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Genetic diversity assessment of *Trichoderma* spp. isolated from various Egyptian locations using its gene sequencing marker, rep-PCR, and their cellulolytic activity

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Abstract

Background The phylogenetic relationships and phylogeny of twenty-six *Trichoderma* species collected from various Egyptian locations were investigated. The genetic diversity among the examined isolates was tested using the rep-PCR marker. *Trichoderma* species were screened for their cellulase activities.

Results Three isolates demonstrated highly significant FPase activities, namely MNF-MAS-Tricho 1, MNF-MAS-Tricho 2, and MNF-MAS-Tricho 3 (0.50, 0.39, and 0.49 IU ml^{-1}, respectively). MNF-MAS-Tricho1 showed the highest significant CMCase activity (0.80 IU ml^{-1}). Concerning β-glucosidase, MNF-MAS-Tricho 1 was the highest (0.78 IU ml^{-1}), while MNF-MSH-Trich 11 and MNF-MAS-Tricho 15 were the lowest (0.36 IU mL^{-1}). The percentage of polymorphism ranged from 46.15 to 83.33%. (GTG)5 marker produced the greatest number of polymorphic loci (13 loci out of 18 loci) with about 83.33% polymorphism, followed by rep-10 with 69.2% polymorphism. Furthermore, the polymorphism information content (PIC) estimates ranged between 0.285 for Rep-10 and 0.340 for (GTG) 5 with an average of 0.306. The tested primers exhibited high discriminating and resolving powers.

Conclusion The findings of this investigation were used to classify *Trichoderma* species, evaluate their genetic variability using ITS sequencing, rep-PCR, and measure their cellulase activities. These markers can facilitate more rapid and less complicated studies of *Trichoderma* population dynamics and evaluate their establishment after release into agricultural environments. The results will help to evaluate the genetic diversity of *Trichoderma* in future research.

Keywords *Trichoderma*, Cellulase activities, Genetic variability, ITS, Polymorphism, Rep-PCR

Background

Providing adequate food for the world's expanding population is one of agriculture's primary objectives, and biotechnology plays a key role in this regard. The huge losses of agricultural products annually represent a serious challenge for all the world (Sari et al. 2021). Biological control refers to the use of biological substances to reduce biological diseases that affect plants and boost agricultural yields (Guzman et al. 2023). *Trichoderma* is an ecologically friendly fungus that also

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functions as a plant growth stimulant and a biological control agent. Through a variety of processes, including hyphal contacts, competitive antibiosis, mycoparasitism, and enzyme release, *Trichoderma* spp. inhibit the development and survival of pathogens. *Trichoderma* fungi are of relevance for biocontrol methods due to their comprehensive arsenal of enzymes, including hemicellulases and cellulases that can break down lignocellulosic biomass (Alshammari et al. 2024).

Cellulase is a mixture of enzymes used for hydrolyzing cellulose and converting lignocellulosic materials into basic sugars. Hydrolysis of the cellulosic material to glucose is thought to require three different enzymes: exo-1,4-beta-glucanase, endo-1,4-beta-glucanase, and beta-glucosidase (Van Dyk and Pletschke 2012). Cellulose is broken down by a combination of endo-1,4- and exo-1,4-beta-glucanases into cello-oligosaccharides, which are then broken down by beta-glucosidase into glucose (Shida et al. 2016). The main supplier of cellulolytic enzymes is now *Trichoderma* spp. (Bharathiraja et al. 2017).

Trichoderma is a widespread genus found in a variety of environments, including soil, forests, and root ecosystems (Hassan et al. 2019). Molecular markers were employed efficiently to exploit genetic diversity and establish phylogenetic relationships. Repetitive-element polymerase chain reaction (rep-PCR) is one of the effective markers in resolving species differences among microbial species (Kaur et al. 2017). Rep-PCR is used to differentiate between strains from each other due to its ability to detect qualitative differences between nucleotides between different isolates. This is because rep-PCR uses oligonucleotide primers complementary to repetitive sequences spread throughout the genome of living organisms. The result of amplicon differences was found in specialized locations around genes (Mohapatra et al. 2007). The use of such techniques has also helped in studying environmental diversity, evolutionary relationships, and distinguishing between genetically close species (Rai et al. 2015).

The present study aimed to assess the efficiency of rep-PCR markers in addressing the *Trichoderma* genetic diversity between 26 *Trichoderma* isolates obtained from various Egyptian locations and to detect their degrading activities of lignocellulosic substances.

Methods

Samples and isolate collection

A total of 26 isolates were gathered from different locations in Egypt and identified based on ITS sequencing (Table 1) (El-Sobky et al. 2019). Rep-PCR primer sequences are presented in Table 2.

Evaluation of cellulase activity

Quantitative cellulase activity was achieved according to Zhang (2010) and Pandey et al. (2014), and *Trichoderma* isolates were tested for cellulase production by growing in 250 ml flasks containing 50 ml of Mandel's and Reese medium containing 1% Avicel (Sigma, Germany) (Martínez et al. 2021). Cellulase activity is measured as (FPase, CMCase and β-glucosidase) from extracellular protein and free sugar produced from *Trichoderm*a isolates in Mandel's Media (Zhang 2010).

Evaluation of cellulase activity

Mandel's and Reese medium cultures' supernatants were used to quantify the amount of cellulase activity. For the production of cellulase, conidial spores of each strain (10^5 spores/ml) were propagated in flasks (250 ml), including 50 ml of Mandel's and Reese medium containing 1% Avicel. An 8-day incubation period was determined in the flasks using a rotary shaker set at 28 °C and 85 r/min. Cellulase activity was measured from the supernatants obtained by filtering and centrifuging the cultures for 20 min (at 11,000 g, 4 °C) after the incubation time (Pandey et al. 2014). Cellulase activity is measured as FPase, CMCase, and β-glucosidase from extracellular protein and free sugar produced in (SmF) cultures of *Trichoderm*a isolates in Mandel's Media with Avicel 1% according to Ghose (1987); Zhang (2010).

