Iraqi Journal of Science, 2023, Vol. 64, No. 3, pp: 1119-1128
DOI: 10.24996/ijs.2023.64.3.8

ISSN: 0067-2904

Genetic Diversity of *Trichoderma* and *Hypocrea lixii* Iraqi Strains by Using Random Amplified Polymorphic DNA (RAPD) Technique

Israa Malallah Handel
The Southern Technical University-Technical Institute, Basrah, Iraq

Received: 17/8/2022 Accepted: 15/1/2023 Published: 30/3/2023

Abstract

As the diversity and characteristics of *Trichoderma* species are difficult to determine using morphological methods, henceforth molecular tools are crucial. This study utilized random amplified polymorphic DNA (RAPD) technique to investigate the genetic diversity of *Trichoderma* with sexual phase *Hypocrea* and to identify similarities and differences in the phylogenetic tree. Nine Iraqi *Trichoderma* strains (four strains of *T. atroviride*, one strain of *Hypocrea lixii*, two strains of *T. gamsii* and two strains of *T. longibriantium*) were examined in this research. The genomic DNA of each species was extracted and amplified with each of the five primers. 197 bands were obtained by using five oligodeoxynucleotide primers of which 98.47% were polymorphic and about 1.52% were monomorphic. When primers OPA4, OPA8, and OPA10 were used, the genetic variability was about 100%. Whereas, after using primers OPA7 and OPA5, the obtained genetic variability was 95.7% and 92.6% respectively. Gel images of the RAPD were processed with photo-cap program which detected the bands of isolates based on the ladder. The detected bands were then clustered based on the Jaccard method in Past software that showed *T. atroviride*, *T. gamsii*, *Hypocrea lixii*, *and T. longibrachiatum* isolates were grouped as clades and lineages. Although all strains belonged to the same species and group in one clade, they differed in size and number of bands. The Jaccard cluster analysis showed that the three isolates of *T. atroviride* were closely related to each other, while the four isolates of *T. atroviride* in one cluster were same as *Hypocrea* lixii, the isolates of *T. gamsii* and two strains of *T. longibrachiatum* formed one cluster. Thus, the high reliability of RAPD markers could be applied to identify *Trichoderma* species and create genetic maps instead of other DNA-based methods which are not only costly but time-consuming too.

Keywords: *Trichoderma*, RAPD, *Hypocrea*, Diversity.

التنوع الجيني لسلالات *Trichoderma* و *Hypocrea lixii* العراقية باستخدام تقنية التضخيم الوراثي متعدد الأشكال العشوائي

اسراء مال الله حنظل

الجامعة التقنية الجنوبية ، المعهد التقني ، البصرة ، العراق

الخلاصه

Email: israa.malallah@stu.edu.iq

في هذه الدراسة استخدمت تقنية الحمض النووي متعدد الأشكال المضخم العشوائي (RAPD) للتحقق ومعرفة التنوع الجيني لعزلات *Trichoderma* مع الطور الجنسي *Hypocrea* وتحديد أوجه التشابه والاختلاف في شجرة الوراثية، وذلك بسبب صعوبة تحديد التباين والتنوع لأنواع *Trichoderma* باستخدام الطرق المورفولوجية. في هذا البحث تم فحص تسع سلالات *Trichoderma* عراقية (أربع سلالات من *T. atroviride* ، سلالة واحدة من *Hypocrea lixii* ، سلالتان من *T. gamsii* وسلالتان من *T. longibrantium*). استخرج الحمض النووي الجينومي لكل نوع وتم تضخيمه مع كل من البادئات الخمسة، تم الحصول على 197 حزمة من التضخيم باستخدام الخمسة من البادئات oligodeoxynucleotide منها 98.47 ٪ كانت متعددة الأشكال بينما كان حوالي 1.52 ٪ أحادي الشكل. عند استخدام البادئات OPA4 و OPA8 و OPA10 كان التباين الجيني حوالي 100 ٪ ، بينما باستخدام البادئين OPA7 و OPA5 كان التباين الجيني الذي تم الحصول عليه 95.7 ٪ و 92.6 ٪ ، على التوالي. تمت معالجة الصور الناتجة من التضخيم لتقنية RAPD باستخدام برنامج photocap الذي بدوره بين حزم العزلات بناءً على المعلم، وادخلت هذا الحزم في برنامج Past و بطريقة Jaccard. بينت النتائج ترابط العزلات *T. atroviride* و *T. gamsii* و *Hypocrea. lixii* و *T. longibrachiatum* ضمن شجرة وراثية واحدة على الرغم من أن جميع السلالات تنتمي إلى نفس النوع ، إلا أنها تختلف في الحجم وعدد الحزم. أظهرت نتائج التحليل العنقودي بإستخدام مؤشر جاكار أن العزلات الثلاث لـ *T. atroviride* كانت مرتبطة ارتباطاً وثيقاً ببعضها البعض، في حين أن العزلة الرابعة من *T. atroviride* شكلت عنقوداً مفرداً في الشجرة وكذلك *Hypocrea lixii* والتي شكلت عنقود اخر ، اما عزلات *T. gamsii* فقد ارتبطت معا بنفس العنقود في حين السلالتين لـ *T. longibrachiatum* ارتبطتا كلاهما بعنقود واحد. بالتالي يمكن اعتبار الموثوقية العالية للتقنية RAPD في تحديد أنواع *Trichoderma* وإنشاء خرائط وراثية، بدلا عن استخدام الطرق المعتمدة على الDNA التي تكون مكلفة وتستغرق وقت اطول.

