INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
22–0015/2023/27–6–371–380
DOI: 10.17957/IJAB/15.1937
http://www.fspublishers.org

Full Length Article

Isolation and Molecular Identification of Rhizospheric Fungi Associated with *Opuntia cochenillifera* from Semiarid in Alagoas, Brazil

João Manoel da Silva[1*], Viviane Araujo Dalbon[2], Regla Toujaguez la Rosa Massahud[3], Juan Pablo Molina Acevedo[4], Mayra Machado de Medeiros Ferro[4], Tania Marta Carvalho dos Santos[4], Elizabeth Simões do Amaral Alves[5] and Gaus Silvestre de Andrade Lima[4]

[1]*Agronomy Department, Universidade Estadual do Piauí, Campus Dep. Jesualdo Cavalcanti. Piauí, Brazil*
[2]*Universidade de Córdoba, Montería, Colombia*
[3]*Campus de Engenharias e Ciências Agrárias, Universidade Federal de Alagoas, Brazil*
[4]*Corporación Colombiana de Investigación Agropecuária C. I Turipana, AGROSAVIA, Colombia*
[5]*Departamento de Biociência Animal, Universidade Federal Rural de Pernambuco, Brazil*
[*]For correspondence: agrobio.jm@gmail.com
Recieved 11 January 2022; Accepted 25 April 2022; Published 15 June 2022

Abstract

The aim of this study was isolation and identification of rhizospheric fungi in association with cacti [*Opuntia cochenillifera* (Linnaeus) Miller] in an area under salinization and desertification, as well as to model their chemical and mineralogical attributes. The experimental area was located in the municipality of Ouro Branco-AL, Brazil. To determine the microbial population, rhizospheric soil was collected from a depth of 0–20 cm. The dilution was carried out to fractions 10^{-3}, 10^{-4} and 10^{-5} and inoculated in Petri dishes containing selective culture media. A count of colony forming units (CFU), and morphological identification was performed. The isolates were subjected to DNA extraction, followed by amplification through PCR. The sequences were analyzed with Staden Package and MEGA softwares, followed by the BLASTn search in GenBank. Through the phylogenetic analysis of the rDNA ITS, the species belonging to *Coprinellus*, *Paecilomyces*, *Aspergillus*, *Penicillium,* and *Neurospora* were identified. These genera are in the literature for their metabolic richness, being applicable to biotechnological processes for growth promotion in plants, as well as other processes of industrial interest. © 2022 Friends Science Publishers

Keywords: *Aspergillus;* Eurotiales; Internal transcribed spacer; Microbial diversity; *Paecilomyces; Penicillium;* Soil fungal diversity

Introduction

Due to its natural condition, the soil is an environment conducive to a vast diversity of organisms, such as fungi and bacteria. Part of these microorganisms live in symbiosis with various species of plants, whether cultivated or not, and which have potential for plant production (Javaid 2009; Javaid and Mehmood 2010). Beneficial microorganisms such as actinomycetes, common bacteria and filamentous fungi have been studied for agricultural growth, making it possible to use their functions to develop processes. The action of the soil microbiota confers benefits such as biological N fixation, phosphate solubilization and biological control of phytopathogens and agricultural pests, which has already been demonstrated in several studies (Silva *et al.* 2017; Barros *et al.* 2019; Sharf *et al.* 2021). However, for the maintenance of soil microbiota, vegetation and consequently their chemical attributes, it is necessary to adopt conservation practices in view of the cultivation system and plants adopted in the area/region. The Brazilian Semiarid region has suffered from the exponential growth of environmental degradation, especially with regard to the soil, where the changes suffered in the landscape are observed, especially the damage caused by bad agricultural practices (Nascimento *et al*. 2018). This huge degradation process has been caused by the misuse of the same or of water, resulting in salinization, desertification and reduction of biological diversity in many ecosystems and agroecosystems, which has resulted in a constant concern with the recovery of degraded areas and conservation of still resistant environments (Nascimento *et al*. 2018, 2021).

The semiarid vegetation is constituted by several plants, being the Cactaceae considered endemic in this region. In this way, this botanical family has been used as a resource for providing food, destined especially for animals.

To cite this paper: Silva JMD, VA Dalbon, RTR Massahud, JPM Acevedo, MMDM Ferro, TMCD Santos, ESDA Alves, GSDA Lima (2022). Isolation and molecular identification of rhizospheric fungi associated with *Opuntia cochenillifera* from Semiarid in Alagoas, Brazil. *Intl J Agric Biol* 27:371–380

On the other hand, the practice of conservation management in such areas is deficient, leaving at the mercy of the plant species themselves the maintenance of the propagation of descendants so that the species is maintained, which causes another factor that provides the degradation processes environmental (Prăvălie 2021). The investigation of soil microorganisms with a view to prospecting for agricultural, pharmaceutical and industrial purposes is constant and dynamic. Soil is understood as a living compartment, housing a vast community of organisms, including fungi, which have the ability to establish harmonious relationships with plants. However, when it comes to studies related to the interactions between microorganisms and cactuses in the semiarid region, most of the works have been focused on preserved areas (Silva *et al.* 2019a), which means that real data are often not explored.

Given the importance of the relationships between this group of microorganisms and plants, there is a certain interest to know the processes related to them. Regarding to their agricultural, industrial and pharmaceutical characteristics, there is ecological and economic importance. Therefore, tools such as morphology are essential for the identification of these microorganisms. However, at certain times, the morphology is not enough to identify these organisms at the species level. Thus, molecular techniques developed over the years from the Polymerase Chain Reaction (PCR), and DNA sequencing have been of great value for the study and identification of organisms (Khan and Javaid 2021, 2022a). Thus, among the molecular markers used to identify fungi, the ITS region (Internal Transcribed Spacer) stands out, which separates the 18S and 28S rDNA genes and can be amplified with primers anchored to these two regions (Fungaro 2000). These regions have been used in phylogenetic studies due to the fact that they are conserved among species and with low variations at the genus level (Lee and Taylor 1992). This gene region has been described by scientists in the study of soil fungal diversity. Berutti *et al*. (2017) state that by using the ITS region it is possible to estimate the structure of the microbial community of arbuscular mycorrhizal fungi as a function of their environmental relationships. Schoch *et al*. (2012), studying regions to identify soil living fungi, explain that the ITS region was more successful in identifying these fungi. For Rittenour *et al*. (2014), the ITS region provided greater accuracy in identifying fungi of the Ascomycota phylum. There are many recent examples of using ITS marker for identification of *Pyricularia oryzae* (Javaid *et al.* 2019), *Curvularia lunata* (Khan and Javaid 2020), *Alternaria radicina* (Javed and Javaid 2021), *Penicillium echinulatum* and *Mucor fragilis* (Khan and Javaid 2022b, c).

