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Influence of fungal Endophytes on growth and yield of rice (*Oryza sativa* L.)

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Abstract
A field experiment was conducted at University of Agricultural Sciences, GKVK, Bengaluru, to study the effects of fungal endophytes on growth, yield and yield attributes of Rice (*Oryza sativa* L.). The results revealed that the plants colonized by fungal endophytes had significantly improved grain and straw yield of rice by enhancing the plant height, tiller and panicle production and also by increasing the individual grain weight. However, *Aspergillus nidulans* and *Penicillium funiculosum* colonized plants had significantly higher harvest index (0.32). Hence this study suggests that fungal endophytes could be used as a bio-stimulants for rice.

Keywords: Bio-stimulants, *Aspergillus nidulans*, *Oryza sativa* and *Penicillium funiculosum*

Introduction

Rice is the staple food of more than half of the world's population. Among the rice growing countries, India has the largest area (44 million hectares) and it is the second largest producer (131 million tonnes) of rice next to China (211 million tonnes). The rice productivity in India is 2.71 tonne per ha while the world average is 5.14 tonne per ha (IRRI, 2011). At the current population growth rate (1.5 per cent), the rice requirement of India by the year 2025 would be around 125 million tonnes. The rice production has to be enhanced to meet the food requirement of the growing population, by improving the rice growth and development. Rice growth depends on the combination of genotype, environment and management practices. At present, lots of fertilizer and pesticides are being used to raise the crop productivity which results reduction in soil fertility. To overcome these problems one of the eco-friendly approach is endophytic microbes which can be used as bio-stimulants. Endophytes have pivotal role in improving the plant growth and development, which in turn improves the sustainable yield.

Endophytes are microorganisms (fungi, bacteria, protozoa, virus or algae) those live part of their life or complete life inside living plant without causing negative effect on the host plants. The distribution of endophytes is ubiquitous and has been reported in all most all tissues of plant including leaves, stems, roots, flowers and fruits. These endophytes have intimate relation with host plant and this interaction provides benefits to both host plants and endophytic microorganisms. For host plants, endophytes can enhance plant nutrient uptake, promote host plant growth, enhance tolerance to abiotic stresses, inhibit infection by plant pathogens, and eventually increase biomass yield of the plants (Rodriguez *et al*., 2008; Mei and Flinn, 2010) [10, 9]. There has been an increase in attention to endophytic fungi in the past few years because of their beneficial properties to plants. Endophytes could enhance the fitness of plant by acquisition/supply of nutrients *via* siderophores, nitrogen and phosphorus-assimilating enzymes or release of organic acids and improves the absorption of water, supporting the growth of other bacteria/fungi near the rhizosphere or in plant system. Hence, endophytes have a vital role in plant growth promotion. Even endophytes could present in rice plant such as *Chaetomium cupreum*, *Colletotrichum* spp., *Curvularia lunata*, *Fusarium solani*, *Penicillium* spp., *Fusarium oxysporum*, *Trichoderma harzianum* and *Penicillium* sp., *Aspergillus niger*, *Aspergillus flavus*, *Pythium* spp., *Rhizopus* sp (Leewijit, 2016) [6].

Few reports documented about those endophytic fungi which improved the growth of rice plant such as *Phialemonium dimorphosphorum*, *Trichoderma* sp. *Aspergillus niger* and *Fusarium* sp. (Doni *et al*., 2014; Gupta *et al*., 2015; Kandar *et al*., 2018) [2, 3, 5]. However, study on fungal interaction with rice under field condition is less explored. Hence present study was carried to evaluate the effect of fungal endophytes on growth and yield of rice crop.

Materials and Methods

Mass production of fungal endophytes

Fungal endophytes such as *A. flavus, A. nidulans, E. endophytica,* and *P. funiculosum* were cultured individually on PDA media, supplemented with 100 µg/L of ampicillin and grown at 30 ºC (Fig. 1). After 20 days of growth, conidia were harvested from plates by adding 10 mL of sterile water and gently scraping off conidia with a sterile glass slide. The suspension was filtered through four layers of sterile cotton cheese cloth gauze. The concentration of final spore suspensions was adjusted to 10^{-5} conidia/mL with the help of haemocytometer. This suspension was used for the field experiment.

Field experiment setup

Description of location and experimental site

The field experiment was conducted at experimental plot of K-block, Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru during summer, February-June 2021. The farm is geographically situated at the altitude of 920 m above sea level 13.07 N latitude and 77.59 E longitude. The soil type here is sandy loamy soil in texture and alkaline in reaction. During crop growth period, the average rainfall was about 48.5 mm, mean relative humidity was 86 per cent and the average maximum and minimum temperature was 31.06 ºC and 18.28 ºC, respectively.

Experimental treatments and design

The experiment comprised of five treatments such as T1: Control, T2: *A. flavus*, T3: *A. nidulans*, T4: *E. endophytica*, and T5: *P. funiculosum* with four replications under Randomized Complete Block Design (RCBD). All the treatments received 100% RDF (Recommended Dose of fertilizer), FYM and NPK.