Genomic DNA extraction

The Norgen Plant/Fungi DNA Isolation Kit (Sigma, Thorold, Canada) was utilized for DNA extraction from *Trichoderma* isolates after incubating them for 5 Days at 28 °C on Capek Dox broth as previously reported by Hassan et al. (2019).

ITS markers-based analysis and phylogenetic reconstruction

Reconstruction of the phylogenetic tree among 26 *Trichoderma* isolates was performed using the neighbor-joining method as implemented in MEGA 11 (Tamura et al. 2021). Bootstrapping was used to determine the relative robustness of each tree branch, generating 1000 bootstrapped trees from the resampled data.

Rep markers efficiency and genetic diversity assessment

The PCR settings for the *Trichoderma* isolates used in this research were standardized in order to facilitate repetitive sequence analysis. Using six repeating sequence primers (Table 2), the genomic DNA of the *Trichoderma* isolates was amplified according to Mazrou et al. (2020). The presence or absence of a

Table 1 The NCBI BLAST query for *Trichoderma* isolated from different location in Egypt

Isolates	Species	Query coverage %	E value	Ident %	Accession number
MNF-MAS-Tricho1*	*Trichoderma harzianum*	100.00	0.00	100.00	MH688753
MNF-MAS-Tricho 2*	*Hypocrea lixii/Trichoderma harzianum*	100.00	0.00	99.00	MH688857
MNF-MAS-Tricho 3*	*Trichoderma longibrachiatum*	100.00	0.00	99.00	MH707326
MNF-MAS-Tricho 4*	*Trichoderma harzianum*	99.00	0.00	99.00	MH712434
MNF-MAS-Tricho 5*	*Hypocrea lixii/Trichoderma harzianum*	100.00	0.00	99.00	MH697665
MNF-MAS-Tricho 6*	*Trichoderma harzianum*	100.00	0.00	100.00	MH699073
MNF-MAS-Tricho 7*	*Trichoderma asperellum*	99.00	0.00	100.00	MH688914
GIZ-MAS-Tricho 8[1]	*Trichoderma sp.*	99.00	0.00	99.00	OR805522
MNF-MAS-Tricho 9*	*Trichoderma harzianum*	100.00	0.00	99.00	MH702379
MNF-MAS-Tricho 10*	*Trichoderma harzianum*	99.00	0.00	100.00	MH697404
MNF-MAS-Tricho 11*	*Hypocrea lixii/Trichoderma harzianum*	100.00	0.00	100.00	MH697405
MNF-MAS-Tricho 12*	*Hypocrea lixii/Trichoderma harzianum*	100.00	0.00	99.00	MH697536
MNF-MAS-Tricho 13*	*Hypocrea lixii/Trichoderma harzianum*	99.00	0.00	99.00	MH697554
MNF-MAS-Tricho 14*	*Trichoderma sp.*	99.00	0.00	99.00	MH697555
MNF-MAS-Tricho 15*	*Trichoderma sp.*	100.00	0.00	99.00	MH697561
MNF-MAS-Tricho 16	*Hypocrea lixii/Trichoderma harzianum*	100.00	0.00	100.00	MH697561
MNF-MAS-Tricho 17*	*Trichoderma harzianum*	99.00	0.00	100.00	MH697573
MNF-MAS-Tricho 18*	*Hypocrea lixii/Trichoderma harzianum*	99.00	0.00	99.00	MH697572
MNF-MAS-Tricho 19*	*Hypocrea lixii/Trichoderma harzianum*	100.00	0.00	99.00	MH697574
MNF-MAS-Tricho 20*	*Trichoderma harzianum*	100.00	0.00	100.00	MH697684
MNF-MAS-Tricho 21[1]	*Trichoderma harzianum*	99.00	0.00	100.00	OR794113
MNF-MAS-Tricho 22[1]	*Trichoderma harzianum*	99.00	0.00	99.00	OR794112
MNF-MAS-Tricho 23*	*Trichoderma harzianum*	100.00	0.00	99.00	MH697609
GIZ-MAS-Tricho 24[1]	*Trichoderma harzianum*	100.00	0.00	100.00	OR793989
ASI-MAS-Tricho 25[1]	*Trichoderma harzianum*	99.00	0.00	100.00	OR793988
ASI-MAS-Tricho 26[1]	*Trichoderma harzianum*	99.00	0.00	99.00	OR793967

Whereas, [1]New isolates (Current study), *Old isolates (El-sobky et al. 2019)

Table 2 Primers names and sequences of ITS and rep-PCR markers

Primer name	Primer sequence (5′ → 3′)
ITS1	TCG GTA GGT GAA CCT GCG G
ITS4	CCT CCG CTT ATT GAT ATG C
EF1-728F	CAT CGA GAA GTT CGA GAA GG
EF1-986R	TAC TTG AAG GAA CCC TTA CC
(GTG)5	GTG GTG GTG GTG GTG
BOXA1	CTA CGG CAA GGC GAC GCT
IS-4G	AGAGAGAGAGAGAGAGG
Rep-10	CAG CAC ACA CAC ACA CA
Rep-13	AGA GAG AGA GAG AGA GG
Rep-16	TCT CTC TCT CTC TCT CC

