Recent Research in Science and Technology 2011, 3(6): 70-75
ISSN: 2076-5061
www.scholarjournals.org

www.recent-science.com

RRST-Biochemistry

Indole Aecetic Acid (IAA) Levels of Cladodes and Roots in Effect of Mycorrhizal and Actinorhizal Inoculation on *C. equisetifolia* under Glasshouse Condition

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Article Info

Article History

Received : 11-01-2011
Revised : 26-04-2011
Accepted : 27-04-2011

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Abstract

Casuarina equisetifolia is one of the economically important plant which will be used for firewood, medicinal property, N_2 fixation, coastal environment preservation. In our present experiment carried out to find out the effect of mycorhizal and actinorhizal (*Frankia*) on *C. equisetifolia* on Indole Acitic Acid (IAA) level both cladodes and roots. The results recorded shows that the combined inoculated (VAM + Ectomycorhizal (*Psilothus tinctorius*) + Actinorhizal (*Frankia*) enhancing the quality and quantity of IAA both in cladodes and roots, than the other individual treatments of symbionts. Since the *C. equisetifolia* is a tall plant longer height shoot, farmers can utilize this symbionts to the firewood production and N_2 fixation.

Key Words: *Casuarina equisetifolia*, VAM, *Frankia*, *Psilothus tinctorius*, IAA

Introduction

Casuarina grows in a diverse range of environments, from tropical forest to arid woodlands and coastal dunes. Mycorrihizae were hypothesized to play an important role in plant growth in several dune restoration studies. *Casuarina* are capable of forming symbiotic nitrogen fixing associations with *Frankia* and may contribute significantly to the nitrogen economy of ecosystem. On the basis of morphology and cultural characteristics, the actinomycete endophytes of non-legumes are placed in one family *Frankiaceae* with a single genus, *Frankia*. Analysed and identified auxin by GC – Mass in mycorrhizal fungi [8].

Levels of IAA, Cytokinin and gibberllin like activity in actinomycetes and leguminous nodules are higher than in the roots [13]. There have been frequent speculations about the nodule auxins and cytokinins [16, 4]. Berry et al. [5] identified five indole compounds secreted by *Frankia* under defined cultural conditions by using gas chromatography and mass spectrometry technique

Indole compounds secreted by *Frankia* were reported by Berry et al. [5] and Hansen and Wheeler [9]. Phillips and Torrey [13] demonstrated the production of IAA, Cytokinin and Gibberllin like (GA) substances by *Rhizobium* in culture [18]. Orchid mycorrhizal fungi belonging to the endomycrrhizal group of fungi secreted indole acetic acid (IAA) in pure culture [14].

Silver et al. [19] found low IAA concentration in the root nodules of both *Myrica* and *Casuarina* and stated that it is due to an active IAA oxidizing system in the root nodules. It was suggested that negative geotrophic response of the roots associated with the nodules of *Myrica* and *Casuarina* can be explained by this activity producing a suboptimal IAA concentration. Dullart [6, 7] estimated spectrofluorometrically, in the acid ether soluble fraction of methanol extracts of *A. glutinosa* root nodules, indole – 3 acetic acid (IAA) in a concentration of 0.6 to 1.0 mg/kg fresh weight of nodules.

The increase in the vasculature, greater lignifications of the styl, early flowering and other reproductive and anatomical changes observed in the VA mycorrlizal plants may all be due to the altered hormonal balance [10]. Chromatography has been used by Madej and Haggblom [9] to 2 detect IAA in *P. tinctorius* and 3 species of *Suillus*.

Hence the present study aims to analyse the (IAA) Levels of Cladodes and Roots in Effect of Mycorrhizal Inoculation on *C. equisetifolia* under Glasshouse condition by using HPLC (Hitachi Co., L 6200) technique.

