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Characterization of *Trichoderma* species isolated from different ecosystems in Myanmar

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ABSTRACT

The identification of *Trichoderma* species, existing in different ecosystems in Myanmar was investigated by using morphology and molecular based techniques. A total of 25 isolates of *Trichoderma* spp. were isolated from the rhizosphere soils collected from different ecosystems. Serial dilution plate technique was used to recover *Trichoderma* spp. from soil samples. On the basis of colony growth rate, the 21 isolates were observed the same growth rate (30 mm d^{-1}) at 28 ±2 °C while the lowest growth rate (27.34 mm d^{-1}) was observed in isolate Tri17-Mmy. All of isolates were classified into three different groups on the basis of their colony characters and the microscopic observations. The macroscopic and microscopic characters of these isolates were compared with the reported literature and confirmed the group-I isolates as *Trichoderma harzianum* (12 isolates), group-II as *Trichoderma viride* (8 isolates) and group-III as *Trichoderma longibrachiatum* (5 isolates). Sequence characterized amplified region (SCAR) markers were used to characterize the isolates by PCR. Among these isolates, 12 isolates of *Trichoderma* species showed a specific band of 220 bp only in *T. harzianum* and 8 isolates also showed a specific band of 990 bp only in *T. viride*.

Keywords: Morphology, molecular characterization, Myanmar, SCAR markers, *Trichoderma*

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1 Introduction

Microorganisms in soil are critical to the maintenance of soil function in both natural and managed agricultural soils because of their involvement in such key processes as soil structure formation, decomposition of organic matter, cycling of carbon, nitrogen, phosphorus and sulphur. Despite the importance of these organisms for ecosystem functioning, relatively little is known about the relationship between plant species composition and the diversity of soil microorganisms (Wardle et al., 1999). Soil microorganisms are mostly saprophytic, thus they use plant exudates or decomposing plant material for food. A reduction in food quantity and a change in food quality caused plant diversity. It encourages activity and diversity of soil microbial communities (Wardle and Lavelle, 1997; Broughton and Gross, 2000; Hooper et al., 2000). Microorganisms like *Trichoderma* also play key roles in suppressing soil borne plant diseases and promoting plant growth (Garbeva et al., 2004). The genus *Trichoderma* is one of the most common genera isolated from soil and generally found widely in agricultural soil, prairie, forest, salt marsh and desert soil in all climates. These general saprophytes are highly interactive in root, soil and foliar environments. They are fast-growing filamentous deuteromycetes which can sporulate abundantly and have been described to constitute up to 3% of the total fungus propagules in forest soil (Harman et al., 2004).

Trichoderma was first described more than two hundred years ago by Pearsoon in 1794 and was later

envisaged into four genera. The identification and characterization of *Trichoderma* was worked out into a monograph (Rifai, 1969), in which this genus was classified into nine aggregates. Kiffer (2011) have recognized a total of thirty-six species under the genus *Trichoderma*. Identification based on morphological characters consent a relatively simple method for classification of *Trichoderma* as genus, but the species perceptions are complex to construe and there is considerable confusion over the application of specific names. The system was based on species morphology and on the concept of species aggregates. Bissett (1991) proposed a revised classification of the genus *Trichoderma* based on key morphological characteristics such as colony color, phialides, conidia and conidiophores that are still used to identify *Trichoderma* species. Most of the *Trichoderma* species have a rapid growth over different substrates with a large number of green conidia; unusually white (Kirk et al., 2001).

Nowadays, molecular techniques are gaining importance in characterization of microbial population. It is said that he taxonomic confirmation of species of the genus *Trichoderma*, based only on morphological markers are limited and of low accuracy, due to the plasticity of its characteristics (Hebert et al., 2003). Therefore, molecular techniques must be combined with adopting a variety of parameters in order to identify species correctly (Druzhinina et al., 2010). These techniques are also not influenced by environment, independent of growth stage and reproducible when compared to conventional methods and helps in characterization of potential biocontrol agents in future. However, Random Amplified Polymorphic DNA (RAPD) techniques have been utilized to generate unique Polymerase Chain Reaction (PCR) products in filamentous fungal species or strains of interest to be converted into species or strain by using specific sequence characterized amplified region (SCAR) markers (Lecomte et al., 2000). SCAR markers differ from RAPD markers in that SCAR primers are designed based on known DNA sequences of the organism of study. This allows for the development of characterization assays to amplify specific fungal DNA in laboratory cultures as well as field samples containing mixed DNA by annealing primers specifically to fungal sequences. Moreover, most of the commercial products of *Trichoderma* strains are exotic strains in Myanmar. The maximum performance of *Trichoderma* isolates could not be obtained due to incompatibility of exotic strains in local use. Collection and identification of native *Trichoderma* strains are very important in order to produce commercially to be successfully integrated into mainstream agriculture and to achieve efficient root colonization. Characterization and identification of *Trichoderma* species have not been investigated extensively in Myanmar. Therefore, the present investigation was taken to identify *Trichoderma* species by morphological characters and to analyze the genetic diversity of *Trichoderma* species from different ecosystems in Myanmar.

2 Materials and Methods

2.1 Collection and identification of *Trichoderma*

This experiment was carried out at Department of Horticulture, Yezin Agricultural University (YAU), during March 2018 to December 2018.

2.1.1 Collection of soil samples

The soil samples were taken from a depth of 10 to 15 cm of top soil from the crop growing field of 24 research stations and Horticulture Section under Department of Agricultural Research (DAR), Yezin, Nay Pyi Taw (Fig. 1). The collected soil samples were sieved (2 mm mesh) to remove gravel and plant debris, then made air dried for 24 h at room temperature.

2.1.2 Isolation and identification

Isolation of *Trichoderma* spp. from soil was done by serial dilution technique. The *Trichoderma* spp. was cultured from soil suspensions (Kumar et al., 2013). Ten grams of each soil sample were taken and added to 90 mL of sterilized distilled water and then, mixed a rotary shaker at 180 rpm for 30 min. Each suspension was serially diluted to obtain dilution factor from 10^{-1} to 10^{31}. From each of dilution, 1 mL of the suspension was taken with the help of a micropipette and transferred into sterilized plates. Rose Bengal Agar (RBA) (Madigan et al., 2009) was poured into the plate seeded with 1 mL of soil suspension. The plates were incubated at room temperature for 7 d. Purification of *Trichoderma* was done and maintained on PDA slant at 10 °C for further use.

