The Pharma Innovation Journal 2022; 11(6): 17-23

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(6): 17-23

www.thepharmajournal.com
Received: 16-04-2022
Accepted: 24-05-2022

N Swaroopa Rani
Department of Plant Pathology, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad, Telangana, India

N Balram
Department of Plant Pathology, Regional Agricultural Research Station, Polasa, Jagtial, Telangana, India

Bharati N Bhat
Department of Plant Pathology, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad, Telangana, India

SNCVL Pushpavalli
Department of Molecular Biology and Biotechnology, Institute of Biotechnology, Rajendranagar, PJTSAU, Hyderabad, Telangana, India

Corresponding Author:
N Swaroopa Rani
Department of Plant Pathology, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad, Telangana, India

Morphological characterization of endophytes associated with rice

N Swaroopa Rani, N Balram, Bharati N Bhat and SNCVL Pushpavalli

Abstract
Endophytes are the microorganisms that inhabit the internal parts of plants without showing any apparent symptoms in the host plant. The present work aims to study the endophytic fungal communities associated with three rice varieties *viz*., Jagtial Rice-1, Telangana Sona, and Tetep. A total of 30 endophytic fungi were isolated using tissue transplanting method from leaf, stem and root of the healthy plant at heading stage by using PDA medium. Based on the morphological and microscopic characteristics, the isolated endophytes were identified as *Fusarium* spp., *Aspergillus* spp., *Chaetomium* spp., *Curvularia* spp., and *Nigrospora* spp. The isolates were studied for their cultural characteristics like colony color, texture and growth rate on four different media. The results revealed that colony color and texture endophytes did not differ significantly on four types of culture media. However, there was difference in growth rate. The majority of isolates showed highest radial growth rate on OMA followed by MEA, PDA and CZA. Among the 30 isolates, J3 and R5 had the highest and lowest radial growth rates of 7.08 and 3.19 mm/day respectively.

Keywords: Rice, endophytes, fungi, isolation, morphology, growth rate, different growth media

Introduction
Endophyte is defined as an important group of widespread and diverse plant symbionts that live asymptomatically and sometimes systemically within plant tissues without causing symptoms of disease (Promputtha *et al.*, 2005; Porras-Alfaro and Bayman, 2011) [12, 10]. Endophytes can be used in agriculture as a tool to improve crop performance due to their capability of colonizing the internal tissues of the plants and their ability to promote plant growth and to control plant diseases (Yuan *et al*., 2009) [14]. In the past two decades, a great deal of information on the diversity and the role of endophytes in agriculture have been collected (Yuan *et al*., 2009) [14]. Endophytic fungi have been associated with a variety of crops, including wheat (*Triticum aestivum*), maize (*Zea mays*) and soybean (*Glycine max*) (Dingle and McGee, 2003; Istifadah and McGee, 2006; Larran *et al*., 2010) [3, 4, 5].

Rice (*Oryza sativa* L.) is one of the most important cereal crops of the world and is staple food for more than half of the world's population. There are relatively few studies focused on the endophytic fungi associated with rice varieties. Furthermore, there have been very few studies on rice endophytes from India, and most workers have reported bacterial endophytes from rice and their use as plant growth promoter (Sharma *et al*., 2015) [13]. Hence, the present investigation was undertaken to study endophytic fungi associated with three rice varieties in terms of their existence and characteristics.

Materials and Methods
Sample collection and processing
Healthy rice plant samples of three rice varieties, (Jagtial Rice-1(JGL-24423), popularly cultivated variety, Telangana Sona (RNR-15048), popular and blast resistant variety, Tetep, a highly blast resistant land race) were collected at heading stage from Regional Agricultural Research Station, Polasa, Jagtial, Telangana during *Kharif*, 2021.The plants were uprooted and placed in sterile polythene bags and transferred to laboratory for further analysis. Endophytes were isolated from the leaf, stem and root.

Surface sterilisation
Surface sterilisation was performed according to modified protocol as given by Potshangbam *et al.* (2017) [11]. The freshly collected plant samples were properly washed under running tap water to remove the adhering soil and associated undesirable particles, then soaked in distilled

water with a few drops of tween 20 for 10 minutes. Leaves, stems and roots were cut into 1 cm segments and washed twice with distilled water before being surface sterilised with 80 percent ethanol for 1 minute (leaf), 2 minutes (stem), and 3 minutes (root). The samples were treated with 4 per cent sodium hypochlorite, rinsed with sterile distilled water, then treated for 1 minute with 70 per cent alcohol followed by 8–10 rinses with sterile distilled water and dried under sterile condition.