Materials and Methods

Extraction and identification of IAA from shoots and roots

The extraction and identification of IAA were carried out according to Saukurai *et al.,* (1985). The samples of shoots, roots or nodules were stored in 80% ethanol containing 0.3% ascorbic acid. They were homogenized after the addition of 200 µl of indolebutylic acid (IBA) as an internal standard. The homogenate was filterd. The residue was resuspended in 80% ethanol, then kept in dark for 1h until filtration. Both filtrates were combined and reduced to a small volume (20 ml) with an evaporator. The solution was mixed with polyvinyl poly pyrrolidone (PVPP) in a small beaker. The suspension was filterd. The filtrate was adjusted to pH 3.5 and washed by three successive partitioning with the same volume of petroleum

ether. The IAA extracted from the water layer was evaporated and the residue was dissolved in 5 mM sodium acetate. This solution was used for identification by mass spectrometry.

HPLC Separation

IAA sample was determined by HPLC (Hitachi Co., L 6200) equipped with a fluorometric detector (wave length 280 nm, 350 nm). The sample was loaded on a Deversil packed column ODS – 5 (Nomura chemical Co., Japan) and eluted by a linear gradient of acetonitrile (0 -3- min 0-30 %, 30 -40 min 30 -4- %) in 20 mM sodium acetate buffer (p H 3.5) with a flow rate of 1 ml/ min.

Results

IAA levels in cladodes

The amounts of IAA in the treated plants are presented in table 1. The IAA level increased along with age in all plants. A maximum of 74.70 ng/g; (+66%) was found in triple inoculated (V+E+F) plants, followed by E+F (74.40 ng; +65%), V+F (63.96 ng; +42%), *P. tinctorius* (57.57 ng; +28%), V+E (55.87 ng; +24%), E+F (61.1 ng; +36%), *Frankia* (51.40 ng; +14%) and *G. fasiculatum* (47.89 ng; +7%) inoculated plants.

Table 1. Level of IAA in cladodes of mycorrhizal and actinorhizal inoculated *C. equisetifolia.*

Treatment	Amount of IAA µg/g dry wt. in different zones										
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	Total
Control	4.40 ±0.26	4.03 ±0.25	1.27 ±0.15	4.03 ±0.25	5.13 ±0.35	9.17 ±0.50	3.37 ±0.31	1.30 ±0.20	5.53 ±0.15	6.67 ±0.12	44.90 ±2.54
G. fasiculatum (V)	4.03 ±0.25	4.33 ±0.15	9.53 ±0.15	4.23 ±0.15	5.03 ±0.25	2.37 ±0.15	3.80 ±0.10	3.17 ±0.21	5.37 ±0.35	6.03 ±0.06	47.89 ±1.82
P. tinctorius (E)	4.17 ±0.12	3.33 ±0.25	8.33 ±0.15	4.57 ±0.25	9.93 ±0.15	3.27 ±0.15	2.97 ±0.06	3.47 ±0.21	9.40 ±0.40	8.13 ±0.61	57.57 ±2.35
Frankia (F)	5.10 ±0.20	3.43 ±0.25	9.00 ±0.10	5.30 ±0.40	4.87 ±0.12	4.17 ±0.29	3.07 ±0.40	2.70 ±0.56	5.13 ±0.35	8.63 ±0.25	51.40 ±2.92
V+E	4.03 ±0.45	3.57 ±0.35	8.67 ±0.32	5.23 ±0.32	8.27 ±0.35	5.20 ±0.26	4.03 ±0.45	2.83 ±0.55	5.07 ±0.31	8.97 ±0.35	55.87 ±4.03
V+F	5.17 ±0.25	3.80 ±0.20	5.43 ±0.45	9.13 ±0.67	7.93 ±0.31	5.03 ±0.06	5.20 ±0.30	8.30 ±0.60	8.10 ±0.30	5.87 ±0.31	63.96 ±3.45
E+F	6.37 ±0.31	4.00 ±0.40	5.83 ±0.45	10.33 ±0.55	9.37 ±0.70	6.47 ±0.47	6.33 ±0.35	8.33 ±0.55	9.37 ±0.50	8.00 ±0.20	74.40 ±4.48
V+E+F	6.30 ±0.26	4.17 ±0.15	6.03 ±0.25	10.40 ±0.53	9.37 ±0.06	6.40 ±0.10	6.33 ±0.06	8.60 ±0.20	8.60 ±0.20	8.47 ±0.12	74.70 ±1.60

2^3 – Factorial experiment showing the significance of IAA in mycorrhizal and actinorhizal inoculated *C. equisetifoloia* cladodes.