1. Introduction

Trichoderma species are widespread in soil, forest and root ecosystems, and have a variety of applications, including controlling diverse soil-borne pathogens, enhancing plant growth, inducing plant resistance, producing enzymes and antibiotics, and decomposing wastewater sludge [1], [2]. It is monophyletic that has the genus *Hypocrea* as a teleomorphsim [3]. Genus *Trichoderma* is considered a natural biocontrol agent for aggressive plant pathogenic fungi that are widely distributed fungus all over the world and occur nearly in all soil [4]. As the biocontrol mechanism of *Trichoderma* includes parasitism, antibiosis, competition and enzyme activity on cell walls, the biofertilizer involves plant growth enhancement, soil nutrient acquisition, and inducing plant defense responses [5]. A well-known biological control mechanism of *Trichoderma* spp. has made it widely used in agricultural applications [6]. During interaction with plants, they create a variety of secondary metabolites with antibiotic properties that induce pathogen inhibition [7]. Furthermore, *Trichoderma* spp. is good competition in the rhizosphere due to its capacity to tolerate a high level of reactive oxygen species (ROS), which then facilitates the activity of cell wall-degrading enzyme (CWDE) against pathogens [8]. This results in *Trichoderma* promoting seed germination and encouraging the plants to grow with more dry mass, greener leaves and increased photosynthesis [9]. The positive effects of

Trichoderma on plants result in increased crop yields and increased plant protection. *Trichoderma* also boosts growth and maintenance of resistance, stimulating primary meristematic tissues in young plant parts through interaction with growth-regulating hormones and iron-chelating compounds [10]. As a result, *Trichoderma* strains can be used to protect and stimulate plants in sustainable agriculture [9], [11].

The taxonomy and identification of *Trichoderma* species have remained problematic and not easy to differentiate morphologically. Hence, the use of the appropriate molecular tool is vital for characterizing them [12]. A molecular tool is now in existence for the fast, accurate and specific detection of *Trichoderma* spp. [2]. Based on the new level of precision offered by molecular techniques, fungi taxonomy is virtually restarted, and unprecedented success with standardization and unification is achieved [13]. These molecular tools include restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA markers (RAPD), sequence analysis of ribosomal DNA, microsatellite markers and coding sequence variation phylogenetic markers [3], [14]. Gautam *et al.* [15] pointed out more recently DNA-based methods in taxonomy, particularly rRNA sequences or rDNA, strain-specific fluorescent oligonucleotides, and the polymerase chain reaction (PCR). Each of these methods has specific applications and advantages [16]. In light of the simplicity, specificity, and sensitivity associated with PCR-based assays, PCR-based methods have found widespread use for identifying pathogens, including *Trichoderma* species [17]. Molecular characterization of the potential biocontrol agents using RAPD-PCR assists in determining their diversity and characteristics [18]. RAPD is valuable for determining the diversity of *Trichoderma* species as it has proven effective in identifying mysterious genomes and requires only minimal amounts of DNA [19]. Furthermore, it is a superior marker because it is more specific and usually detects only a single locus [14]. Therefore, this study invested RAPD-PCR to investigate eight strains of *Trichoderma* with sexual phase *Hypocrea* and produce an accurate identification of similarities and differences through the phylogenetic tree.