It is imperative to consider the conservation and recovery of degraded environments or those undergoing degradation processes of social and ecological importance. Thus, the study of the population and microbial diversity of soils in salinized and desertified areas are of paramount importance to foster data that corroborate actions that contribute to their recovery processes, especially for agrarian development. Therefore, the objective of this study was to isolate and identify rhizospheric fungi associated with cacti from the semiarid region of Alagoas, Brazil regarding their association with the chemical attributes of the soil.

Materials and Methods

Chemical attributes of the soil

Soil samples were collected at two points in the rural area of the municipality of Ouro Branco-AL, Brazil, which were recorded using a Global Processing System (GPS) equipment (Point A O: 37° 24' 45.9" S: 9° 4' 47.3"; Point B O: 37° 24' 51.0' S: 9° 4' 38.3"), in an area that is in the process of desertification and salinization (Nascimento *et al*. 2018). In the area there is an abandoned plantation of forage cactus (*Opuntia cochenillifera*), and currently its propagation occurs spontaneously.

Isolation and estimative of fungal population

From each point samples were collected at a depth of 0–20 cm from the surface layer of the rhizosphere of *O. cochenillifera*. The samples were placed in brown paper bags, identified and sent to the laboratory for chemical and biological analysis. For the isolation and counting of microorganisms, the decimal serial dilution method was adopted, followed by inoculated on selective microbial culture medium. Fungi were isolated by serial decimal dilution (10^{-3}), plating in Martin culture medium and incubated for five days to count the colony forming units (CFU g^{-1}) to estimate the population of fungi associated with the rhizosphere of cacti and subsequent subculturing and purification of the obtained isolates.

For the chemical analysis of the soil, the following parameters were used: Mehlich^{-1} Extractor, 1.0 *M* KCl Extractor, Ca acetate extractor at pH 7.0, Welkley-Black method, Base saturation, and aluminum saturation. The data provided a comparison between chemical and microbiological attributes for modeling the conditions of the area in the process of desertification and salinization. Microbiological data were subjected to analysis of variance using Sisvar software (Ferreira 2014), and the means were grouped by the Skott-Knott test at 5% level of significance.

Morphomolecular characterization of fungal isolates

For DNA extraction, the strains were grown in potato dextrose (BD) culture medium and after five days the mycelium was filtered and washed in sterile distilled water and put to dry at room temperature. Afterwards, maceration of a fragment of the mycelial mass in liquid N was performed, and later, extraction was performed by adding 1

mL of CTAB buffer (2%), and 2 μL of 2% β-mercapto-methanol. The liquid obtained was transferred to 2 mL microtubes and placed in a water bath for 30 minutes at 65ºC, followed by centrifugation at 13000 rpm for 5 minutes and 4ºC.

The supernatants were transferred to new 1.5 mL microtubes and an equal volume of chloroform-isoamyl alcohol (24:1 v/v) was added to the recovered supernatant, homogenized and centrifuged at 13,000 rpm for 10 min. Again supernatants were transferred to other microtubes and equal volumes of ice-cold isopropanol added. The system was kept at -20ºC for 1 h. Then the microtubes were centrifuged at 13,000 rpm for 10 min. The precipitated DNA was washed with 70% ethanol and 50 μL of Ultrapure water was added. After extraction, electrophoresis was performed to confirm the existence of DNA.

The PCR reaction was performed to amplification of ITS1 and ITS4 regions from rDNA in total volume of 30μL using the Taq DNA polimerase buffer 1X, 1,5 m*M* of MgCl 2; 0,4 μM of each primer ITS1 (5' – TCCGTAGGTGAACCTGCGG – 3') and ITS4 (5' – TCCTCCGCTTATTGATATGC – 3'), 0,2 m*M* of dNTPs, and 0, 2 U of Taq DNA polimerase, and 25ng of the extracted DNA.

PCR was performed in an Applied Biosystems Thermocycler (2720 Thermal Cycler) under the following conditions: initial denaturation was 95°C for 2 min, followed by 38 cycles of denaturation at 95°C for 1 min, annealing a 55°C for 30s, extension at 72°C for 45 s, and a final extension at 72°C for 10 min. PCR products were separated by 1.0% agarose gel electrophoresis with 1X Tris Borate EDTA, stained with ethidium bromide (5 mg mL^{-1}) and visualized in a UV transilluminator.

After amplification the products were sent to Magrogen® for sequencing, which was performed using the Sanger technique. The chromatograms obtained from the PCR products of the sequences were visualized, analyzed and edited using the Staden Package software (to obtain the consensus sequence), and aligned using the MUSCLE tool (Edgar 2004), implemented by the MEGA v. 6 program (Tamura *et al*. 2013; Kumar *et al*. 2018). Sequences from previous studies, available at GenBank (https://www.ncbi.nlm.nih.gov/genbank) (Table 1) were retrieved and contrasted by the BLASTn tool and included in the analyzes for similarity comparison, where they were those with proximity > 99% were adopted. The *Metarhizium anisopliae* fungus was adopted as an outgroup.

The construction of the phylogenetic tree was carried out by adopting the Neighbor-Joining method, which consists of finding pairs of operational taxonomic units aiming to minimize the total length of the branch at each stage of phylogenetic grouping (Saitou and Nei 1987). In addition, the morphological characteristics were compared to confirm the inferences related to the species, according to morphological identification keys according to Luz and Inácio (2009).

Results

A fungal population of 8.6×10^3 CFU g^{-1} was obtained for the first collection point (Point A), which has a deactivated well due to the advanced salinization process. At the second collection point, a population of 9.4×10^3 UFC g^{-1} was obtained, which corresponds to an abandoned agricultural area, where there had been corn cultivation for years, but without success in production, currently existing populations of *O. cochenillifera* and other species of spontaneously growing cacti. The data obtained for the microbial populations of the two areas did not differ statistically by the Skott-Knott test ($P \leq 0.05$).

