



**COLEOPTILE AND SECONDARY LEAF SHEATH ELONGATION AS INFLUENCED
BY ENDOPHYTIC FUNGI ASSOCIATED WITH BAMBOO**

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Received 7th Dec. 2016; Revised 11th Feb 2017; Accepted 22nd Apr 2017; Available online 1st Nov. 2017

ABSTRACT

Gibberellic acid like activity of the fungal endophytes associated with bamboo (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Cladosporium cladosporioides*, *Monascus ruber*, and *Penicillium citrinum*) was elucidated in this study through the elongation of coleoptile and second leaf sheath of *Oryza sativa* (rice). Coleoptile treated with *A. flavus* crude extract had the longest coleoptile of 11.21mm, followed by *A. ochraceus* crude extract with 9.12mm. Meanwhile, only *P. citrinum* crude extract caused secondary leaf sheath elongation to 44.50mm and *A. ochraceus* crude extract with 40.86mm whereas inhibitory activity of the *A. niger*, *A. flavus*, *A. ochraceus*, *C. cladosporioides* and *Monascus ruber* were noted.

Keywords: Coleoptile, Secondary Leaf Sheath Elongation, Endophytic Fungi, Bamboo

INTRODUCTION

Fungal endophytes of group of microorganisms that co-exist and could possibly form symbiotic relationship with one another. Some of which have the ability

to produce compounds which could affect the growth of the plants. Generally the relationship between the plant and its endophytes is one of a symbiotic nature

whereby the endophytes colonize the internal tissues of the plant [1, 2, 3, 4, and 5]. It has been reported that fungal endophytes are valuable sources of new bioactive compounds [6]. According to Ryan [7], endophytes promote plant growth by several mechanisms which include the production of phytohormones such as auxin, cytokinins, abscissic acid and gibberellins. Gibberellins are associated with various plant growth and development processes such as seed germination, stem and hypocotyl elongation, leaf expansion, floral initiation, floral organ development, fruit development and induction of some hydrolytic enzymes in the aleurone of cereal grains [8].

In the present study the gibberellic acid like activity of the fungal endophytes (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Cladosporium cladosporioides*, *Monascus ruber*, and *Penicillium citrinum*) were evaluated through the elongation of coleoptile and secondary leaf sheath of *Oryza sativa*. This is in line with the study of Paguia and Valentino [9] and Maiquez et al. [10] wherein similar group of fungal endophytes were used to evaluate their phytohormone-like activities.

MATERIALS AND METHODS

Extraction of Fungal Endophytes

Fungal isolates (*A. flavus*, *A. niger*, *M. ruber*, *P. citrinum*, *A. ochraceus*, and *C. cladosporioides*) were grown in Potato Dextrose Broth for 7 days. After which, the fungal mycelia were removed from broth using sterile forceps and were placed in Petri plates to dry over night at room temperature. For ethanol extraction, ten grams (10 g) of dried mycelial mat were submerged and extracted in one hundred milliliter 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.

For the crude extracts, mycelial mat of the fungal isolates was prepared by inoculating potato dextrose broth with the test fungal endophytes. Then the fungal cultures were incubated for 7 days at room temperature. Then, the mycelial mat together with the supernatant were homogenized using blender and it was placed in an amber bottle in a refrigerator.

Evaluation of the Gibberellin-like activity

For the evaluation of the gibberellic acid - like activity, rice seeds were utilized as test plant. The length of coleoptile and the length of the second leaf sheath were measured (Murakami, 1959).

Coleoptile Elongation

Viability test was carried out prior to the tests by soaking rice seeds in distilled water for two hours. Floating seeds were 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 fungal extracts. The length of coleoptiles was measured using a digital vernier caliper.