2. Material and Methods

2.1 Fungal Cultures

Eight isolates anamorphic and one teleomorphic stage from various sources of the Iraqi environment were used in this study. All isolates belonged to one genus, *Trichoderma*, classified into four species, which are (*T.gamsii, Hypocrea lixii, T. longibrachiatum,* and *T. atroviride*) (Figure 1).

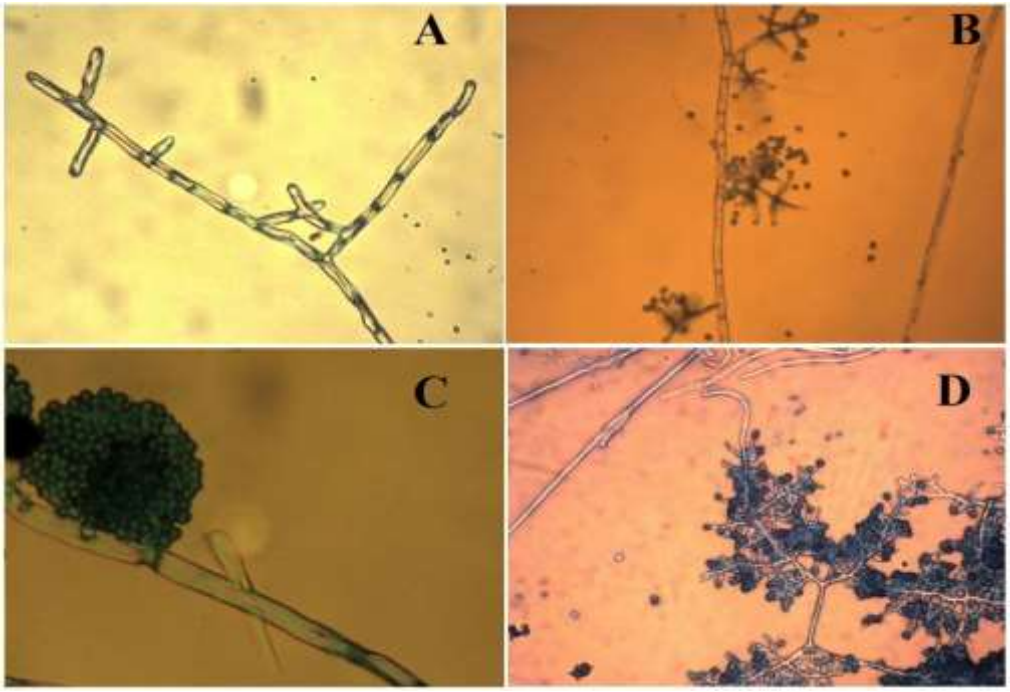

Figure 1: Species under study: (A) *T.gamsii* (B) *T.atroviride* (C) *Hypocrea lixii* and (D) *T.longibrachiatum*

These strains were taxonomic in IMI by author concurrence CABI.org. (Table 1).

Table 1: Samples of *Trichoderma* species strains with IMI identification number [20]

Species Name	Strain	No. In IMI	Species Name	Strain	No. In IMI
T. atroviride	First strain	IMI501472	*T. gamsii*	Second strain	IMI501469
T. atroviride	Second strain	IMI501467	*Hypocrea lixii*	One strain	IMI501470
T. atroviride	Third strain	IMI501466	*T. longibrachiatum*	First strain	IMI502638
T. atroviride	Fourth strain	IMI501762	*T. longibrachiatum*	Second strain	IMI502639
T. gamsii	First strain	IMI501468			

2.2 DNA Extraction and PCR Amplification

Pure cultures of *Trichoderma* isolates were reserved on potato dextrose agar (PDA) slants, mycelia were transferred to flasks of potato – dextrose broth and incubated for 7 days at 25◦C without shaking. Mycelia were harvested by filtration through a piece of filter paper and then washed with distilled water before extracting genomic DNA, according to Hermosa *et al*. [21]. The quality and quantity of DNA were estimated using a NanoDrop spectrophotometer.