Data from the rhizospheric microbial community associated with cactuses from the semiarid region of Alagoas, Brazil show that they follow the impacts suffered by the soil in the same way, that is, degraded environments, although they present their considerable microbial populations (filamentous fungi, common bacteria and actinomycetes), they have a decline in these communities, which is related to their chemical attributes.

Thus, the soil analyzes (Table 2) showed high Na^+ values, observing a Percentage of Exchangeable Sodium (PES) of 6.27 for Point B and 17.34 for Point A, where a deactivated well is located. Therefore, it is understood that, although at points relatively close to each other, the soil attributes present differences, which may be based on the fact that soil drilling causes elevation and release of sodium and other salts that were in depth, making it the free ones in the arable layer, which with inadequate agricultural management, regarding the efficiency of water use, provided acceleration in the soil salinization process. Thus, it is possible to state that the soil in the region under study has a sodic saline character (PES > 15; pH 5.2).

Furthermore, the region also has low levels of organic matter (10.6 g kg^{-1} for Point A and 15.5 g kg^{-1} for Point B). Therefore, this characteristic contributes to the low microbial population, since the low content of organic matter makes the availability of nutrients for cell development and multiplication of microorganisms scarce. Therefore, it also explains the fact that there is a greater population of filamentous fungi, as they have resistance structures (spores) which gives them greater ability to survive in an environment without water and organic matter. Furthermore, filamentous fungi can have a symbiotic character such as mycorrhizae, with a certain affinity for colonization of the root system of plants.

The species presented here (Fig. 1) have been described in previous studies, being isolated from the most distinct environments, such as *Penicillium*, which has been described in the literature for promoting plant growth, either through the production and synthesis of hormones vegetables such as gibberellins or through indirect mechanisms that act on plant nutrition, such as phosphate solubilization.

Table 1: Chemical attributes of the soil collected at two points in the municipality of Ouro Branco, Alagoas, Brazilian Semiarid

Determinations	Point A	Point B
pH in water	5.2	5.8
Na (mg dm^{-3})	10	5
P (mg dm^{-3})	11	25
K (mg dm^{-3})	58	75
Ca ($cmol_c$ dm^{-3})	1.32	1.32
Mg ($cmol_c$ dm^3)	0.72	0.97
Al ($cmol_c$ dm^3)	0	0.05
H + Al ($cmol_c$ dm^{-3})	3.39	2.43
CEC* efective ($cmol_c$ dm^{-3})	2.34	2.55
CEC total ($cmol_c$ dm^{-3})	2.62	4.93
MO** (g kg^{-1})	10.6	14.5
V*** (%)	40	51
m (%)	5	2
Ca saturation (%)	23.5	26.8
Mg saturation (%)	12.8	19.7
K saturation (%)	2.7	3.9
Na saturation (%)	0.7	0.4

*CEC: cation exchange capacity; **OM: Organic Matter; ***V: Base saturation

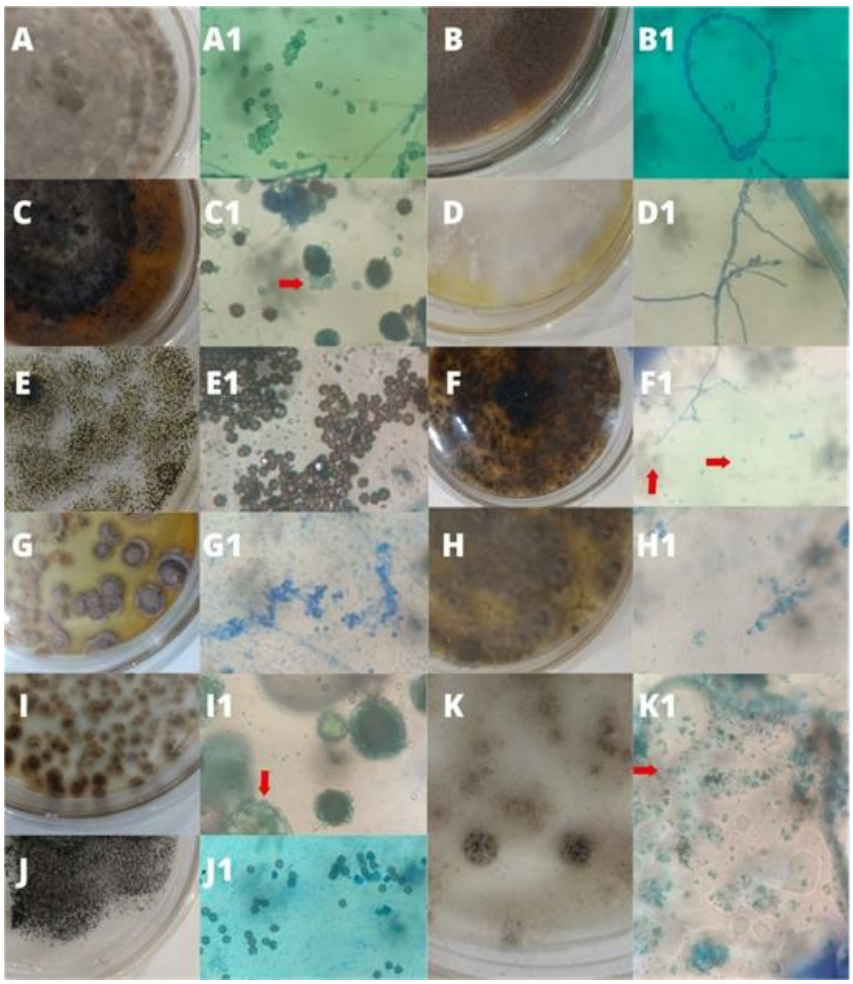

Fig. 1: Morphology of fungi isolated from cacti rhizosphere. A: *Penicillium* spp., B: *Aspergillus* spp., C: *Coprinellus radians*, D: *Aspergillus* spp., E, *Neurospora* spp., F: *Coprinellus radians*, G: *Aspergillus* spp., H: *Penicillium* spp., I: *Paecilomyces* spp., J: *Penicillium* spp., K: *Paecilomyces* spp.; A1: *Penicillium* spp., B1: *Aspergillus* spp., C1: *Coprinellus radians*, D1: *Aspergillus* spp., E1, *Neurospora* spp., F1: *Coprinellus radians*, G1: *Aspergillus* spp., H1: *Penicillium* spp., I1: *Paecilomyces* spp., J1: *Penicillium* spp, K1: *Paecilomyces* spp.

