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Indole -3-acetic Acid Production by Some Fungi Associated with *Cajanus cajan* Linn. in Presence and Absence of L- Tryptophan

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Rhizosphere, rhizoplane, phyllosphere, phylloplane and endophytic mycoflora of *Cajanus cajan*, Linn. was isolated. The potential of the isolates for plant growth promotion was evaluated by screening them for Indole -3 acetic acid (IAA) production. 112 species belonging to 32 genera were screened during the present study. 23.62 % of the isolates could produce IAA, in medium containing L- tryptophan. Screening of IAA production was carried out colorimetrically as well as on nitrocellulose membrane.

Key words: Fungi, *Cajanus cajan* Linn., Indole 3 acetic acid, L- tryptophan.

Microorganisms are known to produce extracellular metabolites (Datta & Basu, 2000). Bacteria and fungi have been recorded to produce auxins (IAA) as secondary metabolites (Kampert M. *et al.* 1975, Sterzelczyk E. *et al.*, 1984). Soil fungi as well as the fungi associated with various parts of the plants have been recorded to promote the growth of the plant (Stein A. *et al.* 1990). With this knowledge, it was thought interesting to isolate and screen the hyphomycetous fungi associated with *Cajanus cajan*, Linn, an important pulse crop in Maharashtra, India. The study aims at understanding the role of such fungal species in promotion of the growth of the selected crop plant.

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Fungi were isolated from different regions like rhizosphere, rhizoplane, phyllosphere, phylloplane and endophytic region. L- Tryptophan being the precursor of IAA, the selected fungal species were screened for the production of IAA in a medium amended with L-Tryptophan. The ability of these species to produce IAA in absence of L- Tryptophan was also studied.

MATERIAL AND METHODS

Collection of samples

Rhizosphere and rhizoplane soil samples and leaves, stem, root samples of *Cajanus cajan* were collected regularly at the interval of one month through out the growing season of plant.

Isolation of Fungi

Fungi associated with rhizosphere, rhizoplane, were isolated using serial dilution method (Warcup, 1960) and phylloplane by washed disc method (Dubey & Maheshwari, 2002). The phyllosphere fungi were isolated by method described by Johnson & Curl (1972) and

endophytic mycoflora from the cut pieces of all parts of the plant was isolated by using the method described by Sharma *et al.*, (2009).

Media used

The isolation of fungi were carried out on nine different media, namely, Czapek's dox agar, Potato dextrose agar, Potato sucrose agar, Saboraud's agar, Waksman's special agar, Rose Bengal agar, Corn meal agar, Malt extract agar, Richard's agar supplemented with 80µg/ml streptomycin. The isolated microorganisms were maintained on slants on the respective media (Manoharachary *et al.* 2006)

Screening of fungi for Indole -3- Acetic Acid (IAA) production

Indole-3- acetic acid (IAA) production of these fungi was tested in nitrogen free Ashby's broth supplemented with 0.005M Tryptophan as the precursor of IAA. The test organisms were inoculated in the broth. After 10 days of incubation the concentration of IAA in culture broth was determined by colorimetric method using Salkowaski reagent (Weber 1951). 1ml of the supernatant was mixed with 2ml of Salkowaski reagent and the intensity of red color developed within 30 minutes was checked on colorimeter at 530 nm wavelength. IAA production was calculated using a standard curve prepared from standard solutions of Indole acetic acid. The IAA production of these fungi was also tested on Nitrocellulose membrane (Weber 1951).