For PCR amplification program, five different random primers, OPA-010, OPA-08, OPA-07, OPA-05, and OPA-010 (operon technologies) were used (Table 2). The following PCR program was used with an initial denaturing at 94°C for 7 min, followed by 45 cycles of 94°C for 1 min, 35°C for 2 min, and 72°C for 3 min with a final 7 min extension at 72°C were implemented in a thermocycler (Esco). Amplification products were detected in 1.2 % agarose gel stained with ethidium bromide and visualized on a UV transilluminator. A 100bp ladder and negative control (mixture of reagents as the other samples but without DNA template) were loaded on wells used for comparison.

Table 2: Primers and their sequences used in this study

No.	Primer name	Nucleotide sequence
1	OPA-10	**5'-GTGATCGCAG-3'**
2	OPA-08	**5'-GTGACGTAGG-3'**
3	OPA-07	**5'-GAAACGGGTG-3'**
4	OPA-05	**5'-AGGGGTCTTG-3'**
5	OPA-04	**5'-AATCGGGCTG-3'**

2.3 Cluster Analysis

The RAPD data was used to assess the genetic similarity of isolates using the Jaccard coefficient in Past software which took the RAPD marker value of the photo-cap program and used it to calculate the genetic similarity. Afterward, based on the data, a dendrogram was constructed.

3. Result and Discussion

Five random primers OPA-010, OPA-08, OPA-07, OPA-05, and OPA-04 were used to

show an intra-inter specific variety of *Trichoderma* isolates. These primers generated 197 bands in this study. Fewer bands number were obtained with primer OPA7 showed 23 bands, while using primer OPA4 produced a larger number of bands was 57 bands. A percentage polymorphism was calculated when using OPA10, OPA8 and OPA4 primers were 100% while others were 95.7 % with primer OPA7 and 92.6% with OPA5 (Table 3).

Table 3: The number of RAPD bands, monomorphic bands, polymorphic bands, and polymorphic loci of RAPD primers were seen in nine isolates.

Primer	Total Bands	Monomorphic Bands	Polymorphic Bands	Polymorphic loci (%)	Volume Bands pb
OPA10	39	0	39	100	211-1150
OPA8	50	0	50	100	207-1349
OPA7	23	1	22	95.65	250-644
OPA5	27	2	25	92.59	378-650
OPA4	57	0	57	100	200-1429

Amplification with OPA7 primer generated 23 bands between (250pb – 644pb) and a monomorphic band with value (306 pb) appeared in strain 9 and OPA5 had 27 bands between (378pb – 650pb) with monomorphic in strain 9 (387pb) and 650kb in strain10. OPA10 primer generated 39 bands ranging between (211pb- 1150 pb), OPA8 primer gave 50 bands between (207pb – 1349pb), OPA4 primer generated 57 bands ranged (200pb -1429pb). The results of OPA7 were as followed, iso1, iso2, iso3, and iso4 with value 344pb, iso6, iso7, and iso9 with value 306pb and the value 500pb collected the iso1 and iso5. As well as the primer OPA5 produced the least number of bands connected the least number of isolates like the band with approximation value 338pb connected between iso4, iso7 and iso9, also the value 155pb combined between iso7, and iso9, while the iso1 and iso6 joined with value 293pb. Lastly, the value 650pb was shared between iso2 and iso5. With primer OPA10, the approximation value 356pb was scored in iso4, iso6 iso7 and iso9. Whereas, the approximation value 592pb was found in iso4, iso6, and iso7. Also the iso4, iso7 and iso1 shared the approximation value of 211pb. The primer OPA8 scored the higher number of connected isolates with an approximation value of 271pb for iso2, iso3, iso7, and iso9 and the approximation value 354pb for iso4, iso6, iso7, and iso9. On the other hand, the iso1 iso2 and iso4 had the same approximation value 550pb. The last primer OPA4 also gave a lot of similarities between isolates. The most effective approximation value was 260pb that connected the isolates iso1, iso7, iso8, and iso9 connected also with value 328, followed by approximation value 299pb for iso4, iso7, and iso8. The iso2, iso3, and iso5 shared two approximation values (279pb and 350pb) (Figure 1).