Table 12: Retrieved isolates from GenBank for phylogenetic analisys of ITS from rDNA

Isolate	Species/Genera	Place	GenBank
F02	*Penicillium* spp.	Brazil	OK210351
F04	*Aspergillus* spp.	Brazil	OK210353
F05	*Coprinellus radians*	Brazil	OK210350
F07	*Aspergillus* spp.	Brazil	OK210342
F08	*Neurospora* spp.	Brazil	OK178929
F09	*Coprinellus radians*	Brazil	OK178928
F10	*Aspergillus* spp.	Brazil	OK210345
F11	*Penicillium* spp.	Brazil	OK210326
F14	*Paecilomyces* spp.	Brazil	OK210352
F15	*Penicillium* spp.	Brazil	OK210344
F17	*Paecilomyces* spp.	Brazil	OK210347
HURB 18573	*Penicillium* spp.	Brazil	NR172038
974-SAB SP2 2	*Penicillium* spp.	Brazil	MT820349
439010	*Penicillium* spp.	USA	MW313849.1
BFM-L104	*Paecilomyces lilacinus*	China	AB369489
YCG1(1)	*Aspergillus* spp.	China	KM268709
UFMGCB10058	*Coprinellus radians*	Brazil	KU954342
HCH-13	*Coprinellus* spp.	México	MK307658
PanB1A	*Coprinellus radians*	Panamá	JQ922136
URM7046	*Aspergillus niveus*	Brasil	KM613137
CGMCC_3.03920	*Aspergillus allahabadii*	China	MH292843
MS-Deb-PCB	*Aspergillus allahabadii*	India	MN339985
isolate 80	*Neurospora* spp.	South Africa	KY587330
isolate PG2	*Neurospora* spp.	South Africa	KY606539
B65-ITS1_K18	*Coprinellus radians*	Saudi Arabia	MN753979
SZ211	*Aspergillus versicolor*	China	MH509421
MEFC092	*Aspergillus* spp.	China	MK732127
3-F9	Aspergillus *versicolor*	China	MW081327
RCZ2D-2	*Penicillium daleae*	Niger	MW260092
KP1	*Penicillium* spp.	India	JQ387731
M1861	*Paecilomyces formosus*	USA	KC157764
Yu2-2	*Paecilomyces* spp.	China	MG827159
2723	*Paecilomyces sinensis*	Colombia	EU272527
RHi	*Penicillium janthinellum*	Malaysia	KM246752
DTO 249-D2	*Penicillium raperi*	Netherlands	KC797647
XI19	*Penicillium* spp.	China	KX008645
KVL 96-31**	*Metarhizium anisopliae*	Greece	AF363470.1

*CEC: cation exchange capacity; **OM: Organic Matter; ***V: Base saturation

Red arrows indicate spores and/or conidia. Based on the results obtained, the genus *Coprinus* (*Coprinellus*) (Agaricales: Psathyrellaceae) can be confirmed for isolates F05 and F09 through phylogenetic inference (100%) (Fig. 2) and morphology, as well as compared to studies already published as Huang and Bau (2020), where morphogenetic similarities can be verified. Isolates F02, F11 and F15 also showed agreement between morphology and phylogenetic inference.

Isolates F04, F10 and F07 were similar to the genus *Aspergillus* (Eurotiales: Trichocomaceae). For isolates F14 and F17, the phylogenetic correspondence comprised the grouping of the genus *Paecilomyces* (Sin. *Purpureocillium*) (Eurotiales: Trichocomaceae), which is a polyphyletic genus, as pointed out by Luangsa *et al*. (2004), occurring in the subclasses Sordariomycetidae and Eurotiomycetidae. This genus has been described in the literature as an important biocontrol agent, more specifically as a nematophagus acting in the integrated pest management, and may be a strong candidate in the production of bioinputs for agricultural development.

Isolate F08 corresponded to the genus *Neurospora* (Saccharomycetes: Sordariales). This filamentous ascomycete fungus is an important eukaryote, being understood as a model organism in biological studies in various areas such as health and biotechnology.

Discussion

Soils in semiarid regions have, by nature, higher levels of sodium, a characteristic explained by the low presence of primary minerals, which is caused by low weathering. It was possible to observe the presence of some fungal species, which have biotechnological potential for agriculture. Also, the first report of the presence of the *Coprinellus* fungus for the region is highlighted. Studies have shown the wealth of fungi associated with plants from dry environments, stimulating the prospecting of these microorganisms with typical vegetables from arid and semiarid habitats (Freire *et al.* 2015). In the present study, 11 morphologically distinct isolates were obtained, making it possible to identify the genera: *Aspergillus*, *Penicillium*, *Paecilomyces, Neurospora* and *Coprinellus*, making it necessary to identify the isolates obtained through molecular

