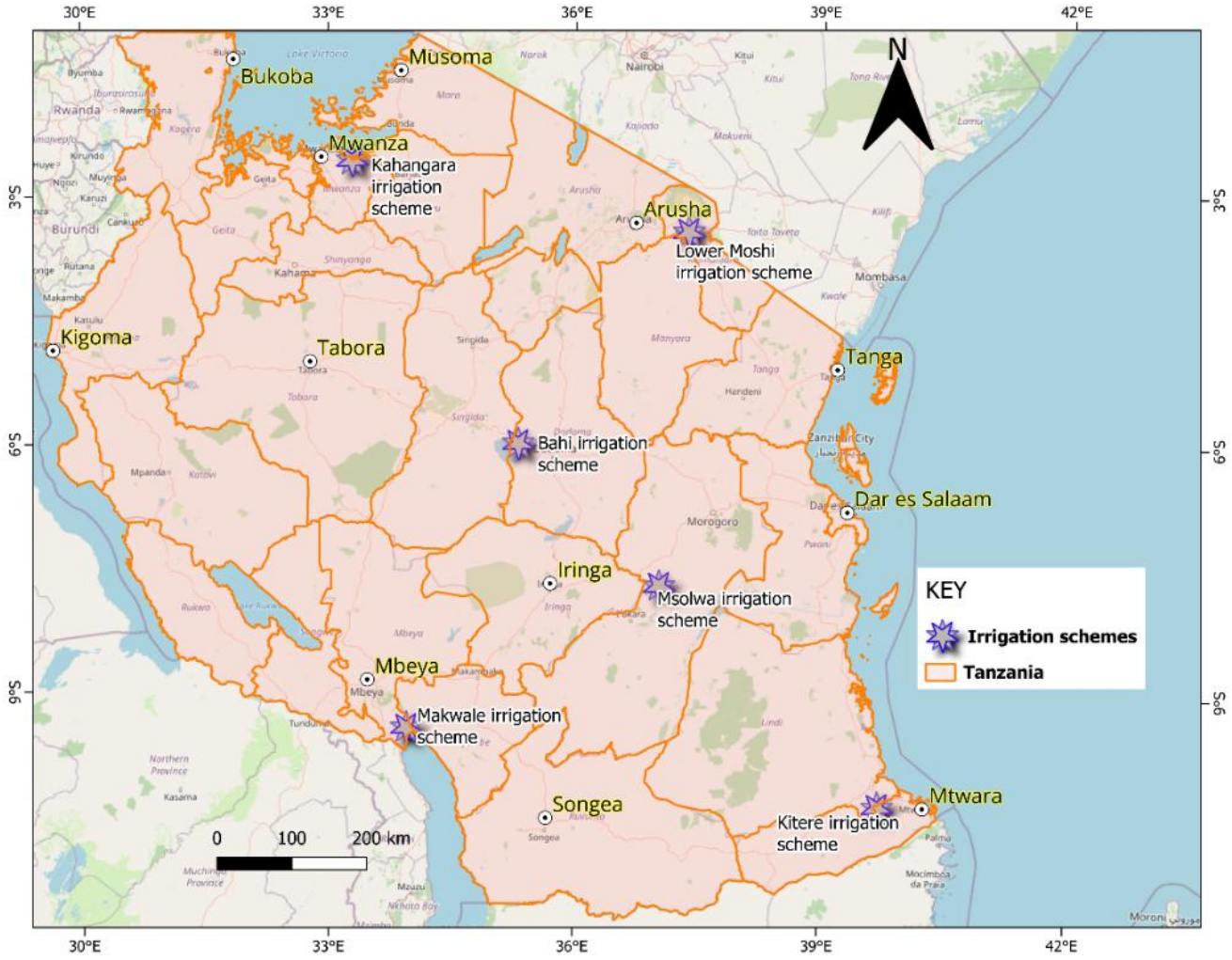


Figure 1. Map showing the soil sampling locations in six irrigated lowland rice agroecological zones of Tanzania. Map was generated in QGIS (version 3.0)

2.2 DESCRIPTION OF SAMPLING SITES

The identification of the sampling zones was based on the Agroecological zones of Tanzania document by the Ministry of Agriculture (http://wwww.kilimo.go.tz) accessed on 30th July, 2023. The Kahangara irrigation scheme is found in Magu district and lies within the Semi-arid agroecological zones. It is found between Latitude 2.6° to 3.0° South and Longitude 33.0° to 33.5° East with an Altitude ranging from 1,100 to 1,300 meters above sea level (Masuki and Mbogoni, 2016). The scheme covers approximately 2,288 hectares of low-lying plains characterized by moderate rainfall (500-700 mm) and sandy loam soils (Magu District Profile, 2022).

The Bahi irrigation scheme is found in the Central Plateaux as and is located in Bahi District. The scheme lies approximately between latitudes 5.0° and 5.5° South and longitudes 35.7° to 36.1° East, on the Dodoma plateau (Bahi Socio-economic profile, 2008). It covers an area of 1,200 hectares, and it supports small to medium-scale irrigation farmers focused on crops such as rice, maize, and pulses. The scheme is located at an elevation of 900 to 1,150 meters above sea level. Soils are black cotton (Vertisols) with high clay content (Bahi Socio-economic profile, 2008). The region experiences a mean annual temperature ranging from 21°C to 26°C and rainfall ranging from 500 mm to 700 mm. During soil sampling time, the area showed typical dry-season conditions.

Msolwa irrigation scheme in Kilombero district represented the Alluvial zone. The scheme is found between the Kilombero River in the south-east and the Udzungwa Mountains in the north-west, dominated by alluvial soils, predominantly sandy clay and sand clay loam (Kalala *et al.,* 2017). The scheme covers approximately 70 to 100 hectares of irrigated land. It lies between latitudes 8.5° to 8.7° South, longitudes 36.5° to 36.7° East, and altitudes 300 to 350 meters above sea level. The mean annual rainfall in the valley is 1200- 1400 mm, and the average minimum and maximum temperatures are 20.5- 31.6 °C. During soil sampling, weather conditions were characterized by daytime temperatures ranging from 20 to 24 °C, with no

rainfall, indicating a dry season in this region (Kilombero Sugar Company Limited weather data).

Kitere irrigation scheme in Mtwara district represented the Coastal zone. The scheme is located at 10°20'28" South and 39°41'33" East. It has a tropical humid climate with moderate to high rainfall, typically ranging between 800 and 1,900 mm annually (Kalala *et al*., 2017). The soil texture is predominantly clay, and the average temperatures generally range from about 25 to 30.5°C. Soil was sampled during the dry season.

Makwale irrigation scheme in Kyela district represented the Southern and Western highlands zone. The scheme covers 100 hectares and is found within a latitude of 6.1° and 6.5° South and a longitude of 35.0° and 35.5° East. The scheme is located between the altitudes of 400 and 1200 meters above sea level and is dominated by sandy clay loam (Ngailo *et al.,* 2017). The mean annual temperature of the Makwale irrigation scheme ranges between 20 and 26°C**.** Soil was sampled during the dry season.

Lower Moshi irrigation scheme in Moshi district represented the Northern highlands zone. The scheme covers about 1,100 hectares of irrigated land and is found within 3.40° South, 37.36° East at an elevation of 775 meters above sea level. The average annual temperature is 18.0°C- 30.7°C (Bell and Kimaro**,** 2015). The soils in this zone are often fertile volcanic soils mixed with sandy loam and clay. The soil sample was collected during the dry season.

2.3 SOIL SAMPLING

Before soil sampling, a research permit was provided by the Sokoine University of Agriculture and endorsed by the President's Office Regional Administration and Local Government (PO-RALG). Upon soil collection, village and ward leaders were involved. Farmer's consent was requested.

From each site, twelve (12) acres from each lowland rice agroecosystem were selected for soil sampling. A zone-based soil sampling method was used, in which each sampling site was divided into distinct zones to accommodate soil variabilities (Fleming *et al.,* 2000). This method was selected since it is suitable for the area between 2–15 acres (Fleming et al., 2000). An area of 12 acres was subdivided into subzones of 1 acre. In order to capture field diversity, a systematic grid sampling technique was used, in which a 15 m × 15 m plots were established while avoiding the boundaries. At each grid intersection point, circular sampling units with an 8 m diameter were established. Within each circular unit, sampling positions were taken using a zig-zag approach to ensure spatial representation within the circle at a depth of 10–30 cm using a soil auger.

Soil samples from all subsampling points within each circle were pooled together using the quartering method, and a 1 kg composite sample was created for each circular unit. A total of 72 samples were collected from the selected agroecological zones, stored in sterile zipped plastic bags, sent to the Plant Pathology Laboratory at Sokoine University of Agriculture (SUA), Tanzania, and stored at 4°C**.**

2.4 ANALYSIS OF SOIL PHYSICOCHEMICAL PROPERTIES

Soil texture, electric conductivity (EC), pH, organic matter content and texture were determined. For soil electrical conductivity (EC), pH, and organic matter contents (OM), soil samples were dried at room temperature and processed according to the protocol developed by Ehyaie and Behbahanizade (1993). The 500 mg of soil samples were passed through a 2 mm sieve, and sterile distilled water was added to prepare a saturated paste, stirred briefly and allowed to equilibrate for 24 h at room temperature.

For the soil pH, a ratio of 1:1 (w/v) soil-to-water was prepared. Saturated soil sample was measured using a digital pH meter (744 Metrohm) to obtain the pH for soil samples. The electrical conductivity (EC) of each saturated paste extract was measured using a digital EC/TDS analyzer (WTW 3310 model, Germany). Soil

organic carbon was determined by oxidation method (Nelson and Sommer, 1982) and soil texture by the Hydrometer method (Bouyoucos, 1962).

2.5 ISOLATION OF *TRICHODERMA*

Trichoderma isolates were obtained using serial dilution technique on rose Bengal agar (RBA) as described by Chaitanya *et al.* (2018). Briefly, 20 g of dried and ground soil sample was dissolved in 100 mL of distilled water and put on an orbital shaker for 20 min. Serial dilution was prepared by diluting 1 ml of the stock sample into 9 mL of distilled water to obtain dilutions 10^1 to 10^4. Alquito (1 mL) from each dilution was pipetted on petri dishes (90 mm) containing rose Bengal agar incubated at 28^{o}C for 5-7 days.

Culture plates were examined daily for fungal colony growth. The suspected *Trichoderma* colonies was sub cultured onto a fresh Potato Dextrose Agar (PDA) plate and incubated at 28^{o}C for 7 days to obtain pure culture for morphological identification. Distinct morphological characteristics were observed for identification, and the isolates were inoculated in PDA slants in Bijou bottles and stored at 4 °C.

2.6 MORPHOLOGICAL CHARACTERIZATION OF *TRICHODERMA* ISOLATES

Morphological characterization of *Trichoderma* species were carried out by observing both macroscopic and microscopic features of the fungal colonies. For macroscopic features, growth rate, colors of the colonies and conidiation patterns were examined as per Gezgin *et al.,* (2023). Briefly, a 5 mm mycelia plug from the colon margin of active growing mycelia was cut using a cork borer before starting to produce conidia and inoculated at the center of the PDA plate. The inoculated agar plates were incubated at 28 ^{o}C for 7 days.

Culture was observed daily for observation of color and conidiation pattern. For colony growth rate, a 5 mm mycelial plug from the margin of growing fungal colonies was cut and placed at the center of a 90 mm PDA plate in triplicate. The culture plates were then incubated in triplicate at 28 ^{o}C for 72 h. Average colony diameter (mm) was recorded.

For microscopic features, slides were prepared, and mycelia from each isolate were taken from a PDA plate and spread onto a clean slide mounted with a drop of stain (lactophenol cotton blue), covered with a cover slip, and then observed under a light microscope (Leica) using 40X magnification. Morphological characterization was conducted using qualitative traits, including Conidiophores' branching patterns, shape of phialides, presence of chlamydospores and intercalary phialides.