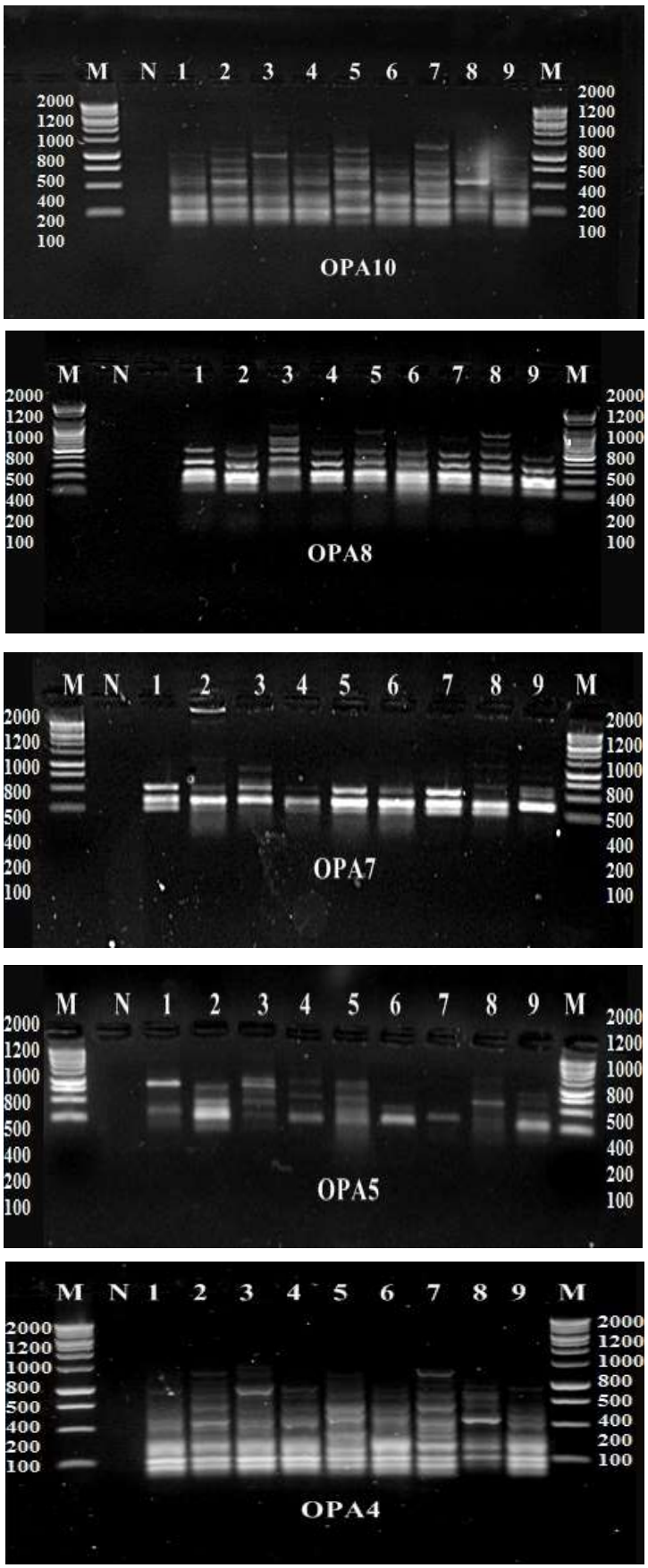

Figure 1: RAPD-PCR profile of the nine *Trichoderma* strains amplified with primers OPA010, OPA08, OPA07, OPA05, and OPA04. Lane M, 100 bp DNA ladder, N, negative control, lane 1-9 nine samples of *Trichoderma* strains.

