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Effect of two root endophytes (*Campylospora parvula* and *Tetracladium setigerum*) on the growth of the wheat plant

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Abstract

Endophytic aquatic hyphomycetes, *Campylospora parvula* and *Tetracladium setigerum*, recovered from the roots of riparian plants, *Pilea scripta* and *Debregessia* sp., respectively, were used to treat wheat (*Triticum aestivum* L.) through pot experiments for assessing their effect on the growth and development of the plant. Both endophytes were effective in enhancing the growth of the test plant significantly ($p<0.01$). The increment in plant growth was measured in terms of length (shoot and root), diameter (shoot and root), total fresh weight and total dry weight of test plants in comparison with the control plants. Both endophytes served as significantly affective ($p<0.01$) wheat growth promoters. The present findings strongly support the utilization of these endophytic fungi as plant growth promoters. In the future, these strains deserve to be used as bio-fertilizers to increase the yield of agricultural crops.

Keywords – Bio-fertilizer – plant growth promoter – pot experiment – root endophytes – wheat.

Introduction

Mutualism affects the diversity and productivity of the ecosystem. Nearly 300,000 plant species harbor one or more endophytes (Smith et al. 2008). Endophytism is defined as the asymptomatic association of healthy plant tissues with microorganisms without specific effects on the host (Petrini 1991, Schulz et al. 1998, Hyde & Soytong 2008, Dissanayake et al. 2018). Endophytes (fungi and bacteria) produce different types of growth hormones or induce the host plants to secrete hormones which stimulate the growth and development of the host by improving the nutrient metabolism (Schulz & Boyle 2005, Saengket et al. 2021).

Aquatic hyphomycetes constitute an important ecological community in freshwater streams, mainly in litter decomposition and mediating energy flow in stream ecosystems (Ingold 1942, Bärlocher 2005, Gessner et al. 2007). A broad spectrum of fungi, including aquatic hyphomycetes, colonizes the roots of aquatic plants (Sati & Belwal 2005, Bärlocher 2006, Kohout et al. 2012, Ghate & Sridhar 2017, Sridhar 2019, 2021). Endophytic aquatic hyphomycetes produce a wide range of secondary metabolites, such as antifungal, antibacterial, plant growth promoters and different cell wall degrading enzymes (Bärlocher & Kendrick 1976, Gulis & Stephenovich 1999, Sati & Arya 2010a, b, Sati & Singh 2014). However, the investigation into the bio-prospective impact of these fungi as plant growth promoters is poorly understood.

Therefore, the objective of the present study was to evaluate the effects of two root endophytic, aquatic hyphomycetes (*Campylospora parvula* and *Tetracladium setigerum*) on plant

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growth promotion by pot experiments under controlled glass house conditions using wheat (*Triticum aestivum*), as test plants.

Materials & Methods

Isolation of root endophytes

The root samples of angiospermic plants were collected from the streams at different localities in Nainital, Kumaun Himalaya, India. The collected root samples were washed thoroughly under running tap water for 2-3 hours. They were treated with a 2 % sodium hypochlorite solution (2-5 minutes) for surface sterilization. These samples were washed again with sterilized water and then cut into small pieces (1-2 cm) using a sterile scissor and placed into Petri dishes containing 20 ml of sterilized water. The root segments in Petri dishes were incubated at 20 ± 2 °C for 3–7 days and examined regularly. Observations were made using a compound microscope to confirm mycelial growth and conidial liberation into the water. Floating conidia were picked up by a sterile needle on glass slides, and semi-permanent slides were made. Pure cultures were prepared by transferring conidia onto 2% malt extract agar (MEA). Germinated conidia were grown and maintained on MEA slants at 4 °C. The isolated fungi were identified based on morphological characteristics with the help of pertinent literature and in consultation with experts.