2.1.3 Morphological characterization

All the isolates of *Trichoderma* spp. were subjected to morphological characterization based on the characteristic of colony, conidiophores, conidia and phailide. For measuring the radial growth rate, all the 25 isolates of *Trichoderma* were inoculated at the center of 90 mm PDA plates. The mycelium discs (5 mm) was removed from actively growing cultures of each isolate and kept at the centre of petriplate containing PDA and three replications were maintained. The cultures were incubated at 28±2 °C for three days. After 3 d of incubation, linear growth (mm) of *Trichoderma* isolates was recorded. Linear growth measured by averaging three diameters taken from each colony. Average linear growth rate was measured by the following formula (Elad et al., 1981).

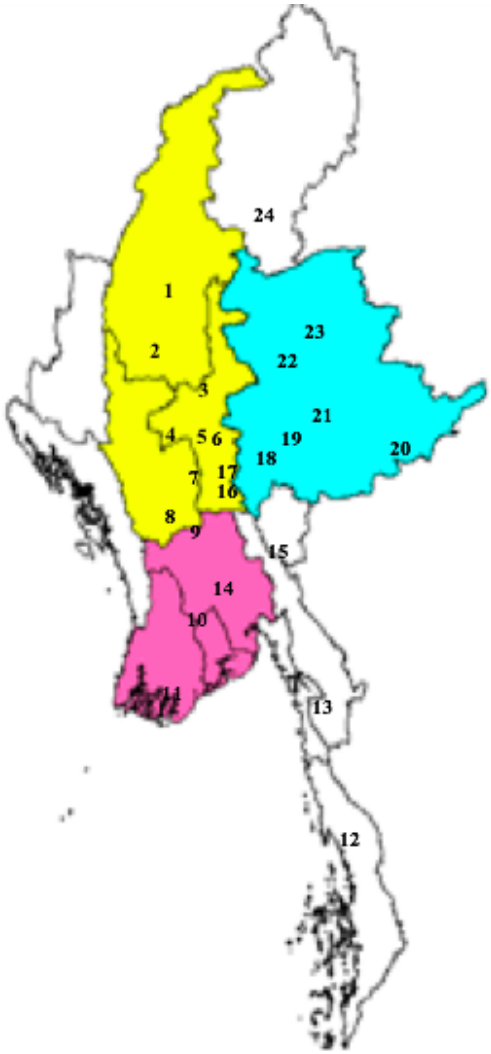

Figure 1. Collection of soil samples in different ecosystem in Myanmar.
1. Pangon,
2. Zaloke,
3. Kyauktada,
4. Myingyan,
5. Kyaukse,
6. Myithar,
7. Nyaungoo,
8. Magway,
9. Kinpondaung,
10. Letpadan,
11. Myaungmya,
12. Dawae (7) miles,
13. Azin-2,
14. Thegon,
15. Loikaw,
16. Tatkon,
17. Sebin,
18. Aungban,
19. Taryaw,
20. Kyineton,
21. Htonebo,
22. Kyaukme,
23. Naung Mon, and
24. Mo Hynin

$$ALGR = \frac{C_3 - C_0}{3} \quad (1)$$

Where, $ALGR$ = average linear growth rate (mm d^{-1}), C_3 = colony diameter after 3 d of inoculation, C_0 = initial colony diameter of inoculation. Colony characteristics were observed at five days after incubation.

2.1.4 Microscopic studies

Microscopic features of isolates such as branching and apex of the conidiophores; disposition and shape of the phialides and shape of the conidia were taken as parameters for differentiation of species. The photographs were taken under ×400 magnification.

2.2 Molecular characterization and assessment of genetic diversity

Molecular characterization and assessment of genetic diversity of *Trichoderma* were carried out at Plant Biotechnology Section, Biotechnology, Plant Genetic Resources and Plant Protection Division, Department of Agricultural Research (DAR), Yezin during January 2019 to June 2019.

2.2.1 Culturing of *Trichoderma* species mycelium

Mycelia of *Trichoderma* species isolates were produced in potato dextrose broth. Erlenmeyer flasks (250 mL) containing 100 mL of potato dextrose broth were inoculated with four pieces of 5 mm myceial discs removed from actively growing monosporic cultures. Cultures were maintained at 28 °C for three days. Mycelial mats were harvested by filtration, washed three times with sterile distilled water and drying in oven at 65 °C for 24 hours. The drying mycelia were kept at −20 °C.

2.2.2 DNA Extraction

Genomic DNA for *Trichoderma* species were extracted from frozen mycelium of *Trichoderma* species based on Cetyltrimethyl ammonium bromide (CTAB) mini extraction method of Cullings (1992) with partial modification. Three grams of the frozen mycelium of each isolate were put into 1.5 mL tube. Before using, DNA

extraction buffer (100 mM TRIS HCL pH 8.0, 1.4 M NaCl, 50 mM EDTA pH 8.0 and 2% CTAB) was pre-heated at 65 °C for 30 min. And then, DNA extraction buffer was added to this and grinded and mixed well and incubated in a water bath at 65 °C by gentle shaking for one hour. After incubation, the mixture was centrifuged at 11000 rpm for 10 min at 25 °C. The supernatant was transferred into 1.5 mL tube and the equal volume of chloroform and Isoamyl alcohol (24:1 v/v) were added and mixed gently for 20 min and were centrifuged at 11000 rpm for 10 min at 25 °C. The upper aqueous phase was taken out and 0.6 volume of Isopropanol and 0.1 volume of 3M Sodium acetate (pH 5.2) were added to it and the mixture were kept overnight at −20 °C. Next day, the samples were centrifuged at 11000 rpm for 10 min at room temperature and the supernatant were discarded and the pellets obtained were washed with 70% ethanol (twice) and dried. After that, TE buffer were added to pellet containing nucleic acid and stored at −20 °C in small aliquots. The nucleic acid was given RNAase treatment to obtain pure DNA suitable for PCR amplification.