Treatment	IAA in different zones									
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Main effects										
VAM										
Ecto	2.61	1.92	255.18*	0.33	0.12	804.17*	3.26	28.97*	0.36	6.65**
Frankia	1.05	10.50*	917.22*	2.37	295.17*	605.39*	2.78	39.03*	197.88*	35.68*
	9.53*	7.71**	098.44*	13.37*	0.91	434.78*	1.56	16.29	2.11	64.16*
2–Way interactions										
V + E	2.61	4.66**	005.79*	12.00*	125.77*	273.64*	7.72**	19.54*	2.88	87.76*
V + F	11.43*	1.16	318.87*	216.75*	100.44*	297.12*	58.45*	407.93*	87.19*	10.61*
E +F	75.26*	0.02	383.04*	330.75*	229.59*	126.78*	153.06*	411.27*	194.48*	44.25*
3–Way interactions										
V + E + F	70.24*	0.38	417.32*	337.78*	229.59*	133.12*	153.06*	443.05*	124.47*	53.75*

Significane at $p < 0.001$
Significane at $p < 0.05$

IAA level of roots

Variation in the IAA levels in the roots of experimental plants are presented in table 2. The maximum amount of IAA (71.8 ng/g; +91%) was recorded in the triple (V+E+F) inoculated plants followed by E+F (58.23; +55 %), V+F (54.65 ng; +45%), V+E (51.34 ng; +37%), *Frankia* (48.13 ng; +28%), *P. tinctorius* (45.60 ng; +21%) inoculated plants. *G. fasciculatum* inoculated plants contained only IAA (40.60 ng; +8%).

The mass spectra and HPLC analyses of IAA in *C. equisetifolia* seedlings confirmed the presence of IAA (Fig. 1 – 5)

Table 2. Level of IAA in roots of mycorrhizal and actinorhizal inoculated *C. equisetifolia*

Treatment	Amount of IAA µg/g dry wt. in different zones										
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	Total
Control	3.20 ±0.17	3.13 ±0.12	2.20 ±0.10	3.17 ±0.15	3.20 ±0.20	7.77 ±0.15	2.90 ±0.36	1.17 ±0.15	4.93 ±0.23	6.00 ±0.20	37.60 ±1.83
G. fasiculatum (V)	0.53 ±0.25	3.53 ±0.06	2.17 ±0.15	3.17 ±0.15	3.30 ±0.10	8.97 ±0.15	3.13 ±0.15	1.40 ±0.20	5.20 ±0.20	6.20 ±0.20	40.60 ±1.45
P. tinctorius (E)	4.07 ±0.12	3.50 ±0.10	3.03 ±0.46	3.43 ±0.15	3.53 ±0.06	9.13 ±0.15	3.70 ±0.10	3.30 ±0.30	5.40 ±0.20	6.53 ±0.32	45.62 ±1.90
Frankia (F)	4.27 ±0.12	4.17 ±0.15	2.63 ±0.15	3.63 ±0.15	3.80 ±0.10	9.33 ±0.15	4.23 ±0.20	3.73 ±0.12	5.87 ±0.12	6.47 ±0.06	48.13 ±1.26
V+E	4.57 ±0.12	4.33 ±0.29	3.20 ±0.69	3.87 ±0.12	4.03 ±0.15	9.70 ±0.10	5.13 ±0.12	3.87 ±0.31	5.97 ±0.29	6.67 ±0.12	51.34 ±2.31
V+F	4.73 ±0.15	4.77 ±0.12	4.30 ±0.17	4.17 ±0.15	4.20 ±0.10	10.47 ±0.12	5.67 ±0.14	4.17 ±0.15	5.30 ±0.10	6.87 ±0.12	54.65 ±1.30
E+F	5.03 ±0.15	5.10 ±0.10	4.60 ±0.17	4.40 ±0.17	4.57 ±0.25	11.17 ±0.15	5.97 ±0.29	4.93 ±0.23	5.43 ±0.15	7.03 ±0.20	58.23 ±1.86
V+E+F	6.30 ±0.10	6.79 ±0.15	5.97 ±0.29	6.80 ±0.26	6.00 ±0.20	12.17 ±0.15	7.07 ±0.21	6.13 ±0.21	6.37 ±0.06	8.20 ±0.20	71.80 ±1.77

2^3 – Factorial experiment showing the significance of IAA in mycorrhizal and actinorhizal inoculated *C. equisetifoloia* cladodes.