A total of 105 *Trichoderma i*solates suspects were initially obtained and after detailed morphological characterization, only 67 distinct isolates were found to represent separate morphological groups. Observed morphological features were compared with the verification keys provided by Bissett (1984, 1991a, 1991b), Rifai (1969), and Samuels *et al.* (2004). The differences between them were termed as character states, and each character state was assigned a rank using a one-hot encoding method for use in morphological analysis and clustering.

2.6 MOLECULAR CHARACTERIZATION OF *TRICHODERMA* ISOLATES

2.6.1 GENOMIC DNA EXTRACTION

Genomic DNA from selected *Trichoderma* isolates was extracted using the cetyltrimethylammonium bromide (CTAB) method described by Ramachandran *et al.* (2022), with slight modifications. Pure cultures of each isolate were grown on potato dextrose agar (PDA) plates (20 mL medium in 9-cm Petri dishes) and incubated at 28°C in the dark until the mycelium completely covered the plate surface.

Approximately 50–100 mg of fresh mycelial biomass was harvested by scraping the fungal mat from the agar surface and washed three times with sterile distilled water. The mycelia were frozen in liquid nitrogen and ground into a fine powder using a pre-chilled mortar and pestle. The powdered mycelium was transferred into sterile microcentrifuge tubes and incubated at −20°C for 1 h.

A volume of 700 µL pre-warmed CTAB extraction buffer (1.4 M NaCl, 50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 2 % CTAB, and 1% β-mercaptoethanol) was added to each sample. The mixture was vortexed thoroughly and incubated at 65°C for 30 min in a water bath with occasional mixing.

Following incubation, 700 µL chloroform: isoamyl alcohol (24:1, v/v) was added to each tube. The mixture was vortexed vigorously until emulsified and centrifuged at 12,000 rpm for 15 min. The aqueous phase (approximately 600 µL) was carefully transferred into a new sterile microcentrifuge tube. To remove RNA contamination, 5 µL RNase A (10 mg mL^{-1}) was added, mixed gently, and incubated at 37°C for 1 h.

DNA was precipitated by adding cold isopropanol (420 µL), followed by gentle inversion of the tubes. Samples were incubated on ice for 30–45 min and centrifuged at 12,000 rpm for 5 min to pellet the DNA. The resulting DNA pellet was washed with 70 % ethanol, centrifuged again, and air-dried at room temperature.

The dried DNA pellet was resuspended in 50 µL Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM EDTA) and stored at −20°C until further use. DNA concentration was measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific) at 260 nm (A260), and DNA purity was evaluated using the A260/A280 absorbance ratio. Samples with A260/A280 ratios between 1.8 and 2.0 were considered sufficiently pure for downstream molecular analyses

2.6.2 PCR AMPLIFICATION OF THE DNA

ITS1F (5'-TCCGTAGGTGAACCTGCGG-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′) universal primers that amplifies 600bp (Hoggard *et al.,* 2018) were used to amplify the entire ITS (ITS1-5.8S-ITS2) region of *Trichoderma* rDNA. PCR amplification of *Trichoderma* isolates was performed using conventional PCR (thermocycler model) in a final reaction volume of 50 µl. The reaction mixture included 5 µl of diluted (1:10) DNA extract containing 50 ng/µl of template of genomic DNA), 0.2 µM of each primer, 0.2 mM dNTPs, 2.5 mM $MgCl_2$,

PCR reaction buffer (10 mM Tris-HCl, 50 mM KCl; pH 9.0), and 1.25 units of DNA polymerase.

The PCR conditions were as follows: an initial denaturation of 94 °C for 1 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 54.2 °C for 45 s, extension at 68 °C for 1 min, and final elongation at 68 °C for 6 min. The PCR products were analyzed by gel electrophoresis in a 1% agarose gel in 0.5 TAE buffer (Tris Acetate _EDTA), stained with ethidium bromide using 1Kb DNA ladder marker at 120 volts for 90 min and were visualized in a UV transilluminator (Uvitec- BXT-26.M).

2.6.3 PHYLOGENETIC INFERENCES

The sequenced products of 67 *Trichoderma* isolates were assembled and edited using bioinformatics software. The quality of the nucleotide sequences was checked using Sequence Scanner software version 2, and only 48 *Trichoderma* isolates' sequences had high quality for further analysis. Consensus Sequences of forward and reverse amplicons were generated using Bio Edit software version 7.2 and analyzed for homologies to the known sequences using the BLASTn program in the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST) as per Kumar *et al.* (2024).

A similarity of ≥ 97 % and an E-value of (0.0) was used as a threshold to select the homologous generated by BLASTn. Phylogenetic inferences were performed using GenBank sequences, 48 query *Trichoderma* sequences and Protocrea *farinose* and *Protocrea pallida* as outgroup *(*GenBank access numbers: MH863119 and EU703924, respectively) (Sudhir *et al.,*2024) Sequences were aligned in MEGA (Molecular Evolutionary Genetics Analysis) software version 12, and multiple sequence alignment was performed by using the MUSCLE program.

All ambiguous positions were removed for each sequence pair (pairwise deletion option). The evolutionary distances were computed using the maximum composite likelihood method, and the phylogenetic tree was built based on the maximum

likelihood algorithm using the Kimura-2-parameter distance model (Kumar *et al.,* 2024; Tamura *et al.,* 2022). Confidence values were assessed from 1000 bootstrap replicates of the original data. The phylogenetic tree was edited by using the online tool ITOL (Version 4) (Letunic and Bork. 2016)

2.7 DETERMINATION OF *TRICHODERMA* SPP. ABUNDANCE AND DIVERSITY

Isolation frequency (IF) was calculated to determine the common *Trichoderma* species in soil samples, and fungal relative density (RD) was calculated to determine *Trichoderma* species abundance within the fungal community. These parameters were determined by using equations 1 and 2 as described by González *et al.* (2008).

$$IF\ (\%) = \frac{ns}{N} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$RD\ (\%) = \frac{ni}{Ni} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Whereby: n_s is the number of soil samples where the respective spp. of fungus occurs; N is the total number of soil samples; n_i is the number of Trichoderma species in a given sample of soil; N_i is the total number of fungal isolates obtained in soil samples

Species diversity was quantified using Simpson's Diversity index (1-D) (Simpson, 1949) and Shannon's biodiversity index (H) (Shannon and Weaver, 1949), while species dominance was determined by Simpson's dominance index (D) (Simpson, 1949). Pielou's species evenness index (E) (Pielou, 1966) was used to determine the species evenness within and between the agroecological zones. Generally, these indices were applied to describe the diversity and habitat preferences of *Trichoderma* species quantitatively.

$$Shannon\ index\ (H) = -\sum_{i=1}^{s} pi \ln pi \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

$$Simpson\ index\ (D) = 1 - \frac{\sum ni(ni-1)}{N(N-1)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

$$E = H/\ln S \qquad \text{.....} \quad (5)$$

Where p_i is the proportion of the entire community made up of species i; ln is the natural logarithm, 'N' is the total number of individuals of all species; 'S' is species richness; 'E' is Pielou's species evenness index; and n_i is the number of individuals of each species.

3.0 STATISTICAL ANALYSIS

All analysis was conducted using RStudio software (Version 4.4.1), where by descriptive statistics were used to assess the relative density and isolation frequencies of *Trichoderma* species, and their significant differences were assessed by Principal Coordinate analysis (PCoA). Pearson correlation was used to determine the relationship between soil physicochemical properties and *Trichoderma* diversity, and their significance was evaluated at $P < 0.05$. The effect of soil physicochemical properties on the *Trichoderma s*pecies distribution was evaluated by Canonical correspondence analysis (CCA) (Li *et al.,* 2022). All data in percentages were transformed into arcsine values (Gomez and Gomez, 1984) before analysis.

4.0 RESULTS

4.1 ECOLOGICAL CHARACTERISTICS OF THE AGROECOLOGICAL ZONES

Selected irrigation schemes covered a range of agroecological zones, from Central plateaux, Semi-Arid Areas, humid coastal areas, Alluvial lowlands, to highland zones. Average temperatures of these zones range from 17 °C in cooler highland areas to about 30.5 °C in warmer coastal areas. Average annual rainfall in the zones varies widely from low (500–700 mm) semi-arid zones to high (1,200– 1,900 mm) in coastal, alluvial, and highland regions. Soil texture varied from sandy loams and sandy clay loams typical in drier semi-arid locations, clay-rich Vertisols found in the Central plateaux zone, to volcanic soils mixed with sandy loam and clay in the

Northern Highlands. Alluvial zones and the Southern and Western Highlands had fertile soils with good texture and structure (sandy clay loam), while Semi-arid zones had low-fertility sandy loam soils. Altitudes of these zones range from near sea level in coastal and alluvial areas up to nearly 2000 meters above sea level in highland zones.

4.2 *TRICHODERMA* ISOLATE'S MORPHOLOGICAL CHARACTERISTICS

4.2.1 COLONY COLOR

All *Trichoderma* isolates initially appeared as translucent, water-soaked inocula during the first 1–2 days of incubation on PDA. Between days 3 and 7, colonies developed abundant cottony white aerial mycelia, which subsequently differentiated into distinct pigmentation patterns. Colony colors ranged from whitish, pale green, green, and dark green to pale yellowish, reddish, and yellow-green. Reverse colony colors ranged from whitish and creamy to yellow, turmeric brown, and pale green (**Appendix A**).

4.2.2 CONIDIATION PATTERN AND MYCELIAL TEXTURE

The isolates exhibited variation in conidiation patterns on PDA. Some isolates displayed effuse conidiation, characterized by a uniform distribution of conidia across the colony surface, whereas others exhibited zonate conidiation, forming distinct concentric rings of sporulation. Several isolates showed granular conidiation, characterized by the formation of localized pustules or compact tufts of conidiophores. Despite the differences in conidiation, all isolates produced floccose mycelia with abundant cottony aerial growth. Colony elevation was consistently flat, while colony growth form was circular with entire margins (**Appendix A**).

4.2.3 COLONY RADIAL GROWTH CHARACTERISTICS

All *Trichoderma* isolates demonstrated rapid growth on PDA, with colony diameters ranging from 67 to 85 mm after 72 h of incubation. However, variation in average

growth rates was observed among isolates originating from different agroecological zones. Isolates from the Alluvial and Northern Highlands zones exhibited the highest average colony diameters of 74.60 mm and 74.45 mm, respectively. These were followed by isolates from the Coastal zone (73.00 mm), Semi-arid zone (72.00 mm), Southern and Western Highlands zone (70.00 mm), and Arid zone (68.00 mm).

4.2.4 CONIDIOPHORE, PHIALIDE, AND CONIDIAL CHARACTERISTICS

Microscopic examination revealed substantial variation in conidiophore architecture, phialide morphology, and conidial characteristics among the isolates (**Appendix A**). Conidiophores were generally long and moderately to highly branched, with two to three levels of branching. Most isolates exhibited a dendritic branching pattern, whereas a few displayed verticillate branching. In contrast, isolates BH3-1 and BH5-1 lacked branching altogether. Phialides occurred either singly or in clusters of two to six and were observed in terminal as well as intercalary positions.

Their shapes varied from ampulliform (flask-shaped) to lageniform (bottle-shaped), with some isolates exhibiting intermediate forms. Chlamydospores were present in certain isolates, indicating the capacity to produce resistant survival structures under unfavorable conditions. Conidial morphology was also diverse, ranging from globose and subglobose to elliptical and ovoid forms (**Figure 2; Appendix A**). Such variation in microscopic characteristics reflects the phenotypic diversity of the isolates and provides important diagnostic features for species identification and differentiation within the genus *Trichoderma*.

