International Journal of Pharma and Bio Sciences

INDOLE-3-ACETIC ACID FROM CONTAMINANT FUNGUS AND POTENTIAL APPLICATION FOR CELL CULTURES OF *ALTERNANTHERA SESSILIS*

KARTHIKEYAN SUBBARAYAN*, NITHYA VARADHARAJAN AND RAJAGOPAL KALYANARAMAN

Department of Biotechnology, School of Life Sciences, VELS University, Chennai – 600117, India.

**** Corresponding author*** karthisubbarayan@gmail.com

ABSTRACT

Microbes use Indole 3-acetic acid (IAA) to interact with plants including phytostimulation and circumvention of basal plant defence mechanism. Fungal contamination is the most predominant in *Alternanthera sessilis* tissue culture than bacteria. *Colletotrichum* Sp. produced 25 mg/l of IAA in presence of tryptophan (400 mg/l) in Czapex medium. *In vitro* studies were performed to utilize the IAA for callus induction of *A. sessilis*, an important medicinal plant used in Siddha system of medicine. In Murashige and Skoog medium supplemented with 9.0 µM 2,4-D + 2.8 µM IAA, callus induction was maximum. Callus induction on 1.8 µM fungal IAA + 1.0 µM IAA (HiMedia) shows the potential application of fungal IAA in plant cell cultures.

KEYWORDS

Indole 3-acetic acid, *Alternanthera sessilis*, Contamination and *Colletotrichum* Sp.

INTRODUCTION

Plant tissue culture offers the possibility of making available the propagules irrespective of the time with assured better health status and also in producing large number of propagules and a multitude of phytopharmaceutical and aromatic chemical in cells lines[1]. *Alternanthera sessilis* grows in the tropical region of the world, especially tropical America, Africa and Asia; leaves and young shoots are eaten as vegetable[2]. The whole plant of *A. sessilis* is used to treat wounds, flatulene, cough, bronchitis and diabetes. This plant is also reckoned as an important ingredient of several compounds of Siddha system of medicine[3]. Plant tissue culture is the technique of growing plant cells, tissue and organ in an artificially prepared nutrient medium under aseptic conditions[4]. The medium contain many different microbial nutrients, both original constituents of the medium and exudates from the plant cells. Thus pathogens, endophytes, epiphytes and incidental contaminants may all occur and may interfere with growth of the plant tissue[5]. The contaminants have deleterious or beneficial effects on the plant culture[4]. Bacterial and fungal contaminants create a major problem in plant tissue culture laboratory. Once the explant have been established *in vitro*, it is essential that the culture to be indexed for the presence of microbial contaminant before being multiplied[6]. Microorganism including bacteria and fungi are able to synthesize indole acetic acid[7, 8]. The cost of components of tissue culture medium has been another concern for most commercial laboratories. Agar, sucrose and growth hormones are the chief constituents which play a significant role in cost of production[9]. IAA is the main auxin in plants, controlling many important

physiological processes including cell enlargement and division, tissue differentiation and responses to light and gravity[10]. The role of microbial IAA in different microorganism plant interaction highlights the fact microbes use this phytohormone to interact with plants as part of their colonization strategy, including phytostimulation and circumvention of basal plant defence mechanism. Tryptophan has been identified as a main precursor for IAA biosynhesis pathway[11]. Although IAA production by several fungi was reported[12, 13], there is no report on utilizing the fungal IAA in plant cell cultures. The present study was therefore undertaken to study the effect of IAA extracted from contaminant fungus and to investigate its effects on callus culture of *A. sessilis*.

MATERIALS AND METHODS

i. Collection of Explants and surface sterilization

Fresh plants of *Alternnanthera sessilis* were collected from Irula Tribe women welfare society (ITWWF), Kanchipuram Dist, Tamil Nadu, India. The leaf explants were washed with tween 20 (2%, v/v) in running water for 10 minutes. Then the explants were surface sterilized using mercuric Chloride (0.1%, w/v) for 1 minute followed by three washes with sterile distilled water.

ii. Callus Culture

Leaf explants from *A. sessilis* were cultured on Murashige and Skoog basal medium (1962) supplemented with 2,4-D (2.0 - 10.5 µM) or combination of 2,4-D and IAA (1.4 - 4.2 µM). The tubes were incubated at 27˚C and 60 % relative humidity under light 16 hr/day photoperiod provided 2000 lux light intensity and observed daily for monitoring growth and contamination. Microbial contaminations were removed immediately from the culture room.

iii. Isolation of fungi and bacteria

Most predominating fungal strains were isolated from contaminant cultures. Pure culture was maintained in Potato Dextrose Agar Medium[14]. Yeast extract dextrose agar medium was used to stimulate bacterial growth.