RESULTS AND DISSCUSSION

Total 112 fungi isolated from various regions of soil and plant parts associated with *Cajanus cajan* Linn, were screened for their ability to produce IAA. 24 species (21.4%) were found to be positive for IAA production. These 24 isolates were tested for their ability to produce IAA in

Table 1. Screening of fungi associated with *Cajanus cajan* Linn for IAA production

S. No.	Name of organisms	Source or Regions of plant	IAA production		
			On Nitrocellulose membrane	With tryptophanT	*Without tryptophan t*
1	*Aspergillus aculeatus*	3,4	+	+	-
2	*A. clavatus*	1,2,4	-	-	-
3	*A .carneus*	1,2,4	-	-	-
4	*A. candidus*	1,2,4	-	-	-
5	*A .cremeus*	3,4	-	-	-
6	*A .cervinus*	3,4	-	-	-
7	*A .flavus*	1,2,3,4,5	+	+	+
8	*A. flavipes*	1,2,3	+	+	-
9	*A. fumigatus*	3,4	-	-	-
10	*A .giganteus*	3,4	-	-	-
11	*A .glaucus*	3,4	-	-	-
12	*A .restrictus1*	5	+	+	-
13	*A .ochraceous*	1,2	-	-	-
14	*A. raperi*	1,2	-	-	-
15	*A .terricola*	4	+	+	-
16	*A .wentii*	*1,2,4*	+	+	+
17	*A .nidulans*	*5*	-	-	-
18	*A .sulphureus*	*1,2*	-	-	-
19	*A .niger*	*1,2,3,4,5*	+	+	+
20	*A .restrictus2*	*1,2,5*	-	-	-
21	*A .sparsus*	*1,2*	-	-	-
22	*A. terreus*	*1,2,5*	+	+	+
23	*A .ustus*	*1,2*	-	-	-
24	*A .kanaguansis*	*3*	-	-	-
25	*A. ornatus*	*3*	-	-	-
26	*A. koningii*	*1,2*	-	-	-

27	*Acremonella sp.*	*3*	-	-	-
28	*Alternaria longipes*	*1,2*	+	+	+
29	*A. citri*	*3,5*	-	+	-
30	*A. humicola*	*1,5*	-	-	-
31	*A. alternata*	*2,5*	-	-	-
32	*A. tennuis*	*5*	+	+	+
33.	*A.tennuissima*	*5*	-	-	-
34.	*A. brassicola*	*4,5*	-	-	-
35.	*A. dianthicola*	*5*	-	-	-
36	*A. longissima*	*1,3,4*	-	-	-
37	*Botrytis sp.*	*4*	-	-	-
38	*Bispora sp.*	*4*	-	-	-
39	*Basipetalospora sp.*	*4*	-	-	-
40	*Cladosporium oxysporm*	*3*	-	-	-
41	*C.sphaerospermum*	*1,2,3*	-	-	-
42	*C.macrocarpum*	*2,4*	-		
43	*C. lignicola*	*5*	+	+	+
44	*C. herbarum*	*4,5*	-	-	-
45	*C. cladosporoides*	*1,5*	-	-	-
46	*C. spongiosum*	*5*	-	-	-
47	*C. variabile*	*5*	-	-	-
48	*Cladosporium sp.*	*1,3,4,5*	-	-	-
49	*Cephalosporium sp.*	*3*	-	-	-
50	*Curvularia. eragrostidis*	*5*	-	-	-
51	*C. trifolli*	*5*	-	-	-
52	*C. lunata*	*1,5*	-	-	-
53	*C. pallescens*	*1,2,3,5*	+	+	-
54	*C. intermedia*	*5*	-	-	-
56	*C.ovoidea*	*1,4*	-	-	-
57	*C.leonesis*	*1,4*	-	-	-
58	*Drecslera hawaiiensis*	*5*	+	+	+
59	*D. australiensis*	*5*	+	+	+
60	*F.avanaceum*	*5*	-	-	-
61	*F. equiseti*	*1,2*	+	+	+
62	*F. moniliforme*	*4,5*	+	+	-
63	*F. nivale*	*1,2,5*	-	-	-
64	*F.oxysporum*	*1,2,4*	+	+	+
65	*F. solani*	*2,4,5*	+	+	+
66	*F. semitectum*	*1,2*	-	-	-
67	*F.stillboides*	*3*	-	-	-
68	*F. tabacinum*	*3,5*	-	-	-
69	*F. tricinctum*	*3*	-	-	-
70	*F. udum*	*5*	-	-	-
71	*F. lateritium*	*3*	-	-	-
72	*F.dimerum*	*4*	-	-	-
73	*F.xyllaroides*	*1,2*	-	-	-
74	*F.hetrosporum*	*1,3,4*	-	-	-
75	*Gleosporium sp.*	*3,4*	-	-	-
76	*Gliomastix sp.*	*3,4*	-	-	-
77	*Gilmaniella sp.*	*5*	-	-	-
78	*Humicola grisea*	*3*	-	-	-
79	*Monodyctis sp.*	*2,3,4*	-	-	-
80	*Monilia sp.*	*2,3,4*	-	-	-
81	*Mamnaria sp.*	*1,2,3,4*	-	-	-
82	*Papulospora sp.*	*1,3,4,5*	+	+	-
83	*Pacelomyces sp.*	*3,4*	-	-	-