Leaf Sheath Elongation

For the elongation of the second leaf sheath, rice seeds were allowed to germinate in a dish lined with filter paper flooded with distilled water. After 24 hours of incubation, seeds with 1mm coleoptiles were transferred to a filter paper immersed in 9ml of different treatment and control solutions. The seeds were allowed to grow under ordinary daylight conditions at 25-28°C. Each treatment was added with 0.5 ml of distilled water for every 24 hours. The secondary leaf sheath was measured using a digital vernier caliper after 7 days of incubation.

RESULTS

Coleoptile Elongation

Coleoptile elongation of *Oryza sativa* germinated seeds are shown in Table 1 and Figure 1. Among the coleoptiles treated with fungal extracts, *A. flavus* crude extract-

treated coleoptiles had the longest coleoptile of 11.21mm, followed by *A. ochraceus* crude extract- treated coleoptiles with 9.12mm while the least length of coleoptile was observed in *C. cladosporioides* crude extract-treated coleoptiles with 7.05mm and *P. citrinum* ethanol extract - treated coleoptiles with 7.13mm. Statistically, the length of the coleoptile of *O. sativa* treated with commercial gibberellic acid and *A. flavus* crude extract were comparable with one another, whereas those treated with ethanol extracts of *A. flavus* (7.69mm), *A. niger* (9.06mm), *M. ruber* (8.62mm) and crude extracts of *M. ruber* (8.28mm), *P. citrinum* (7.87mm), *A. ochraceus* (9.12mm) were significantly higher than the untreated coleoptile. Thus, this signifies the potential hormone like activity of the endophytic fungi which led to the increase in the coleoptile growth of *O. sativa*.

Secondary leaf sheath elongation

Presented in Table 2 and Figure 2 are the results for the secondary leaf sheath elongation. *Oryza sativa* treated with *P. citrinum* crude extract had the longest secondary leaf sheath with 44.50 mm and *A. ochraceus* crude extract with 40.86mm. On the other hand, *P. citrinum* ethanol extract registered the least length of secondary leaf sheath with 13.01mm and *C. cladosporioides*

ethanol extract with 13.69mm which is noticeably lower than the untreated ones. Significant differences were observed among the treatment means. Statistically, only

P.citrinum crude extract exhibited elongation promoting effect to the secondary leaf sheath whereas inhibiting activity were recorded on the remaining fungal extracts.

Table 1: Means of the coleoptile elongation in crude and ethanol extract

TREATMENTS	Coleoptile Elongation (mm)
<i>A. flavus</i> CE-treated coleoptile	11.21 ^a
<i>A. flavus</i> EE- treated coleoptile	7.99 ^{cdef}
<i>A. niger</i> CE-treated coleoptile	7.69 ^{defg}
<i>A. niger</i> EE- treated coleoptile	9.06 ^{bc}
<i>M. ruber</i> CE-treated coleoptile	8.28 ^{bcd}
<i>M. ruber</i> EE- treated coleoptile	8.62 ^{bcd}
<i>P. citrinum</i> CE-treated coleoptile	7.87 ^{def}
<i>P. citrinum</i> EE- treated coleoptile	7.13 ^{fg}
<i>A. ochraceus</i> CE-treated coleoptile	9.12 ^b
<i>A. ochraceus</i> EE- treated coleoptile	7.19 ^{efg}
<i>C. cladosporioides</i> CE-treated coleoptile	7.05 ^{fg}
<i>C. cladosporioides</i> EE- treated coleoptile	7.12 ^{fg}
Positive control (gibberellic acid)	12.19 ^a
Negative control (distilled water)	6.63 ^g

*Treatments with different letters are significantly different.