2.2.3 Quantification of DNA

The DNA concentration and purity of the samples were determined with Nano Drop Spectrophotometer. Working solutions having 100 ng μL^{-1} were prepared for optimization of PCR reaction.

2.2.4 PCR reactions and electrophoresis

Two primers were used during the PCR method for identifying the *Trichoderma* spp. Table 1. PCR reactions were performed with Bio Rad PCR machine. Each 10μL reaction volume containing amplified products were carried out in a C-1000 Touch™ thermal cycler (Bio Rad, USA) in a volume of 10 μL, containing 1 μL of 10× PCR Buffer (Mg^{2+}), 1 μL of dNTP mixture (TaKaRa), 0.5μL of forward and reverse primer (10 μM), 0.1 μL Taq DNA (TaKaRa), 2 μL of DNA (50 ng μL^{-1}) and 4.9 μL of ddH_2O. The PCR amplification reactions were performed in C1000TM Thermo Cycler Bio-Rad, using the following PCR program for *Trichoderma harzianum* and *Trichoderma viride*: 1 cycle at 94 °C, 5 min; 40 additional cycles consisting of 94 °C for 45 s, 36 °C for 1 min, 72 °C for 2 min and final extension 72 °C for 5 min. Amplified DNA products were analyzed by electrophoresis in 1.2% agarose gel run in TAE buffer. The gels were stained with ethidium bromide (5 μg mL^{-1}). 1 kb ladder (Bangalore Genie Pvt. Ltd, Bangalore) was used as a standard marker. DNA was visualized and photographed by Gel Doc system (UVitec, Cambridge, England).

3 Results and Discussion

3.1 Categorization based on radial growth

There is profuse inter- and intra-specific variability occurs in *Trichoderma*. Papavizas (1985) has stated that different species of *Trichoderma* have their own ecological preferences. However, the species of *Trichoderma* are distributed worldwide. In this study, it is evident that wide occurrence of *Trichoderma* is supported by the fact that it has variable ability for their growth and other functional abilities. Average linear growth rate of different isolates on potato dextrose media was varied significantly difference from each other (Table 2). It was found that the growth rate of 21 *Trichoderma* isolates (30 mm d^{-1}) except Tri2-Ty, Tri17-Mmy, Tri3-Kt and Tri18-Sb were no significant difference from each other. The lowest growth rate (27.34 mm d^{-1}) was observed in isolate Tri17-Mmy which was significantly lower than those of all *Trichoderma* isolates. However, the growth rates of four isolates were significantly different from each other. Based on observations recorded at 3 days incubation period, isolates were observed as medium to fast growing nature and there was presence of noticeable difference in their growth rate (Fig. 2). Cultural characteristics comprising growth rate, colony colour and colony appearance were regarded as taxonomically useful characteristics for *Trichoderma* (Samuels et al., 2002). Studies revealed that all twenty-five rhizosphere isolates differ in cultural characteristics with most isolates exhibiting rapid growth, effuse conidiation and/or loosely arranged conidia in pustules.

3.2 Morphological characterization

Morphological characterization was conventionally used in the identification of *Trichoderma* species, and it remains as a potential method to identify *Trichoderma* species. Three different groups were characterized according to cultural characters and microscopic observations. A total of 12 isolates namely, Tri3-Kt, Tri5-Lk, Tri9-Nm, Tri10-Hb, Tri12-Pg, Tri13-Kbt, Tri14-Az-2, Tri15-Ktd, Tri17-Mmy, Tri18-Sb, Tri20-Mgy and Tri24-Tkh shared common culture characters and were categorized as Group-I. Colony texture was effuse to dense conidiation over the center of colonies then toward the margin. They formed 3-4 concentric rings with dense conidial production, with dark green color and yellow to dull yellowish on reverse side (Fig. 3). Conidiophores were characterized by highly branched divergent and dendritic. Longest branches form near the base of the hypha and nearest the main axis. Branches toward the tip and secondary branches tended to be held at 90° with respect to the axis from which they arise.

Table 1. Sequence of SCAR primers and universal ITS primer used for species identification

Primers	Sequences	Species	bp	References
HAR-5	5′-CTTTTGGTTTGACACGGTTCT-3′ 5′-AAGCTTTGAAGTTGCGAGGA-3′	*Trichoderma harzianum*	220	Parmar et al. (2015)
VIRI-7	5′-TACGCTCCAGGCTACCACTT-3′ 5′-GAGATGAGCTCCTTGCTGCT-3′	*Trichoderma viride*	900	Parmar et al. (2015)

Table 2. Average linear growth rates cultured on Potato Dextrose Agar (PDA) at 3days after incubation period

Isolate codes	Growth rate (mm d^{-1})
Tri1-Ab	30.00a
Tri2-Ty	29.11b
Tri3-Kt	28.09c
Tri4-Mg	30.00a
Tri5-Lk	30.00a
Tri6-Kks	30.00a
Tri7-Mhn	30.00a
Tri8-Kkm	30.00a
Tri9-Nm	30.00a
Tri10-Hb	30.00a
Tri11-Zl	30.00a
Tri12-Pg	30.00a
Tri13-Kbt	30.00a
Tri14-Az-2	30.00a
Tri15-Ktd	30.00a
Tri16-Dw-7	30.00a
Tri17-Mmy	27.34d
Tri18-Sb	27.78cd
Tri19-Tk	30.00a
Tri20-Mgy	30.00a
Tri21-No	30.00a
Tri22-Mt	30.00a
Tri23-Lpd	30.00a
Tri24-Tkh	30.00a
Tri25-Dhor	30.00a
LSD (0.05)	0.27
F-test	**
CV (%)	1.1