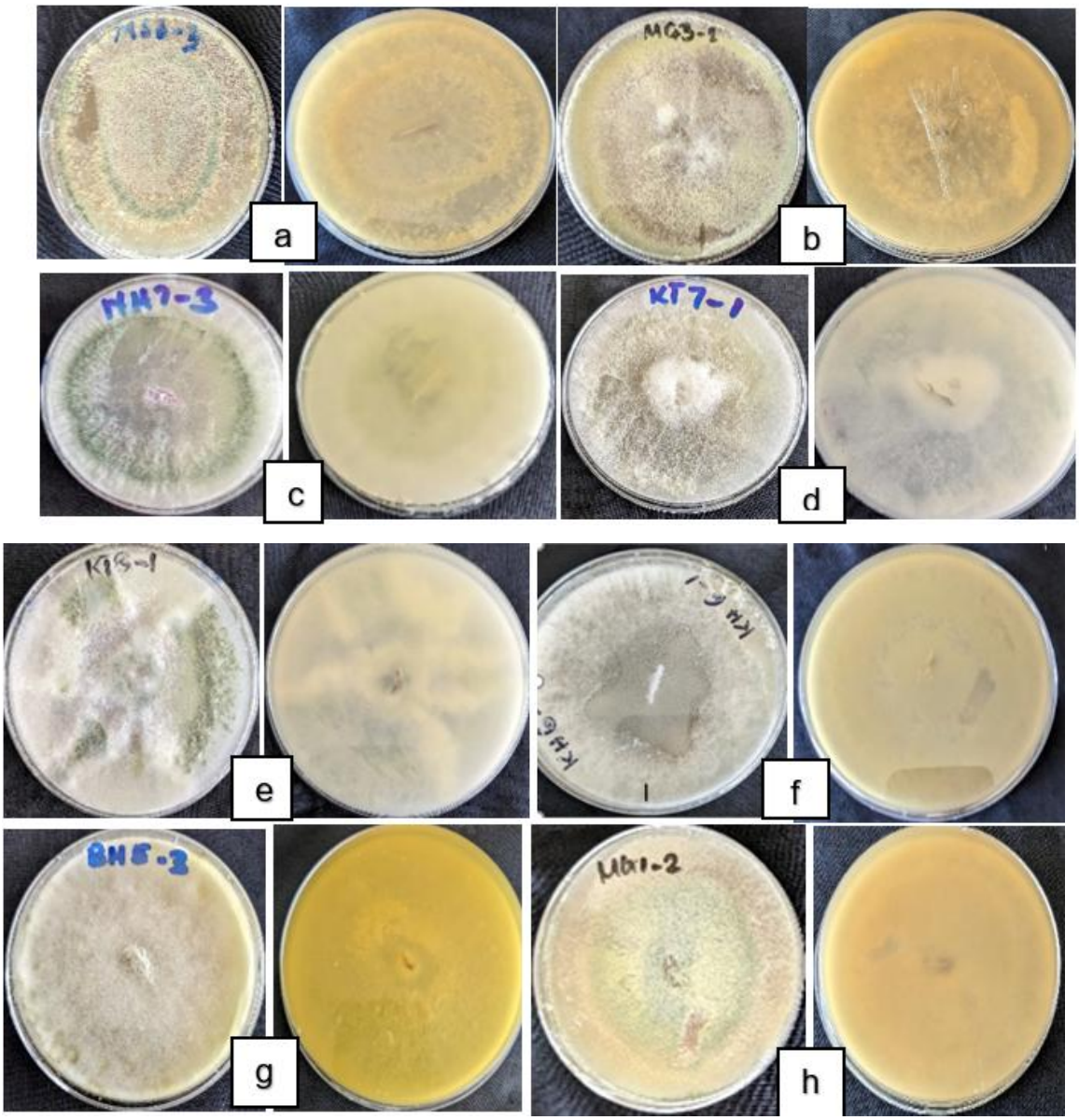


Figure 2 a-h. Colony morphology and reverse appearance of *T. asperellum, T. erinaceum, T. virens, T. paraviridescens, T. pleuroti. T. koningiopsis, T. longibrachiatum, and T. harzianum*

4.3 *TRICHODERMA* ISOLATES MORPHOLOGICAL CLUSTERS

Based on the morphological characteristics of isolates presented in Appendix A, three clusters were developed using Jaccard's similarity analysis:

Cluster I colonies exhibited effuse conidiation, with some isolates showing the presence of 1-2 concentric rings with the production of yellow-green to dark green-colored pigmentation. The conidiophore was rebranched up to three dendroid branches, regularly arranged. Branches arose at right angles in pairs, with lower branches being taller and upper branches shorter, forming a pyramid shape. Conidia were globose to subglobose. Phialides were lageniform to ampulliform, born in the whorl of 2-5 with the presence of intercalary phialides. Chlamydospores were present in some isolates.

Cluster II displayed whitish mycelium, and some exhibited one broad concentric ring with yellow diffusible pigments. Conidiophore in this group was not branched, phialides were lageniform and singly arranged, conidia were ovoid, and no chlamydospores were observed.

Cluster III displayed a filamentous and feathery appearance with green to dark green pigmentation. Conidiophore branching was verticillate, and branch arrangement was irregular. Phialides were ampulliform and present in whorls of 2-4 with no solitary phialides or chlamydospores; conidia were globose.

Based on the above morphological features, cluster I was related to *T. harzianum*, cluster II to *T. longibrachiatum,* and cluster III to *T. virens* (Bisset, 1984, 1991a, 1991b; Samwels et al.,2004)

4.4 MOLECULAR CHARACTERIZATION AND PHYLOGENETIC STUDIES OF *TRICHODERMA* ISOLATES

Based on Nucleotide BLAST analysis and pairwise alignments of the ITS rRNA sequences, 9 species of *Trichoderma* were identified corresponding to; *T. harzianum* (36 isolates)*, T. virens* (4 isolates)*, T. longibrachiatum (*2 isolates*), T. erinaceum (*1 isolate*), T. atroviride (*1 Isolate*), T. asperellum (1 isolate), T. koningiopsis (1 isolate), T. paraviridescens (1 Isolate), and T. Pleuroti* (1 isolate). These results are related to the results reported by Atanasova *et al*., 2023; Zhang et

al., 2021). The identification, origin, and NCBI GeneBank accession numbers of all the isolates are given in **Appendix B**. The phylogenetic tree (Figure 4) identified 9 species in four distinct clades (*Harzianum, Viridae, Longibrachiatum,* and *Virens* clades) with bootstrap values higher than 70%. Visual representation of their distribution across the irrigation schemes in the six agroecological zones is presented in Figure 3

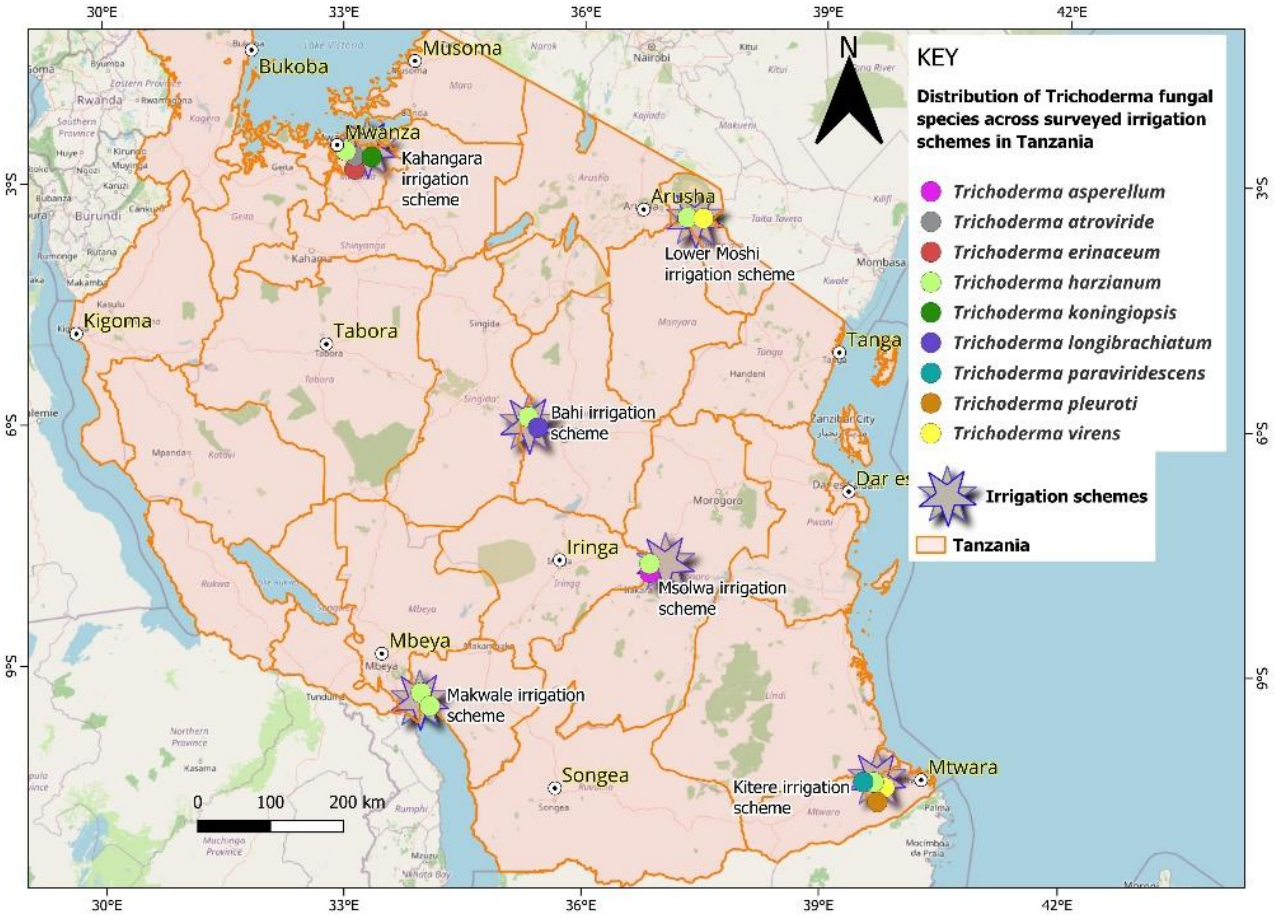


Figure 3. Distribution of *Trichoderma* species across the lowland rice irrigation schemes in the six agroecological zones.