particular band at the rep-PCR genetic marker was used for screening all genotypes. Following genotype screening, GenAlex v6.5 was used to estimate the percentage of polymorphisms. Also, the iMEC program was used to calculate gene diversity. Gene diversity was calculated according to the following formula $[H = 1 - \Sigma P_i^2]$ where p_i is the allele frequency for the i-th allele, and the summation is over all available alleles. Polymorphism information content (*PIC*, Botstein et al. 1980) is calculated according to the following formula $[\ PIC = 1 - \Sigma P_i^2 - \Sigma\Sigma P_i^2 P_j^2]$, where $p_{\ i}$ and $p_{\ j}$ are the population frequency of the i-th and j-th allele. The first summation is over the total number of alleles, whereas the two subsequent summations denote all the i and j where $i \neq j$. Discriminating power (D) is calculated according to the following formula$D = 1 - C$, For the i-th pattern of the given j-th primer, present at frequency p_{i} in a set of varieties, the confusion probability is $C = \Sigma\ c_{\mathrm{i}} = \Sigma\ p_{\mathrm{i}}\ \frac{Npi-1}{N-1}$, where for N individuals, C is equal to the sum of all c_{i} for all of the patterns generated by the primer. Resolving power (R) calculated according to the following equation: $\mathrm{R} = \Sigma \mathrm{I}_{\ b}$, where $I_{\ \mathrm{b}}$ or band informativeness is represented on a scale of 0–1 and is defined as $I_{\ b} = 1 - [2 \times |0.5 - p|]$,

where p is the portion of the samples containing the observed band. *PIC* measures the ability of a marker to detect polymorphisms and consequently has substantial importance in selecting markers for genetic studies (Serrote et al. 2020). On the other hand, D is the likelihood that two randomly selected individuals would have distinct banding patterns and can thus be distinguished from one another. The capacity of a marker to differentiate between genotypes is known as its resolving power.

Rep markers-based phylogenetic analysis

Phylogenetic analysis based on the Unweighted Pair Group Method utilizing the arithmetic average (UPGMA) was carried out to ascertain the genetic relationships among the isolates of *Trichoderma*. This technique was run using the SAHN (sequential, hierarchical, agglomerative, and nested clustering) process developed in NTSYS-pc software version 2.0 (Exeter Software, Setauket, NY) (Roldán-Ruiz et al. 2000). Jaccard's coefficient was used because it is the most appropriate coefficient for dominant molecular markers. To illustrate the degree of genetic similarity among isolates and among sampling sites, two different heat maps were generated based on genetic distance.

Data analysis

Determination of significant differences was calculated through the utilization of the least significant difference test (also known as the Duncan Test), employing a statistical analysis system computer program developed by IBM Corp. in 2017, specifically IBM SPSS Statistics for Windows, Version 25.0, located in Armonk, NY. Within all tables, mean values that exhibit statistical significance are designated with distinct letters, denoting their significance at $P < 0.05$.

Results

Evaluation of cellulase activity

It is known that the complete hydrolysis of cellulose requires the cooperation of exoglycanases, endoglucanases, and beta-glucosidase enzymes. Hence, cellulase activities (FPase, CMCase, and β-glucosidase) of all isolates were evaluated in Mandel's media supplemented with 1% Avicel as sole carbon source under submerged fermentation conditions as presented in Table 3.

For FPase, activity, the isolates MNF-MAS-Tricho1, MNF-MAS-Tricho 3, and MNF-MAS-Tricho 2 exhibited the highest activity with 0.50, 0.49, and 0.39 (IU ml^{-1}), respectively. Meanwhile, MNF-MAS-*Tricho* 16 and MNF-MAS-*Tricho*17 showed the lowest FPase activity (0.24 IU ml^{-1}). Regarding CMCase activity, MNF-MAS-*Tricho*1, MNF-MAS-*Tricho* 4, and MNF-MAS-*Tricho* 2 showed the highest activity with 0.80, 0.70 and 0.62 (IU ml^{-1}), respectively. In addition, the lowest CMCase activities were recorded for the isolates MNF-MAS-*Tricho* 8 and MNF-MAS-*Tricho*24 (0.44 IU ml^{-1}). Concerning β-glucosidase activity, the isolate MNF-MAS-*Tricho* 1 was the highest (0.78 IU ml^{-1}), while MNF-MSH-*Tricho*11 and MNF-MAS-*Tricho15* were the lowest (0.36 IU ml^{-1}).

Table 3 Total cellulase enzymes activity (FPase, CMCase and β-glucosidase) produced in (SmF) cultures of *Trichoderma* isolates grown in Mandel's media with Avicel 1%