Divergent phialides were typically arranged in whorls of 3-5 and held at 90° with respect to the hyphae from which they arose, or solitary. Those in whorls were typically flask-shaped, enlarged in the middle, sharply constricted below the tip to form a narrow neck and slightly constricted at the base (Fig. 4). Conidia shape was globe to subglobe (Fig. 5). Samuels et al. (2002) reported that the characteristic of *Trichoderma harzianum* was white in initially stage and further become dark green in color. They formed 3-4 concentric rings with dense conidial production and in undulating concentric rings toward the edge; no pustules formed. Reverse colony color was yellow to dull yellowish color. Conidiophores were spread to the top and smooth or rounded, wide near the base. Branches toward the tip and secondary branches tended to be held at 90° with respect to the axis from which they arise. Phialides were arising mostly in crowded but had an angle with conidiophore, and had whorls of 2-6 on the terminal branches with respect to the hyphae from which they arose or solitary. It was ampulliform to flask-shaped, arising mostly in crowded but had an angle with conidiophores. Conidia was globe, subglobe to ellipsoidal, no scar, apex broadly rounded, base more narrowly rounded (Samuels et al., 2002). These characteristics of the species *T. harzianum* sect, *Pachybasium* were same as described by Gams and Bissett (1998). In this result, the characters of the isolates in Group-I, were matching with this species as reported by Gams and Bissett (1998) and Samuels et al. (2002). Therefore, Group-I isolates (Tri3-Kt, Tri5-Lk, Tri9-Nm, Tri10-Hb, Tri12-Pg, Tri13-Kbt, Tri14-Az-2, Tri15-Ktd, Tri17-Mmy, Tri18-Sb, Tri20-Mgy and Tri24-Tkh) were *Trichoderma harzianum*.

Similarly, a total of 8 isolates namely, Tri1-Ab, Tri2-Ty, Tri4-Mg, Tri8-Kkm, Tri11-Zl, Tri21-No, Tri23-Lpd and Tri25-Dhor shared common culture characters and were also categorized as Group-II. Colony texture grew as one irregular lawn with diluted and widely spread conidiation. It showed no sporulation around the inoculum point, but dense mycelia toward the edge. Their colony showed dark green to dark bluish green sporulation. Colony reverse was amber or uncoloured (Fig. 3). Conidiophores were usually long, infrequently branched, verticillate. Phialides were frequently paried, lageniform. It was typically arising single directly from the main axis or at the tip of a short lateral branch or in the whorls of 2-3 at the tips of short branches, cylindrical to somewhat swollen in the middle and sometimes with an elongated neck, straight, hooked or sinuous (Fig. 4). Conidia shape was globes to obovoid (Fig. 5). Sekhar et al. (2017) reported that colony color of *Trichoderma viride* showed dark green to dark bluish green sporulation, colony reverse was amber or uncoloured, conidiophore usually long, infrequently branched, verticillate conidiophores. Phialides were frequently paried, lageniform convergent or divergen. Conidial shape was globose to ellipsoidal.

Formation of chlamydospore was infrequent or frequent producing terminally and intercalary. Based

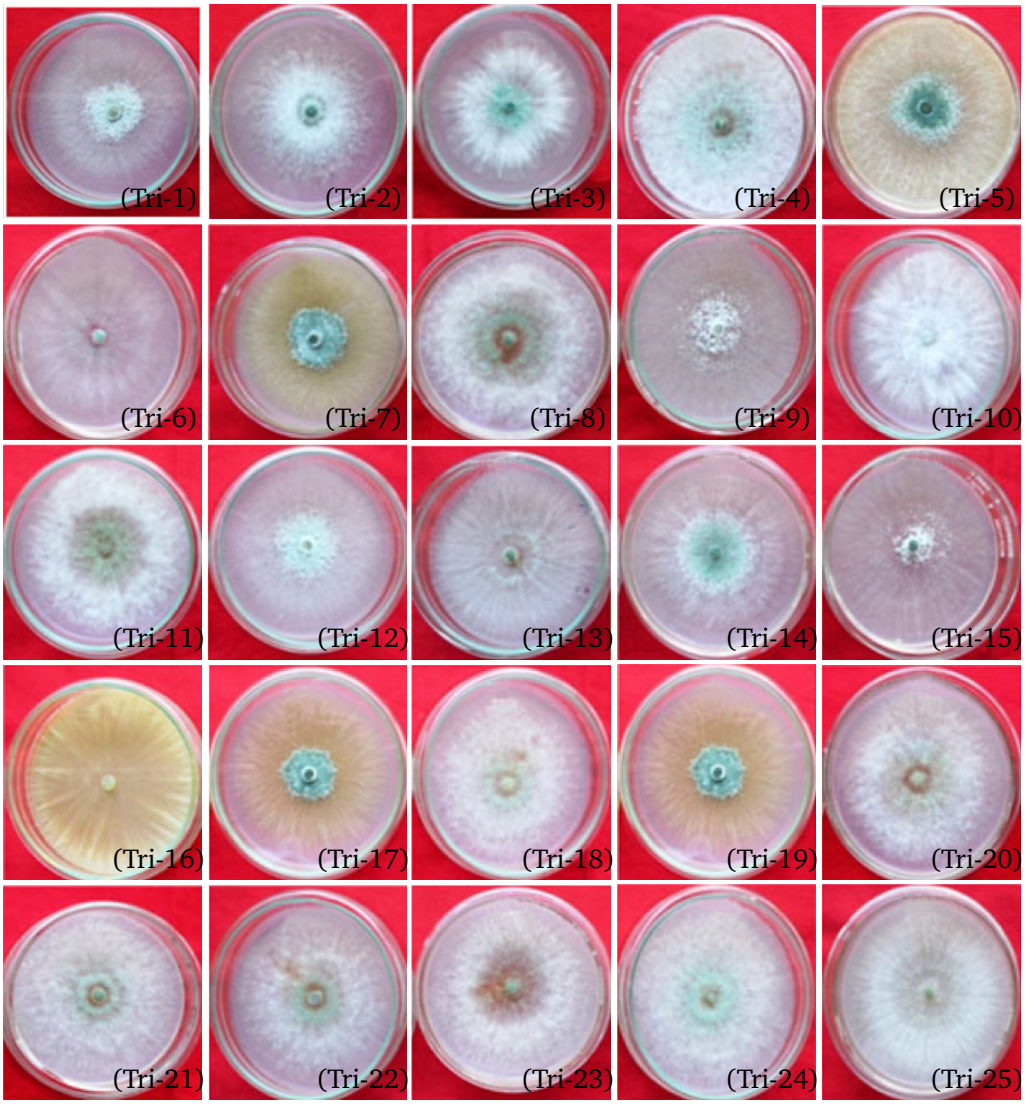

Figure 2. Different growth rates of *Trichoderma* isolates cultured on Potato Dextrose Agar (PDA) at 3 days after incubation period