iv. Identification of fungal and bacterial contaminants

Contaminants were purified using standard microbiological methods. The fungi were identified with the help of keys provided[15, 16, 17]. The sterile Mycelia that grew out from the tissue were subcultured and exposed to light to induce sporulation[18]. Bacteria was characterized by biochemical tests and staining techniques such as Gram stain, motility, gelatinase, oxidase and O/F (oxidation/fermentation) [19,20].

v. Indole-3-acetic acid production

The cultures were inoculated into Czapex medium[21] with different concentrations of tryptophan (0 – 0.8 mM) and incubated in darkness at 26˚C for 5 days. Then the cultures were harvested and centrifuged at 10,000 rpm for 5 minutes. 5 ml of 0.01 M ferric chloride in 35 % perchloric acid was added to 1 ml of supernatant. After 30 minutes of incubation, optical density measured at 540 nm[22].

vi. Effect of fungal IAA on callus induction

Appropriate concentrations and combinations of synthetic IAA (HiMedia, India) and IAA of fungal source were used for callus induction. To analyze the potential of fungal IAA in plant cell cultures, IAA (HiMedia) was used as control.

RESULTS

1. Establishment of callus culture

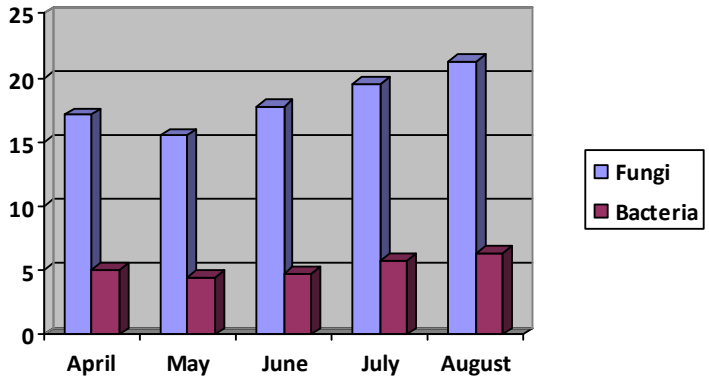
Totally Green friable callus was obtained from leaf explants of *A. sessilis* and data was analyzed on 25th day of inoculation. Maximum survival rate (84.5%) with 1.9 ± 0.17 g average fresh weight of callus was observed on MS with 9.0 µM 2,4-D + 2.8 µM IAA. Data was obtained after 25 days of inoculation and tabulated in Table 1

Table 1
Effect of hormone growth regulators on callus formation from the leaf explants of A. sessilis on MS medium.

Growth regulator µM		Survival rate %	Average Fresh weight (g)	Average Dry weight (g)
2,4-D	IAA			
2.0	-	48.3	0.98 ± 0.41	0.21 ± 0.04
4.5	-	57.6	1.09 ± 0.53	0.24 ± 0.04
6.0	-	61.2	1.32 ± 0.36	0.29 ± 0.03
7.5	-	75.8	1.51 ± 0.48	0.37 ± 0.02
9.0	-	81.4	1.78 ± 0.28	0.48 ± 0.04
10.5	-	72.3	1.40 ± 0.24	0.34 ± 0.08
9.0	1.4	78.2	1.73 ± 1.26	0.36 ± 0.02
9.0	2.8	84.5	1.90 ± 0.17	0.56 ± 0.07
9.0	4.2	76.8	1.69 ± 0.14	0.46 ± 0.02

2. *Percentage occurrence of bacterial and fungal contaminants*

Graph 1
Percentage distribution of contaminants



The percentage occurrence of contaminants of the tissue culture process was studied from the month of April to August 2009[23]. The fungal and bacterial contaminants were isolated and identified. The percentage of distribution of contaminants is shown in Graph 1. It is evident from Table 1 that fungal contamination was more in the cultures. Total incidence of fungi in cultures was 18.2% and bacteria it was recorded as 5.3%.

3. *Isolation and identification of fungi and bacteria*

A total of 8 fungal and 5 bacterial genera were identified. Contaminants are shown in Table 2. The most predominantly occurring fungi were *Colletotrichum* Sp. and *Penicillium* sp. The most dominating bacteria were *Bacillis* sp., and *Pseudomonas* sp.

Table 2
Fungal and bacterial contaminants observed in the callus culture of A. sessilis.