Cultural Practices

Prior to planting, the land was ploughed and harrowed. Later, land was levelled with plank and divided into 20 plots, each with a plot size of 2.0 m X 1.5 m. Small raised bunds were formed around each plot to mark individual treatment and irrigation channels were formed between the replications. Further, individual plots were leveled and the flat beds were converted into ridges and furrows at 30 X 10 cm distance. Rice seed was directly sown by dibbling in lines in the dry field at a depth of 3-5 cm. Immediately after sowing, light irrigation was applied through border strip method. Subsequent irrigation was given as and when needed for proper growth and development of the crop. Recommended dose of fertilizers (120 kg N, 50kg P2O5 and 50 kg K2O ha-1) was applied in all the treatments through urea, diammonium phosphate and muriate of potash. Entire dose of phosphorus and potassium and half of nitrogen were applied as basal. The remaining dose of nitrogen was top dressed equally at active tillering and panicle initiation stage.

Recording observations

Observations pertaining to the growth parameters were recorded at 35 DAS, 70 DAS and at harvest of crop. Yield attributes and yield data were collected at the time of harvest. The plant height was recorded from five hills using a meter scale from the base of the plant to the tip of the longest leaf or the panicle and expressed in centimetres. The number of tillers were counted and recorded from the five hills within the net plot. Similarly, panicle length, number of panicles were recorded from five hills of each plot. The grains were separated by threshing the crop collected from each net plot separately and were dried under sun for three days. Later winnowed and cleaned, then weight of the grains per net plot was recorded. Straw obtained from each net plot area after threshing was sun dried for four days and then weighed and expressed in q ha^{-1}. Biological yield and harvest index were calculated.

Statistical analysis

The data obtained from field experiments were statistically analyzed using and Randomized Complete Block Design (RCBD) respectively. The statistical analysis was done by using WASP: 2.0 (Web Agri Stat Package 2) statistical tool (www.icargoa.res.in/wasp2/index.php) and mean were separated by Duncan's Multiple Range Test (DMRT).

Results and Discussion

The fungal endophytes influenced the plant height and tiller numbers of rice. At 35 DAS, plant height was significantly higher in *A. nidulans* inoculated plants followed by *P. funiculosum*, *A. flavus* and *E. endophytica.* The least was observed in control plants. Similar significant trend was recorded during 70 DAS. Whereas during harvest, *A. nidulans*, *P. funiculosum* and *A. flavus* treated plants had higher plant height and showed significantly on par with each other. The results indicated that endophytes inoculated plants improve length of the plant. It might be due to production of phytohormones. Several reports documented that fungi such as *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium corylophilum*, *Penicillium cyclopium*, *Penicillium funiculosum* and *Rhizopus stolonifer* has ability to produce gibberellin and indole actic acid (IAA) (Hasan, 2002) [4]. Mattos *et al.* (2008) [8] reported that the endophytic *Burkholderia kururiensis* promoted rice plant growth by production of the plant auxin, IAA. Our results are aligning with Kandar *et al.*, (2018) [5] who also reported that *Phialemonium dimorphosphorum*, *Gaeumannomyces graminis*, and *Gaeumannomyces amomi* significantly increases the plant height of rice seedlings.

Tiller number is an important attribute for grain yield. The number of tillers were significantly maximum in plants colonized by *A. nidulans* followed by *P. funiculosum*, *A. flavus* and *E. endophytica* at 35 DAS and 70 DAS. During harvesting stage, these endophytes are on par with each other except *E. endophytica* which is the lower number of tillers as compared to other endophytes but significantly higher tillers compared to un-inoculated plants. Cytokinin has pivotal role in increasing number of branches and spikelets in rice panicle for the benefit of grain yield has been corroborated more recently (Ashikari *et al*., 2005) [1]. Earlier studies are reported that fungal endophyte, *Piriformospora indica* has ability to produce cytokinin (Vadassery *et al*., 2008) [11] which might be helpful to increase the tillers in plant system.

The results pertaining to yield attributes of rice plants influenced by fungal endophytes were represented in Table 2. The plants inoculated by fungal endophytes recorded higher panicle length compared to un-inoculated plants and all the endophytes showed significantly on par results with each other.

In rice plant, panicle numbers are directly proportional to tiller number. The present study support this statement.

Endophyte (*A. nidulans)* colonized plants recorded higher panicle number per hill followed by *P. funiculosum*, *A. flavus* and *E. endophytica.* Similar trend was observed in 1000 seed weight of rice. Improvement in yield attributes might be due to increased nutrient supply by fungal endophytes to the host plant. These results are also inline the findings of Yuan *et al*. (2010) [13] who reported that endophytes could enhance the fitness of plant by acquisition/supply of nutrients *via* siderophores, nitrogen and phosphorus- assimilating enzymes or release of organic acids and improve the absorption of water, supporting the growth of other bacteria/fungi near the rhizosphere or in plant system which in turn improve the assimilation of photosynthates and sink translocation and eventually produced larger sized and more grains of higher weight that ultimately increased the yield. Plant inoculated with plant growth promoting fungi improved the plant growth by increasing the nutrient uptake particularly nitrogen and phosphorus (Yadav *et al*., 2009) [12].