Isolates	Enzymes activities (IU mL^{1})		
	Fpase	CMCase	β-Glucosidase
MNF-MAS-Tricho1	0.50^{k*}	0.80^{j}	0.78^{k}
MNF-MAS-Tricho2	0.39^{j}	0.62^{h}	0.55^{i}
MNF-MAS-Tricho3	0.49^{k}	$0.55^{e,f,g}$	0.51^{h}
MNF-MAS-Tricho4	$0.30^{e,f}$	0.70^{i}	0.53^{hi}
MNF-MAS-Tricho5	$0.30^{e,f}$	$0.49^{a,b,c,d}$	$0.41^{c,d}$
MNF-MAS-Tricho6	0.36^{h}	0.56^{g}	$0.43^{d,e}$
MNF-MAS-Tricho7	0.33^{g}	0.55^{efg}	0.61^{j}
GIZ-MAS-Tricho8	$0.25^{a,b}$	0.44^{a}	$0.41^{b,c,d}$
MNF-MAS-Tricho9	$0.27^{b,c,d}$	$0.51^{c,d,e}$	$0.40^{a,b,c,d}$
MNF-MAS-Tricho10	$0.30^{e,f}$	$0.48^{a,b,c}$	0.50^{g}
MNF-MAS-Tricho11	$0.28^{c,d,e}$	$0.47^{a,b}$	0.36^{a}
MNF-MAS-Tricho12	$0.29^{d,e,f}$	0.45^{a}	$0.45^{e,f}$
MNF-MAS-Tricho13	$0.28^{c,d,e}$	$0.46^{a,b}$	0.51^{h}
MNF-MAS-Tricho14	$0.27^{b,c,d}$	$0.47^{a,b}$	$0.37^{a,b}$
MNF-MAS-Tricho15	$0.28^{c,d,e,f}$	0.45^{a}	0.36^{a}
MNF-MAS-Tricho16	0.24^{a}	0.46^{a}	$0.41^{c,d}$
MNF-MAS-Tricho17	0.24^{a}	$0.49^{a,b,c,d}$	$0.37^{a,b}$
MNF-MAS-Tricho18	0.33^{g}	$0.48^{a,b,c,d}$	$0.47^{f,g}$
MNF-MAS-Tricho19	$0.26^{a,b,c}$	0.46^{a}	0.61^{j}
MNF-MAS-Tricho20	$0.25^{a,b}$	$0.50^{b,c,d}$	$0.53^{h,i}$
MNF-MAS-Tricho21	0.44^{j}	$0.52^{d,e,f}$	$0.47^{f,g}$
MNF-MAS-Tricho22	$0.26^{a,b,c,d}$	$0.51^{c,d,e}$	$0.38^{a,b,c}$
MNF-MAS-Tricho23	0.30^{f}	$0.54^{e,f,g}$	$0.53^{h,i}$
GIZ-MAS-Tricho24	$0.25^{a,b}$	0.44^{a}	$0.41^{b,c,d}$
ASI-MAS-Tricho25	0.33^{g}	$0.50^{b,c,d}$	$0.38^{a,b,c}$
ASI-MAS-Tricho26	$0.27^{b,c,d}$	$0.47^{a,b}$	$0.37^{a,b}$

* Within columns, values with the same letter do not differ significantly *(P < 0.05)*

ITS markers-based analysis and phylogenetic reconstruction

Among the 26 *Trichoderma* isolates, three were the most frequent, including *Hypocrea lixii*/ *T. harzianum*, *T. harzianum*, and *Trichoderma* sp. The average sequence length and average % GC content varied among these frequent isolates, and the wide range of sequence length was the highest in *Trichoderma* sp. compared to *Hypocrea lixii*/*T. harzianum*, *T. harzianum* (Fig. 1a). On the other

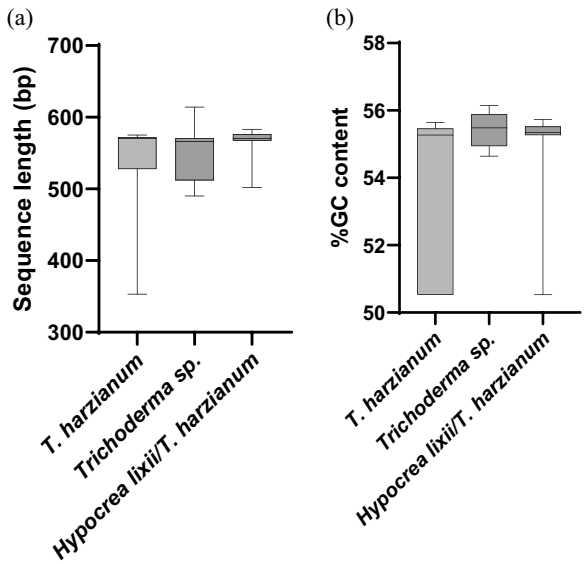

Fig. 1 Average sequence length (bp), (**a**) and average percent of GC content (**b**) of the most frequent *Trichoderma* species

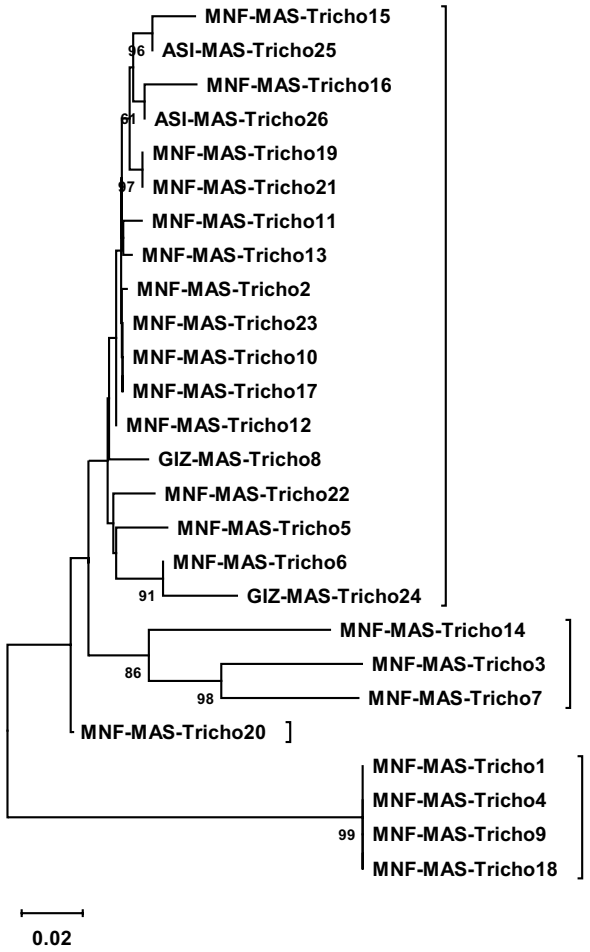

Fig. 2 Phylogenetic tree of 19 *Trichoderma* isolates based on ITS sequence using the neighbor-joining method. Bootstrap values > 50% are shown below branches

hand, the % GC content showed a wide range in *T. harzianum* (Fig. 1b), while *Trichoderma* sp. showed the highest average %GC content compared to *Hypocrea lixii*/*T. harzianum* and *T. harzianum*.