Validation of surface sterilisation method

The effectiveness of the surface sterilization process was checked at every isolation process. This was done to ensure that the microorganisms isolated were truly endophytes. The absence of microbial growth on PDA media plates impregnated with 50 µl aliquots of the final rinsed water proved the efficacy of the surface sterilizing method. The fungus separated through this particular isolation technique is considered as true endophyte when no microorganisms are present at the imprinting path (Potshangbam *et al*., 2017) [11].

Isolation of endophytic fungi

The indigenous methods followed for isolation of endophytes was direct plate impression of sterilized tissues. The samples, leaf, stem and root were carefully made into thin slices removing the outer cover and placed on potato dextrose agar (PDA). Further, the plates were incubated at 28 ± 2°C for 2 weeks until the observation of growth of the fungi (Coombs and Franco, 2003) [2]. The endophytic fungal colonies growing from plant parts were sub-cultured into fresh PDA medium and incubated. The endophytic fungi were identified to genus level based on their macroscopic and microscopic characteristics using identification keys.

Morphological characterization

Morphological characterization of the endophytic fungal isolates on four types of culture media was carried out by growing each isolate in triplicate on potato dextrose agar (PDA), oat meal agar (OMA), czapek dox agar (CZA) and malt extract agar (MEA). The pure cultures of fungal endophytes isolated were grown on potato dextrose agar (200g potato, 20g dextrose, 20g agar/L distilled water), oat meal agar (20g oats, 20g dextrose, 20g agar/L distilled water), czapek dox agar (synthetic) and malt extract agar (synthetic) media to study their growth characteristics such as mycelium type, cultural characteristics like colony color, texture and growth rate and conidia using trinocular microscope, Olympus CX41. The statistical analysis was carried out by using online tool, OPSTAT (http://14.139.232.166/opstat/).

Results and Discussion

Isolation of fungal endophytes

Healthy rice plant samples of three rice varieties, Jagtial Rice-1, Telangana Sona, and Tetep were collected at heading stage from Regional Agricultural Research Station, Polasa, Jagtial, Telangana during *Kharif*, 2021 and 30 endophytic fungi were isolated on PDA medium. The maximum number of fungal endophytes (11 nos.) were obtained from Jagtial Rice-1 (7 from the root, 3 from the leaves, and 1 from the stem), followed by 10 isolates from Tetep (5 from the roots, 2 from the leaves, and 3 from the stem) and least 9 isolates from Telangana Sona (5 from root, 3 from leaves and 1 from stem). The root harboured the highest proportion of endophytes (17), followed by the leaves (8), while the stem harboured the lowest (5) fungal endophytes (Table 1).The current findings are in consistent with those of Naik *et al.* (2006) [8], who reported that overall colonisation rates were 40.3 percent in roots and 25.83 percent in leaves during the winter season, and 20.15 percent in roots and 8.66 percent in leaves during the summer season.

Based on morphological characteristics (Barnett and Hunter, 1972), ten endophytes were identified as *Fusarium* spp., nine as *Aspergillus* spp., six as *Chaetomium* spp, three as *Curvularia* spp., and two as *Nigrospora* spp. (Figure 1). Ten isolates, namely J2, J3, J8, J9, J10, J11, T3, T4, T7 and R8 were identified as *Fusarium* spp. based on these characters, like colony growth (4.25 to 7.08 mm/day), with white floccose (cottony) to creamy aerial mycelium producing fusiform multi-celled macroconidia and cylindrical to oval, 1-2 celled microconidia (Figure 2a. and table 1-4) (Leewijit *et al.*, 2010 and Barnett and Hunter, 1972) [1, 10]. Nine isolates, namely J1, J6, T2, T5, T6, T9, R1, R2 and R6 were identified as *Aspergillus* spp. based on characters, like colony growth (3.45 to 6.74 mm/day) on PDA, with velvety or powdery surface growth, showing various shades of green and yellow with floccose or powdery texture and conidia produced from the phialides on the conidiophore vesicles (Figure 2b and table 1-4) (Leewijit *et al.*,2010 and Barnett and Hunter, 1972) [1, 10]. Six isolates, namely J4, T1, T10, R3, R4 and R5 were identified as *Chaetomium* spp. based on the characters like colony growth (3.19 to 5.5 mm/day) and producing red globose to subglobose ascomata and pale greenish to dark olive-brown lemon-shaped ascospores (Figure 1c and table 1-4.) (Leewijit *et al*., 2010 and Barnett and Hunter, 1972) [1, 10]. Three isolates, namely J7, T8 and R9 were identified as *Curvularia* spp. based on characters like colony growth (3.99 to 5.56 mm/day), greyish appearance of colony with conidiophores producing curved brown colour with 3 to 4 septa (Figure 2d and table 1-4) (Leewijit *et al*., 2010 and Barnett and Hunter, 1972) [10, 1]. Two isolates, namely J5 and R7 were identified as *Nigrospora* spp. based on characters like colony growth (5.11 to 6.74 mm/day) with white to black appearance producing 1-celled shiny black ellipsoidal conidia (Figure 2e and table 1-4) (Barnett and Hunter, 1972) [1]. Leewijit *et al*. (2010) [10] isolated endophytic fungi from two rice varieties by tissue transplanting method and found that they belong to eight genera: *Aspergillus* spp., *Chaetomium* spp., *Colletotrichum* spp., *Curvularia* spp., *Fusarium* spp., *Penicillium* spp., *Phytophthora* spp., *Rhizopus* spp. and *Trichoderma* spp.