The RAPD technique proved to be more efficient than other molecular techniques for determining the genetic composition of *Trichoderma*, based on its ability to detect DNA polymorphisms between closely related strains [19]. Besides, RAPD-PCR can identify differences along the entire genome of the fungus, not just in specific regions. As a result, this system helps characterize fungi isolates over a prolonged period [22]. Several studies have evaluated the genetic diversity of *Trichoderma* using RAPD markers. Skoneczny *et al*.[23] collected *Trichoderma atroviride* strains in Poland and used RAPD markers to determine genetic diversity, as well as to identify loci which proved to be useful for assessing genetic variation between strains in this fungus. Lakhani *et al.* [22] have analyzed the genetic variation of two parent strains (*Trichoderma harzianum*, *Trichoderma viride*) and their respective fusants, obtained by protoplast fusion via RAPD. Based on RAPD markers, Abbasi *et al*. [24] examined genetic differences between the mutant and wild types of *T. harzianum*. Wild type has been distinguished from mutants, Th9 from Th17, and also phenotypically superior mutants from other mutants by RAPD analysis. Recently, the RAPD profiles and antagonistic potential of eight strains of *Trichoderma* from five different regions of Egypt have been studied by Hewedy *et al*.[19] to evaluate the genetic diversity among these strains.
Concerning the identification of the similarities and differences through the phylogenetic tree of *Trichoderma*, the relationship among the isolates was evaluated by cluster analysis of data based on the dendrogram that was generated by Jaccard similarity for the results obtained from all nine isolates were grouped into four main clusters (A, B, C, D). Cluster A contained three strains of *T. atroviride* while cluster B contained one strain of *T. atroviride* and the C cluster included one strain represented by *Hypocrea* lixii, the cluster D contained two strains of *T. gamsi* and was linked with two strains of *T. longibrachiatum* which formed one group (Figure 2).

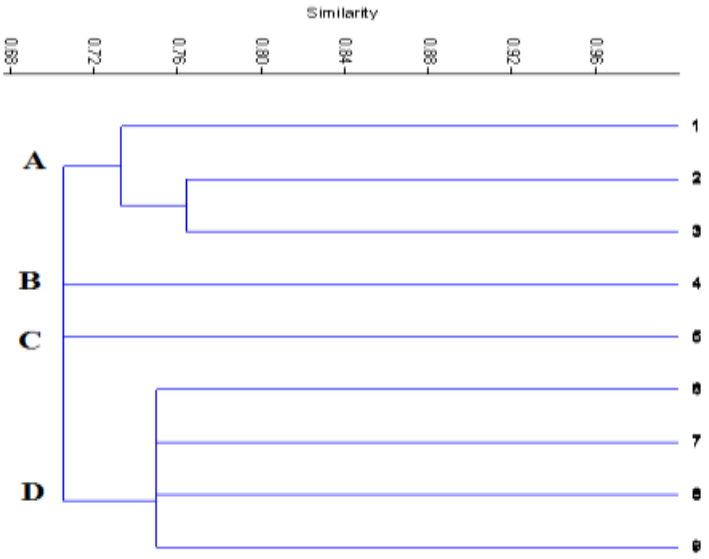

Figure 2: Dendrogram of *Trichoderma* species by using Jaccard similarity method in Past program with 0, 1 data for absence and presence of bands.

The present investigation examined the genetic variability of isolates of *Trichoderma* using the RAPD primers OPA 04, 05, 07, 08, and OPA10. The differences between classification with RAPDP and sequences in detecting DNA polymorphism included the absence or presence of bands resulting from primers due to the ability of linkage or failure to anneal some nucleotide sequences [16]. A set of primers used in the RAPD procedure enabled us to identify each of the examined *Trichoderma* strains based on their unique banding

profiles. The advantage of this technique over morphology-based methods is that it is less time-consuming and only requires small amounts of DNA to be extracted [25]. Primer variants have proven extremely useful for intraspecific discrimination, allowing the differentiation of isolates that are close relatives and belong to the same genus [26]. The results obtained from all nine isolates in the dendrogram were grouped into four main clusters (A, B, C, D). This can be explained by the high genetic variation observed among the isolates of *Trichoderma* combined with the fact that many of these isolates are the result of mutagenesis by ultraviolet light [27]. Consequently, cluster analysis performed by Jaccard showed that the isolates of *T. atroviride* were closely related to each other, and the fourth isolate of *T. atroviride* formed a cluster along with Hypocrea lixii, *T. gamsii* isolates, and two strains of *T. longibrachiatum*. This result was close to the one of García-Núñez *et al*.[28] who used sequences to draw a phylogenetic tree that connected four strains of species *Hypocrea lixii* in one cluster that is connected closely to *T. longibrachiatum*. Besides, studies by Kim *et al*. [29] and Ben Amira *et al*. [30] indicated that *T. harzianum* and *T. atroviride* are closely connected.