The phylogenetic among the 26 isolates revealed the presence of four main groups (Fig. 2). The first group included most of the isolates and included Tricho 2, 5, 6, 8, 10, 11, 12, 13, 15, 16, 17, 19, 21, 22, 23, 24, 25, and 26. The second group included Tricho 7, 3, and 14, while the third group harbored Tricho 1, 4, 9, and 18. However, Tricho 20 formed one distinct group.

Genetic diversity of trichoderma isolates based on rep markers

Among the 26 *Trichoderma* isolates, three were the most frequent, including *Hypocrea lixii*/*T. harzianum*, *T. harzianum*, and *Trichoderma* sp. The average sequence length and average % GC content varied among these frequent isolates, the wide range of sequence length was the highest in *Trichoderma* sp. compared to *Hypocrea lixii*/*T. harzianum*, *T. harzianum* (Fig. 1a). On the other hand, the % GC content showed a wide range in *T. harzianum* (Fig. 1b), while *Trichoderma* sp. showed the highest average % GC content compared to *Hypocrea lixii*/*T. harzianum* and *T. harzianum*.

The phylogenetic tree revealed the presence of four main groups (Fig. 2). The first group included isolates Tricho10, 15, 19, 21, 25, and 26. The second group included Tricho7, 12, 16, and 17, while the third group included Tricho 5, 6, 11, and 22. The fourth group harbored Tricho1, 4, 9, and 18. However, Tricho14 branched out of the tree as the most distinct isolate.

Genetic diversity of trichoderma isolates based on rep markers

Ten rep primers were employed to amplify the genomic DNA of the *Trichoderma* isolates, and six of these primers ((GTG) 5, BOXA1, IS-4G, rep-10, rep-13, and rep-16) that presented strong band resolution were chosen for the present study. The primers generated 84 rep-PCR bands, and the size of the amplicons ranged from 100 to 3200 bp (Fig. 3). The primer (GTG) 5 produced the maximum number of bands (18 bands), and 13 of these bands were polymorphic (83.33%), whereas the primer rep-13 produced the minimum number of bands (12 bands), and out of these bands 8 were polymorphic (66.66%). The polymorphism percentages ranged from 46.15 to 83.33%, as indicated in Table 4.

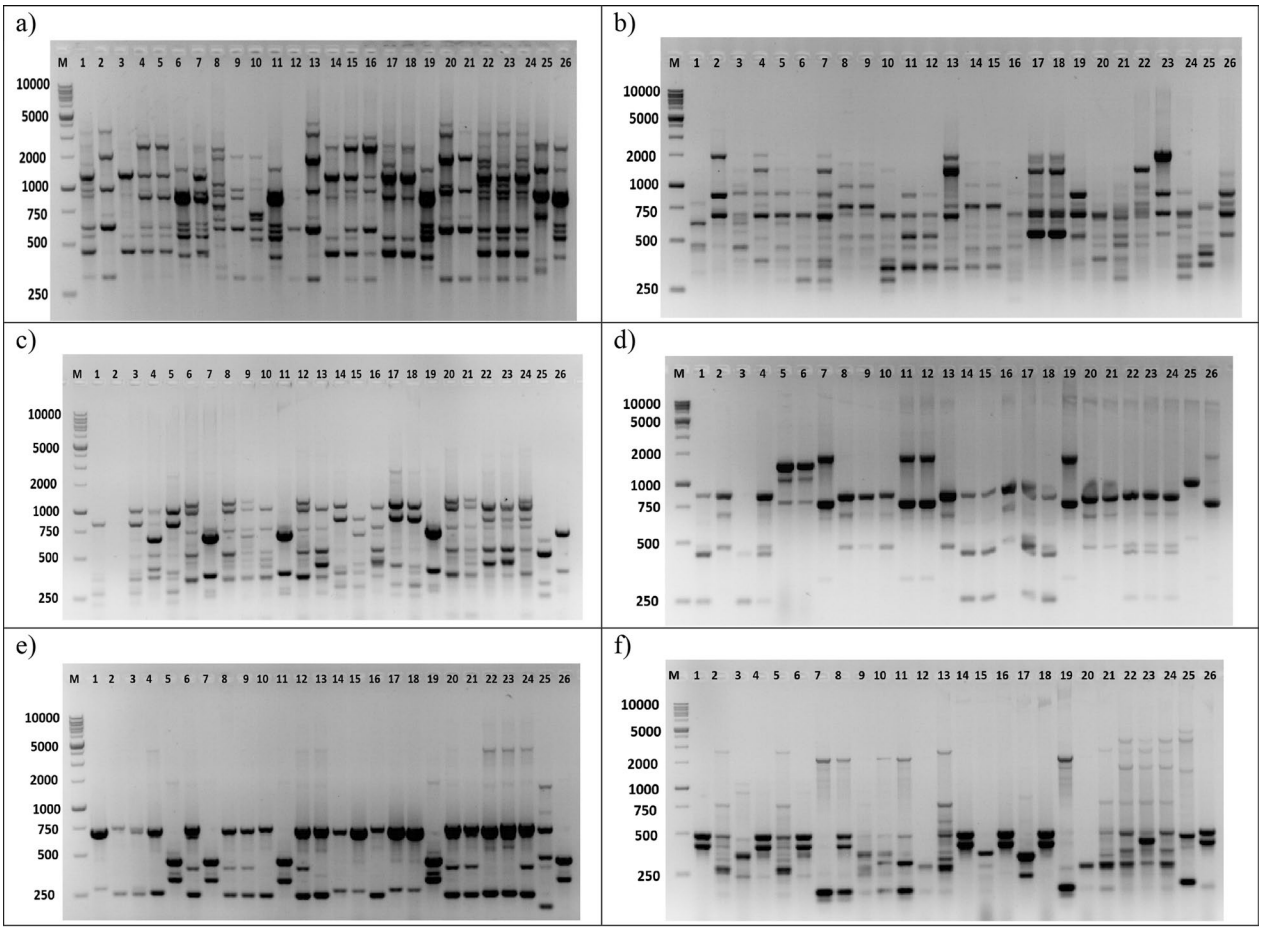

Fig. 3 Rep-PCR profile of 26 *Trichoderma* isolates generated by primers **a** (GTG)5, **b** BOXA1, **c** IS-4G, **d** rep-10, **e** rep-13, and **f** rep-16, whereas M: Positions and sizes of 1 kbp DNA ladder