*CE=crude extract; EE=ethanol extract

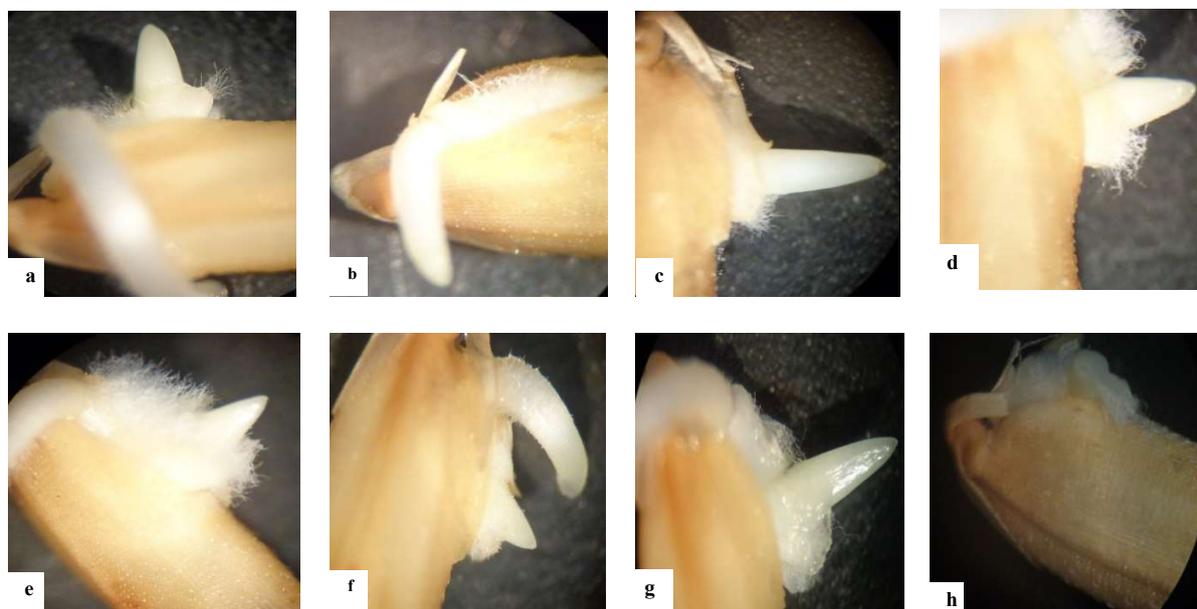


Figure 1: Coleoptiles treated with (a) *A. flavus*CE (b) *A. niger* CE (c) *M. ruber* CE (d) *P. citrinum* CE (e) *A. ochraceus* CE (f) *C. cladosporioides*CE (g) Gibberellic acid (h) distilled water.

Table 2: Means of the second leaf sheath elongation in crude and ethanol extract

TREATMENTS	Second Leaf Sheath Elongation (mm)
<i>Aspergillus flavus</i> CE-treated secondary leaf sheath	33.61 ^{cd}
<i>Aspergillus flavus</i> EE- treated secondary leaf sheath	15.64 ^e
<i>Aspergillus niger</i> CE- treated secondary leaf sheath	37.08 ^{bcd}
<i>Aspergillus niger</i> EE- treated secondary leaf sheath	31.92 ^d
<i>Monascus ruber</i> CE- treated secondary leaf sheath	34.28 ^{cd}
<i>Monascus ruber</i> EE- treated secondary leaf sheath	36.73 ^{bcd}
<i>Penicillium citrinum</i> CE- treated secondary leaf sheath	44.50 ^b
<i>Penicillium citrinum</i> EE- treated secondary leaf sheath	13.01 ^e
<i>Aspergillus ochraceus</i> CE- treated secondary leaf sheath	40.86 ^{bc}
<i>Aspergillus ochraceus</i> EE- treated secondary leaf sheath	18.98 ^e
<i>Cladosporium cladosporioides</i> CE treated secondary leaf sheath	34.36 ^{cd}
<i>Cladosporium cladosporioides</i> EE treated secondary leaf sheath	13.69 ^e
Positive control (gibberellic acid)	54.39 ^a
Negative control (distilled water)	35.19 ^{cd}

*Treatments with different letters are significantly different.