4. Conclusion

Five RAPD primers were used to test the level of polymorphism of *Trichoderma* strains. These primers efficiently showed the similarity and variability of the species under study. Although the strains belonged to one species, the discrimination among them was obvious. The RAPD technique proved to be effective for the classification of *Trichoderma* species as well as the identical isolates in the same species. Based on these characteristics, RAPD markers can be considered sensitive, simple, efficient, and powerful tools for genetic diversity analysis between and among *Trichoderma* isolates and could be used to determine the most appropriate isolates for a variety of beneficial uses.

References

[1] Z. X. Zhu and W. Y. Zhuang, "Trichoderma (Hypocrea) species with green ascospores from China," *Persoonia-Molecular Phylogeny Evol. Fungi*, vol. 34, no. 1, pp. 113–129, 2015.

[2] S. H. Lee, H. J. Jung, S.-B. Hong, J. I. Choi, and J.-S. Ryu, "Molecular markers for detecting a wide range of Trichoderma spp. that might potentially cause green mold in Pleurotus eryngii," *Mycobiology*, vol. 48, no. 4, pp. 313–320, 2020.

[3] L. Blaszczyk, M. Siwulski, K. Sobieralski, J. Lisiecka, and M. Jedryczka, "Trichoderma spp.–application and prospects for use in organic farming and industry," *J. plant Prot. Res.*, vol. 54, no. 4, 2014.

[4] Q. Wu *et al.*, "Identification of a novel fungus, Trichoderma asperellum GDFS1009, and comprehensive evaluation of its biocontrol efficacy," *PLoS One*, vol. 12, no. 6, p. e0179957, 2017.

[5] R. Lahlali *et al.*, "Biological control of plant pathogens: A global perspective," *Microorganisms*, vol. 10, no. 3, p. 596, 2022.

[6] N. A. Zin and N. A. Badaluddin, "Biological functions of Trichoderma spp. for agriculture applications," *Ann. Agric. Sci.*, vol. 65, no. 2, pp. 168–178, 2020.

[7] M. Katoch, D. Singh, K. K. Kapoor, and R. A. Vishwakarma, "Trichoderma lixii (IIIM-B4), an endophyte of Bacopa monnieri L. producing peptaibols," *BMC Microbiol.*, vol. 19, no. 1, pp. 1–10, 2019.

[8] S. L. Woo and O. Pepe, "Microbial consortia: promising probiotics as plant biostimulants for sustainable agriculture," *Front. Plant Sci.*, vol. 9, p. 1801, 2018.

[9] S. Haouhach *et al.*, "Three new reports of Trichoderma in Algeria: T. atrobrunneum,(South) T. longibrachiatum (South), and T. afroharzianum (Northwest)," *Microorganisms*, vol. 8, no. 10, p. 1455, 2020.

[10] M. Á. Matas-Baca *et al.*, "Morphological and molecular characterization of a new autochthonous

Trichoderma sp. isolate and its biocontrol efficacy against Alternaria sp.," *Saudi J. Biol. Sci.*, vol. 29, no. 4, pp. 2620–2625, 2022.

[11] N. A. Nafady *et al.*, "Effective and Promising Strategy in Management of Tomato Root-Knot Nematodes by Trichoderma harzianum and Arbuscular Mycorrhizae," *Agronomy*, vol. 12, no. 2, p. 315, 2022.

[12] Z. Haque, M. S. Iqbal, A. Ahmad, M. S. Khan, and J. Prakash, "Molecular Characterization of spp. Isolates by Internal Transcribed Spacer (ITS) Region Sequencing Technique and its Use as a Biocontrol Agent," *Open Biotechnol. J.*, vol. 14, no. 1, 2020.

[13] F. Cai and I. S. Druzhinina, "In honor of John Bissett: authoritative guidelines on molecular identification of Trichoderma," *Fungal Divers.*, vol. 107, no. 1, pp. 1–69, 2021.

[14] M. M. Hassan, M. A. Farid, and A. Gaber, "Rapid identification of Trichoderma koningiopsis and Trichoderma longibrachiatum using sequence-characterized amplified region markers," *Egypt. J. Biol. Pest Control*, vol. 29, no. 1, pp. 1–8, 2019.

[15] A. K. Gautam *et al.*, "Current insight into traditional and modern methods in fungal diversity estimates," *J. Fungi*, vol. 8, no. 3, p. 226, 2022.

[16] R. Saragih *et al.*, "Exploration, identification, and in vitro antagonism test of Trichoderma spp. against Ganoderma spp. at PT Bumitama Gunajaya Agro palm oil plantation, Central Kalimantan," in *IOP Conference Series: Earth and Environmental Science*, 2022, vol. 976, no. 1, p. 12043.