Table 4 Polymorphism of rep-PCR marker across 26 *Trichoderma* isolates

Primer	Total bands	Mono. (%)	Poly. (%)	No. of alleles	*H*	*PIC*	*D*	*R*
(GTG)5	18	16.67	83.33	19	0.434	0.340	0.899	9.769
Rep-10	13	30.80	69.20	11	0.344	0.285	0.952	4.538
Rep-13	12	33.34	66.66	12	0.355	0.292	0.947	4.000
BoxA1	13	53.85	46.15	12	0.424	0.334	0.908	6.692
Rep-16	15	33.34	66.66	14	0.358	0.294	0.946	6.077
IS-4G	13	38.50	61.50	16	0.355	0.292	0.947	7.231
Mean	14	34.41	65.59	14	0.378	0.306	0.933	6.385

Mono. = *Monomorphism bands; Poly* = *Polymorphism bands; H* = expected heterozygosity; *PIC* = polymorphism information; Content; *D* = discriminating power; and *R* = resolving power

The generated dendrogram based on Jaccard's similarity coefficient divided the *Trichoderma* isolates into two different clusters (Fig. 4). The first cluster contained only *Trichoderma* MNF-MAS-Tricho 25, while the second cluster contained most other *Trichoderma* isolates. The second cluster contained two sub-clusters, and the first one contained *Trichoderma* isolates: MNF-MAS-Tricho 5, MNF-MAS-Tricho 7, MNF-MAS-Tricho 11, MNF-MAS-Tricho 19, and MNF-MAS-Tricho 23. The second sub-cluster contained two groups of *Trichoderma* isolates. The first group contained *Trichoderma* isolates MNF-MAS-Tricho 2, MNF-MAS-Tricho 13,

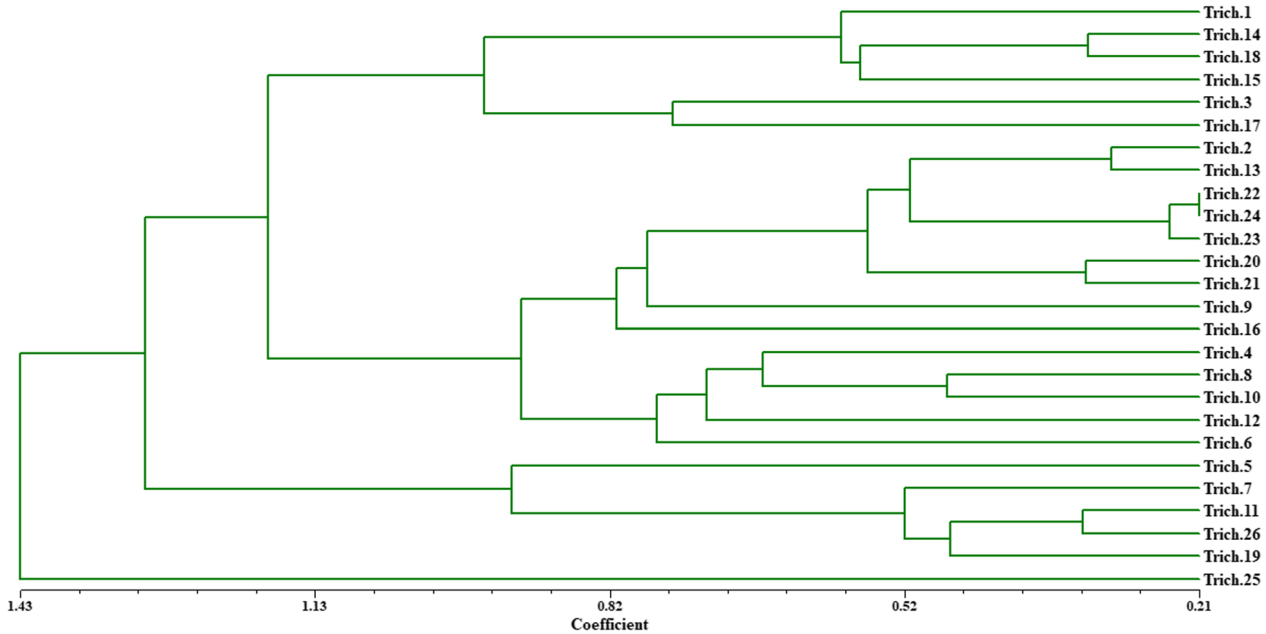

Fig. 4 The dendrogram of 26 *Trichoderma* isolates that generated by six rep-PCR primers

MNF-MAS-Tricho 20, MNF-MAS-Tricho 21, MNF-MAS-Tricho 22, and MNF-MAS-Tricho 23. The second group contained the other *Trichoderma* isolates MNF-MAS-Tricho 1, MNF-MAS-Tricho 3, MNF-MAS-Tricho 4, MNF-MAS-Tricho 6, GIZ-MAS-Tricho 8, MNF-MAS-Tricho 9, MNF-MAS-Tricho 10, MNF-MAS-Tricho 12, MNF-MAS-Tricho 14, MNF-MAS-Tricho 15, MNF-MAS-Tricho 16, and MNF-MAS-Tricho 18.