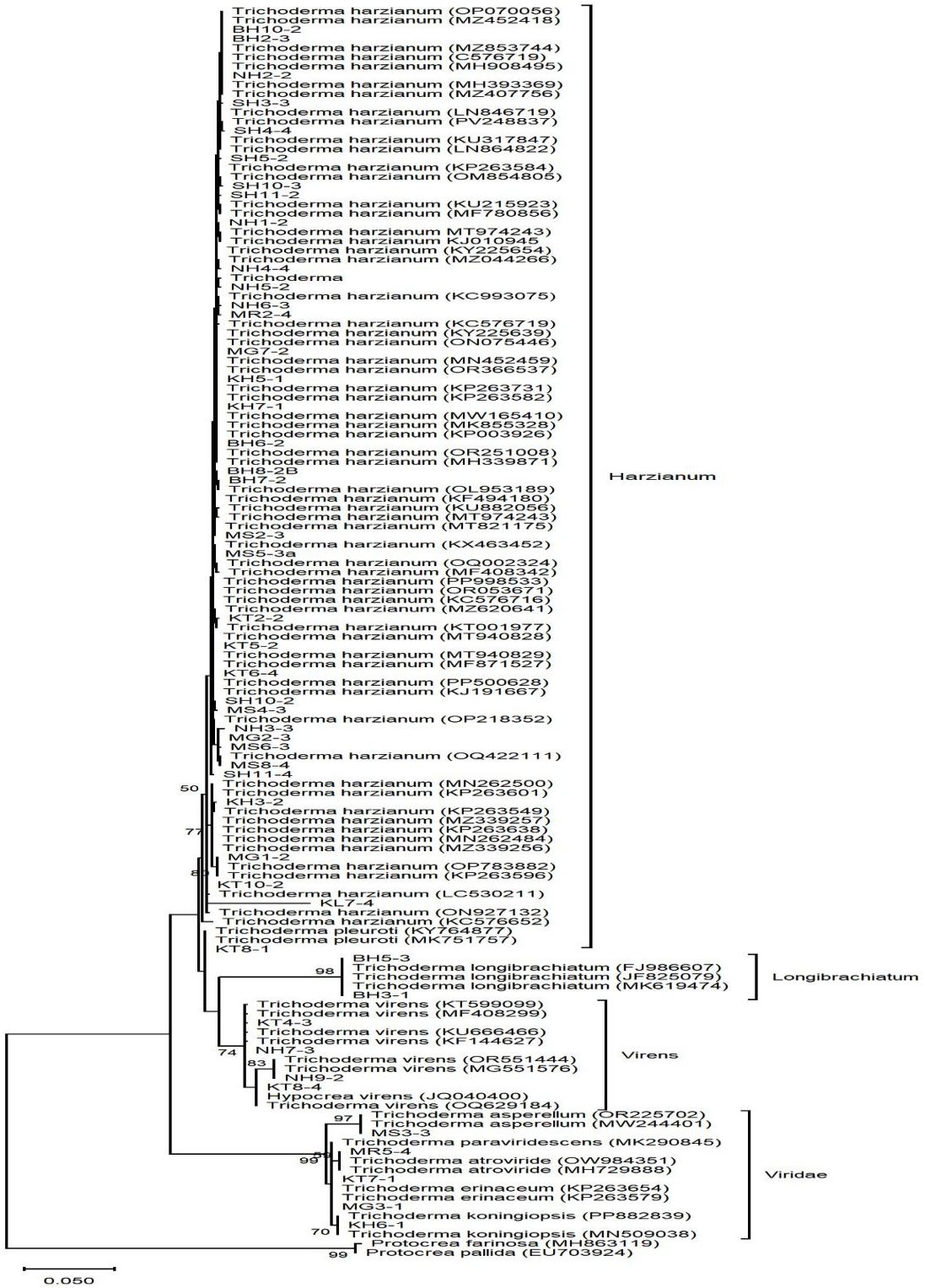


Figure 4. Maximum Likelihood phylogenetic tree based on ITS sequences of 48 *Trichoderma* isolates from this study and reference sequences retrieved from GenBank. The tree was constructed using the Kimura 2-parameter model with 1,000

bootstrap replicates. *Protocrea farinosa* (MH863119) and *Protocrea pallida* (EU703924) were used as outgroups to root the tree, and bootstrap values are shown at the nodes.

4.4.1 *HARZIANUM* CLADE

The isolates KT2-2, KL7-4, KT5-2, MS2-3, KT6-4, MS3-3, KT10-2, MS4-3, BH2-3, MS5-3A, KH7-1, MS6-3, KH5-1, MS8-4, MR2-4, MR5-4, MG1-2, MG2-3, MG3-1, MG7-2, KH3-2, BH6-2, BH7-2, BH8-2b, BH10-2, SH3-3, SH4-4, SH5-2, SH10-2, SH10-3, SH11-2, SH11-4, NH1-2, NH2-2, NH3-3, NH4-4, NH5-2, KH5-1, MG2-3 and NH6-3 collectively represented a range of *T. harzianum* species in the *harzianum* clade. From days 2-4, the isolates displayed a watery whitish color, then progressed to a range of colors (light green, yellow green, to dark green). Reverse colony colors varied widely and included creamy, light green, yellow green, reddish, bright yellow, white, and yellowish shades.

Conidiation patterns ranged from effuse types (evenly distributed with varying density, often denser at the center or margin to aggregated patterns forming ring-like structures of one or more concentric rings; some isolates displayed granular, pustulate conidiation patterns. Growth forms were exclusively circular with entire margins and flat elevations. The colony texture was described as cottony or cottony floccose, characterized by fine to coarse granules and sometimes pustulate formations. Conidiophores were highly branched with dendroid branching pattern, regular arrangements, and phialide shape ranging from ampulliform, lageniform, and lageniform to ampulliform. Conidia shape was subglobose to globose.

4.4 .2 *LONGIBRACHIATUM* CLADE

Trichoderma isolates BH3-1 and BH5-1 represented species in the *Longibrachiatum* clade. The isolates displayed a yellow-green colony color with yellow reverse. Mycelia texture was floccose, and the conidiation pattern was effuse. The growth form was circular. Conidiophore was less and/or rarely branched with a solitary lageniform phialide arising alongside the conidiophore. Conidia were ovoid.

4.4.3 *VIRENS* CLADE

Isolates KT4-3, KT8-4, NH7-3, and NH9-2 were placed in the *Trichoderma* virens clade. Initially, the colony color of the isolates was observed to be whitish to light green, watery in the center, and gradually became deep grass green in color and looked soft with feathery/ filamentous. The conidiophores were erect, less branched, irregularly branched, and singly or vertically arranged at different levels. Phialides were flask-shaped, coverage toward the main branch, emphasizing the penicillate branching, and the conidia were globose to sub-globose.

4.4.4 *VIRIDAE* CLADE

This clade comprises *T. asperellum* (MS 3-3), *T. paraviridescens* (KT7-1), *T. erinaceum* (MG3-1), *T. atroviride* (MR5-4), and *T. koningiopsis* (KH6-1). For MS3-3, the colony changed from cottony white to yellowish/ light green; the reverse color was light green, and the growth form was circular. Mycelia were cottony with 1–2 concentric rings. Conidiophores were highly branched in compact form, and the phialides arose singly or in clusters of 4-6, mostly at the tips of conidiophore branches, with Lageniform to ampulliform shape. Conidia were globose to subglobose. The colony color changes after 2 days from yellowish green to green, and the reverse remained creamy. For the isolate MR5-4 mycelium appeared floccose. Conidiophores were highly branched, arising in compact form, and the phialides were solitary or paired on the terminals with an ampulliform shape. Conidia were globose to subglobose. Isolate MG3-1 had a yellow-green colony with creamy reverse, mycelia were floccose with an effuse conidiation pattern. Growth form was circular. Conidiophores were highly branched and irregularly arranged. Phialide shape was Lageniform to ampulliform and conidia shape was elliptical.

The isolate KT7-1 had a whitish to light green colony with creamy reverse. Mycelia texture was floccose and effuse, and the growth form was circular. The conidiophore was highly branched, dendroid, with a regular branching pattern. Phialides were Lageniform, and conidia were subglobose to obovoid. Isolate KH6-1 had a whitish colony, turmeric brown reverse, mycelia texture was floccose, and the growth form was circular. The conidiophore was highly branched, dendroid, with a regular arrangement. Phialides were lageniform, and conidia shape were elliptical.

4.5 SOIL PHYSICOCHEMICAL CHARACTERISTICS WITHIN AGROECOLOGICAL ZONES

Soil physicochemical characteristics varied among the agroecological zones (**Table 1**). Soil texture ranged from sandy clay loam in Alluvial zone, the Semi-arid zone, and Southern and Western highlands zones to sandy clay in the Northern highlands zone and clay in the Central plateaux and Coastal zone. Soil pH in the alluvial zone and Northern highlands zone was between very strongly acidic and slightly acidic, semi-arid had very strongly acidic to slightly alkaline soil, the central plateaux and Southern-western zone had slightly acidic soils, while the coastal zone had slightly acidic to alkaline soils.

Organic matter (OM) contents were remarkably high in the alluvial zone and the Southern and Western highlands zone, medium in the central plateaux zone and the coastal zone, and low in the semi-arid zone and the Northern Highlands zone. As for electric conductivity (EC), all soils were slightly saline with values ranging from 2.22 $dS \cdot m^{-1}$ to 4.58 $dS \cdot m^{-1}$. Specifically, the alluvial zone had high values; the southern-western highlands zone, semi-arid zone, coastal zone, and Northern highlands zone had lower values.

Table 1: soil physicochemical characteristics

Zone	Soil texture	pH	EC (dS/m)	O.M (%)
Alluvial	Sandy clay loam	3.36-6.13	4.58	3.08-3.18
Semi-arid	Sandy clay loam	4.57-7.13	2.36	1.20-1.45
Central plateau	Clay	5.7-6.7	3.01	2.01-2.09
Southern and western highlands	Sandy clay loam	6.08-6.35	2.77	2.88-3.31
Northern Highlands	Sandy clay	5.24-6.11	2.1	1.07-1.99
Coastal	Clay	6.44-7.31	2.25	2.0-2.35

4.6 *TRICHODERMA* SPECIES OCCURRENCE, DISTRIBUTION, AND ABUNDANCE AMONG AGROECOLOGICAL ZONES (AEZ) AND IRRIGATION SCHEMES

High species richness was observed in the Kahangara and Kitere irrigation schemes, each harboring four species, while the Msolwa, Lower Moshi, and Bahi irrigation schemes contained two species each. The lowest species richness was

recorded in the Makwale irrigation scheme, where only one species was identified. Variation in the recovery of *Trichoderma* species across agroecological zones was assessed based on their isolation frequencies (**Figure 6a**). Across all zones, *T. harzianum* exhibited the highest overall isolation frequency (38.89 %), whereas *T. koningiopsis* showed the lowest frequency (4.17 %).

The highest isolation frequency of *T. harzianum* was recorded in the Makwale irrigation scheme (100 %), while the lowest frequency was observed in the Kitere irrigation scheme (41.7 %). *T. atroviride* and *T. koningiopsis*, isolated from the Kahangara irrigation scheme in the semi-arid zone, exhibited the lowest isolation frequencies, each accounting for 8.33 %. Principal Coordinate Analysis (PCoA) revealed significant differences in the distribution of *Trichoderma* species among agroecological zones. Coastal and Central Plateaux zones were clearly separated from the other zones (Fig. 6b). No significant differences in isolation frequencies were observed among the Northern Highlands, Southern and Western Highlands, Alluvial, and Semi-arid zones.

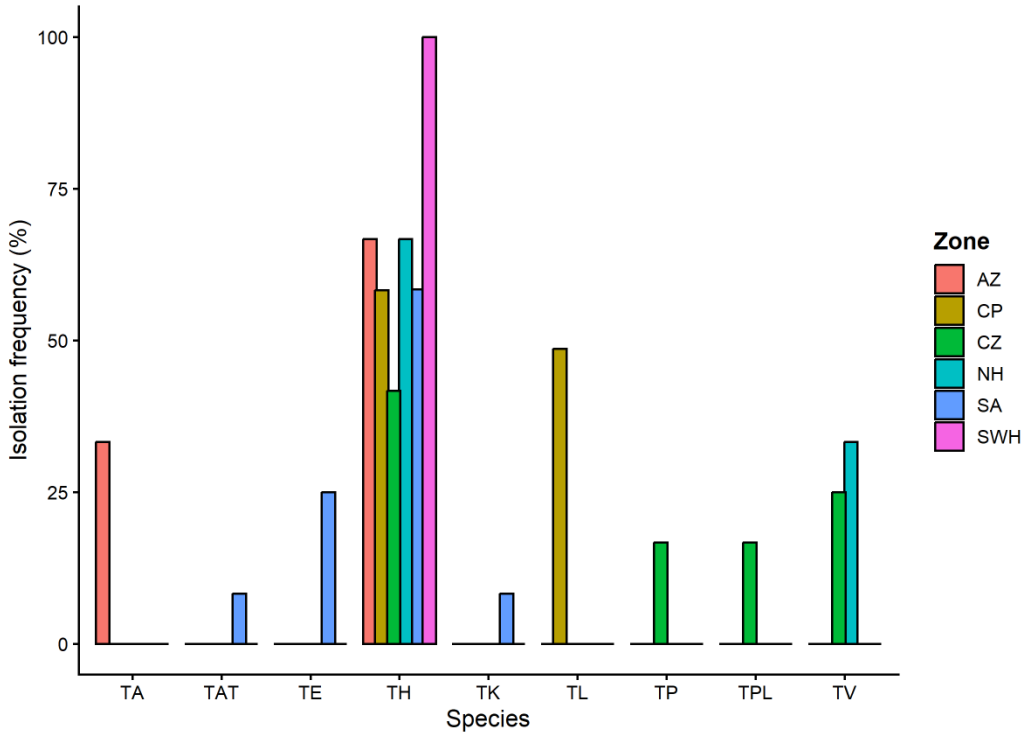


Figure 6a. Isolation frequencies of *Trichoderma* species in the six agroecological zones. The zones AZ, CP, CZ, NH, SA, and SWH are Alluvial, Central plateaux, Coastal, Northern highlands, Semi-arid and Southern and Wesrern highlands. The species; Th- *T. harzianum,* Tas- *T. asperellum,* Tat- *T. atroviride,* Te-*T. erinaceum,* Tk- *T. koningiopsis,* Tl- *T. longibrachiatum,* Tv- *T. virens,* Tap- *T. paraviridescens,* and Tp- *T. pleuroti*