The mycelia growth rate and colony characteristics of the 30 fungal endophytes were studied on four different media *viz.*, Potato dextrose agar (PDA), Oat meal agar (OMA), Czapek dox agar and Malt extract agar (MEA) at 28±2 °C. The mycelium type of all the isolates was septate. For a particular isolate, there was no significant difference in color on four different media under study. However, there was change in color of mycelium of different isolates. A few variations were observed in upper surface of colony color on four media. *Fusarium* spp. upper surface colony color varied from white to pinkish white and lower surface varied from light yellowish to creamish white. *Aspergillus* spp. upper surface colony color varied from light green to dark yellowish brown and lower surface varied from pale brown to light red. *Chaetomium* spp. upper surface colony color varied from light yellow to dark red and lower surface varied from light brown to light red.

Curvularia spp. upper surface colony color varied from light grey to dark grey and lower surface varied from pale brown to light red. *Nigrospora* spp. upper surface colony color varied from white to light black and lower surface varied from light yellowish to creamish white.

Among the four media evaluated, the highest and lowest growth rates of 7.08 and 3.19 mm/day were recorded on OMA and CZA, respectively. Among the 30 isolates J3 and R5 showed the highest and lowest growth rates, respectively, of 7.08 and 3.19 mm/day.

Among ten isolates of *Fusarium* spp., isolate J3, showed the highest mycelial growth rate of 7.08 mm/day on OMA and isolate J11, showed the least growth rate of 4.25 mm/day on CZA medium. On PDA, isolate J3, showed highest growth rate of 6.07 mm/day, whereas isolate J11, showed lowest growth rate of 4.28 mm/day. On OMA, isolate J3, showed highest growth rate of 7.08 mm/day, whereas isolate J11, showed lowest growth rate of 5.31 mm/day. On CZA, isolate J3, showed highest growth rate of 6.07 mm/day, whereas isolate J11, showed lowest growth rate of 4.25mm/day. On MEA, isolate J3, showed highest growth rate of 7.08 mm/day, whereas isolate J11, showed lowest growth rate of 4.25 mm/day.

Among nine isolates of *Aspergillus* spp., isolate T6, showed the highest mycelia growth rate of 6.74 mm/day on OMA and isolate J1, showed the least growth rate of 3.45 mm/day on CZA. On PDA, isolate T6, showed highest growth rate of 5.81mm/day, whereas isolate T5, showed lowest growth rate of 3.99mm/day. On OMA, both the isolates, T6 and R1showed highest growth rate of 6.74mm/day, whereas isolate J1, showed lowest growth rate of 4.55 mm/day. On CZA, isolate T6, showed highest growth rate of 5.80 mm/day, whereas isolate J1, showed lowest growth rate of 3.45 mm/day. On MEA, isolate T6, showed highest growth rate of 5.81mm/day, whereas isolate R2, showed lowest growth rate of 4.12mm/day.

Among six isolates of *Chaetomium* spp., isolate R4, showed the highest mycelial growth rate of 5.56 mm/day on OMA and isolate R5, showed the least mycelia growth rate of 3.19 mm/day on CZA. On PDA, isolate R4, showed highest growth rate of 4.42 mm/day, whereas isolate R5, showed lowest growth rate of 3.75 mm/day. On OMA, both the isolates, R4 and T1 showed highest growth rate of 5.56mm/day, whereas isolate R5, showed lowest growth rate of 4.12 mm/day. On CZA, isolate R4, showed highest growth rate of 4.91mm/day, whereas isolate R5, showed lowest growth rate of 3.19 mm/day. On MEA, isolate R4, showed highest growth rate of 5.11mm/day, whereas isolate R3, showed lowest growth rate of 3.75 mm/day.