on these features, these isolates were identified as *Trichoderma viride*. In this study, the characters of the isolates in Group-II agreed with this species as reported by Sekhar et al. (2017). Group-II isolates (Tri1-Ab, Tri2-Ty, Tri4-Mg, Tri8-Kkm, Tri11-Zl, Tri21-No, Tri23-Lpd and Tri25-Dhor), therefore, were *Trichoderma viride*. Likewise, a total of 5 isolates namely, Tri6-Kks, Tri7-Mhn, Tri16-Dw-7, Tri19-Tk and Tri22-Mt shared common culture characters and were also categorized as Group-III. Colony colour was mottled with white flecks often within conspicuous wefts of yellow hyphae on the dark green conidial mass colony continuous, confluent pulvinate aggregates conidia tending to form concentrative rings. The reverse color was colorless to yellowish green (Fig. 3). Conidiophores were characterized with long, main branches and relatively few, short, side branches. The final branches were very simply constructed. Conidiophores hyaline smooth walled, arising from the substratum to form irregular tufts or arising primarily from the aerial mycelium in older colonies; main branches long and straight. Phialides were solitary or in verticils of 2 or 3; usually broadly lageniform (Fig. 4). Conidia typically ellipsoidal to oblong smooth (Fig. 5). Rifai (1969) and Samuels (1996) reported that colony colour of *Trichoderma longibrachiatum* was conidial mass dark green, sometimes mottled with white flecks, colony continuous, confluent pulvinate aggregates. Colony reverse was colorless to yellowish green, Conidia were tending to form concentrative rings. *T. Longibrachiatum*, to which *T. longibrachiatum* belongs, is characterized by conidiophores with short, rarely rebranched side branches and smooth-walled ellipsoidal to oblong conidia.

Phialides often arose singly, directly from the main axis, were cylindrical or narrowly flask-shaped and slightly wider in the middle than at the base lateral branches often comprised a single phialide subtended

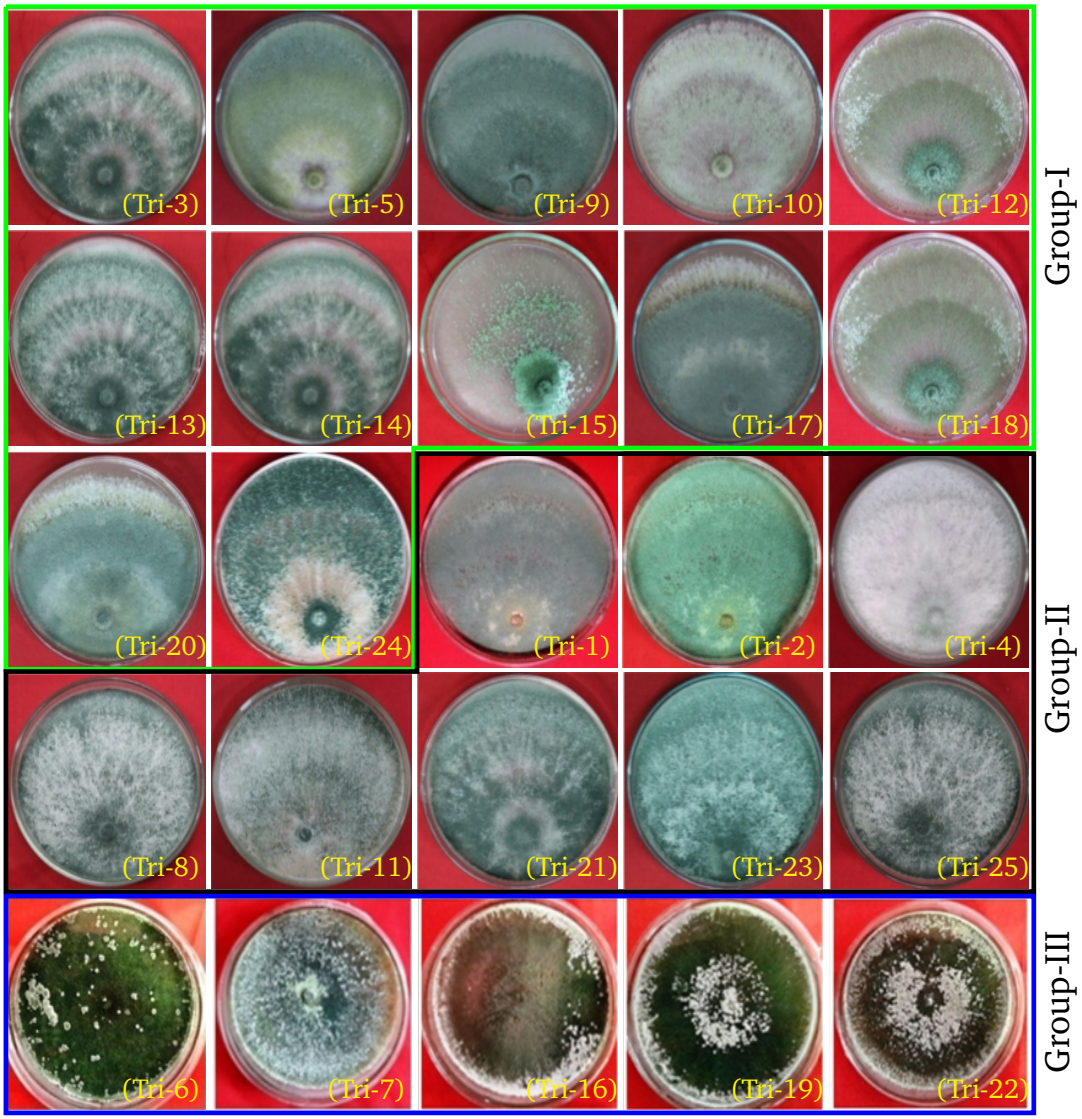

Figure 3. Grouping of 25 isolates of *Trichoderma* species (on the basis of macroscopic characters)

by an acropleurogenous phialide. Conidia were ellipsoidals to oblong, smooth and green. The characters of the isolates in Group-III were matching with this species as reported by Rifai (1969) and Samuels (1996). Group-III isolates (Tri6-Kks, Tri7-Mhn, Tri16-Dw-7, Tri19-Tk and Tri22-Mt) thus, were *T. longibrachiatum*.