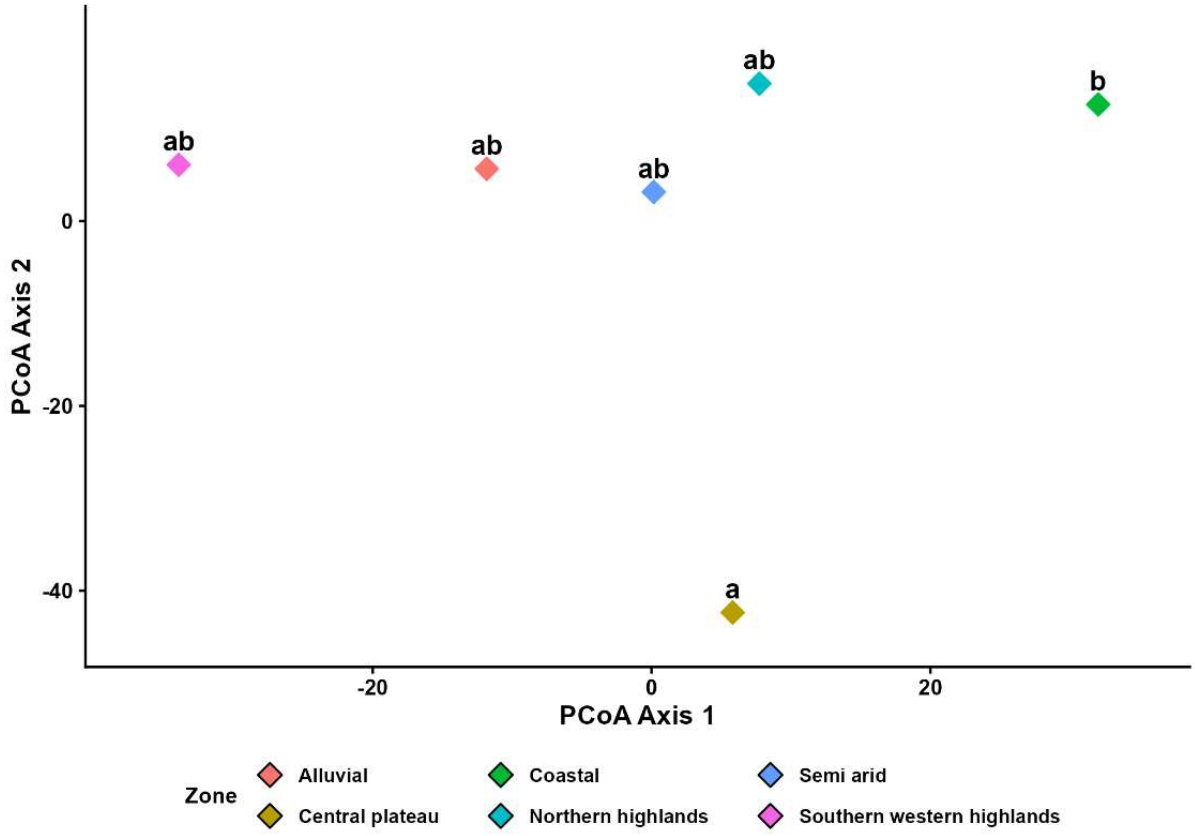


Figure 6b. Principle Coordinates Analysis (PCoA) showing the variation in *Trichoderma* community isolation frequencies across six agroecological zones. Ellipses indicate 95% confidence intervals around group centroids, reflecting homogeneity or variation within zones. Lowercase letters (a, b, and ab) represent results from post-hoc multiple comparison tests on PCoA scores: groups sharing at

least one letter are not significantly different, while groups with no letter overlap differ significantly.

For the relative density (RD), the alluvial zone had the highest RD of *Trichoderma* species, while the Coastal zone and Semi-arid zone had the lowest. The PCoA plot separated the zones, with the Alluvial zone displaying a notably distinct RD compared to other zones (**Figure 7b**). When the RD by irrigation scheme was assessed, *T. harzianum* was the most prevalent species in all schemes, followed by *T. virens*, while *T. pleuroti* and *T. paraviridescens* showed the lowest RD.

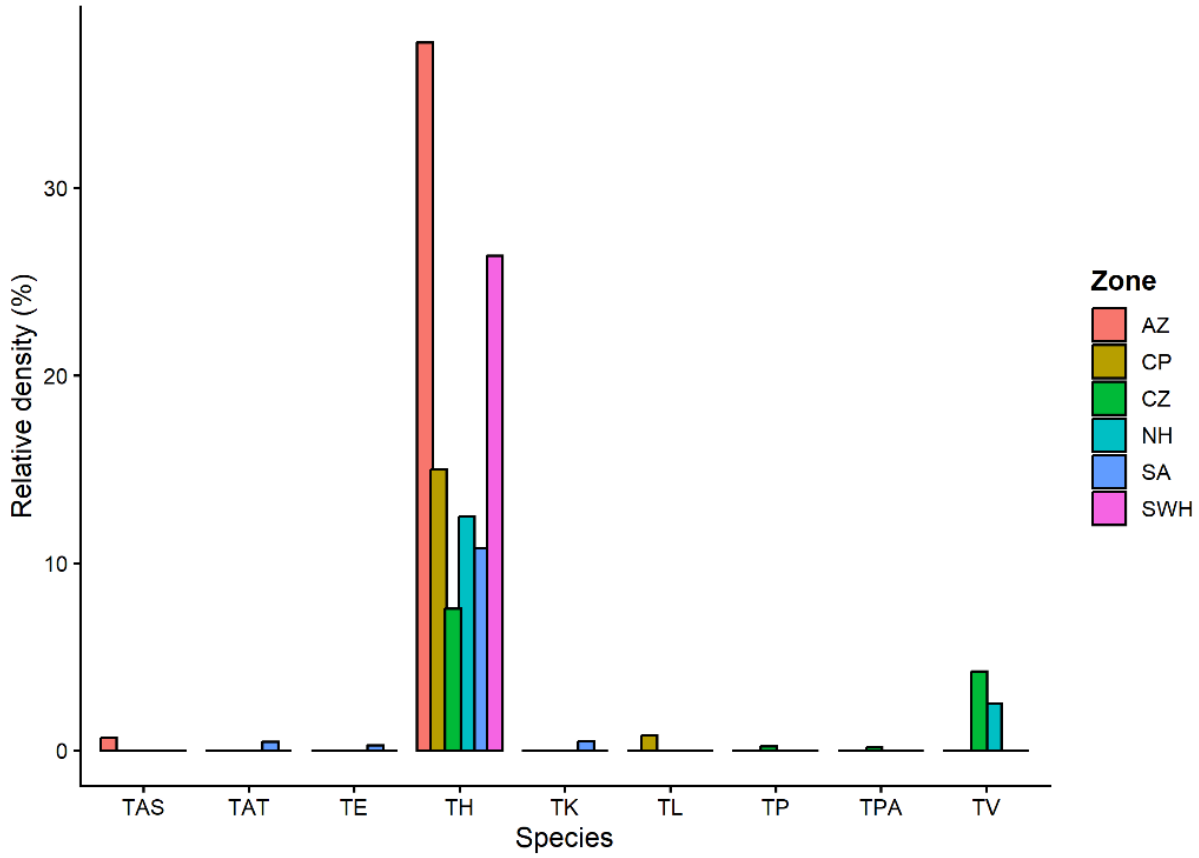


Figure 7a. Relative density of *Trichoderma* species in the six agroecological zones: The zones AZ, CP, CZ, NH, SA, and SWH are Alluvial , Central plateaux, Coastal, Northern highlands, Semi-arid and Southern and Western highlands**. Th-** *T. harzianum,* **Tas**- *T. asperellum,* **Tat**- *T. atroviride,* **Te**- *T. erinaceum,* **Tk**- *T. koningiopsis,* **Tl**-*T. longibrachiatum,* **Tv**- *T. virens,* **Tap**- *T. paraviridescens,* **Tp**-*. pleuroti*

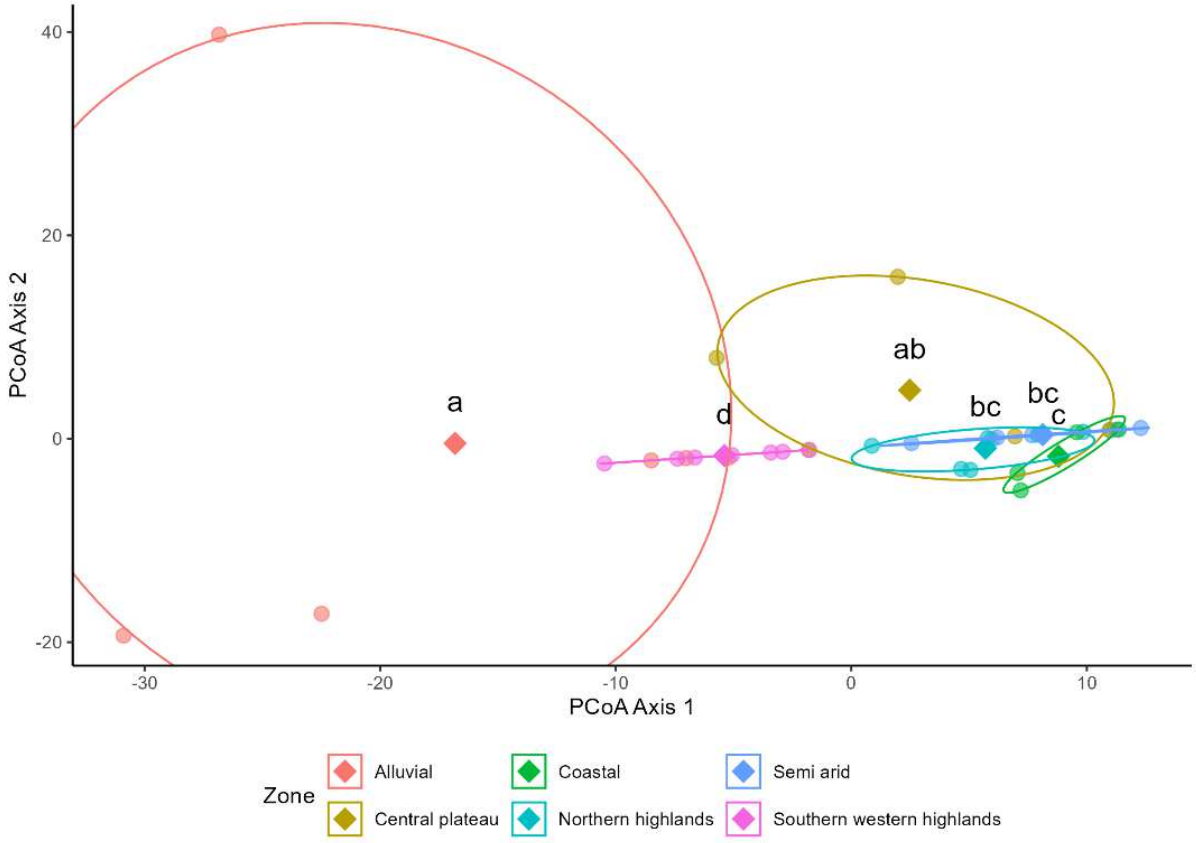


Figure 7b. Principal Coordinates Analysis (PCoA) showing the variation in the *Trichoderma* community relative density across six agroecological zones**.** The axes (PCoA 1 and 2) summarize the primary variation in relative density among samples. Ellipses indicate 95% confidence intervals around group centroids, reflecting homogeneity or variation within zones. Lowercase letters (a, d, c, ab and bc) represent results from post-hoc multiple comparison tests on PCoA scores: groups sharing at least one letter are not significantly different, while groups with no letter overlap differ significantly.