*CE=crude extract; EE=ethanol extract

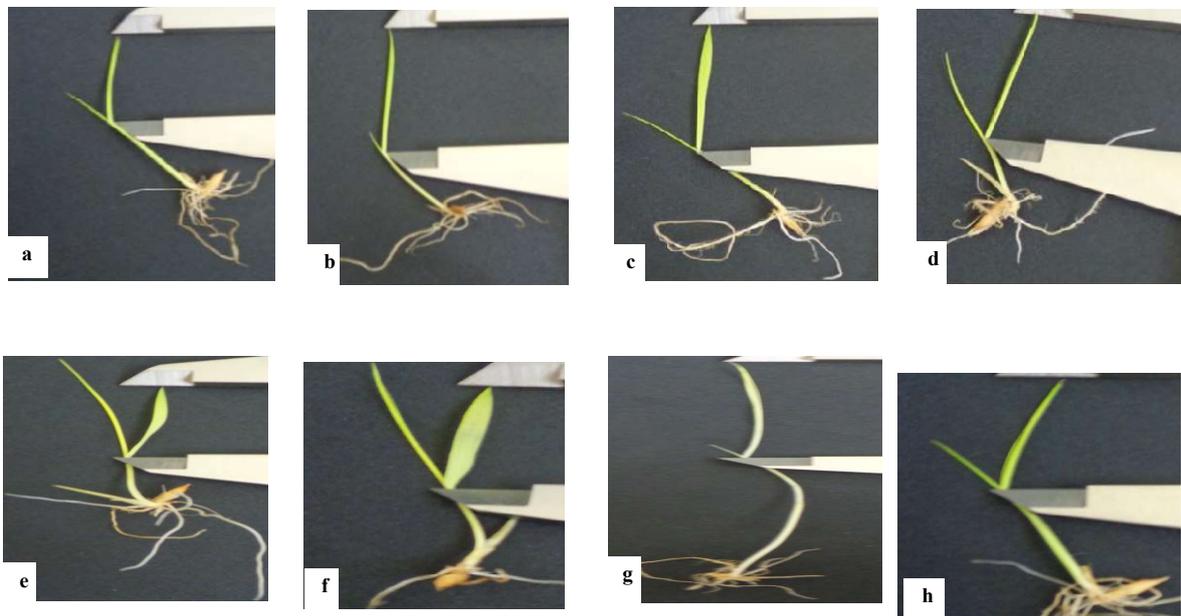


Figure 2: *O. sativa* seedlings treated with (a) *A. flavus* CE (b) *A. niger* CE (c) *M. ruber* CE (d) *P. citrinum* CE (e) *A. ochraceus* CE (f) *C. Cladosporioides* CE (g) Gibberellic acid (h) distilled water

DISCUSSIONS

Similar results were reported by Maiquez et al. [9], and Paquia et al. [10] wherein auxin like activity and seed germination promoting activity were exhibited by the same fungal endophytes. This also coincides with the study of Hasan [11] and Waqas et al. [12], wherein the ability of species of *Aspergillus* in secreting phytohormones (auxin and gibberellic acid) was evaluated. Accordingly, among the metabolites being studied, plant hormones like gibberellins and auxin production are new phenomenon in the endophytic fungi which have been reported to play a pivotal role in plant growth, reproduction, metabolism and response [13, 14, 15].

Hamayun et al., [16] also evaluated the gibberellic acid production potential of *Penicillium sp.* and *Aspergillus sp.*, wherein physiologically active GA3, GA4, and GA7 were detected. In addition, *P. citrinum* is already known for producing mycotoxin citrinin and cellulose digesting enzymes like cellulase and endoglucanase, as well as xylulase. Lastly, its Gibberellins producing ability has already been proven [17].

Meanwhile the inability of the fungi to enhance the growth of the coleoptile in

ethanol extract were also evident in the previous study of Maiquez et al [9] and Paquia et al [10].

CONCLUSION

Ethanol extracts of *A. flavus*, *A. niger*, *M. ruber* and crude extracts of *A. flavus*, *M. ruber*, *P. citrinum*, *A. ochraceous* promoted coleoptile elongation and *P. citrinum* crude extracts influenced the elongation of secondary leaf sheath.

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