Preparation of inoculum

Mycelial agar blocks (5 mm) were cut from the margin of a pure culture of isolated endophyte and transferred into 250 ml Erlenmeyer flasks containing 60 ml of sterilized malt extract broth (MEB) medium. These flasks were incubated at 20±2°C. After ten days, broths were filtered through Whatman No. 1 filters and the filtrate was used as inoculum for the pot experiments. For controlled experiments, un-inoculated flasks were prepared in the same manner and kept under the same conditions.

Preparation of soil and pots

Soils were collected from the Thakur Dev Singh Bisht Campus, Kumaun University, Nainital, India. They were mixed thoroughly and sieved to remove pebbles and other debris. The soil was then made nutrient deficient by mixing with sand (1:3) and used to fill the pots.

Sterilization and sowing of seeds

Seeds of wheat (*Triticum aestivum* L.; Poaceae) were surface sterilized by soaking them in a 1% sodium hypochlorite solution for 1-5 minutes and then rinsing them with sterilized water. Seeds were treated with fungal and medium broths and kept overnight for test and controlled experiments, respectively. The seeds were then sown in their respective pots for germination and growth under the glass house (28±2^{0}C).

Inoculation to the test plants

The pot experiments were conducted in glass house conditions by sowing wheat seeds. Wheat seeds were sown in each pot, containing about 3 kg of soil. After ten days of germination, 50 ml of fungal broth (inoculum) and medium broth were applied to test and control plants, respectively. Subsequent successive treatments were applied after an interval of seven days until the completion of the experiment in the glass house (average temperature and relative humidity were 28 ±2 °C and 37 %, respectively). All pots were watered with sterile distilled water periodically. The experimental plants of *T. aestivum* were harvested after eight weeks of growth (Fig 1A). The following parameters were used to assess the role of endophyte on plant parameters:

i. Shoot and root length
ii. Shoot and root diameter

iii. Total fresh weight
iv. Total dry weight
v. Number of leaves

The dry weight of the plant was measured after drying the samples at 70 °C for 48 h in an oven (Bohm 1979). Data was recorded for each set of test and control plants separately in triplicate. An analysis of variance (ANOVA) was conducted among fungal-treated and non-treated plants.

Results

On the basis of morphological analyses, the endophytic root isolates were identified as *Campylospora parvula* Kuzuha and *Tetracladium setigerum* (Grove) Ingold belonging to aquatic hyphomycetes. *Campylospora parvula* and *Tetladiumrac setigerum* were isolated from the healthy roots of *Pilea scripta* and *Debregessia* sp., respectively.

Effect of treatment on the test plant

The results of the present investigation are summarized in Table 1. Both endophytes were effective for growth promotion in wheat (Fig. 1 and 2). The effects of applied filtrates on the test plants were recorded after eight weeks of plant growth, starting from the seeds sown. The growth and biomass of potted plants were enhanced in test plants significantly ($p<0.01$) compared to control plants (Table 2).