Among three isolates of *Curvularia* spp., isolate R9, showed the highest growth rate of 5.56 mm/day on OMA and isolate J7, showed the least growth rate of 3.99 mm/day on MEA. On PDA, isolate R9, showed highest growth rate of 4.92mm/day, whereas isolate J7, showed lowest growth rate of 4.40 mm/day. On OMA, isolate R9, showed highest growth rate of 5.56 mm/day, whereas isolate J7, showed lowest growth rate of 4.91mm/day. On CZA, isolate R9, showed highest growth rate of 4.91mm/day, whereas isolate J7, showed lowest growth rate of 4.72 mm/day. On MEA, isolate R9, showed highest growth rate of 4.91mm/day, whereas isolate J7, showed lowest growth rate of 3.99mm/day.

Among two isolates of *Nigrospora* spp., isolate R7, showed the highest mycelia growth rate of 6.74 mm/day on OMA and isolate J5, showed the least mycelia growth rate of 5.11 mm/day on CZA. Isolate R7, showed the highest growth rate of 5.81, 6.74, 5.31 and 5.82 mm/day on PDA, OMA, CZA and MEA respectively. The isolate J5, showed the least mycelial growth rate of 5.56, 6.40, 5.11 and 5.56 mm/day on PDA, OMA, CZA and MEA respectively.

The mycelial growth rate of the endophytic isolates was influenced by the different culture media under study. The nutrient composition of a given culture medium is an important factor that influences the growth rate as it allows the fungi to grow without restrictions and express phenotypes (Meletiadis *et al*. 2001) [7]. Okunowo *et al*. (2010) [9] also observed minimum mycelia growth of *Myrothecium roridum* on czapek dox agar which may be due to the presence of chloride ion in the test medium.