Fungi	Bacteria
Colletotrichum	*Bacillus*
Curvularia	*Pseudomonas*
Phoma	*Staphylococcus*
Fusarium	*Klebsiella*
Aspergillus	*Agrobacterium*
Penicillium	
Candida	
Cladosporium	

4. ***Production of Indole-3-acetic acid from predominant fungi***

Predominantly isolated fungi such as *Colletotrichum* sp. and *Pencillium* sp. were well supported by literature for IAA production[24]. Hence production of IAA by these two candidates with different concentration (0-0.8 mM) of tryptophan was determined by colorimetric analysis (Table 3).

Table 3
of Indole-3-acetic acid with different concentrations of tryptophan Production

Tryptophan mM	IAA µM	
	Colletotrichum Sp.	*Penicillium* Sp.
0	31.0 ± 1.6	28.5 ± 2.1
0.25	64.4 ± 1.9	56.1 ± 1.8
0.5	98.5 ± 1.4	91.8 ± 1.7
0.8	99.6 ± 1.7	93.4 ± 1.9

Among the two fungal strains, *Colletotrichum* sp. found to more producer of IAA. Although medium containing 0.8mM tryptophan produced IAA slightly higher than 0.5mM tryptophan, economically 0.5mM tryptophan was standardized as better concentration for mass IAA production.

Table 4
Callus formation in MS medium containing 9.0 µM 2,4-D and different concentrations of IAA (HiMedia) and IAA from fungal source.

IAA		Survival rate %	Average Fresh weight (g)	Average Dry weight (g)
HiMedia µM	Fungal source µM			
2.8	-	84.5	1.90 ± 0.17	0.56 ± 0.05
1.9	0.9	81.1	1.86 ± 0.21	0.53 ± 0.04
1.0	1.8	78.6	1.83 ± 0.21	0.52 ± 0.09
-	2.8	74.3	1.79 ± 0.19	0.48 ± 0.08

5. Effects of fungal IAA on callus induction

The leaf explants of *A. sessilis* inoculated in MS medium containing fungal IAA shows similar induction of friable callus as like IAA (HiMedia) (Table 4). Combination of 1.8 µM fungal IAA + 1.0 µM IAA (HiMedia) shows better induction of callus could be used for further cell culture studies in order to enhance of plant secondary metabolites.

DISCUSSION

Callus induction was reported in MS medium supplemented with 1 mg/l of BAP and 1 mg/l of 2, 4 D from leaf explants of *A. sessilis*[25]. The growth of callus was found to be more in IAA and 2,4-D containing medium which correlates with the studies in *Momordica charntia*. Callus and cell culture of important medicinal plants would be exploited for commercial production and isolation of secondary metabolites from its callus and cell culture[1]. The callus induced from fungal IAA amended medium could be a selected cell lines for metabolite production which can be used further for cell suspension cultures. Although literature reveals that microbial IAA used for plant development, it is the first report on fungal IAA for callus induction.

In the present studies more percentage of fungal contaminants was reported with contrast with the studies in sugarcane tissue culture where more bacterial contaminants were isolated[23].Thus it appears that the occurrence of microbes are influenced by the type of host tissue and chemicals present in the plant and its natural habitat. Presence of few endophytes in this host could be due to the presence of antibacterial compounds or due to surface sterilants.

Fungal contaminant *Pythium violae* and *Alternaria dauci* contamination in carrot tissue culture were reported[6]. *Pseudomanos stutueri* produced 188 ± 25 and 134 ± 10 µg/l with or without tryptophan[26]. In present study *Colletotrichum* Sp. produced 25 mg/l of IAA in presence of tryptophan (400 mg/l) in the medium.

CONCLUSION

Thus indole 3-acetic acid from the cultures of *Colletotrichum* Sp. can be utilized for plant growth regulation. Fungal IAA could be used for further cell culture studies in order to enhance of plant secondary metabolites. If this fungal IAA further could also be a very efficient elicitor for plant secondary metabolite production, this method can also be an economical way of producing biotic elicitors. In conclusion, fungal can further be initiated towards purifying and standardizing for cell suspension cultures for enhanced production of secondary metabolites.

ACKNOWLEDGEMENT

Authors are thankful to the Management of Vel's Educational Trust, Chennai, Tamilnadu, India, for providing the infrastructure for the present study.

REFERENCE

1. Ramachandra Rao S and Ravishankar GA, Plant cell cultures: Chemical factories of secondary metabolites. Biotechnology Advances. 20 (2): 101-153, (2002).
2. Chandrika UG, Svanberg U and Jansz ER, *In vitro* accessibility of β-carotene from cooked Sri Lankan green leafy vegetables and their estimated contribution to vitamin A requirement. Journal of the Science of Food and Agriculture. 86 (1): 54-61, (2006).
3. Jalalpure SS, Agrawal N, Patil MB, Chimkode R and Tripathi A, Antimicrobial and wound healing activities of leaves of *Alternanthera sessilis* Linn. International Journal of Green Pharmacy. 2008: 141-144, (2008).
4. Devi CS and Srinivasan VM, Studies on Various Atmosperic Microorganisms Afffecting the Plant Tissue Culture Explants. American Journal of Plant Physiology. 1 (2):205-209. (2006).
5. Bradbury JF, Isolation and preliminary study of bacteria from plants. Rev. Plant Pathol. 49:213-218, (1970).

