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Seed germination promoting activity of fungal endophytes in Rice (*Oryza sativa* L.) seeds

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ABSTRACT

The seed germination promoting potentials of six endophytes associated with bamboo was assessed using rice seeds as test plants. Rice seeds were treated with crude and ethanol extracts of the Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Cladosporium cladosporioides, Monascus ruber and Penicillium citrinum and the % seed germination at day 1, 3, and 5 were computed. Results revealed the ability of the all fungal endophytes crude and ethanol extracts to increase the rate of seed germination at all incubation periods which are comparable to the commercial gibberellic acid. Hence, their ability to promote seed germination.

INTRODUCTION

Fungal endophytes are fungal organisms that live within the plant tissues without causing any symptoms of disease which are associated with roots, stems or leaves [1, 2, 3]. Several studies have already recognized its importance as sources of bioactive compounds. They can also produce various important secondary metabolites, bioactive natural products, phytohormones, therapeutic and immunosuppressant compounds which could be of potential in the field of medicine, agriculture and pharmaceutical industry [4, 5, 6, 7, 8].

Endophytic fungi perform various symbiotic association with plants and play an important role in plant growth as well as opportunistic ones in their host plants [9, 10, 11]. They obtained their nutrients from the host plants and protection towards abiotic and biotic factors. In return the endophytes release metalotiles against plant pathogens and has a tremendous potentials in secreting phytohormones wich could lead to an improved growth of the host plants [12,13, 14, 15, 16, 17].).

Valentino et al [18] have identified and isolated several species of endophytic fungi from the micropropagated bamboo lateral stem. These include *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Clasdoporium cladosporioides*, *Monascus ruber*, and *Penicillium citrinum*. Thus the conduct of the study to evaluate the hormone like activity of the selected endophytic fungi. This will provide baseline information on their potentials in exhibiting seed germination promoting activity in rice seeds which could further lead to the utilization of the endophytic fungi as sources of phytohormones which is necessary in the micropropagation of plants.

MATERIALS AND METHODS

This study evaluated the seed germination promoting activity of six of endophytic fungi associated with bamboo. The test endophytes were obtained from the collection of the author.

Ethanol extraction

Fungal isolates was cultivated in Potato Dextrose broth for 7 days. After which, the fungal mycelia were air dried at room temperature. Ten grams of dried mycelial mat were submerged and 95% ethanol for 48 hours at room

temperature. Then this was filtered using filter paper (Whatman no. 1). The solvents were separately evaporated in rotary evaporator under reduced pressure at fifty degree Celsius (60°C) to yield ethanol extracts .

Crude Extraction

Endophytic fungi were grown in Potato Dextrose Broth for 7 days. After which, the fungal growth together with the supernatant were osteorized and it was placed in amber bottles and were kept in a refrigerator until needed.

Seed Germination

Viability test was carried out prior to the test by soaking rice seeds in distilled water for two hours. Floating seeds was discarded and the viable seeds were used for the test. Thirty viable rice seeds were soaked in different treatments for 24 hrs and then it was placed in a dish lined with filter paper flooded with 5 ml of different treatments and was incubated at 25-28°C. Percentage seed germination was computed after 1st, 3rd and 5th day of incubation.

RESULTS AND DISCUSSION

Interactions between host plant and endophytic microorganisms which give rise to symbiotic relationship of plants and the endophytes has already been proven in several studies usually give the positive effects on the crop health [17, 18, 19 20, 21, 22]. Endophytes were found to promote plant growth by improving of soil structure, secreting stimulating enzymes, increasing the nutrient availability, and induced the defense mechanism of plant and production of phytohormones [22, 23, 24]. Some endophytes synthesize plant growth hormones such as indole-3-acetic acid, cytokinines and gibberellins that promote plant growth [1, 10, 25]

Presented in Table 1 the mean percentages of seed germination treated with different crude and ethanol extracts of fungal endophytes. At Day 1, Gibberellic acid had the highest percentage of seed germination of 96.67% followed by *Aspergillus ochraceus* ethanol extract of 95.56% and 93.33% both by *Aspergillus flavus* and *Cladosporium cladosporioides* ethanol extracts. Meanwhile *Aspergillus flavus* crude extract had the least of 71.11% and *Cladosporium cladosporioides* crude extract of 77.78%. For the 3rd and 5th day of incubation, 100% of seeds treated with gibberellic acid were successfully germinated. Whereas, those treated with crude and ethanol extracts of fungal endophytes ranges from 96.67%-88.89% and 98.89% to 89.9% after three and five days of incubation, respectively. Statistically, *Aspergillus flavus* and *Cladosporium cladosporioides* crude extracts were significantly lower than the positive control at day 1, while *Aspergillus niger* ethanol extracts is statistically lower than the positive control after 3 and 5 days f incubation. Meanwhile, the rest of the fungal extracts were comparable to the commercial gibberellic acid. Hence, their potential in increasing the rate of seed germination and could be a probable source of gibberellic acid.

Results of the study, coincides with those of Bhagobaty & Joshi [26], Hamayun et al.[27] , Khan et al, Hasan [28] wherein plant endophytes secrete plant growth hormones such as cytokinins, auxins and gibberellins which promote seed germination and growth in crop plants. Addionally, several studies revealed the potential of endophytic funi in ehnhing the seed germination and growth of plants [29, 30, 31] As stated by Jerry [31], endophytic fungi help to degrade cuticle cellulose during seed germination and thereby available carbon for growing seedling, which improves seed germination, vigour and establishment [26, 27, 28]. Among phytohormones, gibberellic acid most function for cell division and elongation, activation of embryo, weakening of endosperm layer and mobilization of endosperm food reserves [32]. While germination, GA counteracts the effects of abscisic acid (ABA) and thus releasing the seed dormancy which positively regulates the germination [33, 34, 35].

Table 1. Mean percentage of seed germination of rice seeds treated with different extracts of fungal endophytes

TREATMENTS	Seed germinated		
	Day 1	Day 3	Day 5
Aspergillus flavus crude extract	71.11^{c}	95.55^{a}	95.55^{ab}
Aspergillus flavus ethanol extract	93.33^{a}	97.78^{a}	98.89^{a}
Aspergillus niger crude extract	92.22^{a}	94.44^{a}	94.44^{ab}
Aspergillus niger ethanol extract	88.89^{ab}	88.89^{b}	89.99^{b}
Monascus ruber crude extract	92.22^{a}	95.56^{a}	95.57^{ab}
Monascus ruber ethanol extract	92.22^{a}	95.56^{a}	95.57^{ab}
Penicillium citrinum crude extract	86.67^{ab}	95.56^{a}	98.89^{a}
Penicillium citrinum ethanol extract	92.22^{a}	97.78^{a}	97.78^{a}
Aspergillus ochraceus crude extract	90.00^{a}	96.67^{a}	98.89^{a}
Aspergillus ochraceus ethanol extract	95.56^{a}	96.67^{a}	98.89^{a}
Cladosporium cladosporioides crude extract	77.78^{bc}	96.67^{a}	97.78^{a}
Cladosporium cladosporioides ethanol extract	93.33^{b}	95.56^{a}	97.78^{a}
Gibberellic acid (50ppm)	96.67^{a}	100.00^{a}	100.00^{a}

**Treatments with same superscript are not significantly different from one another*

CONCLUSION

The six endophyting fungi showed seed germination promoting activity at all incubation time in rice seeds which is comparable to the commercial gibberellic acid. It is therefore recommended also to screen the phytohormonelike activity of these fungal isolates in vivo and further isolation of the secondary metabolites responsible

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