4.7 DIVERSITY OF *TRICHODERMA* SPECIES BY AGROECOLOGICAL ZONES (AEZ) AND IRRIGATION SCHEMES

Table 2 shows Shannon's biodiversity index (H), Simpson's dominance index (D), Simpson's diversity index (1-D), and evenness (E) for the *Trichoderma* species

within irrigation schemes of the six agroecological zones. The Coastal Zone recorded the highest species diversity and evenness, with a Shannon index (H') of 1.21, an evenness value (J) of 0.88, and a Simpson's dominance index (D) of 0.34. Semi-arid zone (Kahangara irrigation scheme) exhibited H' = 0.94, E= 0.68, and D = 0.52, indicating relatively high diversity with low evenness.

The Northern highlands zone (Lower Moshi irrigation scheme) and Central plateaux zone (Bahi irrigation scheme) had moderate diversity and high evenness with H = 0.60, E=0.86, D = 0.59, and H = 0.60, E=0.86, D = 0.41. The alluvial zone (Msolwa irrigation scheme) had low diversity and low evenness with H = 0.41, E = 0.21, and D = 0.76. The Southern and Western Highlands zone (Makwale irrigation scheme) had no diversity, being completely dominated by a single species with H = 0, E= 0, and D = 1. The diversity across all agroecological zones was relatively high but not evenly distributed, indicated by H' = 1.21, J =0.51, and D 0.57.

Table 2: *Trichoderma* species diversity across agroecological zones and irrigation schemes.

Zone(s)	Irrigation Scheme	Total_ individuals_(N)	Shannon Diversity Index (H')	Simpson's Dominance _Index(D)	Simpson's Diversity_ Index(1-D)	Evenness (E)
All zones		48	1.21	0.57	0.43	1.51
Alluvial	**Msolwa**	7	0.41	0.76	0.25	0.21
Semi-arid	**Kahangara**	12	0.94	0.52	0.48	0.68
Arid	**Bahi**	7	0.60	0.41	0.59	0.86

Southern -western	**Makwale**	6	0	1	0	0
Northern highlands	**Lower Moshi**	8	0.60	0.59	0.41	0.86
Coastal	**Kitere**	8	1.21	0.34	0.66	0.88

4.8 CORRELATION OF *TRICHODERMA* SPECIES DIVERSITY WITH SOIL PHYSICOCHEMICAL CHARACTERISTICS

Pearson correlation analysis revealed significant relationships between soil physicochemical properties and species diversity indices (**Table 3**). pH showed a positive correlation with Shannon diversity, Simpson diversity, and species evenness, but a negative relationship with species dominance (D). Soil organic matter (OM) and electrical conductivity (EC) correlated positively with Simpson's dominance (D), and negatively with species diversity and evenness, indicating that increased soil organic matter and high salinity levels reduce *Trichoderma* species diversity and evenness.

Table 3. Correlation analysis of *Trichoderma* species with soil physicochemical characteristics.

Soil factor	Community index	R	*p-value*	Significance
pH	D	-0.053	0.065	ns
pH	DI	0.053	0.065	ns
pH	E	0.045	0.09	ns
pH	H	0.14	0.0023	*
OM	D	0.4	< 0.001	***
OM	DI	-0.45	< 0.001	***
OM	E	-0.7	< 0.001	***
OM	H	-0.14	< 0.001	***
EC	D	0.092	<0.05	*
EC	DI	-0.092	<0.05	*
EC	E	-0.32	<0.001	***
EC	H	-0.11	<0.001	***

*Variables D, DI, E, and H represent Simpson's Dominance index, Simpson's Diversity index, Species evenness, and Shannon diversity index, respectively. * present significant, *** highly significant, and ns present non-significant.*

Further analysis of species distribution using Canonical Correspondence Analysis (CCA) revealed that the assemblage *of Trichoderma* species across irrigation schemes was significantly influenced by soil texture, pH, OM, and EC (**Figure. 8**). The first two canonical axes, CCA1 and CCA2 explained 46.3% and 26.8% of the constrained variation, respectively, demonstrating that soil physicochemical properties accounted for 63.1% of the variation in *Trichoderma* species distribution. The ordination biplot showed clear associations between *Trichoderma* species and environmental gradients.

T. longibrachiatum (TL) was associated with soils rich in organic matter; *T. atroviride* (TAT) and *T. koningiopsis* (TK) were linked to high pH levels; *T. erinaceum* (TE) was influenced by low soil pH; *T. asperellum* (TAS) distribution was affected by low soil salinity; *T. virens* (TV), *T. pleuroti* (TPL), and *T. paraviridescens* (TP) were less affected by these soil factors and clustered nearer to neutral sites; *T. harzianum* (TH) appeared to be cosmopolitan, as its distribution was not strongly linked to any specific soil factors.

Soil texture also influenced the distribution of *Trichoderma* species, with clay soils supporting OM- and EC-associated species, while sandy clay loam and sandy clay soils supported generalists and pH-associated species. The CCA biplot indicated a separation of agroecological zones along soil gradients: alluvial zones (AZ) and Central Plateaux (CP) were more associated with soils high in EC and OM. At the same time, the semi-arid zone (SA) was linked to elevated soil pH. Coastal (CZ), Northern Highlands (NH), and Southern and Western Highlands (SWH) zones showed a weaker influence of these soil variables.

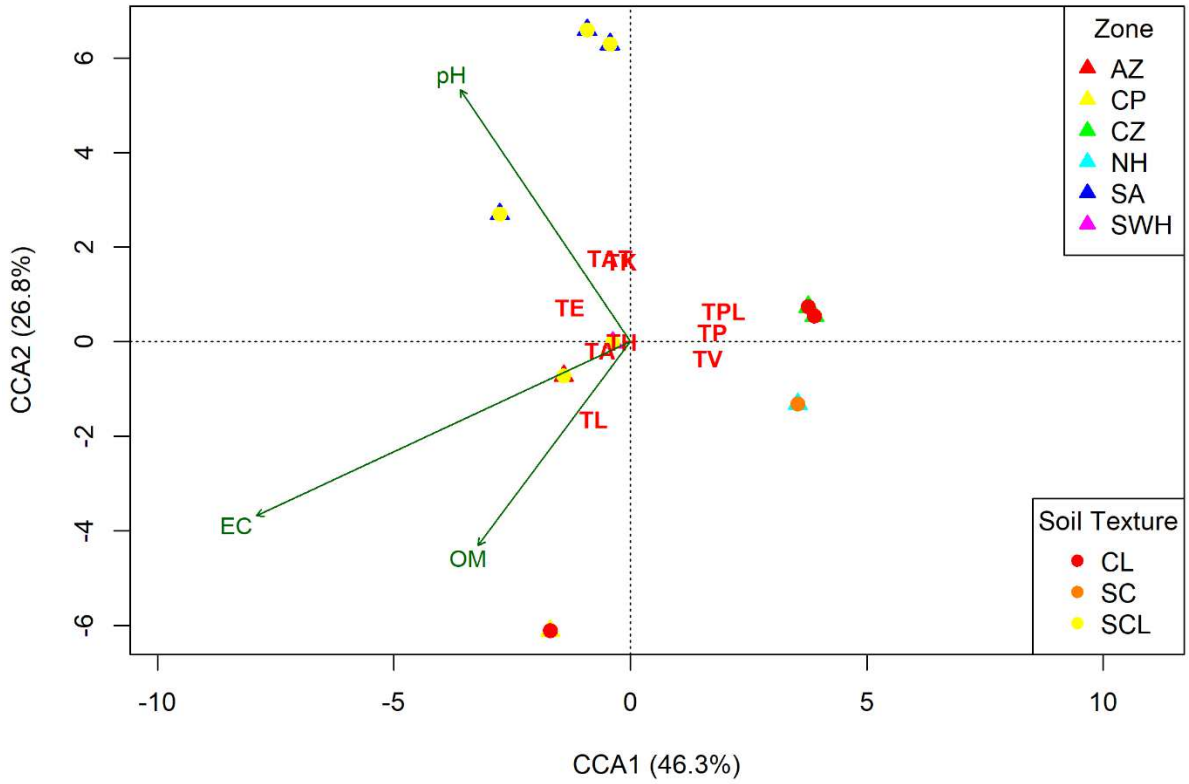


Figure 8. Canonical Correspondence Analysis on the occurrence and distribution of Trichoderma spp. along soil physicochemical gradients. Axes CCA1 & CCA2 represent the main gradients (directions of variation) of soil physicochemical characteristics. The arrows OM (organic matter), pH, and EC (Electrical conductivity) represent soil physicochemical characteristics; the length of each arrow indicates the strength, and the direction of the arrow indicates the influence of that variable on *Trichoderma* distribution. Points AD, SA, SWH, NH, CP, and CZ are the Bahi irrigation scheme (Central plateaux zone), Kahangara irrigation scheme (Semi-arid zone), Makwale irrigation scheme (Southern and western highlands), Lower Moshi irrigation scheme (Northern highlands), Msolwa irrigation scheme (Alluvial zone), and Kitere irrigation scheme (Coastal zone). The red texts are *Trichoderma* species.

4.9 NUCLEOTIDE SIMILARITY OF *TRICHODERMA* SPP. WITHIN IRRIGATION SCHEMES AND AMONG AGROECOLOGICAL ZONES

Intra- and inter-population genetic distances were estimated using the Jukes–Cantor model in MEGA software, and the genetic similarity was obtained. There was higher within-population genetic diversity (π=0.03) and lower between-population diversity (π=0.04). The results revealed both within- and between-population genetic variation among *Trichoderma* isolates from different irrigation schemes and agroecological zone. In general, relatively high within-population nucleotide similarity was observed, with values ranging from 94% to 100%.

The highest similarity (100%) was recorded in the Makwale irrigation scheme (Southern and Western Highlands zone), indicating genetically uniform populations. In contrast, the lowest within-population similarity (94%) was observed in the Kahangara irrigation scheme (Semi-arid zone), suggesting greater genetic variability within that population. Intermediate similarity values were recorded in Lower Moshi (Northern Highlands) and Kitere (Coastal zone) at 97%, Bahi (Central Plateau zone) at 95%, and Msolwa (Alluvial zone) at 96%.

Between-population comparisons also showed relatively high nucleotide similarity, ranging from 93% to 98%. The highest inter-population similarity (98%) was observed between Kahangara (Semi-arid zone) and Makwale (Southern and Western Highlands zone), as well as between Kitere (Coastal zone) and Makwale irrigation schemes. The highest level of similarity (97%) was also observed between Msolwa (Alluvial zone) and Makwale, as well as between Kitere and Lower Moshi irrigation scheme.

Likewise, Kahangara showed 94% similarity with Msolwa (Alluvial zone), Kitere (Coastal zone), and Lower Moshi (Northern Highlands zone). A similarity of 95% was recorded among several comparisons, including Kahangara with multiple zones (Makwale, Lower Moshi, Kitere, and Bahi), as well as Msolwa (Alluvial zone) with Bahi (Central Plateau zone). Msolwa also showed 96% similarity with Lower Moshi

and Makwale irrigation schemes. The lowest inter-population similarity (93%) was recorded between Kahangara (Semi-arid zone) and Bahi (Central Plateau zone), indicating the greatest genetic divergence between these two populations.