The plants colonized by fungal endophytes exhibited significantly higher grain and biological yield as compared to un-inoculated plants. *A. nidulans* and *E. endophytica* treated plants showed significantly higher straw yield followed by *A. flavus*. Our results are confirmed with Gupta *et al*. (2015) [3] who reported that *Trichoderma longibrachiatum*, *Westerdykella aurantiaca*, *Lasiodiplodia* sp. and *Rhizopus delemar* improved the rice grain yield by increasing the yield attributes such as panicle number, spikelet number and length weight of the grain. Harvest index of rice had significantly maximum in *A. nidulans* and *P. funiculosum* colonized plants which indicates that endophytes are more efficient in partitioning dry weight to seed compared to un-inoculated plants.

In conclusion, experimental results indicated that application of fungal endophytes could enhance the growth and yield attributes of rice plants.

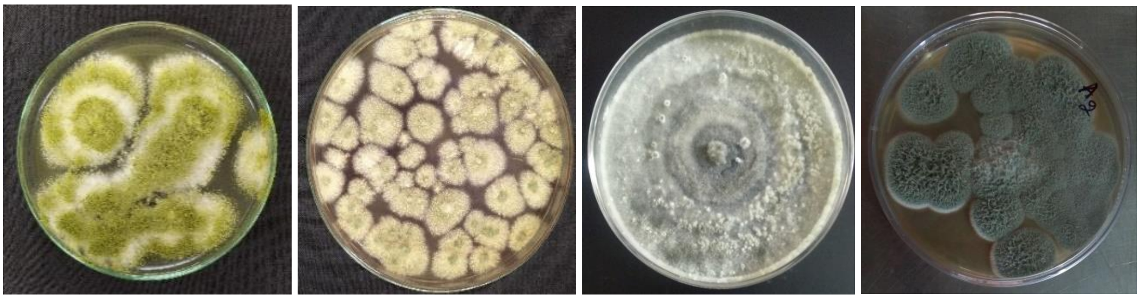

Fig 1: Growth of fungal endophytes such as *A. flavus, A. nidulans, E. endophytica* and *P. funiculosum* on PDA media (left to right)

Table 1: Influence of different endophytic fungi on growth attributes of Paddy at different intervals

	Plant height (cm)			Number of Tillers / hills		
Treatments	**35 DAS**	**70 DAS**	**At harvest**	**35 DAS**	**70 DAS**	**At harvest**
Control	32.02^{d}	69.20^{d}	74.85^{c}	7.86^{d}	20.05^{d}	21.05^{c}
A. flavus	36.83^{b}	76.33^{b}	79.60^{a}	12.26^{b}	24.29^{b}	25.70^{a}
A. nidulans	38.50^{a}	78.08^{a}	81.21^{a}	15.11^{a}	25.25^{a}	26.50^{a}
E. endophytica	35.32^{c}	74.32^{c}	77.83^{b}	10.46^{c}	22.49^{c}	24.02^{b}
P. funiculosum	38.32^{a}	77.55^{ab}	80.33^{a}	14.34^{a}	24.83^{ab}	26.05^{a}
C.D.	1.055	1.579	1.699	1.552	0.958	1.069
SE(m)	0.339	0.507	0.545	0.498	0.308	0.343
C.V.	1.871	1.35	1.385	8.302	2.631	2.784

Table 2: Influence of different endophytic fungi on yield attributes of Paddy

Treatments	**Panicle length (cm)**	**No. of panicle per hill**	**1000 grain weight (g)**	**Grain yield (t/ha)**	**Straw yield (t/ha)**	**Biological yield (t/ha)**	**Harvest Index**
Control	15.67^{b}	15.75^{c}	19.50^{c}	3.38^{b}	9.63^{b}	13.01^{b}	0.26^{b}
A. flavus	17.91^{a}	18.52^{ab}	21.62^{ab}	4.47^{a}	11.02^{a}	15.49^{a}	0.29^{ab}
A. nidulans	18.28^{a}	18.94^{a}	22.40^{a}	4.80^{a}	10.20^{ab}	15.00^{a}	0.32^{a}
E. endophytica	17.78^{a}	17.93^{b}	20.95^{b}	4.51^{a}	10.83^{a}	15.34^{a}	0.30^{ab}
P. funiculosum	18.15^{a}	18.97^{a}	22.08^{ab}	4.72^{a}	9.81^{b}	14.53^{a}	0.32^{a}
C.D.	0.763	0.614	1.249	0.783	1.012	1.242	0.046
SE(m)	0.245	0.197	0.401	0.251	0.325	0.399	0.015
C.V.	2.79	2.186	3.763	11.492	6.306	5.431	9.955

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