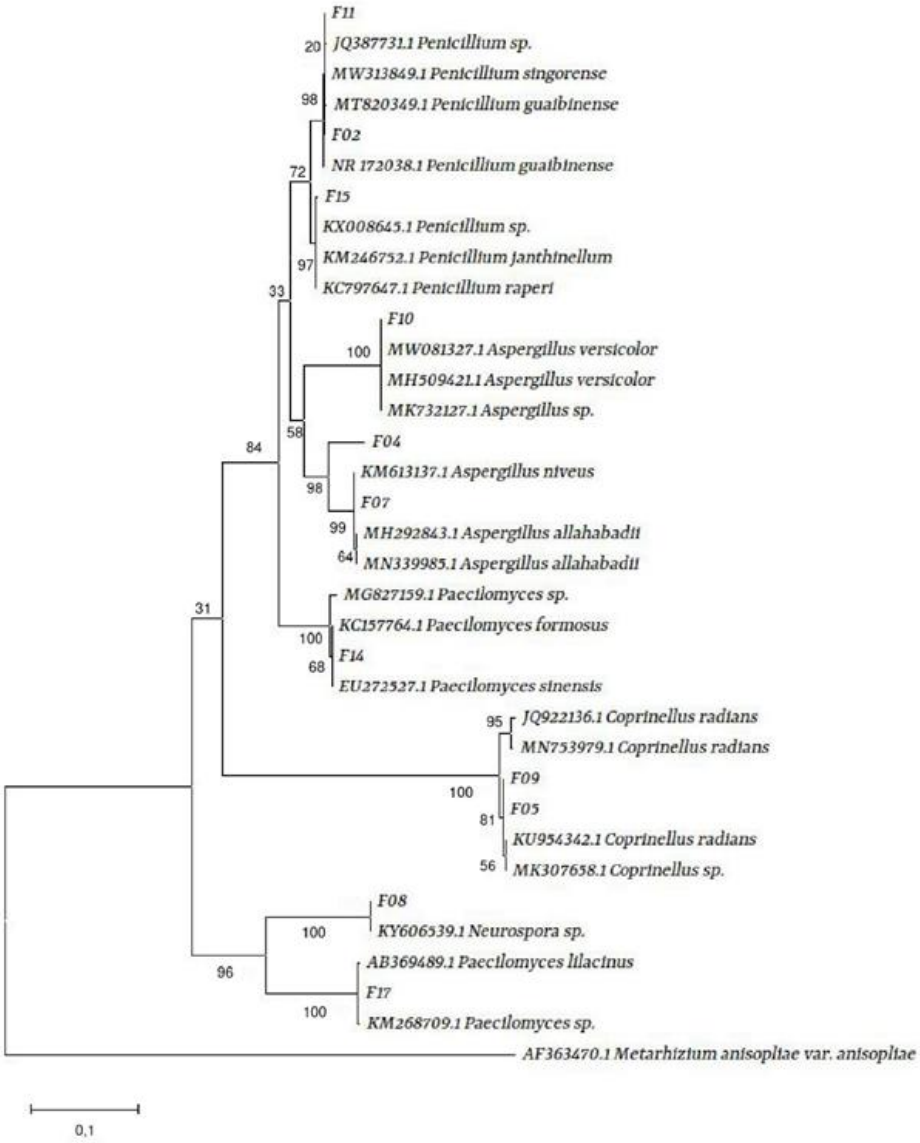

Fig. 2: Phylogenetic tree of maximum like hood (1000 bootstraps) of the sequencing from rDNA ITS (ITS1 and ITS4) in comparison to sequences retrieved from GenBank

techniques to confirm the species. These genera were also described by Freire *et al*. (2015) in cactus species, however living as endophytes. Although there are reports of microbial species in association with the most diverse species of cacti, previous studies are centered on endophyte interactions (Bezerra 2013; Freire *et al*. 2015), that is, there is still a need for more studies on rhizospheric microorganisms that live in associations with cacti. For Six *et al*. (2004), there are five main factors responsible for the stabilization of soil aggregates, with microorganisms being the second most characteristic group. Thus, saprophytic fungi, mycorrhizae and bacteria are relevant factors for soil aggregation (Braida *et al*. 2011).

The establishment of soil microbial life in a given area is mainly influenced by physical and chemical factors such as temperature, pH, luminosity, salinity, energy sources and organic substrates (organic matter), nutrients and the presence or absence of toxic elements. Thus, the different types of soil management exercised can interfere with these factors, changing the microbial population and its activity (Araújo *et al*. 2016; Silva *et al*. 2019b). In this aspect, as demonstrated by Nascimento *et al*. (2018) the anthropic action is a limiting factor regarding the characterization of the areas, as inadequate agricultural practices provided the exponential growth of the desertification process, reducing the botanical diversity and, consequently, the diversity of other organisms and soil microorganisms.

Analyzing the previous literature on the occurrence of rhizospheric fungi associated with cacti, the lack of research in this line is still noticeable, with few publications available. Thus, the recently published works address the isolation and identification of fungal species and genera in association with cacti from the rhizosphere or endophyte associations (Bezerra 2013).

Even so, these works report the fungus-cactus association in areas favorable to the development of these microorganisms, as they are studies carried out in conserved areas or in ecological reserves. Thus, it is important to emphasize the importance of studies aimed at prospecting and characterizing the population and microbial diversity in arid and semi-arid environments in view of the growing needs of increasing plant production.

Another relevant point is the need to encourage actions in programs for the recovery and conservation of degraded areas, with knowledge of microbial diversity being an important aspect, as these microorganisms have biotechnological potential capable of promoting improvements in this aspect. Furthermore, in ecological terms, the interaction between plants and microorganisms creates numerous advantages for both, as well as for the environment, through the cycling of nutrients and organic matter, suppression of pathogens and pests, among others.

Universal primers such as those used in the present study are more commonly applied in amplifying these regions (White *et al*. 1990). The similarity presented here is proposed according to what other works show, as exemplified by Nilsson *et al*. (2008), where the authors state that 2% is an acceptable margin for the study of intraspecific divergence through the ITS region. Paul *et al*. (2013) where this fungus shows changes in the color of the culture medium and the similarity in their reproductive structures, being grouped in branches corresponding to species of the genus *Penicillium* (Eurotiales: Trichocomaceae).

Coprinellus is understood as a coprinoid fungus, of which they are currently distributed in four genera: *Coprinus* Pers 1797, *Coprinellus* P. Karst., Coprinopsis P. Karst and *Parasola* Vilgalys & Hopple. These fungi underwent an adaptive process, which refers to a morphotype that emerged during the evolution of the order Agaricales, characterized by the deliquescence of the hymenophore and cap as part of the sporulation process, accompanied by the presence of pseudo paraphyses and hymenium development (Nagy *et al*. 2011). The fungi grouped as in the taxonomic class Eurotiomycetes (Eurotiales: Trichocomaceae), can be described by their characteristics as having superficial, free and rounded cleistothecia. Its conidia can be filamentous or branched into chains (Luz and Inácio 2009).