3.3 Molecular characterization

The diversity study of native isolated *Trichoderma* strains from the different ecosystems on the basis of morphological and molecular characters revealed that all the *Trichoderma* isolates differed in their morphological behavior while on molecular basis, maximum polymorphism was observed in all isolates. All the bands observed were polymorphic in nature indicating the genetic diversity among *Trichoderma* spp. Twenty-five native *Trichoderma* isolates were screened for the presence/absence of *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma longibrachitum* genes, using PCR-based SCAR markers, HAR-5 and VIRI-5, respectively. Estimation of PCR results for these genes were determined by visualization of amplicons near 900 bp, 220 bp of positive fragments, respectively.

The results of genotypic screening of the 25 native *Trichoderma* isolates are presented in electrophoretic patterns of SCAR markers HAR-5 and VIRI-7 for *Trichoderma harzianum*, *Trichoderma viride*, respectively. During this polymorphic survey, out of the 25 *Trichoderma* isolates, twelve *Trichoderma* isolates (Tri3-Kt, Tri5-Lk, Tri9-Nm, Tri10-Hb, Tri12-Pg, Tri13-Kbt, Tri14-Az-2, Tri15-Ktd, Tri17-Mmy, Tri18-Sb, Tri20-Mgy and Tri24-Tkh) along with HAR-5 amplified 220-bp fragments (Fig. 6) and 8 *Trichoderma* isolates (Tri1-Ab, Tri2-Ty, Tri4-Mg, Tri8-Kkm, Tri11-Zl, Tri21-No, Tri23-Lpd and Tri25-Dhor) along with VIRI-7 amplified 900-bp fragments (Fig. 7), indicating the presence of *Tricho-*

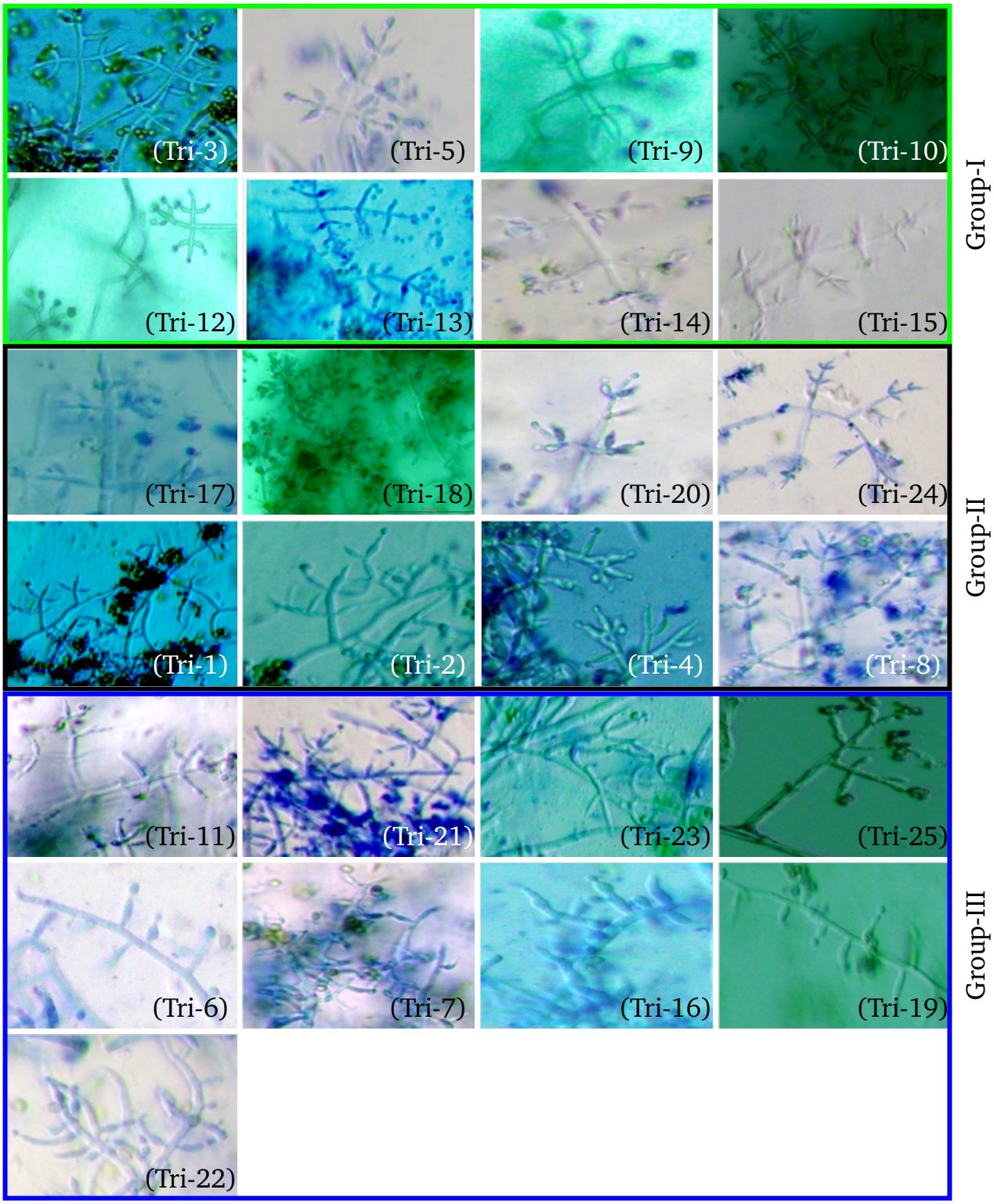

Figure 4. Grouping of 25 isolates of *Trichoderma* species (on the basis of Conidiophore branching and phialide disposition (slide) (×400)