[17] N. Prabhakaran, T. Prameeladevi, M. Sathiyabama, and D. Kamil, "Multiplex PCR for detection and differentiation of diverse Trichoderma species," *Ann. Microbiol.*, vol. 65, no. 3, pp. 1591–1595, 2015.

[18] T. Kamala, S. I. Devi, K. C. Sharma, and K. Kennedy, "Phylogeny and taxonomical investigation of Trichoderma spp. from Indian region of Indo-Burma biodiversity hot spot region with special reference to Manipur," *Biomed Res. Int.*, vol. 2015, 2015.

[19] O. A. Hewedy, K. S. Abdel Lateif, M. F. Seleiman, A. Shami, F. M. Albarakaty, and R. M El-Meihy, "Phylogenetic diversity of Trichoderma strains and their antagonistic potential against soil-borne pathogens under stress conditions," *Biology (Basel).*, vol. 9, no. 8, p. 189, 2020.

[20] I. Handhal, "Taxonomical Morphological and Molecular for some of Trichoderma spp and teleomorph Hypocrea lixii,", Master Dissertation, University of Basrah, 2014.

[21] M. R. Hermosa *et al.*, "Molecular characterization and identification of biocontrol isolates of Trichoderma spp," *Appl. Environ. Microbiol.*, vol. 66, no. 5, pp. 1890–1898, 2000.

[22] H. N. Lakhani, D. N. Vakharia, M. M. Hassan, and R. A. Eissa, "Fingerprinting and molecular comparison among two parental strains of Trichoderma spp. and their corresponding fusants produced by protoplast fusion," *Biotechnol. Biotechnol. Equip.*, vol. 30, no. 6, pp. 1065–1074, 2016.

[23] D. Skoneczny, M. Oskiera, M. Szczech, and G. Bartoszewski, "Genetic diversity of Trichoderma atroviride strains collected in Poland and identification of loci useful in detection of within-species diversity," *Folia Microbiol. (Praha).*, vol. 60, no. 4, pp. 297–307, 2015.

[24] S. Abbasi, N. Safaie, M. Shams-Bakhsh, and S. Shahbazi, "Biocontrol activities of gamma induced mutants of Trichoderma harzianum against some soilborne fungal pathogens and their DNA fingerprinting," *Iran. J. Biotechnol.*, vol. 14, no. 4, p. 260, 2016.

[25] L. Kredics *et al.*, "Molecular tools for monitoring Trichoderma in agricultural environments," *Front. Microbiol.*, vol. 9, p. 1599, 2018.

[26] P. R. B. Filizola, M. A. C. Luna, A. F. de Souza, I. L. Coelho, D. Laranjeira, and G. M. Campos-Takaki, "Biodiversity and phylogeny of novel Trichoderma isolates from mangrove sediments and potential of biocontrol against Fusarium strains," *Microb. Cell Fact.*, vol. 18, no. 1, pp. 1–14, 2019.

[27] R. Belludi, P. S. Sandhu, P. Sharma, and A. S. Sekhon, "Diversity analysis of aflatoxigenic isolates of Aspergillus sp. from major groundnut growing states of India," *Indian Phytopathol.*, pp. 1–10, 2022.

[28] H. G. García-Núñez, Á. R. Martínez-Campos, M. R. Hermosa-Prieto, E. Monte-Vázquez, C. J. Aguilar-Ortigoza, and C. E. González-Esquivel, "Morphological and molecular characterization of native isolates of Trichoderma and its potential biocontrol against Phytophthora infestans," *Rev. Mex. Fitopatol.*, vol. 35, no. 1, pp. 58–79, 2017.

[29] J. Y. Kim, H. W. Kwon, Y. H. Yun, and S. H. Kim, "Identification and characterization of Trichoderma species damaging shiitake mushroom bed-logs infested by Camptomyia pest," *J. Microbiol. Biotechnol.*, vol. 26, no. 5, pp. 909–917, 2016.

[30] M. Ben Amira *et al.*, "Fungal X-Intrinsic Protein Aquaporin from Trichoderma atroviride: Structural and Functional Considerations," *Biomolecules*, vol. 11, no. 2, p. 338, 2021.