Moreno-Gavíra *et al*. (2020) describe the mechanisms by which Paecilomyces species act as plant growth promoters, with their ability to combat phytopathogenic agents such as bacteria (*Xanthomonas campestris*), fungi (*Biscogniauxia, Phytophthora cinnamomi, P. variotii* and *Fusarium moniliforme*) and nematodes (*Rotylenchulus*, *Heterodera*, *Xiphinema*, *Pratylenchus* and *Meloidogyne*). Also according to the authors, the action of these fungi can occur through direct antagonism (antibiosis) or through the induction of systemic resistance, providing an increase in the biometric characteristics of plants inoculated with *P. lillacinus*.

Species belonging to the genus *Penicillium* have also been described as resistance inducers in plants as demonstrated by Elsharkawy *et al*. (2017), who found that a species of *Penicillium* has the ability to induce resistance against *Cucumber Mosaic Virus* in tobacco plants. Hossain *et al*. (2014) found that *Penicillium* spp. promotes plant growth in cucumber plants and protection against Damping-off caused by Rhizoctonia solani and anthracnose caused by *Colletotrichum orbiculare* in cucumber plants inoculated with *Penicillium* spp. isolated, which demonstrates that the species have high adaptability.

According to studies carried out by Gladieux *et al*. (2020), Neurospora species act as a genetic basis for studies in Eukaryotes. Macabeo *et al*. (2020) state that compounds of pharmaceutical and industrial interest were isolated from species of the Neurospora genus and show activity against pathogenic fungi, being the first report describing *N. dagawae* metabolites.

The genus *Coprinus* (which currently houses the species of *Coprinellus* spp.) in turn is comprised within the phylum Basidiomycota, and has greater potential for pharmaceutical and industrial application. Previous studies have shown that species within this genus are capable of producing several enzymes, as described by Pejin *et al*. (2019), where the authors state that *Coprinus* spp. have a high potential for acetylcholinesterase inhibitory activity.

Lim and Choi (2009), studying *C. congregatus*, state that the fungus has high chitinase enzymatic activity. Plantay *et al*. (2019), when isolating infected soil fungi and soil arthropods, they also found the genus *Coprinus*, which makes a parallel with the aforementioned authors, since this enzyme is an indicator for the use of fungi in the biological control of pest insects.

In Brazil, coprinoid fungi (*Coprinus* and *Coprinellus*) were first recorded in Mato Grosso (Pegler 1990). In other states, there are records in Rondônia (Capelari and Maziero 1988), São Paulo (Pegler 1997), Paraná (Meijer 2010), Mato Grosso do Sul (Richardson 2001), Minas Gerais (Rosa and Capelari 2009), and Pernambuco (Alves and Cavalcanti 1996). The most recent records correspond to the states of Paraíba (Gomes and Wartchow 2014, 2018), Ceará (Gomes and Wartchow 2018) and Pernambuco (Melo *et al*. 2016). According to data published by Putzke and Putzke (2017), 64 species are registered in Brazil. Based on the data, the first occurrence of these fungi in the state of Alagoas is registered here.

The genus *Penicillium* receives species of agricultural interest as plant growth promoters, as well as for the

pharmaceutical industry through its diversity in volatile compounds. Hossain *et al*. (2017) describe in their studies the role of the fungus *P. viridicatum* in ethylene signaling, acting as plant growth promoter and systemic resistance inducer in *Arabdopsis* plants.

This genus has been reported, as described by Yadav *et al*. (2018) in different habitats, including extreme environments, in plants, as well as in rotten fruits and vegetables due to the saprophytic character of some species of the genus. *Penicillium* isolated from extreme environments can be used to understand the adaptive processes that allow life in these types of environments as far as their evolutionary processes are concerned. Evidence of its existence in diverse habitats has consequences for the exploration of promising biotechnological and industrial applications.

Research on the composition of the rhizosphere microbiome is becoming more relevant from the perspective of understanding plant-microbe interactions based on ecosystem services and plant adaptation in stressful environments in climate change and food security scenario (Adl 2016; Ahkami *et al*. 2017). These microorganisms play a significant role in plant biogeography, evolution and ecosystem structure. Microbial communities associated with the host influence ecophysiology with regard to nutrition, growth, resistance to biotic and abiotic stresses, and the survival and distribution of plant species (Rey and Schornack 2013; Wani *et al*. 2015), which reinforces the need to intensify studies aimed at rhizospheric fungi associated with cacti, especially in environments in the process of degradation.

In addition to the classic tools for isolation and identification of soil microorganisms, molecular techniques such as rDNA ITS sequencing are efficient in identifying these fungi, making it possible to infer their diversity and distribution. Therefore, based on the results and theoretical support of the previously published literature (Schoch *et al*. 2012; Rittenour *et al*. 2014; Berutti *et al*. 2017) it is possible to state that in the semi-arid Northeast, more specifically in the state of Alagoas, Brazil, there is diversity associated with the rhizosphere of cacti in an area undergoing desertification and salinization.

Conclusion

The species found in the study, according to genetic sequencing and comparison in the literature, have different origins, from the association with marine algae as endophytes in cultivated plants such as coconut and cocoa, which demonstrates high plasticity in terms of adaptation to the environment. The sequencing of the ITS region of the rDNA of filamentous fungi associated with the rhizosphere of cacti allows the identification of potential species for biotechnological applications. Here we have the first record of the genus Coprinellus in the semiarid region of the state of Alagoas, Brazil.

Acknowledgements

The authors are grateful to the Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support for carrying out the research.

Author Contributions

All authors contributed equally to this work

Conflicts of Interest

The authors declare no conflicts of interest.