The rep primers showed a high level of polymorphism among the tested *Trichoderma* isolates. The average number of alleles per locus was 14 and ranged between 11 for Rep-10 and 19 for (GTG) 5 as indicated in Table 4. Moreover, the average of heterozygosity (H) was low (0.378), as anticipated for dominant genetic markers and ranged between 0.434 for (GTG) 5 and 0.355 for Rep-13. Furthermore, the polymorphism information content (PIC) values ranged between 0.285 for Rep-10 and 0.340 for (GTG) 5 with an average of 0.306.

PIC value is considered an efficient parameter to measure the informativeness of a genetic marker. The studied marker was highly informativeness where PIC range is 0.30 to 0.40, and these results are in agreement with those obtained by Roldán-Ruiz et al. (2000). It is worth mentioning that the used primers exhibited high discriminating power (D) and resolving power (R) (Table 4). The discriminating power (*D*) of the studied markers ranged between 0.899 for (GTG)5 and 0.952 for Rep-10 with an average of 0.933, which is considered high discriminating power ($D \geq 50\%$), (Serrote et al. 2020). Concerning the resolving power (*R*), it ranged between 9.769 for (GTG)5 and 7.231 for IS-4G with an average of 6.385.

As sampling was conducted across different geographic sites, it is crucial to address markers' performance across sampling sites. The results showed striking differences in diversity measures across sites. Among the studied sites, Tala and Minuf showed the highest number of bands and band frequency > 50%, (Fig. 5). Moreover, the same two sites showed the highest mean heterozygosity suggesting that samples belong to these sites are more diverse as compared to the remaining sites. Three sites: Sheheen Elkom, Berket Elsabe, and Quweisna, were showed zero heterozygosity, as expected where only one sample per site was collected. On the other hand, Assuit had the highest number of private bands, suggesting that the isolate belongs to this location is unique compared to other sampling sites.

The heat map showing genetic distance among sampling sites is shown in Fig. 6. Four sampling sites Tala, El-Bagour, Al-Sadat, and Minuf formed one block (darker green/red) and showed moderate to low genetic distance. On the other hand, Ashmoun, Assuit, Shebeen, Berket Elsabe, Giza, and Quweisna formed one block (light green) and showed moderate to high genetic distance.

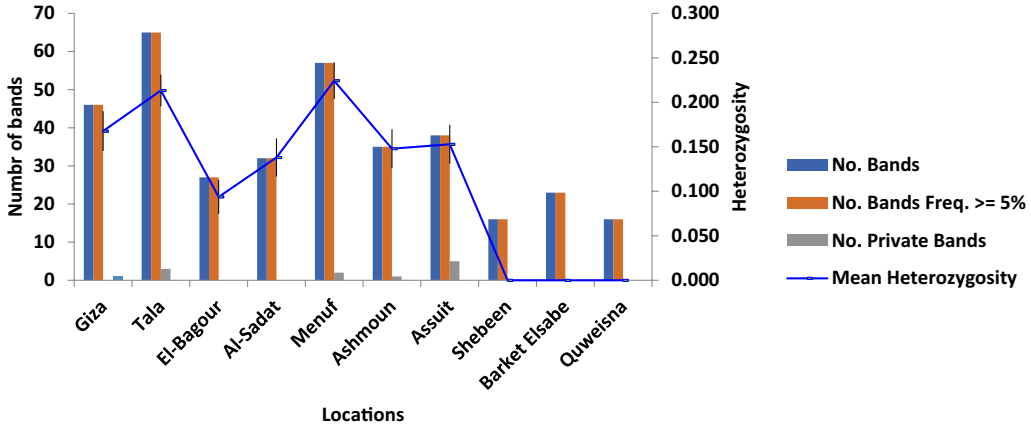

Fig. 5 Band patterns across isolate sampling sites

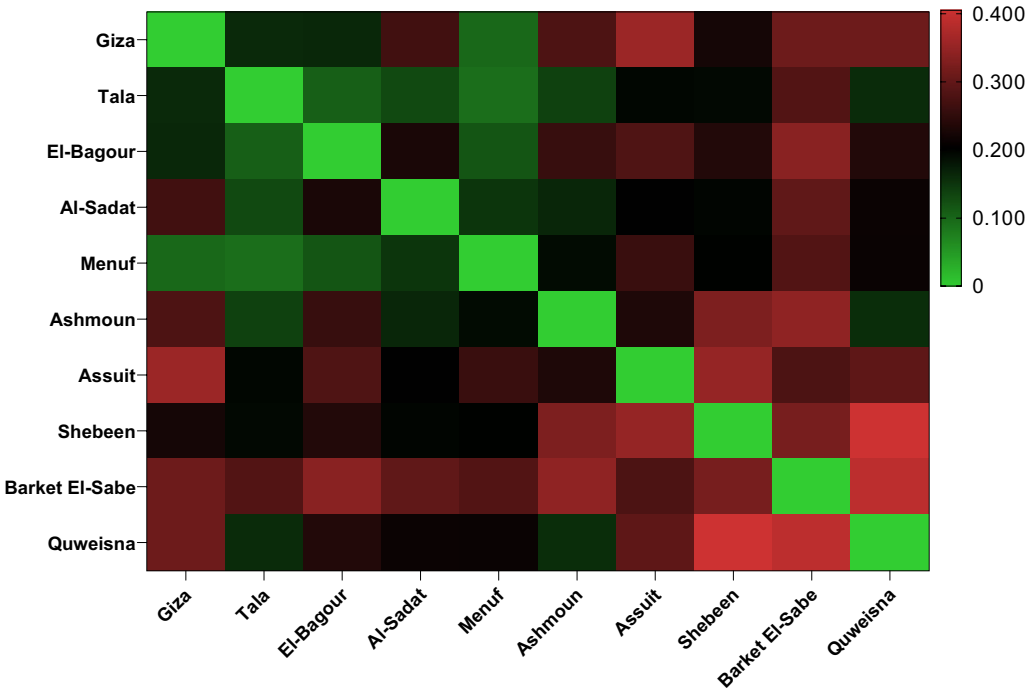

Fig. 6 Heat map representing Nei's genetic distance among 10 *Trichoderma* sampling sites