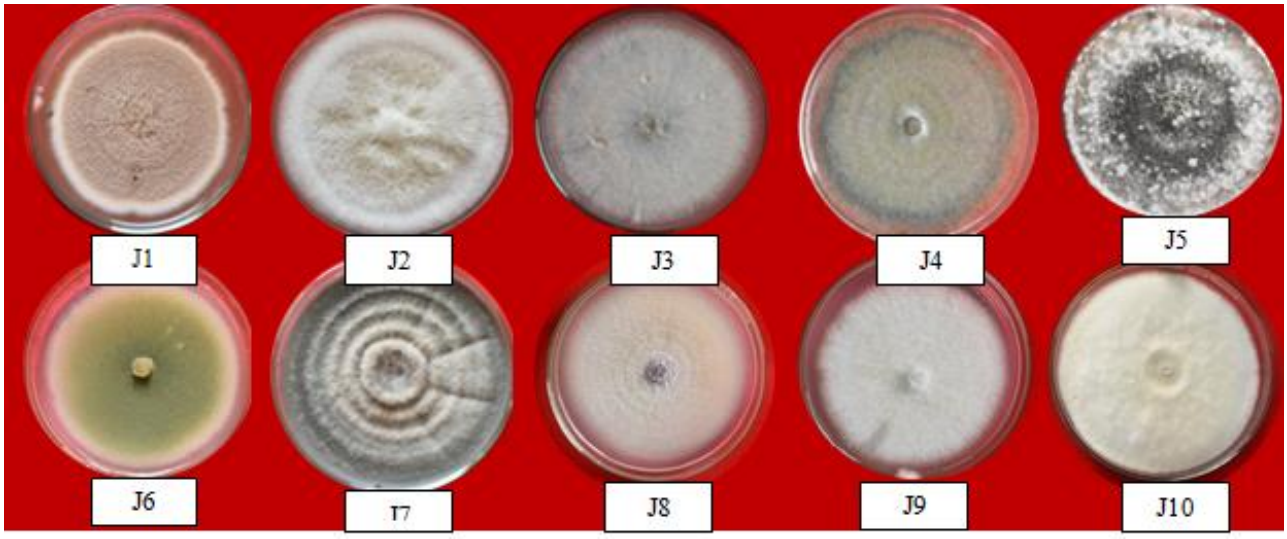

Fig 1: Endophytic fungi isolated from healthy tissues of three rice varieties

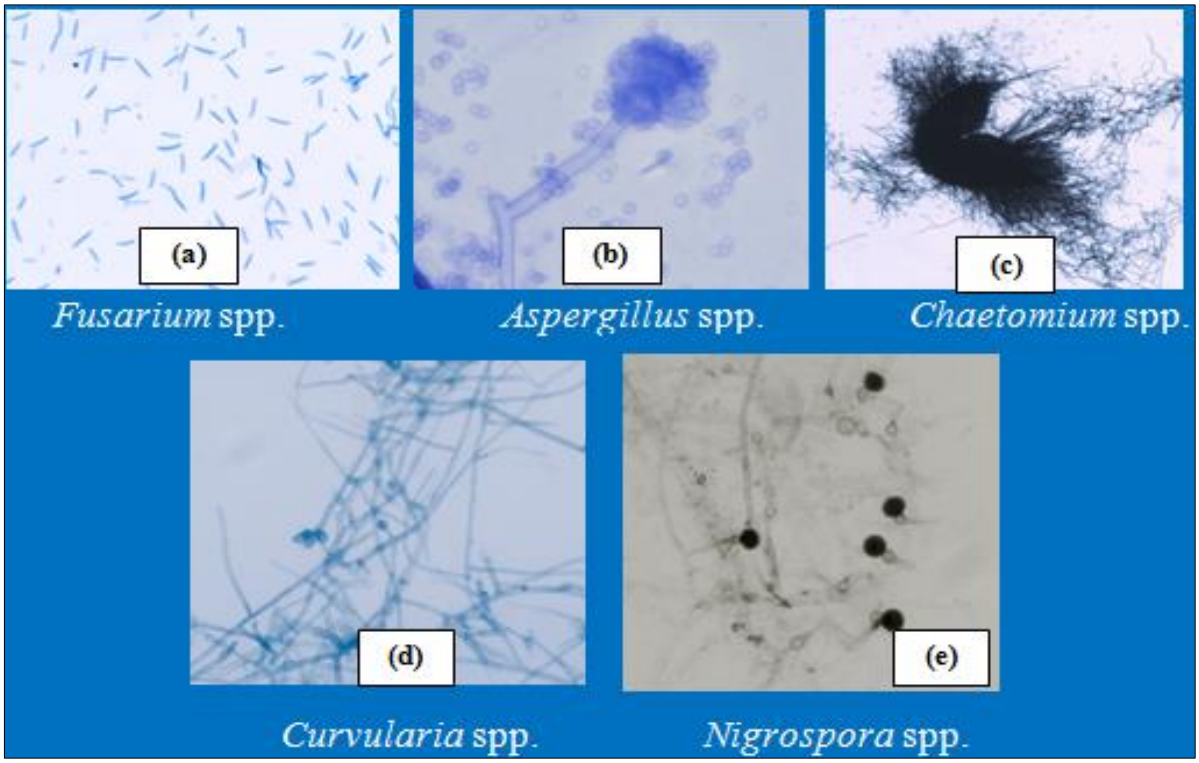

Fig 2: 2(a). Macro and micro conidia of *Fusarium* spp., 2(b). Conidiophores with Sterigmata and Conidia of *Aspergillus* spp., 2(c). Perithecium with lateral hairs of *Chaetomium* spp., 2(d).Conidia of *Cuvularia* spp., and 2(e). Conidia of *Nigrospora* spp.

Table 1: Cultural characteristics and Growth rate of endophytic fungal isolates on PDA