6. Cooper C, Crowther T, Smith BM, Isaac S and Collin HA, Assessment of the response of carrot somaclones to *Pythium violae,* causal agent of cavity spot. Plant pathol., 55: 427-432, (2006).
7. Kaper JM and Veldstra H, On the metabolism of tryptophan by *Agrobactrium tumefaciens.* Biochim Biophy Acta*,* 30: 401-430, (1958).
8. Huddedar SB, Shete AM, Tilekar JN, Gore SD, Dhavale DD and Chopade BA, Isolation, characterization and plasmid pUPI 126-mediated indole-3-acetic acid production in *Acetobacter* strains from rhizosphere of wheat. Appl Biochem Biotech, 21:102-103, (2001).
9. Jain R and Babber SB, Xanthan Gum: An Economical substitute for agar in plant tissue culture media, Plant Cell rep, 25: 81-84, (2006).
10. Lebuhn M and Hartmann A, Method for the determination of indole-acetic acid and related compounds of L-tryptophan catabolism in soils. J chromatogr, 6: 25-27, (1993).
11. Last RL, Bissinger PH, Mahoney DJ, Radwanski ER and Fink GR, Tryptophan mutants in *Arabidopsis* – the consequences of duplicated tryptophan synthase beta genes. Plant Cell. 3: 345-358, (1991).
12. Arshad M and Frankenberger WT, Microbial production of plant hormones. Plant Soil 133: 1–8, (1991).
13. Costacurta A and Vanderleyden J, Synthesis of phytohormones by plant-associated bacteria. Crit. Rev. Microbiol. 21: 1–18, (1995).
14. Rajagopal K, Mahendran TS and Selvakumar S, Preliminary studies on the diversity of fungal endophytes in few *Ficus sp.* Indian. J. Appli. Micro. 8(1): 11-14, (2008).
15. Subramanian CV, *Hyphomycetes (An account of Indian species, except Cercospora)* IARI; New Delhi: 121-128, (1971).
16. Barnett HL and Hunter BB, *Illustrated genera of imperfect fungi*. 3rd edn. Burgers Publishing Company, Minneapotis, Minnesota, (1972).
17. Onions AHS, Allsopp D and Eggins HOW, *Smith's Introduction to Industrial Mycology* 7th edition. Edward Arnold, London: 118-147, (1981)
18. Suryanarayanan TS. Light incubation: A neglected procedure in mycology. – Mycologist 6: 144, (1992).
19. Buckley PM, Dewilde TN and Reed BM, Characterization and identification of bacteria isolated from micropropagated mint plants. *In vitro* cell. Dev. Biol., 31: 58-64, (1995).
20. Klement Z, Rudolph and sauds KDC, Methods in Phyto bacteriology. Akademiaikiado, Budapest, (1990).
21. Tsavkelova EA, Cherdyntseva TA and Netrusov AI, Auxin production by bacteria associated with orchid roots. Microbiol., 74(1): 46-53, (2005).
22. Patten CL and Glick BR, Role of *Pseudomonas putida* Indoleacetic Acid in Development of the Host Plant Root System. Applied and Environmental Microbiology. DOI: 10.1128/AEM.68.8.3795–3801.2002, (2002).
23. Subathra D and Mohana S, Studies on various atmospheric microorganisms affecting plant tissue culture explants. American J. Plant. Physiol. 1(2): 205-207, (2006).
24. Chagué V, Maor R, Sharon A, CgOpt1, a putative oligopeptide transporter from Colletotrichum gloeosporioides that is involved in responses to auxin and pathogenicity. BMC Microbiology. doi:10.1186/1471-2180-9-173, (2009).
25. Singh A, Kandasamy T. and Odhav B, *In vitro* propagation of *Alternanthera sessilis* (sessile joyweed), a famine food plant. 8 (21): 5691-5695, (2009).
26. Lata H, Li XC, Silva B, Moraes RM & Halda-Alija L. Identification of IAA-producing endophytic bacteria from micropropagated *Echinacea* plants using 16S rRNA sequencing. Plant Cell, Tissue and Organ Culture. 85:353-359, (2006).