84	*P. citrinum*	*3*	+	+	+
85	*P. stolonifer*	*1*	-	-	-
86	*P. notatum*	*3,5*	-	-	-
87	*P. jenseni*	*5*	-	-	-
88	*P. nigricans*	*1,2,3,4*	-	-	-
90	*P. fuscum*	*5*	-	-	-
91	*P. glabrum*	*5*	-	-	-
92	*Phoma sp.*	*1,3,5*	-	-	-
93	*Rhizopus oryzae*	*1,2*	-	-	-
94	*R. stolonifer*	*3*	-	-	-
95	*R. pregmaceous*	*5*	+	+	+
96	*R. suinus*	*1,4,5*	-	-	-
97	*R. tritici*	*1,2,3*	-	-	-
98	*Doratomyces sp.*	*3,5*	-	-	-
99	*Stemphyllium sarciniforme*	*3,4*	-	-	-
100	*Scoladobasidium sp.*	*1*	-	-	-
101	*Sporotrichum sp.*	*5*	-	-	-
102	*T. harzianum -1*	*1,2,4*	-	-	-
103	*T. harzianum -2*	*3*	-	-	-
104	*T. polysporum*	*3,5*	+	+	+
105	*T. flavofuscum*	*4,5*	+	+	+
106	*T. atroviride*	*4*	-	-	-
107	*T. pseudokoningii*	*1,2,3*	-	-	-
108	*Scytalidium sp.*	*3*	-	-	-
109	*Verticillium sp.*	*3*	-	-	-
110	*Rhizoctonia solani*	*5*	+	+	+
111	*Nigrospora sp.*	*1,3,4,5*	-	-	-
112	*Sterile mycelium.*	*1,4*	-	-	-

Note: 1 = Rhizosphere, 2 = Rhizoplane, 3=Phyllosphere, 4= Phylloplane, 5 = Endophytic region, '+' = Present & '-' = Absent

Table 2. IAA production in different regions of *Cajanus cajan,* Linn.

Regions of plant	Total isolates	IAA production with tryptophan	IAA production without tryptophan	Non producers (Negative isolates)
Rhizosphere	44	11 (25 %)	7 (15.90%)	26(59.09%)
Rhizoplane	34	9 (26.47%)	7 (20.58%)	18(52.94%)
Phyllosphere	44	8 (18.18%)	4 (9.09%)	32(72.72%)
Phylloplane	43	9 (20.93%)	5 (11.62%)	29(67.44%)
Endophytic	45	18 (40%)	12 (26.66%)	15 (33.33%)

absence of tryptophan (precursor of IAA) and it was observed that 17 species could produce IAA even in absence of tryptophan. All these 24 isolates also showed development of red color on nitrocellulose membrane.