5.0 DISCUSSION

Assessing the biodiversity of agriculturally important fungi such as *Trichoderma* is essential for identifying ecologically adapted strains with potential for biological control and sustainable crop production. Accurate species identification forms the foundation for understanding species distribution, ecological preferences, and functional diversity within agroecosystems (Salwan *et al*., 2021).

In the present study, a combined morphocultural and molecular approach based on ITS sequencing enabled the identification of nine *Trichoderma* species across irrigated lowland rice agroecosystems representing six agroecological zones of Tanzania. The integration of morphological and molecular tools enhanced taxonomic resolution and minimized the risk of misidentification associated with phenotypic plasticity, a common feature within the genus. Similar observations have been reported by Atanasova *et al.* (2023); Li *et al.* (2020), and Zhang *et al*. (2021), who emphasized the importance of molecular phylogenetics in resolving species boundaries within *Trichoderma*.

The occurrence of nine species demonstrates considerable *Trichoderma* diversity within Tanzanian rice-growing environments. Species richness varied among irrigation schemes, with Kitere (Coastal zone) and Kahangara (Semi-arid zone) supporting the highest richness, each harboring four species, while Makwale (Southern and Western Highlands) contained only *T. harzianum*. These differences suggest that agroecological conditions strongly influence species establishment and persistence.

Although geographical separation contributes to community differentiation, local environmental conditions appear to play a more important role in determining species occurrence. Similar patterns have been documented in agricultural soils

worldwide, where soil physicochemical properties and microclimatic conditions act as major determinants of *Trichoderma* community composition (Zhang *et al.,* 2021; Poosapati *et al.,* 2021).

Among all species identified, *T. harzianum* was the most prevalent and widely distributed taxon, occurring in every agroecological zone and exhibiting the highest isolation frequency. These findings align well with Kullnig *et al*. (2000) and Sun *et al.* (2012), who reported *T. harzianum* as the most frequently occurring species in Russia, Siberia, and the Himalayas. Kubicek *et al.* (2003), reported the broad distribution of *T. harzianum* across diverse habitats in regions of Burma, Cambodia, Malaysia, Singapore, Thailand, and Western Indonesia. Likewise, in East Africa, a study by Maina *et al.* (2015) reported that *T. harzianum* was the most abundant species isolated from Machakos County of Kenya.

The ability of *T. harzianum* to persist across soils differing in texture, pH, organic matter content, and electrical conductivity suggests a high degree of ecological plasticity (Singh *et al.,* 2022). This adaptability has been attributed to its capacity to produce diverse extracellular enzymes and secondary metabolites, enabling efficient utilization of varied substrates and tolerance to environmental stress (Szabó *et al.,* 2021). Studies by Wei et al. (2023) and Sbrana et al. (2023) similarly demonstrated that *T. harzianum* maintains activity under saline and drought-prone environments, reinforcing its reputation as a broadly adapted generalist species.

The study further revealed substantial variation in colony morphology among isolates belonging to the same genetically confirmed species. Such variation was particularly evident in *T. harzianum* and *T. virens*. Morphological divergence among genetically related isolates is frequently observed in *Trichoderma* populations and is often associated with environmental influences on phenotypic expression (Matthew *et al.,* 2023). Soil nutrient availability, temperature, moisture conditions, and local adaptation may induce morphological differences without corresponding changes in species identity (Matthew *et al.,* 2023).

Consequently, the discrepancy observed between morphology and molecular identification in several isolates highlights the limitations of relying exclusively on cultural characteristics for species determination and reinforces the necessity of molecular approaches for accurate taxonomic classification.

Species abundance and distribution patterns indicated that environmental filtering plays a major role in structuring *Trichoderma* communities. The higher richness observed in the Coastal and Semi-arid zones may reflect the presence of favorable soil chemical conditions, particularly moderate electrical conductivity and pH values ranging from acidic to slightly alkaline. Such conditions are known to support fungal metabolism, nutrient acquisition, and competitive ability (Tong *et al.,*2021).

Low species richness observed in Makwale suggests that environmental constraints may restrict colonization by less adaptable species, allowing only highly competitive taxa such as *T. harzianum* to dominate. The predominance of a single species under such conditions may indicate ecological filtering, whereby only organisms possessing suitable physiological traits can establish and persist (Zehra, *et al.,* 2017).

Patterns observed through diversity indices provided further evidence that agroecological conditions shape fungal community structure. The Coastal zone exhibited the highest Shannon diversity and evenness values, indicating not only greater species richness but also a more equitable distribution of individuals among species. High evenness suggests reduced competitive exclusion and the coexistence of multiple taxa under relatively favorable environmental conditions. These findings align with Tong *et al.* (2022), who found an even distribution of *Trichoderma* species in the coastal regions, suggesting that soil with moderate to slightly alkaline pH and good moisture retention significantly influenced species diversity and community balance.

The Semi-arid zone on the other hand, exhibited lower evenness despite relatively high species richness, indicating dominance by a few stress-tolerant species.

Environmental stress often reduces community balance by favoring species possessing superior adaptive traits, resulting in lower evenness despite the presence of several taxa (Liu *et al.,* 2023; Poosapati *et al.,* 2021; Rawal *et al.,* 2022).

Remarkably, the Alluvial zone exhibited low species diversity despite having high abundance. This pattern indicates that abundance and diversity are not necessarily positively related. The dominance of *T. harzianum* and *T. asperellum* in this zone suggests that fertile, moisture-retentive alluvial soils may preferentially support a limited number of highly competitive species capable of rapidly exploiting available resources. Similar findings have been reported in other agricultural systems where resource-rich environments often promote dominance by a few opportunistic microbial taxa rather than maximizing species diversity (Okoth and Odhiambo, 2009).

Multivariate analyses further supported the influence of environmental factors on community composition. Principal Coordinate Analysis (PCoA) revealed clear differences among irrigation schemes, indicating that *Trichoderma* assemblages varied substantially across agroecological zones. The distinct separation of Kitere and Bahi irrigation schemes despite some similarities in soil characteristics, suggests that additional factors not directly measured in this study, such as rainfall distribution, humidity, cropping history, and microbial interactions, may contribute to community differentiation. Such factors have previously been identified as important drivers of soil fungal community structure (Montoya-González et al., 2016; Silva et al., 2020).

The significant correlations between diversity indices and soil properties further emphasize the role of environmental conditions in regulating fungal biodiversity. Positive associations between soil pH and both diversity and evenness indicate that neutral to slightly alkaline soils support more balanced *Trichoderma* communities (Kopur *et al*., 2025). Soil pH influences nutrient availability, enzyme activity, and microbial competition, thereby affecting community assembly. A study by Sharma *et al.* (2021) is in line with these findings; they concluded that the abundance of

entomopathogenic fungi (EPF) in Portuguese grapevine fields was influenced by soil pH, where they were more abundant in soils with pH ranging from neutral to alkaline.

On the other hand, high organic matter and electrical conductivity were negatively correlated with species diversity but positively with species dominance, as observed in the Msolwa irrigation scheme (Alluvial zone) and Makwale irrigation scheme (Southern and western Highlands zones). Higher salinity and organic content in these irrigation schemes may have created selective pressures favoring species that are adapted to these conditions, thereby reducing overall biodiversity. A study by Jafari *et al.* (2023) found that the elevated soil salinity and organic matter levels were associated with lower microbial biodiversity because the conditions favored salt-tolerant and organic matter-degrading specialist species, leading to reduced microbial community evenness and diversity.

Soil texture emerged as another important factor influencing species distribution. Clay-dominated soils of the Central Plateaux supported species associated with high organic matter content, whereas sandy clay loam soils of the Semi-arid zone favored species adapted to greater aeration and environmental stress. Soil texture directly affects water retention, nutrient dynamics, aeration, and aggregate formation, all of which influence fungal growth and survival (Lakshman *et al.,* 2024). Consequently, texture-mediated differences in resource availability likely contribute to the distinct species assemblages observed among agroecological zones.

Canonical Correspondence Analysis (CCA) provided additional insights into species-specific ecological preferences. The strong association of *T. longibrachiatum* with soils rich in organic matter suggests an ecological advantage in environments containing abundant decomposable substrates. The species is known for producing a wide range of cellulolytic and hemicellulolytic enzymes that facilitate degradation of complex plant residues (Al-Askar *et al.,* 2022).

Electrical conductivity as an indicator of soil ion concentration and fertility was negatively related to *T. asperellum* (TA) in the alluvial zone, where their distribution

was favored by slightly saline soils. Studies have shown that high EC soils have been associated with higher salt concentration, which in turn has effects on the distribution of soil microbes, including *Trichoderma* (Correa-Delgado *et al.,* 2024). Soil with slight EC gradients generally favors *Trichoderma* abundance and activity by reducing salinity stress and improving soil fertility, driving their distribution.

T. atroviride, *T. koningiopsis*, and *T. erinaceum* exhibited stronger associations with specific soil pH gradients, suggesting narrower ecological preferences than those of *T. harzianum* and *T. virens.* This pattern may be attributed to species-specific physiological adaptations, whereby growth, nutrient acquisition, and extracellular enzyme activities are optimized within particular pH ranges (Kredics *et al.,* 2022). Soil pH also influences microbial competition and resource availability, potentially restricting these species to ecological niches where they are more competitive. Such specialization may enhance their performance under favorable conditions but limit their distribution across broader environmental gradients.

T. harzianum and *T. virens* displayed broad distributions with weak associations to individual environmental variables, supporting their classification as ecological generalists capable of colonizing diverse habitats. These species have been reported to be cosmopolitan (Zhang *et al.,* 2021; Sharma *et al.,* 2024) and persist under contrasting soil conditions, such as different gradients of soil pH, moisture contents, nutrients, and salt concentration (Trotta *et al.,* 2024).

Their ability to produce diverse enzymes, including cellulases, chitinases, and proteases, and secondary metabolites such as peptaibols, gliotoxin-like compounds, and polyketides, enables them to exploit varied nutrient sources and outcompete other microbes (Harman *et al.,* 2021; Kredics *et al.,* 2022). The positioning of *T. pleuroti* and *T. paraviridescens* near the center of the CCA biplot further suggests broad ecological tolerance and reduced dependence on the measured soil factors (Zhang *et al.,* 2021). This distribution suggests that *T. pleuroti* and *T. paraviridescens* may represent relatively generalist taxa whose occurrence is less

influenced by soil chemistry and more by other biotic or unmeasured abiotic factors, such as plant–fungal interactions or localized microclimatic conditions.

The nucleotide similarity analysis revealed that environmental conditions influence not only species distribution but also genetic variation within *Trichoderma* populations. The complete similarity observed in Makwale indicates a genetically homogeneous population, potentially reflecting strong environmental selection and limited introduction of new genotypes. Conversely, the lower similarity observed in Kahangara suggests greater genetic heterogeneity, possibly resulting from adaptation to fluctuating and stressful semi-arid conditions. Such environmental pressures may promote diversification by favoring different adaptive strategies among populations (Velazquez-Muriel *et al.,* 2022).

High nucleotide similarity between geographically distant zones suggests that ecological similarity may be more important than geographical proximity in shaping genetic relatedness among *Trichoderma* populations. This observation supports the concept that environmental selection can maintain similar genotypes across spatially separated habitats when ecological conditions are comparable (Yadav and Yadav. 2021). Conversely, lower similarity between environmentally contrasting zones indicates that divergent selection pressures may contribute to genetic differentiation over time (Velazquez-Muriel *et al.,* 2022).

