Occurrence of dark septate endophytic fungi in the roots of *Pennisetum polystachion* (L.) Schult.

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Occurrence of dark septate endophytic fungi in the roots of *Pennisetum polystachion* (L.) Schult.

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Dark septate endophytes are unique group of endophytic fungi possessing characteristic melanised, septate hyphae. This group of fungi are well known for its host beneficial attributes especially in drought tolerance and survival. *Pennisetum polystachion* is a widely spreading grass weed which is well distributed in the lateritic soils of northern Kerala. In the present study we carried out a microscopic examination of *P.polystachion* roots. Microscopic observation revealed that *Pennisetum polystachion* roots are colonised with endophytic dark septate fungi.

Keywords: Dark septate endophytes, fungi, invasive species, lateritic soil, *Pennisetu*m,perennial grass,weeds

INTRODUCTION

DSEs (dark septate endophytes) are a heterogenous group of fungal root endophytes with characteristic melanized and septate hyphae, which grows inter or intracellularly in the host tissues. They are found in a variety of host plants in diverse habitats and ecosystems. Several, recent reports characterize various capacities of this group of fungi, most of which are related to the better growth performance of host plants (He *et al*. 2019; Li *et al;* 2021; Santos *et al*. 2021). DSEs impart remarkable property of stress tolerance in host plants. They can improve host plant root development and nutrient absorption, which essentially makes the host plant a better performer in stress conditions (Li *et.al* 2019; Liu and Wei, 2019). Inoculation of host plants with DSEs have resulted in increased photosynthesis, a feature which gets diminished in drought conditions.

Such kind of beneficial attributes imparted by DSEs have made them candidate microorganisms that can be used in vegetation restoration attempts, especially to reduce desertification indryland ecosytems (Zou *et al.* 2020; He *etal*. 2021; Hou *et al.* 2021).

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Pennisetum polystachyon (L.)Schult., popularly known as 'Mission grass' is a perennial grass with invasive nature. Its population is seen in a wide variety of soils, including lateritic soils of northern kerala. This plant shows greater stress tolerance when compared to native flora. In the present study, we conducted microscopic analysis of *P.polystachion* roots to explore its endophytic microbiome.

MATERIALS AND METHODS

Study site and sampling of materials

Pennisetum polystachion growing extensively, in the Dharmadam region of Thalassery,Keralawas investigatedin this study.Samples were taken randomly from multiple locations of this region. The plants were uprooted and fine roots were collected in polythene bags. These were brought to lab and roots were washed thoroughly. These roots were cut into small pieces of 1-2 cm in length and were used for microscopic analysis.

Microscopic analysis

The root samples were prepared according to Li *et al*. (2018) with some modifications. The collected root samples were washed thoroughly

with distilled water several times. The samples were treated at 90-100^{0}C in 10% KOH (SRL, India) for 10-15 min. After that roots were washedto remove KOH and the roots were soaked in 2% HCl (SRL, India) for 3-5 minutes. The samples were placed on a clean glass slide and stained with cotton blue (SRL, India). The samples were examined, under 40 X magnification (Olympus CX43, India), after putting a cover glass and gently pressing the preparation to evenly spread the root tissues.

Isolation of fungal endophytes

Isolation of fungal endophytes was carried out on potato dextrose agar medium (Himedia, India) following the methodology given by Li *et al.* (2018) with modifications. The fresh root samples were cut into small pieces after thorough washing in sterile distilled water. These samples were surface sterilized using 70% ethyl alcohol and 5% sodium hypochlorite(each treatment for 5 min) and transferred to potato dextrose agar medium. The samples were incubated in dark at room temperature for 2 days. Mycelia emerging from the root samples were separatedand subcultured to fresh potato dextrose agar medium to generate pure cultures.

Biochemical characterization of fungal isolates Test for IAA production

Fungal isolates were grown in 20ml potato dextrose liquid medium, with or without a 1 mg/L supplement of L-tryptophan. The inoculated liquid medium was incubated in shaker incubator at 25^{0}C, 120 rpm for 4-5 days. To determine the production of IAA, one ml of culture supernatant was mixed with 2 ml of Salkowski reagent (1 ml of 0.5 M $FeCl_2$ in 50ml of 35% $HClO_4$) and incubated in dark for 30 min. Appearance of pink colour indicates the presence of IAA.

Catalase activity

Test tubes with Potato dextrose liquid media were inoculated with fungi and incubated at roomtemperature in shaking incubator at 120 rpm, for 4-5 days. Culture supernatant was taken in a 2 ml microcentrifuge tube and a few drops of 6% H_2O_2 were added into it. Formation of oxygen bubbles confirms the catalase activity of the fungal culture.

Ammonia production

Fungal cultures were inoculated in test tube containing Potato dextrose liquid medium and incubated in shaking incubator (120 rpm) at room temperature for 4-5 days. One ml of the liquid medium was taken into a 2 ml microcentrifuge tube and 0.5 ml of Nessler's reagent was added into it. The development of yellow to brown colour indicates the presence of ammonia.

RESULTS AND DISCUSSION

Microscopic analysis of roots

The prepared root samples were examined under 40 X magnification of compound microscope (Olympus, India). It was found that the roots were colonised by fungi with characteristic melanised and septate hyphae (Fig. 1A). It was also observed that this endophytic fungus is forming microsclerotia like structures (Fig.1B and C).

Isolation of endophytic fungi

Fungal mycelia emerging out from the root samples were carefully separated and subcultured on potato dextrose agar medium. Altogether, six different types of fungal cultures were isolated. Fig. 3 shows the cultures of these fungal types. These cultures need further taxonomic and functional characterization using molecular tools.