S. No	Isolate	Variety name	Tissue part	Colony color	Reverse color	Texture	Organism	*Radial growth rate
1	J1	Jagtial Rice-1	Root	Yellow to brown	Light brown	Powdery	*Aspergillus* spp.	4.12 ± 0.13
2	J2	Jagtial Rice-1	Root	White	White	Light cottony	*Fusarium* spp.	4.41 ± 0.16
3	J3	Jagtial Rice-1	Root	White	Light yellowish	Dense cottony	*Fusarium* spp.	6.07 ± 0.0
4	J4	Jagtial Rice-1	Root	Golden brown to yellow	Golden brown to yellow	Velvety	*Chaetomium* spp.	4.12 ± 0.13
5	J5	Jagtial Rice-1	Root	White and black	Light black	Fluffy	*Nigrospora* spp.	5.56 ± 0.25
6	J6	Jagtial Rice-1	Root	Light green	Light yellow	Powdery	*Aspergillus* spp.	4.41 ± 0.16
7	J7	Jagtial Rice-1	Root	Grey	Black	Fluffy	*Curvularia* spp.	4.41 ± 0.16
8	J8	Jagtial Rice-1	Leaf	White	Yellowish brown	Light cottony	*Fusarium* spp.	4.41 ±0.16
9	J9	Jagtial Rice-1	Leaf	White	Light yellowish	Velvety	*Fusarium* spp.	4.41 ± 0.16
10	J10	Jagtial Rice-1	Leaf	Yellowish white	Yellowish	Fluffy	*Fusarium* spp.	5.82 ± 0.25
11	J11	Jagtial Rice-1	Stem	Light white	Creamish white	Cottony	*Fusarium* spp.	4.32 ±0.0
12	R1	Telangana Sona	Root	Greenish grey	Light green	Powdery	*Aspergillus* spp.	5.56 ± 0.25
13	R2	Telangana Sona	Root	Light green	Greenish white	Powdery	*Aspergillus* spp.	4.12 ± 0.13
14	R3	Telangana Sona	Root	Greyish yellow	Olive yellow	Velvety	*Chaetomium* spp.	4.12 ± 0.13
15	R4	Telangana Sona	Root	Light brown	Olive brown	Velvety	*Chaetomium* spp.	4.42 ± 0.16
16	R5	Telangana Sona	Root	Light grey	Olive yellow	Powdery	*Chaetomium* spp.	3.75 ± 0.11
17	R6	Telangana Sona	Leaf	Geyish green	Light yellow	Powdery	*Aspergillus* spp.	4.92 ± 0.19
18	R7	Telangana Sona	Leaf	White	Light black	Fluffy	*Nigrospora* spp.	5.82 ± 0.25
19	R8	Telangana Sona	Leaf	White	Light yellow	Cottony	*Fusarium* spp.	4.92 ± 0.19
20	R9	Telangana Sona	Stem	Grey	Black	Fluffy	*Curvularia* spp.	4.92 ± 0.19
21	T1	Tetep	Root	Light brown	Light yellow	Velvety	*Chaetomium* spp.	4.41 ± 0.16
22	T2	Tetep	Root	Whitish grey	Yellowish red	Powdery	*Aspergillus* spp.	5.11 ± 0.19
23	T3	Tetep	Root	Whitish	Light yellow	Cottony	*Fusarium* spp.	4.92 ± 0.19
24	T4	Tetep	Root	White	Light brown	Cottony	*Fusarium* spp.	4.41 ± 0.16
25	T5	Tetep	Root	Brownish yellow	Light yellow	Powdery	*Aspergillus* spp.	3.99 ± 0.13
26	T6	Tetep	Leaf	Greyish green	Light yellow	Powdery	*Aspergillus* spp.	5.82 ± 0.25
27	T7	Tetep	Leaf	Light white	Creamish white	Cottony	*Fusarium* spp.	5.82 ± 0.25
28	T8	Tetep	Stem	Grey	Black	Fluffy	*Curvularia* spp.	4.90 ± 0.19
29	T9	Tetep	Stem	Yellow to brown	Light brown	Powdery	*Aspergillus* spp.	5.11 ± 0.19
30	T10	Tetep	Stem	Olive brown	Light yellowish	Velvety	*Chaetomium* spp.	4.41 ± 0.16

Table 2: Cultural characteristics and Growth rate of endophytic fungal isolates on OMA

S. No	Isolate ID	Colony color	Reverse color	Texture	*Radial growth rate
1	J1	Brownish yellow	Light yellow	Powdery	4.56 ± 0.16
2	J2	White	Light yellow	Dense cottony	5.56 ± 0.25
3	J3	White	Light yellow	Dense cottony	7.08 ± 0.00
4	J4	Grey to brown	Light yellow	Fine	5.11 ± 0.19
5	J5	White	Light yellow	Cottony	6.41 ± 0.34
6	J6	Light green	Light red	Velvety	4.92 ± 0.19
7	J7	Grey	Pale brown	Fluffy	4.92 ± 0.19
8	J8	White	Yellowish brown	Cottony	5.56 ± 0.25
9	J9	White	Yellowish red	Cottony	6.41 ± 0.34
10	J10	White	Pale brown	Cottony	6.74 ± 0.34
11	J11	Very light white	pale yellow	Cottony	5.31 ± 0.00
12	R1	Dark grey	Light red	Powdery	6.74 ± 0.34
13	R2	Dark green	Light brown	Floccose	5.11 ± 0.19
14	R3	Creamish yellow	Reddish yellow	Fine	4.56 ± 0.16
15	R4	Grey to brown	Light red	Fine	5.56 ± 0.25
16	R5	Light grey	Yellowish brown	Powdery	4.12 ± 0.13
17	R6	Light green	Light yellow	Flufy	5.56 ± 0.25
18	R7	White	Light yellow	Fluffy	6.74 ± 0.34
19	R8	Pinkish white	Yellowish red	Cottony	6.41 ± 0.34
20	R9	Grey	Light black	Fluffy	5.56 ± 0.25
21	T1	Brown	Light yellow	Fine	5.56 ± 0.25
22	T2	Greenish grey	Creamish white	Velvety	5.82 ± 0.25
23	T3	Whitish	Light yellow	Cottony	5.56 ± 0.25
24	T4	White	Pale brown	Cottony	6.41 ± 0.36
25	T5	Whitish yellow	Brownish yellow	Powdery	4.92 ± 0.19
26	T6	Greyish green	Pale brown	Powdery	6.74 ± 0.34
27	T7	Very light white	pale yellow	Cottony	6.74 ± 0.34
28	T8	Blackish brown	Pale brown	Fluffy	5.54 ± 0.25
29	T9	Yellowish brown	Light yellow	Velvety	5.90 ± 0.59
30	T10	Olive green	Creamish white	Powdery	4.92 ± 0.19