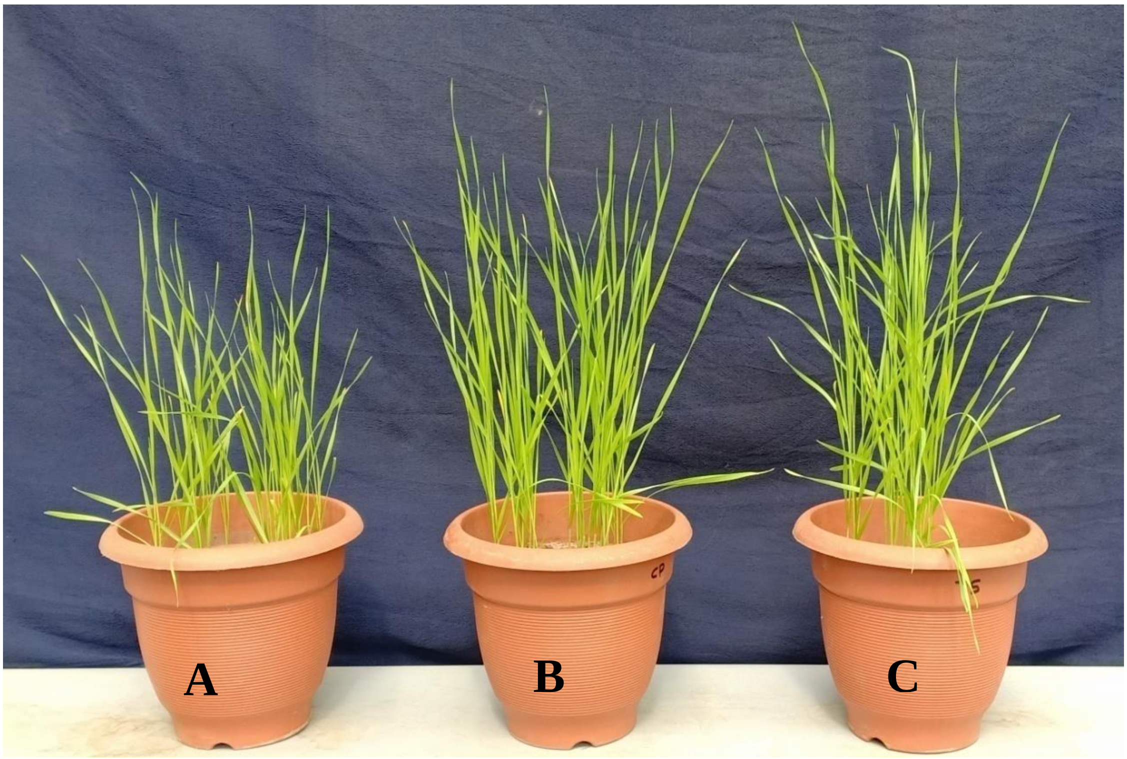

Fig. 1- Effect of endophytic fungal extract treatment on shoot length of wheat plant. A Control. B treated with *C. parvula*. C treated with *T. setigerum*

Table 1 Effect of endophytic fungal extract on the growth of wheat (mean±SEM based on three replicates (9 plants/replicates).

Parameter	Treatments		
	C. parvula	*T. setigerum*	Control
Shoot length (in mm)	380 ±4.3	420 ±3.7	300 ±3.4
Shoot diameter (in mm)	2.06 ±0.09	2.18 ±0.08	1.42 ±0.09

Table 1 Continued.

Parameter	Treatments *C. parvula*	*T. setigerum*	Control
Root length (in mm)	160 ±1.1	200 ±1.6	110 ±0.8
Root diameter (in mm)	1.10 ±0.06	1.14 ±0.07	0.86 ±0.03
Fresh weight of shoot (g/plant)	0.57 ±0.02	0.72 ±0.01	0.40 ±0.04
Fresh weight of root (g/plant)	0.08 ±0.01	0.09 ±0.02	0.05 ±0.01
Dry weight of shoot (g/plant)	0.07 ±0.03	0.09 ±0.01	0.03 ±0.01
Dry weight of root (g/plant)	0.02 ±0.00	0.02 ±0.01	0.01 ±0.01
No. of leaves (after 8 weeks)	3	4	3