Biochemical characterization of fungal isolates

The results of the biochemical tests are summarised in Table 1. Out of the six fungal isolates only PFE6 was positive for IAA production. Fungal isolate PFE4 was found to be positive for both catalase and ammonia production, whereas PFE3 was positive for only ammonia production. Fungal isolates PFE1, 2 and 5 showed no positive results in any of the tests conducted.

This is the first report on the occurrence of dark septate endophytes in *Pennisetum polystachion* (L) Shult., roots. DSEs are a special group attaining much attention in recent times, especially in the context of survival of plants in drought conditions, both native and crop plants. It is even more interesting to note that many plant species have developed specificities in their endophyte interactions (Wearn *et al.* 2012; Geisen *et al.* 2021). *Pennisetum polystachion*, when compared to the native flora, shows luxuriant growth and better adaptability in lateritic soils.

In this study, we could observe and isolate fungi of endophytic origin, but with certain specialised

Table 1. Biochemical activities of endophytic fungi isolated.

	Biochemical tests		
Colony	IAA production	Catalase activity	Ammonia production
PFE 1	-	-	-
PFE 2	-	-	-
PFE 3	-	-	+
PFE 4	-	+	+
PFE 5	-	-	-
PFE 6	+	-	-

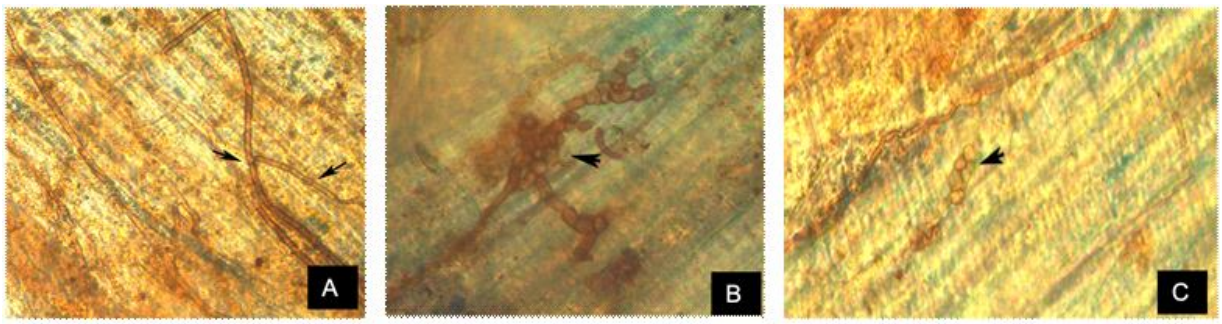

Fig .1: Dark septate endophyte of *Pennisetum polystachion*. A- Colonization of root tissues with fungi bearing melanized hyphae. The arrows point to the septate nature of the mycelium. B & C- Microsclerotia developed by the DSE colonized in the root tissues of *P.polystachion*

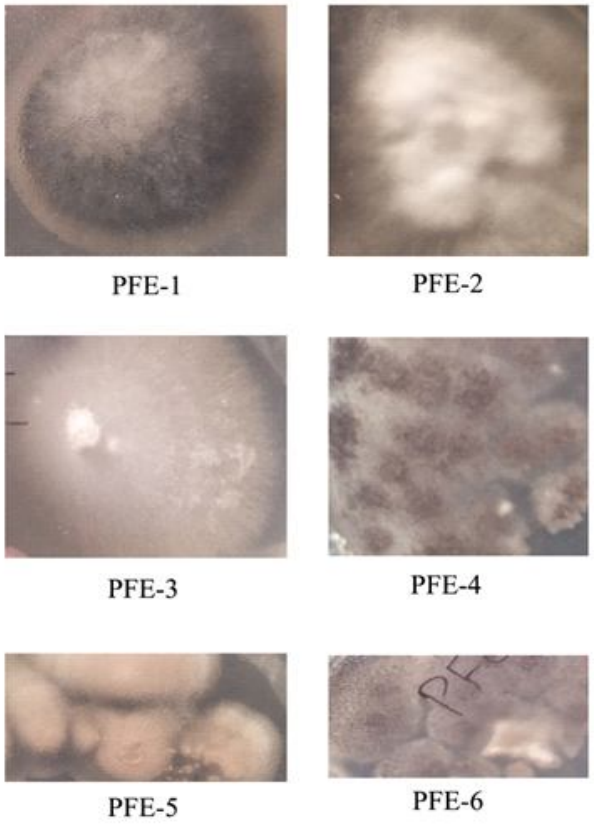

Fig. 2: Endophytic fungi isolated from *P.polystachion,* showing the *in vitro* culture of endophytic fungi isolated from the roots of *P.polystachion.*

features like melanised and septate hyphae, from the roots of *P.polystachion*. It was also recorded that these endophytes develop sclerotia like structures inside the host plant tissue. Such a group of fungi, popularly known as 'dark-septate endophytes' when isolated from a plant source and inoculated in another plant were shown to give some the recipient plant adaptive attributes. Promotion of plant growth in stressful condition, by DSEs, is a feature, which is thought to be helpful in dry and arid land agriculture (He *etal.* 2019; Li.*et.al*, 2021, Santos *et.al* 2021). DSEs make nutrients, become more soluble and available, so that the plants can absorb it easily (Xu *et.al* 2020). Further,it can also be seen that presence of DSEs makes the host plant tolerant towards heavy metal stress (Jin *etal.* 2018; Hou *et al.* 2020; Spagnoletti *et al.* 2020).All these well elucidated features of DSEs, may be operating in *Pennisetum polystachion*, making it a better performer in stressful conditions and thus, successful in invasion. We would also like topoint out a potential of *P.polystachion* DSEs in commercial cropping system and alsofor the restoration of native flora facing desertification or habitat loss.

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