*Data represents mean of three replicates ± SE

Table 3: Cultural characteristics and Growth rate of endophytic fungal isolates on CZA

S. No	Isolate ID	Colony color	Reverse color	Texture	*Radial growth rate
1	J1	Brownish yellow	Light yellow	Powdery	3.45 ± 0.09
2	J2	White	White	Cottony	4.41 ± 0.16
3	J3	White	white	Cottony	6.07 ± 0.00
4	J4	Light yellowish brown	Brown	Velvety	3.45 ± 0.09
5	J5	White	Light blackish white	Cottony	5.11 ± 0.19
6	J6	Light green	Light yellow	Velvety	4.41 ± 0.16
7	J7	Whitish grey	Light reddish black	Velvety	4.72 ± 0.00
8	J8	White	Olive brown	Cottony	4.41 ± 0.16
9	J9	White	Light olive brown	Cottony	4.28 ± 0.13
10	J10	White	Dark yellow	Cottony	5.82 ± 0.25
11	J11	White	Light yellow	Cottony	4.25 ± 0.00
12	R1	Greenish grey	Creamish white	Powdery	4.92 ± 0.19
13	R2	Green and white	Light brown	Floccose	4.12 ± 0.13
14	R3	Cremish yellow	Greenish yellow	Fine	3.75 ± 0.11
15	R4	Whitish brown	Light brown	Raised fluffy	4.92 ± 0.19
16	R5	Light grey	Light yellow	Powdery	3.19 ± 0.08
17	R6	Whitish green	White	Velvety	4.41 ± 0.16
18	R7	White	Light blackish white	Fluffy	5.31 ± 0.00
19	R8	White	Light olive brown	Cottony	4.92 ± 0.19
20	R9	Whitish grey	Dark grey	Velvety	4.92 ± 0.19
21	T1	Light yellowish brown	Light brown	Fluffy	4.41 ± 0.16
22	T2	Greenish grey	Cream	Velvety	4.56 ± 0.16
23	T3	White	Light yellow	Cottony	4.92 ± 0.19
24	T4	White	Light yellow	Cottony	4.41 ± 0.16
25	T5	Brownish yellow	Light yellow	Fluffy	3.99 ± 0.13
26	T6	Greyish green	Light green	Powdery	5.82 ± 0.25
27	T7	White	Light yellow	Cottony	5.11 ± 0.19
28	T8	Whitish grey	Dark grey	Velvety	4.90 ± 0.19
29	T9	Dark yellowish brown	Light yellowish	Velvety	4.56 ± 0.16
30	T10	Golden brown	Light brown	Fluffy	3.65 ± 0.11

*Data represents mean of three replicates ± SE

Table 4: Cultural characteristics and Growth rate of endophytic fungal isolates on MEA

S. No	Isolate ID	Colony color	Reverse color	Texture	*Radial growth rate
1	J1	Yellow	Light yellow	Fine	4.12 ± 0.13
2	J2	White	Light yellow	Cottony	4.41 ± 0.16
3	J3	White	Light brown	Fluffy	7.08 ± 0.00
4	J4	Light yellow	Dusky red	Velvety	4.41 ± 0.16
5	J5	White and black	Creamish white	Fluffy	5.56 ± 0.25
6	J6	Light yellow	Reddish yellow	Powdery	4.92 ± 0.19
7	J7	Brownish grey	pale brown	Velvety	3.99 ± 0.13
8	J8	White	Reddish brown	Cottony	4.41 ± 0.16
9	J9	White	Olive yellow	Cottony	4.41 ± 0.16
10	J10	White	Yellowish	Fluffy	5.82 ± 0.25
11	J11	White	Light yellow	Cottony	4.28 ± 0.25
12	R1	Greenish grey	White	Powdery	5.56 ± 0.25
13	R2	Yellowish green	Reddish yellow	Powdery	4.12 ± 0.13
14	R3	Yellow	Reddish yellow	Fine	3.75 ± 0.11
15	R4	Olive brown	Light brown	Raised fluffy	5.11 ± 0.19
16	R5	Light yellowish	Light yellow	Fine	4.12 ± 0.13
17	R6	Whitish green	Light yellow	Velvety	4.92 ± 0.19
18	R7	Light black	Creamish white	Fluffy	5.82 ± 0.25
19	R8	White	Olive yellow	Cottony	4.92 ± 0.19
20	R9	Brownish grey	Black	Velvety	4.92 ± 0.19
21	T1	Dark red	Dusky red	Raised fluffy	4.92 ± 0.19
22	T2	Pale green	Light brown	Velvety	5.11 ± 0.19
23	T3	Light pink	Dark pink	Cottony	4.92 ± 0.19
24	T4	White	Light yellow	Cottony	4.41 ± 0.16
25	T5	Light yellow	White	Powdery	4.41 ± 0.16
26	T6	Greyish green	Light green	Powdery	5.82 ± 0.25
27	T7	White	Light yellow	Cottony	5.82 ± 0.25
28	T8	Brownish grey	pale brown	Velvety	4.41 ± 0.15
29	T9	Yellowish	Light yellow	Powdery	5.11 ± 0.19
30	T10	Greyish brown	Light red	Velvety	4.41 ± 0.16