Ethics Approval

Not applicable in this paper

References

Adl SM (2016). Rhizosphere, food security, and climate change: A critical role for plant-soil research. *Rhizosphere* 1:1–3

Ahkami AH, RA White III, PP Handakumbura, C Jansson (2017) . Rhizosphere engineering: Enhancing sustainable plant ecosystem productivity. *Rhizosphere* 3:233–243

Alves MH, MAQ Cavalcanti (1996). Coprinaceae en el campus de la Universidad Federal de Pernambuco (Recife, PE, Brasil). *Bol Micol* 11:33–40

Araújo KD, MA Souza, GR Santos, AP Andrade, JVF Neto (2016). Atividade microbiana no solo em diferentes ambientes da região semiárida de alagoas. *Geografia* 25:5–18

Barros VDC, MAL Junior, MVF Santos, AF Costa, AM Arruda, CA Sousa (2019). Diversidade rizobiana em função de solo e clima no semiárido pernambucano. *Pesq Agropec Pernamb* 24:1–6

Berruti A, A Desirò, S Visentin, O Zecca, P Bonfante (2017). ITS fungal barcoding primers versus 18S AMF-specific primers reveal similar AMF-based diversity patterns in roots and soils of three mountain vineyards. *Environ Microbiol Rep* 9:658–667

Bezerra JDP (2013). Diversidade de fungos endofíticos de mandacaru (*Cereus jamacaru* DC., Cactaceae) em áreas sucessionais de Caatinga. *Dissertação (Mestrado em Biologia de Fungos)*, Universidade Federal de Pernambuco, Recife

Braida JA, C Bayer, JA Albuquerque, JM Reichert (2011). Matéria orgânica e seu efeito na física do solo. *In*: *Tópicos em Ciência do Solo*, Vol. VII, pp:221–278. Klauberg Filho O. Sociedade Brasileira de Ciência do Solo

Capelari M, R Maziero (1988). Fungos macroscópicos do estado de Rondônia região dos Rios Jaru e Ji-Paraná. *Hoehnea* 15:28–36

Edgar RE (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucl Acid Res* 32:1292–1297

Elsharkawy MM, JM Abass, SM Kamel, M Hyakumashi (2017). The plant growth promotin fungi *Penicillium* spp. GP16-2 enhace the growth and confers protection against Cucumber mosaic vírus in tobacco. *J Virol Sci* 1:145–154

Ferreira DF (2014). Sisvar: A guide for its Bootstrap procedures in multiple comparisons. *Ciênc Agrotecnol* 38:109–112

Freire KTLS, GR Araújo, JPD Bezerra, RN Barbosa, DCV Silva, VM Svedese, LM Paiva, CM Souza-Mota (2015). Fungos endofíticos de *Opuntia Ficus-indica* (L.) Mill.(*Cactaceae*) sadia e infestada por *Dactylopius opuntiae* (Cockerell, 1896) (Hemiptera: Dactylopiidae). *Gaia Sci* 9:104–110

Fungaro MHP (2000). PCR na micologia. *Biotecnol Ciênc Desenvol* 14:1216

Gladieux P, F Bellis, C Hann-Soden, J Svedberg, H Johannesson, JW Taylor (2020). Neurospora from natural populations: Population genomics insights into the life history of a model microbial eukaryote. *In*: *Statistical Population Genomics*, pp:313–336. Dutheil JY (Ed). Springer, New York, USA

Gomes ARP, F Wartchow (2018). Notes on two coprinoid fungi (Basidiomycota, Agaricales) from the Brazilian semiarid region. *Edinb J Bot* 75:285–295

Gomes ARP, F Wartchow (2014). *Coprinellus arenicola*, a new species from Paraíba, Brazil. *Sydowia* 66:249–256

Hossain M, F Sultana, M Hyakumachi (2017). Role of ethylene signalling in growth and systemic resistance induction by the plant growth-promoting fungus *Penicillium viridicatum* in *Arabidopsis*. *J Phytopathol* 165:432–441

Hossain M, F Sultana, M Miyazawa, M Hyakumach (2014). The Plant Growth-promoting Fungus *Penicillium* spp. GP15-1 Enhances Growth and Confers Protection against Damping-off and Anthracnose in the Cucumber. *J Oleo Sci* 63:391–400

Huang M, T Bau (2020). New findings of Coprinellus species (Psathyrellaceae, Agaricales) in China. *Phytotaxa* 374:119–128

Javaid A (2009). Arbuscular mycorrhizal mediated nutrition in plants. *J Plant Nutr* 32:1595–1618

Javed S, A Javaid (2021). First report of black rot of carrot caused by *Alternaria radicina* in Pakistan. *J Anim Plant Sci* 31:1208–1211

Javaid A, N Mahmood (2010). Growth, nodulation and yield response of soybean to biofertilizers and organic manures. *Pak J Bot* 42:863–871

Javaid A, F Anjum, N Akhtar (2019). Molecular characterization of *Pyricularia oryzae* and its management by stem extract of *Tribulus terrestris. Intl J Agric Biol* 21:1256–1262

Khan IH, A Javaid (2022a). Molecular characterization of *Penicillium italicum* causing blue mold on lemon in Pakistan. *J Plant Pathol* 48:1–2

Khan IH, A Javaid (2022b). *Penicillium echinulatum* causing blue mold on tomato in Pakistan. *J Plant Pathol* 102:1–1

Khan IH, A Javaid (2022c). *Mucor fragilis* causing rot of seychelles pole bean in Pakistan. *Aust Plant Pathol* 76:1–4

Khan IH, A Javaid (2021). Molecular characterization of *Penicillium expansum* associated with blue mold disease of apple in Pakistan. *Pak J Bot* 53:2299–2303

Khan IH, A Javaid (2020). First report of *Curvularia lunata* causing postharvest fruit rot of banana in Pakistan. *Intl J Agric Biol* 24:1621–1624

Kumar S, G Stecher, K Tamura (2018). MEGA7: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Biol Evol* 35:1547–1549

Lee SB, JW Taylor (1992). Phylogeny of five fungus-like protoctistan Phytophthora species, inferred from the internal transcribed spacers of ribosomal DNA. *Mol Biol Evol* 9:683–653

Lim H, HT Choi (2009). Enhanced expression of chitinase during the autolysis of mushrooms in *Coprinellus congregatus*. *J Microbiol* 47:225–228

Luangsa J, NL Hywel-Jones, RA Samson (2004). The polyphyletic nature of Paecilomyces sensu lato based on 18S-generated rDNA phylogeny. *Mycologia* 96:773–780

Luz WC, CA Inácio (2009). *Micologia Avançada. Volume IIA: taxonomia de ascomicetos*. Revisão Anual de Patologia de Plantas: São Paulo, Brazil

Macabeo APG, AJC Cruz, A Narmani, M Arzanlou, A Babai-Ahari, LAE Pilapil, KYM Garcia, M Stadler (2020). Tetrasubstituted α-pyrone derivatives from the endophytic fungus, *Neurospora udagawae*. *Phytochem Lett* 35:147–151