Treatment	IAA in different zones									
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Main effects										
VAM										
Ecto	7.01**	10.66**	0.01	0.00	0.60	105.79*	1.83	1.71	3.20	1.63
Frankia	47.43*	8.96*	9.32*	3.71	6.66**	137.22*	21.53*	143.71*	9.80*	11.63*
	71.86*	71.18*	2.52	11.36*	21.60	180.32*	56.86*	208.03*	39.20*	8.90*
2–Way interactions										
V + E	117.96*	96.00*	13.43*	25.56*	41.66*	274.61*	167.81*	230.21*	48.05	18.18*
V + F	148.49*	177.85*	53.73*	52.17*	60.00*	535.59*	257.53*	284.21*	6.05**	30.72*
E +F	212.28*	257.85*	77.37*	79.36*	112.06*	849.30*	316.41*	448.03*	11.25*	40.90*
3–Way interactions										
V + E + F	278.52*	462.29*	102.82*	195.01*	194.40*	849.30*	337.38*	496.87*	8.45*	58.90*

Significane at $p < 0.001$
Significane at $p < 0.05$

Source of Variation	DF	Mean Square	F - Value	Significance of F
Between different zones	9	89.304	407.498	000
Main effects				
G. fasiculatum (V)	1	1.291	5.889	0.16
P. tinctorius (E)	1	9.520	43.441	0.000
Frankia (F)	1	16.328	74.506	0.000
2–Way interactions				
V + E	1	28.017	127.842	0.000
V + F	1	42.673	94.718	$p < 0.05$
E + F	1	63.243	288.580	$p < 0.05$
3–Way interactions				
V + E + F	1	82.134	374.783	$P < 0.05$

Residual	223	0.219
Total	239	4.163

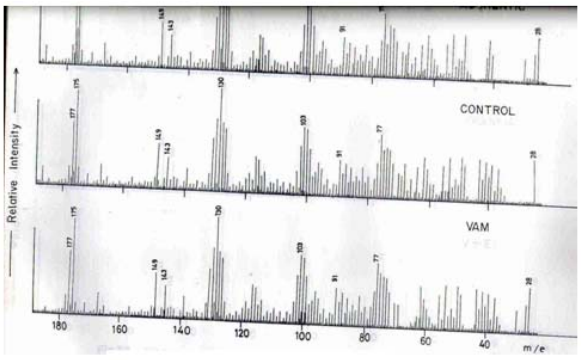

Fig. 1. Mass Spectra of IAA from *Casuarina equisetifolia* inoculated with *Glomus fasciculatum*

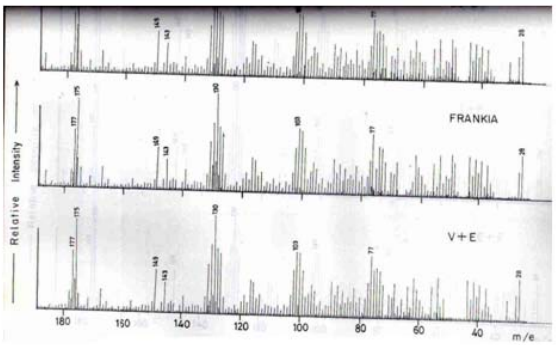

Fig. 2. Mass Spectra of IAA from *Casuarina equisetifolia* inoculated with *Pisolithus tinctorius*, Frankia, *Glomus fasciculatum* + *P. tinctorius*