*Data represents mean of three replicates ± SE

Conclusion

The results presented in this study revealed the endophytic fungi associated with three varieties of rice. All the parts of rice plants were found to be colonized with endophytic fungi and the most frequently occuring genera were *Fusarium*, *Aspergillus*, *Chaetomium*, *Curvularia* and *Nigrospora*. Difference in endophytic colonization has been observed in tissue types. Roots harboured more endophytes followed leaves and stem. The growth of individual isolates varied significantly based on growth media used. Oat meal agar and czapek dox agar were found to be most and least suitable media for growth of the endophytes.

Acknowledgment

The authors are thankful to Regional Agricultural Research Station, Polasa, Jagtial and College of Agriculture, Rajendranagar, PJTSAU for providing facilities and financial assistance during the research work.

References

1. Barnett HL, Hunter BB. Illustrated Genera of Imperfect Fungi. Burgess Publishing Company, Minnesota, 1972.
2. Coombs JT, Franco CM. Isolation and identification of actinobacteria from surface-sterilized wheat roots. Appl. Environ. Microbiol. 2003;69(9):5603-5608.
3. Dingle J, Mc Gee PA. Some endophytic fungi reduce the density of pustules of *Puccinia recondita* f. sp. *tritici* in wheat. Mycol. Res. 2003;107:310-316.
4. Istifadah N, Mc Gee PA. Endophytic *Chaetomium globosum* reduces development of tan spot in wheat caused by *Pyrenophora tritici–repentis*. Australia's Plant Pathol. 2006;35:411-418.
5. Larran S, Monaco C. Status and progress of research in endophytes from agricultural crops in Argentina. In: Arya, A. and A. E. Perello (Eds.) Management of fungal plant pathogens. 2010, 149-151.
6. Leewijit T, Pongnak W, Soytong K, Poeaim S. Isolation of soil and endophytic fungi from rice (*Oryza sativa* L.). Int. J. Agric. Technol. 2017;12:2191-2202.
7. Meletiadis J, Meis JFGM, Mouton JW. Analysis of growth characteristics of filamentous fungi in different nutrient media. J Clin. Microbiol. 2001;39:478-484.
8. Naik, B S, Shashikala J, Krishnamurthy YL. Study on the diversity of endophytic communities from rice (*Oryza sativa* L.) and their antagonistic activities *in vitro*. Microbiol. Res. 2009;164(3):290–296.
9. Okunowo WO, Gbenle GO, Osuntoki AA, Adekunle AA. Mediastudies on *Myrothecium roridum* Tode: A potential biocontrol agent for water hyacinth. J Yeast Fungal Res. 2010;1(4):55-61.
10. Porras-Alfaro A, Bayman P. Hidden fungi, emergent properties: endophytes and microbiomes. Annu. Rev. Phytopathol. 2011;49:291-315.
11. Potshangbam M, Devi SI, Sahoo D, Strobel GA. Functional characterization of endophytic fungal community associated with *Oryza sativa* L. and *Zea mays* L. Front Microbiol. 2017;8:325.
12. Promputtha I, Jeewon R, Lumyong S, McKenzie EHC, Hyde KD. Ribosomal DNA fingerprinting in the identification of non sporulating endophytes from *Magnolia lilifera* (Magnoliaceae). Fungal Divers. 2005;20:167-186.
13. Sharma A, Patni B, Shankhdhar D, Shankhdhar SC. Evaluation of different PGPR strains for yield enhancement and higher Zn content in different genotypes of rice (*Oryza sativa* L.). J Plant Nutr. 2015;38:456-72.
14. Yuan Z, Zhang C, Lin F, Kubicek CP. Identity, diversity, and molecular phylogeny of the endophytic mycobiota in the roots of rare wild rice (*Oryza granulate*) from a nature reserve in Yunnan, China. Appl. Environ. Microbiol. 2009;76(5):1642-1652.