Meijer AAR (2010). Preliminary list of the macromycetes from the Brazilian state of Paraná: Corrections and updating. *Bol Mus Bot Munic* 72:1–9

Melo RFR, RS Chickowski, AN Miller, LC Maia (2016). *Coprophilous Agaricales* (Agaricomycetes, Basidiomycota) from Brazil. *Phytotaxa* 266:1–14

Moreno-Gavíra A, V Huertas, F Diánez, B Sánchez-Montesinos, M Santos (2020). *Paecilomyces* and its importance in the biological control of agricultural Pests and diseases. *Plants* 9:1746–1773

Nagy LG, G Walther, J Házi, C Vágvölgyi, T Papp (2011). Understanding the evolutionary processes of fungal fruiting bodies: Correlated evolution and divergence times in the psathyrellaceae. *Syst Biol* 60:303–317

Nascimento CM, WS Mendes, NEQ Silvero, RR Poppiel, VM Sayão, AC Dotto, NV Santos, MTA Amorim, JAM Demattê (2021). Soil degradation index developed by multitemporal remote sensing images, climate variables, terrain and soil attributes. *J Environ Manage* 227:111316

Nascimento SPG, JM Silva, EO Santos, PVM Silva, JRU Santos, TMC Santos (2018). Impactos ambientais da produção vegetal no processo de desertificação do Semiárido alagoano: O caso de Ouro Branco – AL. *Ciênc Agríc*16:31–35

Nilsson RH, E Kristiansson, M Ryberg, N Hallenberg, KH Larsson (2008). Intraspecific ITS variability in the kingdom fungi as expressed in the internal sequence databases and its implications for molecular species identification. *Evol Bioinform* 4:193–201

Paul NC, JX Deng, JH Lee, SH Yu (2013). New records of endophytic P*aecilomyces inflatus* and *Bionectria ochroleuca* from chili pepper plants in Korea. *Microbiology* 41:18–24

Pegler DN (1997). *The Agarics of São Paulo, Brazil*, p:70. Royal Botanical Gardens, Kew, London

Pegler DN (1990). Agaricales of Brazil Described by JPFC Montague. *Kew Bull* 45:161–177

Pejin B, K Tesanovic, D Jakovljevic, S Kaisarevic, F Sibul, M Raseta, M Karaman (2019). The polysaccharide extracts from the fungi *Coprinus comatus* and *Coprinellus truncorum* do exhibit AchE inhibitory activity. *Nat Prod Res* 33:750–754

Plantay RL, D Papura, C Couture, D Thiéry, PH Pizzuolo, MV Bertoldi, GS Lucero (2019). Characterization of entomopathogenic fungi from vineyards in Argentina with potential as biological control agents against the European grapevine moth *Lobesia botrana*. *BioControl* 64:501–511

Prăvălie R (2021). Exploring the multiple land degradation pathways across the planet. *Earth-Sci Rev* 220:103689

Putzke J, MTL Putzke (2017). *Cogumelos (Fungos Agaricales) no Brasil*. Jair Putzke, e-book

Rey T, S Schornack (2013). Interactions of beneficial and detrimental root-colonizing filamentous microbes with plant hosts. *Genom Biol* 14:121–126

Richardson MJ (2001). Coprophilous fungi from Brazil. *Braz Arch Biol Technol* 44:283–289

Rittenour WR, JH Park, JM Cox-Ganser, DH Beezhold, BJ Green (2014). Internal transcribed spacer rRNA gene sequencing analysis of fungal diversity in Kansas City indoor environments. *Environ Sci Proc Imp* 14:766–774

Rosa LH, M Capelari (2009). Agaricales Fungi from atlantic rain forest fragments in Minas Gerais, Brazil. *Braz J Microbiol* 40:846–851

SaitouN, M Nei (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425

Schoch CL, KA Seifert, S Huhndorf, V Robert, JL Spouge, CA Levesque, W Chen, FB Consortium (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proc Natl Acad Sci* 109:6241–6246

Sharf W, A Javaid, A Shoaib, IH Khan (2021). Induction of resistance in chili against *Sclerotium rolfsii* by plant growth promoting rhizobacteria and *Anagallis arvensis. Egypt J Bioll Pest Cont* 31:16-26

Silva JM, CCN Cristo, YC Montalto, CS Silva, EOA Sena, RB Vigoderis, GSP Barroso, JS Brito Neto, JUL Oliveira, TMC Santos (2019a). Atividade e população microbiana de um podzólico vermelho amarelo sob sistemas de cultivo orgânico e convencional de *Citrus sinensis* (L.) Osbeck. *Rev Ciênc Agrár* 42:340–346

Silva JM, SPG Nascimento, RTR Massahud, TMC Santos, GSA Lima (2019b). Atributos químicos e biológicos do solo: Um estudo no Semiárido Alagoano. *In: Ensaios Interdisciplinares em Ciências agrárias no Nordeste do Brasil*, pp:42–80. Gomes IA. Itacaiúnas, Ananindeua

Silva JM, RRO Teixeira, JR Rocha, TMC Santos (2017). *In vitro* and *in vivo* antagonism of *Screrotium rolfsii Sacc* by strains of *Trichoderma* spp. *Intl J Agric Environ Biores* 2:60–67

Six J, ET Bossuyt, S Degryzr, K Dene (2004). A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. *Soil Till Res* 79:7–31

Tamura K, G Stecher, D Peterson, A Filipski, S Kumar (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 30:2725–2729

Wani ZA, N Ashraf, T Mohiuddin, S Riyaz-Ul-Hassan (2015). Plant-endophyte symbiosis, an ecological perspective. *Appl Microbiol Biotechnol* 99:2955–2965

White TJ, T Bruns, S Lee, J Taylor (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic. *PCR Protoc Guide Meth Appl*, pp:315–322. Innis MA (Ed.) Academic Press, San Diego, California, USA

Yadav AN, P Verma, V Kumar, P Sangwan, S Mishra, N Panjiar, AK Saxena (2018). Biodiversity of the Genus *Penicillium* in Different Habitats. *In: New and Future Developments in Microbial Biotechnology and Bioengineering*, pp: 3–18. Gupta VG, S Rodriguez-Couto (Eds). Elsevier Publishers, London