Discussion

An important function of *Trichoderma* spp. in the contact zone is the inhibition of pathogens through various biocontrol mechanisms, including mycoparasitism, antibiosis, competition for nutrients and sites, metal availability, production of volatile and non-volatile compounds, production of extracellular hydrolytic enzymes, and inactivation of pathogen enzymes (Mazrou et al. 2020). It has been shown that several fungi with conidial density and high growth rates may degrade cellulose more quickly because of the production of the enzyme cellulase such as the genus *Trichoderma*.

Cellulases are enzymes produced by microbes during the hydrolysis process of cellulose, and microbes such as bacteria and fungi are considered good producers of cellulose (Al-Hazmi and Javeed 2016). In general, there are two types of fermentation techniques: solid-state fermentations (SSF) and submerged fermentations (SmF). Both of these techniques have been widely used and studied in cellulase production (Hassan et al. 2019). In this study, twenty-six *Trichoderma* isolates producing

cellulase were measured for their ability to produce cellulase activity using FPase, CMCase and β-glucosidase. It is clear that MNF-MAS-Tricho1, MNF-MAS-Tricho 2 and MNF-MAS-Tricho 3isolates were the most productive. Similarly, it is scientifically proven that the *Trichoderma* fungus is one of the most cellulose-producing species due to its rapid growth and abundance of cells (Hassan et al. 2014). It has also become noteworthy to use the *Trichoderma* fungus in the biological control of many fungal diseases due to the ability of *Trichoderma* to degrade the cell wall of these plant-pathogenic fungi, such as *Pythium* and *Phanerochaete* (Mazrou et al. 2020). The strong ability to respond to diverse environmental signals and the rapid growth of fungi due to the highest density of conidial clusters make *Trichoderma* fungi more effective, or faster, in breaking down the cell wall of other pathogenic fungi through producing cellulolytic enzymes (De Paula et al. 2018). The differences between *Trichoderma* isolates in their activities may be also due to the divergence in their origin, genetic content, and the quantity of cellulase enzymes secreted by the fungus (Ismaiel et al. 2022). This work demonstrates that less costly methods may be used to create cellulolytic enzymes from MNF-MAS-Tricho1, MNF-MAS-Tricho 2 and MNF-MAS-Tricho isolates for future use in laboratories.

Hiett and Seal (2009) employed rep-PCR marker for exploiting the genetic variations of *Trichoderma* isolates. In this study, five rep-PCR primers yielded a total of 179 amplified fragments, and 170 amplicons (94.97%) were polymorphic. Moreover, the dendrogram based on UPGMA cluster analysis differentiated the wild-type from its mutants at 30% similarity level. Our results exhibited high diversity among *Trichoderma* isolates that ranged from 57 to 88%. These results indicated that the rep-PCR is a highly reproducible method for the characterization of fungal species (Hassan 2014).

The use of different methodologies (i.e., Nei's genetic distance, and Jaccard's coefficient) are crucial to address genetic diversity among genotypes. The use of combination of more methods simultaneously helps in a thorough understanding of the similarities among genotypes which previously used in different species (Nemati et al. 2023). In light of these findings, rep-PCR may be used as a reliable marker to uncover genetic diversity in *Trichoderma* isolates.

Remarkably, the geographical-based clustering did not match the isolates geographical origin. This observation was reported before by Rai et al. (2016) who utilized rep-PCR to study the genetic diversity among twenty *Trichoderma* isolates collected from tomato rhizosphere. The lack of distinct clustering in accordance with geographical location is most probably associated with extensive soil/plant movement across the examined agricultural sites. The presence of ASI-MAS-Tricho25 individually supports, in part, this hypothesis, where ASI-MAS-Tricho 25 and ASI-MAS-Tricho 26 collected from Assuit which represent the most far-off collection site. The findings have validated the effectiveness and consistency of rep-PCR as a potent tool for identifying and evaluating the genetic diversity of Egyptian isolates of *Trichoderma* spp.

Conclusion

Trichoderma may be useful as biocontrol agents to reduce disease outbreaks and boost agricultural crop yields. In the present study, MNF-MAS-Tricho1, MNF-MAS-Tricho 2 and MNF-MAS-Tricho 3 were the most promising isolates. They will help to optimize the use of *Trichoderma* spp. for enzyme production in biotechnological industrial uses. Additionally, Rep-PCR was a powerful tool for assessing *Trichoderma* genetic diversity.

Abbreviations

Rep-PCR	Repetitive-element polymerase chain reaction
UPGMA	Unweighted pair group method utilizing arithmetic average.
ITS	Internal transcribed spacer (ITS) region
FPase	Filter-paper cellulase
CMCase	Carboxy-methyl cellulase

Supplementary Information

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Additional file 1. Table (S1). The Trichoderma isolates locations.

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Author contributions

M.A.E. contributed to methodology, formal analysis, investigation, and writing. K.S.A., A.I.F., and A.M.E. contributed to methodology and writing—original draft. M.E.E. contributed to resources, visualization, writing, reviewing, and editing. M.M.H. contributed to visualization, investigation, and supervision. R.A.E. contributed to conceptualization and supervision. All authors read and approved the final manuscript.

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Availability of data and materials

All data and materials are available (Additional file 1: Table s1).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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