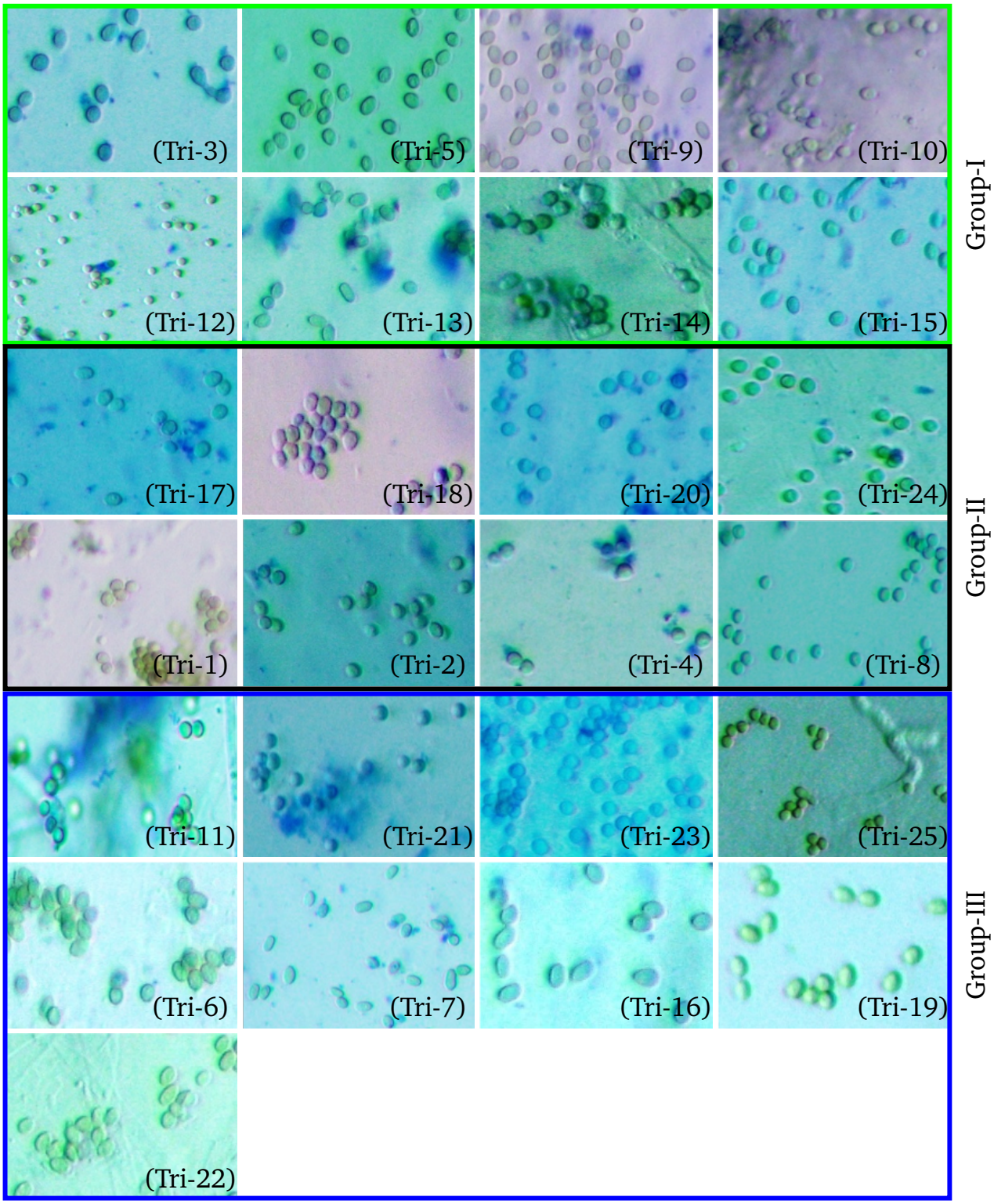

Figure 5. Grouping of 25 isolates of *Trichoderma* species on the basis of conidial shape (×400)