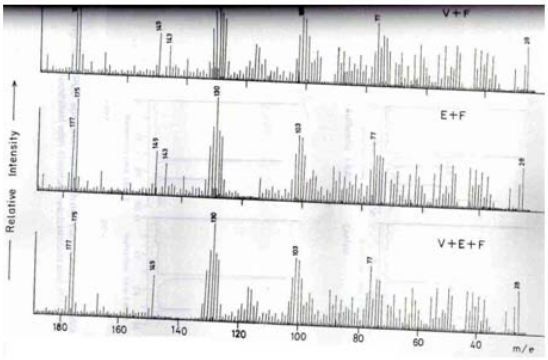

Fig. 3. Mass spectra of IAA from *Casuarina equisetifolia* with various treatments. V + F = *Glomus fasciculatm* + *Frankia*, E + F = *Pisolithus tinctorius* + *Frankia*, V + E + F

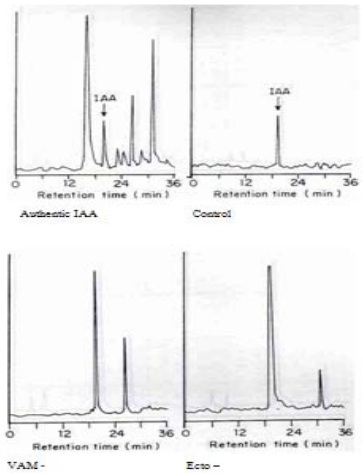

Fig. 4. HPLC spectrum of IAA from *Casuarina equisetifolia* seedlings inoculated with *Glomus fasciculatum* and *Pisolithus tinctorius*

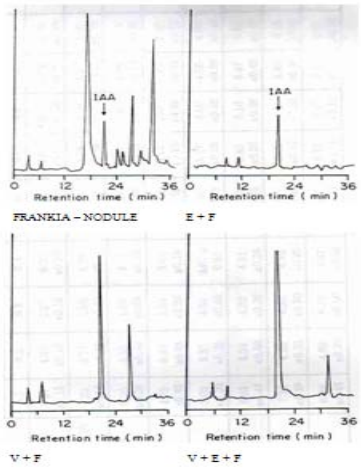

Fig. 5. HPLC spectrum of IAA from *Casuarina equisetifolia* seedlings inoculated with various treatments. V – *Glomus fasciculatum*, E – *Pisolothus tinctorius*, F – *Frankia*.

Discussion

Many of the physiological changes in a plant system are mediated by hormone [12]. IAA was detected from *Pisolithus tinctorius* and three species of *Suillus* [9]. Indole compounds secreted by *Frankia* were shown by Berry et al. [5] and Hensen and Wheeler [9]. Some ectomycorrhizal fungi produce auxin, cytokinin and gibberllin like substances. Silver et al. [18] observed the low IAA concentration in the root nodule of both *Myrica* and *Casuarina* is due to an active IAA oxidize system in the root nodules. The negative geotrophic response of the roots associated with nodules of *Myrica* and *Casuarina* can be explained by this activity producing a suboptimal IAA concentration. Dullart [6] estimated IAA in the acid ether soluble fraction of methanolic extracts of *Alnus glutinos*a root nodules. Levels of IAA, cytokinin and giggerllin – like activity in actinomycete and leguminous nodules were almost higher than in the roots [13]. Berry et al. [5] identified five indole compounds secreted by *Frankia* under defined cultural conditions by using gas chromatography and mass spectrometry techniques.

Allen et al. [1, 2] reported the increase of cytokinin and gibberllin contents in plants tissue associated with the infection of VA mycorrhizal fungi. Barea and Azcon-Aguilar [3] noted that in axenic experiments mycorrhizal fungi produced auxin, gibberlin and cytokinin like substance and stimulated plant growth. IAA was indispensable for the initation of growth of nodules [7].

IAA levels in the cladodes of *C. equisetifolia* increased with age in all plants. Maximum amount of IAA in cladodes and roots were observed in triple (V+E+F) inoculated plants. Mycorrhizal colonization increased the gibberllic acid level in shoots of VAM plants over that of non-mycorrhizal plants [1]. *G. mosseae* produces gibberellins – like substances [3]. Maximum amount of gibberellic acid was observed in triple inoculated (V+E+F) plants both in cladodes and roots

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