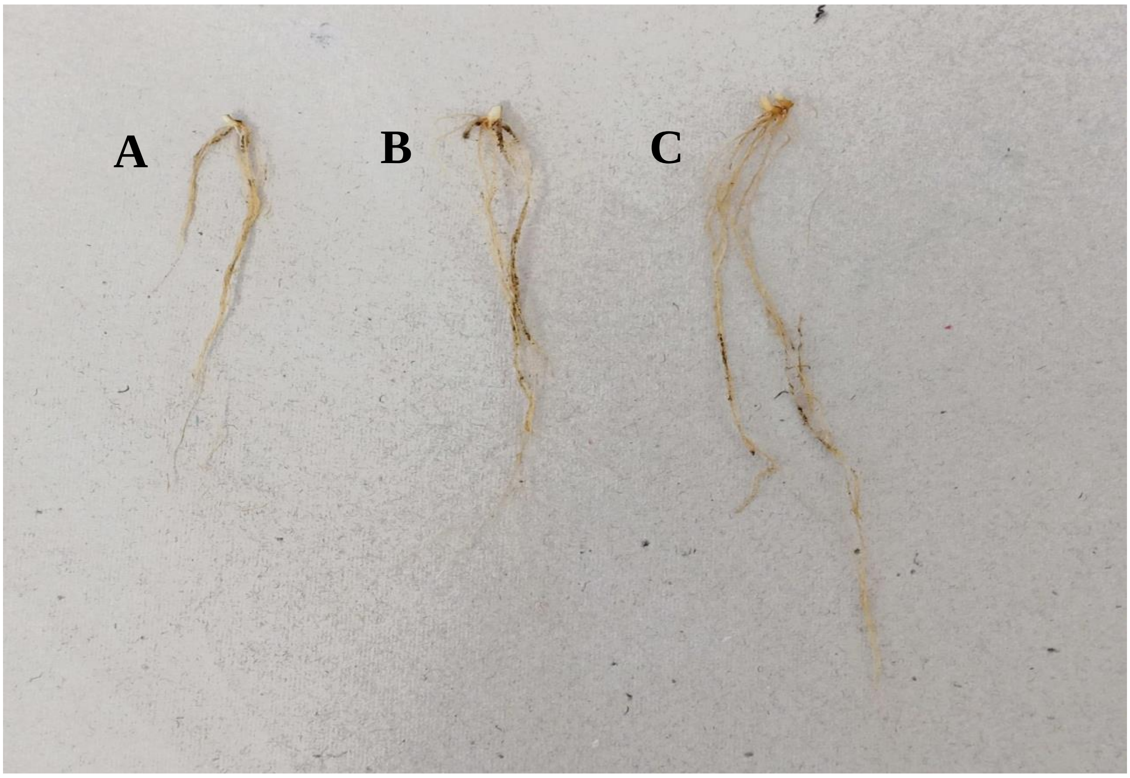

Fig. 2 – Effect of endophytic fungal extract on roots of wheat. A Control. B Treated with *C. parvula*. C Treated with *T. setigerum*

Table 2 ANOVA values for test plants in different parameters (*p<0.01).

Parameter	Sum of Square	df	Mean Square	F	P value
Shoot length	23755.556	2	11877.778	19.796	0.002*
Root length	13266.667	2	6633.333	12.702	0.007*
Shoot diameter	1.024	2	0.512	548.512	0.000*
Root diameter	0.146	2	0.073	96.926	0.000*
Fresh weight of shoot	0.287	2	0.143	127.723	0.000*
Dry weight of shoot	0.006	2	0.003	10.792	0.014
Fresh weight of root	0.008	2	0.004	9.410	0.003*
Dry weight of root	0.000	2	0.000	1.500	0.296
Number leaves	0.667	2	0.333	1.500	0.001*

The Analysis of Variance (ANOVA) among the treated and non-treated plants showed a noticeable difference for all parameters (p<0.01). Based on the results, both endophytic fungi showed significant effects on shoot length, root length, shoot fresh weight, shoot dry weight and total biomass compared with the control pot plants (Table 1). Thus, endophytic fungi have significant potential to enhance plant growth in all tested parameters.

Discussion

Fungal endophytes are representatives of the symbiotic association between fungi and their host plants. A number of endophytic fungi are now gaining importance as they are capable of producing bioactive compounds of agricultural and medicinal importance (Petrini et al. 1992). Some aquatic hyphomycetes have also been reported as root endophytes, but information on their role as endophytes in plant health is quite meager.