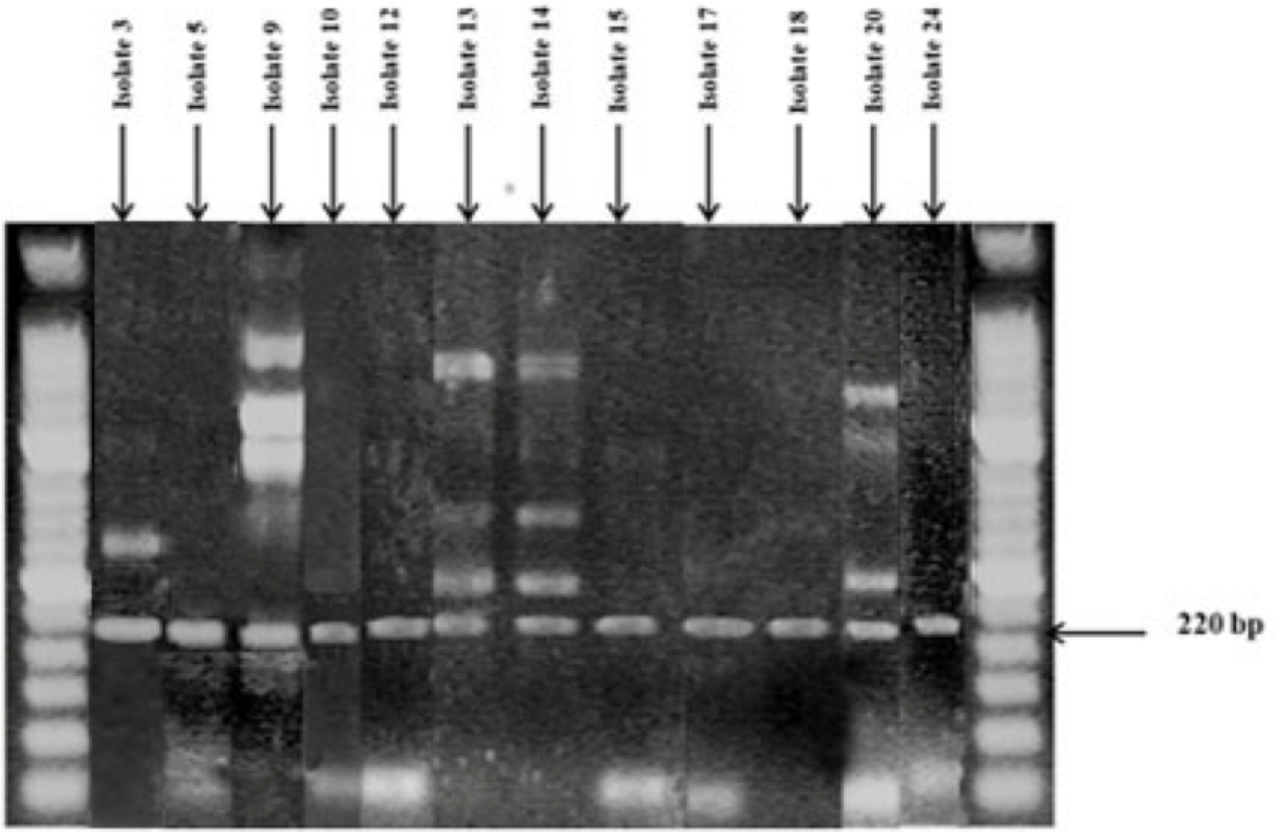

Figure 6. Banding patterns showing the presence of HAR-5 gene (*Trichoderma harzianum*) in native *Trichoderma* isolates amplified 220 bp, size fragment

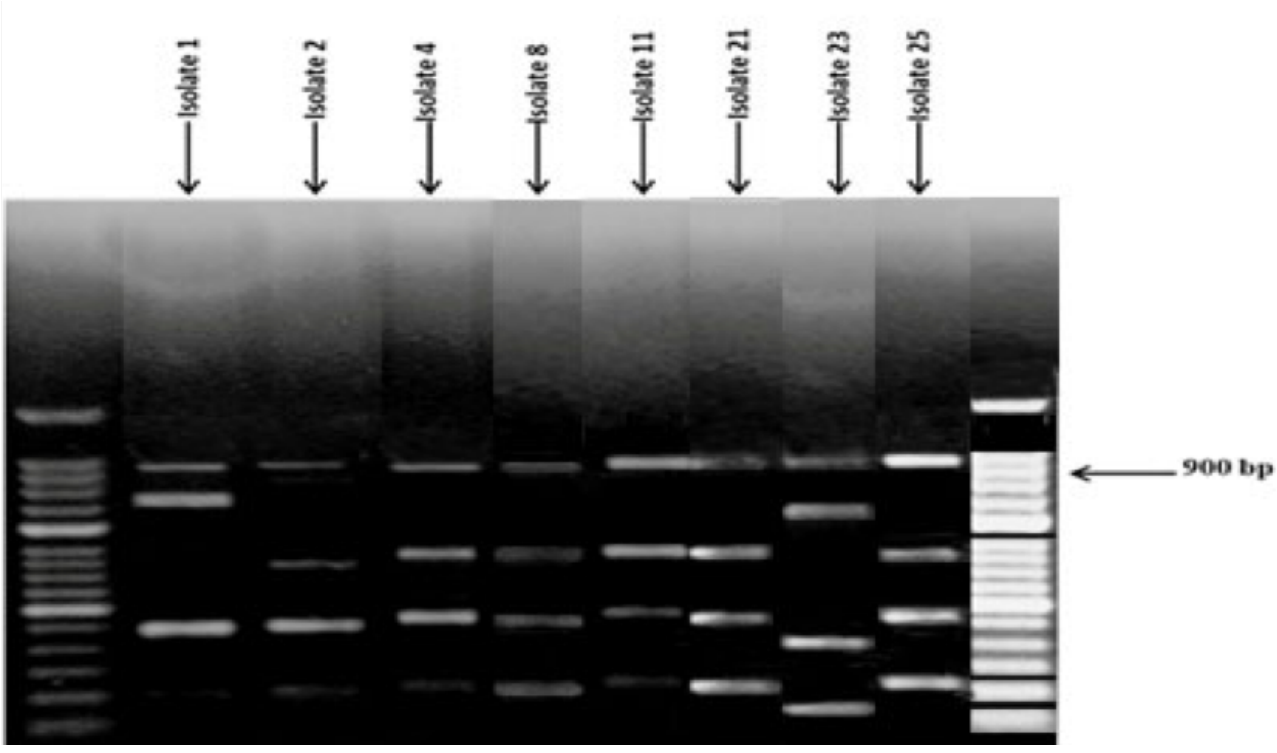

Figure 7. Banding patterns showing the presence of VIRI-7 gene (*Trichoderma viride*) in native *Trichoderma* isolates amplified 900 bp, size fragment

derma harzianum and *Trichoderma viride* genes, respectively. Similar results were also reported by Parmar et al. (2015). Their results showed that *Trichoderma* species were a specific band of 220 bp only in *T. harzanium* and a specific band of 900 bp. only in *T. viride* with the optimized PCR parameters. This sequence characterized amplified region (SCAR) marker was sensitive and could detect small quantities of *Trichoderma* DNA as low as 30 ng with high efficiency. This marker could also clearly distinguish *T. harzianum* and *T. viride* from other isolates of *Trichoderma*.

4 Conclusions

A total of 25 isolates of *Trichoderma* spp. were isolated from rhizosphere soils of different ecosystems. On the basis of colony growth rate, the 21 isolates were observed the same growth rate (30 mm d^{-1}) while the lowest growth rate (27.34 mm d^{-1}) was observed in isolate Tri17-Mmy. Colour and texture of the isolates found to be variable and were classified into three groups based on these characters. The morphological data obtained for different characteristics such as conidiophores, phialides (shape and disposition), and conidia shape coincides with the grouping of isolates based on their colony characters. The Group-I (12 isolates), II (8 isolates) and III (5 isolates) were identified into *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma longibrachiatum*, respectively. PCR amplification and sequence characterized amplified region (SCAR) markers were used to characterize these isolates of *Trichoderma*. SCAR markers were successfully used to amplify genomic DNA extracted from mycelium of these isolates and showed clear differences among the isolates. Morphological grouping and specification matched with molecular grouping of most of the isolates especially with the results of SCAR marker-based analysis. The integration of morphology and molecular based techniques gave fair solution in easing some of the complex problems that are associated with either morphology or molecular based techniques. Among these isolates, 12 isolates of *Trichoderma* species showed a specific band of 220 bp only in *Trichoderma harzianum* and 8 isolates showed a specific band of 990 bp only in *Trichoderma viride*.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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