LIMITATION OF THE STUDY

This study employed a molecular identification approach based primarily on the ITS rDNA region, which is widely used for fungal barcoding and was effective in delineating *Trichoderma* species in the present analysis. The results obtained were consistent and showed no clear taxonomic ambiguity. However, reliance on a single genetic marker may still limit the robustness of phylogenetic inference since ITS alone may not always provide sufficient resolution for fully confirming species boundaries or detecting subtle intraspecific genetic variation. Therefore, future studies are recommended to incorporate multilocus sequence analysis (e.g., tef1-α

and other protein-coding genes) to further validate and strengthen the genetic resolution and confirm the findings of the current study

CONCLUSION

The findings of this study highlight the ecological complexity of *Trichoderma* communities in rice-growing agroecosystems and demonstrate that their distribution, diversity, and genetic structure are governed by interactions between environmental conditions and species-specific adaptive traits. The coexistence of both broadly adapted species and environmentally specialized taxa suggests that *Trichoderma* populations are shaped by ecological filtering processes that promote niche differentiation across agroecological zones. These results reinforce the importance of considering ecological context when exploring microbial resources for sustainable agriculture and biological control.

From an applied perspective, the widespread occurrence and adaptability of *T. harzianum* indicate its potential as a broadly effective biocontrol agent, while the presence of locally adapted species highlights opportunities for developing site-specific biocontrol strategies tailored to particular environmental conditions. Such approaches may improve establishment, persistence, and efficacy under field conditions compared to the use of a single universal strain.

Future research should focus on evaluating the antagonistic activity, plant growth-promoting potential, stress tolerance, and field performance of the identified *Trichoderma* species and strains. Multi-gene phylogenetic analyses and whole-genome approaches could further improve understanding of genetic diversity, adaptive evolution, and functional traits associated with ecological fitness. In addition, studies investigating interactions between *Trichoderma*, rice plants, soil microbiomes, and changing environmental conditions would provide valuable insights for optimizing their use in sustainable crop production. The integration of

ecological, molecular, and functional approaches will be essential for identifying elite strains and developing effective bioinoculants capable of supporting resilient and environmentally friendly rice production systems.

Declarations

Ethical approval: This study did not involve human or animal subjects.

Consent to participate: Not applicable.

Consent to publish: All authors have read and approved the manuscript and consent to its publication

Data availability: The datasets generated and/or analysed during the current study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the accession number SUB26352134. The data can be accessed at https://submitt.ncbi.nlm.nih.gov/subs/genebank/

Competing interests: The authors declare no competing interests

Funding: The authors declare that no funds, grants, or other support were received

Clinical trial number: not applicable

AUTHORSHIP CONTRIBUTION STATEMENT

NFN: Conceptualization, methodology, investigation, data curation, formal analysis, visualization, and writing the original draft. **RRM**: Conceptualization, formal analysis, Supervision, writing reviews, and Editing. **NLK**: Conceptualization, formal analysis, Supervision, writing reviews and Editing

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Appendices

Appendix A. Trichoderma isolates' morphological characteristics

Zone	Isolate code	Colony color	Reverse color	Mycelial texture	Conidiation	Growth form
Alluvial	KL1-1	Yellow green	Reddish	Floccose	Zonation	Circular

Alluvial	KL2-4	Yellow green	Reddish	Floccose	Zonation	Circular
Alluvial	KL6-2	Yellow green	Reddish	Floccose	Zonation	Circular
Alluvial	KL7-4	Yellow green	Reddish	Floccose	Zonation	Circular
Alluvial	MS2-3	Yellow green	Creamy	Floccose	Zonation	Circular
Alluvial	MS3-3	Light green	Light green	Floccose	Zonation	Circular
Alluvial	MS3-4	Light green	Light green	Floccose	Zonation	Circular
Alluvial	MS4-3	Yellow	Creamy	Floccose	Effuse	Circular
Alluvial	MS5-1	Yellow green	Creamy	Floccose	Zonation	Circular
Alluvial	MS5-3a	Yellow green	Creamy	Floccose	Zonation	Circular
Alluvial	MS6-3	Yellow green	Creamy	Floccose	Effuse	Circular
Alluvial	MS8-4	Light green	Light yellow	Floccose	Effuse	Circular
semi-arid	MR2-2	Yellow green	Creamy	Floccose	Effuse	Circular
semi-arid	MR2-3	Yellow green	Creamy	Floccose	Effuse	Circular
semi-arid	MR2-4	Yellow green	Creamy	Floccose	Effuse	Circular
semi-arid	MR4-3	Yellow green	Creamy	Floccose	Effuse	Circular
semi-arid	MR5-1	Green	Creamy	Floccose	Zonation	Circular

semi-arid	MR5-2	Green	Creamy	Floccose	Zonation	Circular
semi-arid	MG1-2	Yellow green	Creamy	Floccose	Effuse	Circular
semi-arid	MG2-3	Yellow green	Creamy	Floccose	Effuse	Circular
semi-arid	MG3-1	Yellow green	Creamy	Floccose	Effuse	Circular
semi-arid	MG7-2	Yellow green	Creamy	Floccose	Effuse	Circular
semi-arid	KH3-2	Yellow green	Creamy	Floccose	Zonation	Circular
semi-arid	KH5-1	Green	Creamy	Floccose	Effuse	Circular
semi-arid	KH5-2	Green	Creamy	Floccose	Effuse	Circular
semi-arid	KH6-1	Whitish	Tumeric brown	Floccose	Effuse	Circular
semi-arid	KH7-1	Light green	Light yellow	Floccose	Zonation	Circular
Central	BH1-2	Yellow green	reddish	Floccose	Zonation	Circular
Central- plateaux	BH2-3	Yellow green	Yellow	Floccose	Zonation	Circular
Central- plateaux	BH3-1	Yellow green	Yellow	Floccose	Zonation	Circular
Central- plateaux	BH4-1	Yellow green	Yellow	Floccose	Zonation	Circular
Central- plateaux	BH5-1	Yellow green	Yellow	Floccose	Zonation	Circular
Central- plateaux	BH6-2	Yellow green	reddish	Floccose	Effuse	Circular

Central- plateaux	BH7-2	Yellow to light green	Creamy	Floccose	Effuse	Circular
Central- plateaux	BH8-2a	Whitish	Creamy	Floccose	Effuse	Circular
Central- plateaux	BH8-2b	Whitish	Creamy	Floccose	Effuse	Circular
Central- plateaux	BH8-2c	Whitish	Creamy	Floccose	Effuse	Circular
Central- plateaux	BH10-2	Yellow green	Creamy	Floccose	Effuse	Circular
Southern-western-highlands	SH3-3	Green	Creamy	Floccose	Zonation	Circular
Southern-western-highlands	SH4-4	Green	Creamy	Floccose	Effuse	Circular
Southern-western-highlands	SH5-2	Green	Creamy	Floccose	Effuse	Circular
Southern-western-highlands	SH10-2	Green	Creamy	Floccose	Zonation	Circular
Southern-western-highlands	SH10-3	Green	Creamy	Floccose	Effuse	Circular
Southern-western-highlands	SH11-2	Green	Creamy	Floccose	Effuse	Circular

Southern-western-highlands	SH11-4	Green	Creamy	Floccose	Effuse	Circular
Northern highlands	NH1-2	Green	Creamy	Floccose	Effuse	Circular
Northern highlands	NH2-2	Green	Creamy	Floccose	Zonation	Circular
Northern highlands	NH2-3	Green	Creamy	Floccose	Effuse	Circular
Northern highlands	NH3-3	Green	Creamy	Floccose	Effuse	Circular
Northern highlands	NH4-4	Green	Creamy	Floccose	Zonation	Circular
Northern highlands	NH5-2	Green	Creamy	Floccose	Zonation	Circular
Northern highlands	NH6-3	Green	Creamy	Floccose	Zonation	Circular
Northern highlands	NH7-3	Light green	Creamy	Floccose	Zonation	Filamentous
Northern highlands	NH9-1	Light to dark green	Creamy	Floccose	Zonation	Filamentous
Northern highlands	NH9-2	Light to dark green	Creamy	Floccose	Zonation	Filamentous
coastal	KT2-2	Green	Creamy	Floccose	Effuse	Circular
coastal	KT4-3	Dark Green	Creamy	Floccose	Zonation	Filamentous
coastal	KT4-4	Dark Green	Creamy	Floccose	Zonation	Filamentous

coastal	KT5-2	Dark Green	Green	Floccose	Granular	Circular
coastal	KT5-4	Dark Green	Green	Floccose	Granular	Circular
coastal	KT6-2	Dark Green	Green	Floccose	Granular	Circular
coastal	KT6-4	Dark Green	Green	Floccose	Granular	Circular
coastal	KT7-1	White to light green	Creamy	Floccose	Granular	Circular
coastal	KT7-4	White to light green	Creamy	Floccose	Granular	Circular
coastal	KT8-1	White to green	Creamy to light Green	Floccose	Granular	Circular
coastal	KT8-4	Dark Green	Creamy	Floccose	Zonation	Filamentous
coastal	KT10-2	Dark Green	Creamy	Floccose	Effuse	Circular

Appendix B: Trichoderma species as identified in NCBI

Isolate code	***Species***	**Irrigation scheme**	**NCBI accession number**
KL7-4	*T. harzianum*	Msolwa	ON764877
MS2-3	*T. harzianum*	Msolwa	KX463452
MS3-3	*T. asperellum*	Msolwa	OR225702
MS4-3	*T. harzianum*	Msolwa	OP218352
MS5-3a	*T. harzianum*	Msolwa	OQ002324

MS6-3	*T. harzianum*	Msolwa	OQ422111
MS8-4	*T. harzianum*	Msolwa	MN262500
MR2-4	*T. harzianum*	Kahangara	KC575619
MR5-4	*T. atroviride*	Kahangara	OW984351
MG1-2	*T. harzianum*	Kahangara	OP783882
MG2-3	*T. harzianum*	Kahangara	OQ422111
MG3-1	*T. erinaceum*	Kahangara	KP263654
MG7-2	*T. harzianum*	Kahangara	MN452459
KH3-2	*T. harzianum*	Kahangara	KP263549
KH5-1	*T. harzianum*	Kahangara	KP263731
KH6-1	*T. koningiopsis*	Kahangara	MN509038
KH7-1	*T. harzianum*	Kahangara	MW165410
BH2-3	*T. harzianum*	Bahi	MZ853744
BH3-1	*T. longibrachiatum*	Bahi	MK619474
BH5-3	*T. longibrachiatum*	Bahi	FJ986631
BH6-2	*T. harzianum*	Bahi	0R251008
BH7-2	*T. harzianum*	Bahi	OL953189
BH8-2b	*T. harzianum*	Bahi	MH339871
BH10-2	*T. harzianum*	Bahi	MZ452418
SH3-3	*T. harzianum*	Makwale	LN846719
SH4-4	*T. harzianum*	Makwale	KU317847
SH5-2	*T. harzianum*	Makwale	KP263584
SH10-2	*T. harzianum*	Makwale	KJ191667
SH10-3	*T. harzianum*	Makwale	OM884805
SH11-2	*T. harzianum*	Makwale	KU215923
SH11-4	*T. harzianum*	Makwale	KP263649
NH1-2	*T. harzianum*	Lower Moshi	MT974243

NH2-2	*T. harzianum*	Lower Moshi	MH393369
NH3-3	*T. harzianum*	Lower Moshi	OQ422111
NH4-4	*T. harzianum*	Lower Moshi	MZ044266
NH5-2	*T. harzianum*	Lower Moshi	CS576719
NH6-3	*T. harzianum*	Lower Moshi	KC993075
NH7-3	*T. viren*	Lower Moshi	KF144627
NH9-2	*T. viren*	Lower Moshi	MG551576
KT2-2	*T. harzianum*	Kitere	KT001977
KT4-3	*T. viren*	Kitere	KT599099
KT5-2	*T. harzianum*	Kitere	MT940829
KT6-4	*T. harzianum*	Kitere	PP500628
KT7-1	*T. paraviridescens*	Kitere	MK290845
KT8-1	*T. Pleuroti*	Kitere	KY764877
KT8-4	*T. viren*	Kitere	JQ040400
KT10-2	*T. harzianum*	Kitere	LC530211