In the present investigation, the role of root endophytic aquatic hyphomycetes in plant health promotion has been studied. The tested endophytic root fungi *C. parvula* and *T. setigerum* isolated from the riparian area of Nainital Kumaun Himalaya were found to be effective in enhancing the growth of wheat in many studied parameters. The test plants treated with culture filtrates had a positive influence on growth as well as biomass compared to the control plants (Fig. 3). The increment in growth of the test plant may be attributed to the production of some of the growth-promoting compounds by the endophytic aquatic fungi.

Fernando & Currah (1996) reported the positive effects of some root endophytic hyphomycetous fungi on the growth of sub-alpine plants. Recently, Sati and Arya (2010a) studied the role of three root endophytic aquatic hyphomycetes, viz., *H. lugdunensis*, *Tetracladium elegans* and *Tetracladium nainitalense*, for plant growth and development and found a significant impact on the growth of test plants. In the present study, it is interesting to note that the test plants inoculated with endophytic fungi grew better than control plants (Fig. 1–3; Table 1), supporting the findings of previous studies. Recently, an attempt has also been made to study the effect of *C. parvula* and *T. setigerum* on the test plant chilli, and it was shown to produce some plant growth-promoting substances that enhanced the plant biomass (Sati & Pant 2020).

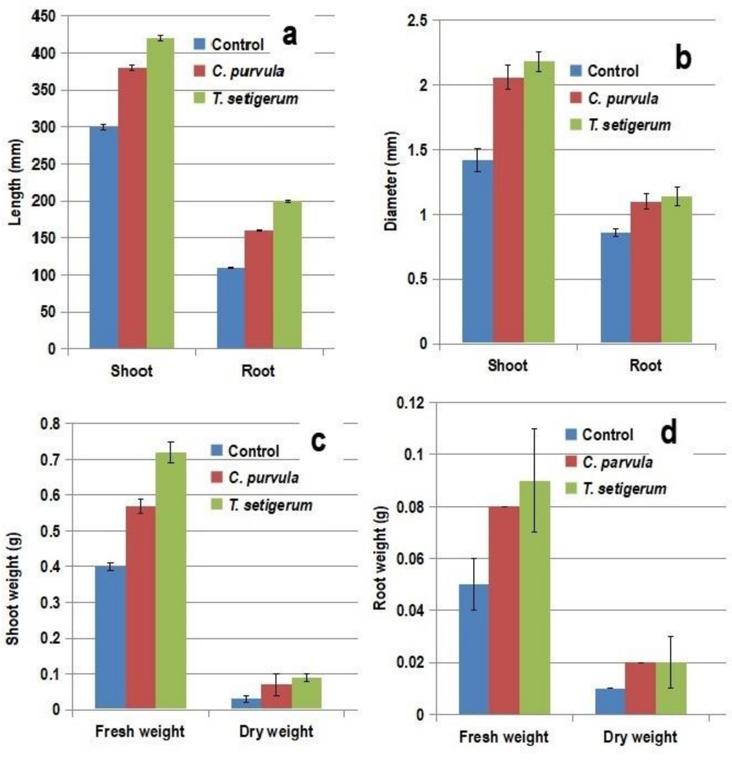

Fig. 3. – Effects of *C. parvula* and *T. setigerum* treatments on wheat. a Effect on shoot and root lengths. b Effect on shoot and root diameter. c Effect on fresh and dry weight of shoot. d Effect on fresh and dry weight of root.

The endophytic fungi include a diverse group of species that differ in the symbiotic associations they form and their ecological functions. These fungi are considered an outstanding source of various bioactive natural compounds that act as antibacterial, antifungal and anti-cancerous compounds. Thus, based on the above findings, using these root endophytic aquatic hyphomycetes as bio-inoculants may be helpful to minimize the application of chemical fertilizer and sustainably promote agricultural yield. Furthermore, the chemical characterization of their metabolites may be an alternative to expensive chemicals used in agriculture.

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