Total isolations from the different sites indicate that almost equal number of species was found to be associated with all parts of the plant. Out of the five different sites studied, 44 species were isolated from rhizosphere region, of these 11 isolates (25%) could synthesize IAA with tryptophan and 7 species (15.9 %) could synthesize IAA without tryptophan. 34 species were isolated from rhizoplane region, 9 isolates (26.47 %) of these could synthesize IAA with tryptophan and 7 species (20.58 %) showed ability to produce IAA in absence of the precursor. 44 species were isolated from phyllosphere region, of these 8

isolates (18.18 %) could produce IAA with tryptophan, and 4 species (9.09 %) showed ability to produce IAA without tryptophan. Out of 43 isolates of phylloplane region, 9 isolates (20.93 %) could produce IAA with tryptophan, and 5 isolates (11.62 %) showed ability to produce IAA without tryptophan. Maximum isolates in the endophytic region i.e., (16 out of 43, i.e.37.20%) could produce IAA with tryptophan and 11species (25.58%) could produce IAA without tryptophan. Of the 43 fungi isolated from endophytic regions 23 species were isolated exclusively from this region. Seven of them were IAA producers. Except for *Aspergillus restrictus* all others i.e. *Alternaria tennuis* , *Cladosporium lignicola*, *Drechslera hawaiiensis*, *Drechslera australiensis*, *Rhizopus pregmaceous* and *Rhizoctonia solani* could produce IAA with and without tryptophan supplement. Two species of *Aspergillus*, namely, *A. flavus* and *A. niger* were isolated from all the sites studied and both produced IAA with and without tryptophan.

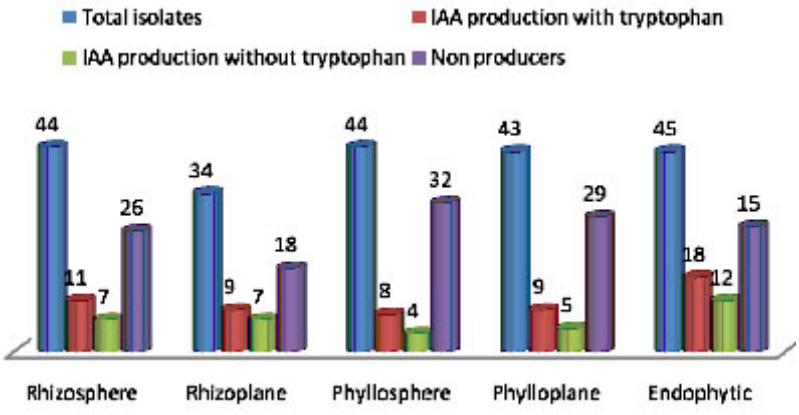

Fig. 1. IAA production in different regions of *Cajanus cajan*

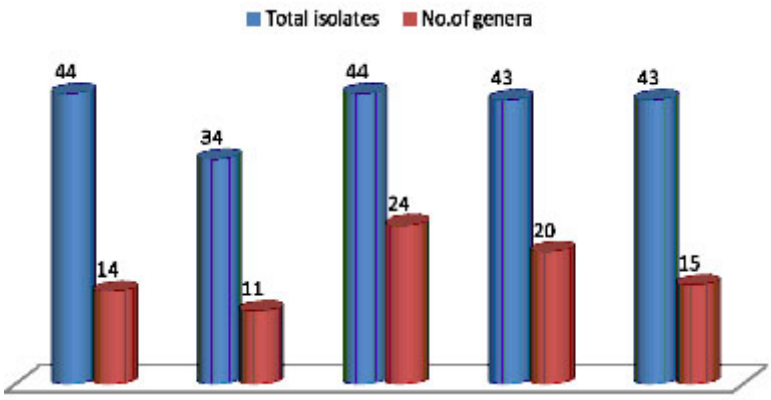

Fig. 2. Number of fungi isolated from each region of *Cajanus cajan*

CONCLUSIONS

All most equal number of species was isolated from the various sites selected. Above 20% of fungi from each region showed definite ability to produce IAA. The present study reveals occurrence of maximum (37.2%) IAA producing species in the endophytic region indicating the possibility of their direct effect on growth of the plant. The study suggests that culture filtrate of selected IAA producing fungi could be used on large scale as a low cost seed treatment or foliar spray after some field trials.

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