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Indole Acetic Acid Production By Fusarium Spp. And Their Growth Promoting Effects On Gram And Cucumber Seeds

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Abstract: ***Laboratory experiments were performed to find out a correlation between growth and indole acetic acid production of five Fusarium isolates obtained from an agricultural field of Murshidabad district in West Bengal, India. The isolates were recovered on a selective medium supplemented with pentachloronitrobenzene and chloramphenicol. The isolates were varied both in growth pattern and conidial structures. Each of the soil isolates was tested for their ability to produce indole acetic acid (IAA) at three different concentrations of tryptophan (1000ppm, 1500ppm and 2000ppm) with respect to a control. All the isolates were found to be potential IAA producers. Both mycelial dry weight and IAA production improved significantly with increasing tryptophan concentrations. The soil isolates were able to produce IAA even in the absence of tryptophan. Among the five Fusarium isolates, two were found largely efficient in production of IAA (350µg/ml) in presence of tryptophan (2000 ppm). Moreover, the effects of the culture filtrates of each Fusarium soil isolates on gram and cucumber seeds were found to be markedly encouraging as both root and shoot lengths were enhanced to a considerable extent as compared to control without exhibiting any inhibitory effects on the seed germination. Therefore, these soil isolates can be successfully exploited in the agricultural fields to increase crop yield.***

Keywords: ***Fusarium, Growth, Conidia, IAA, Tryptophan, Seed germination, Gram and Cucumber seedlings***

I. INTRODUCTION

Production of indole acetic acid (IAA) by non-pathogenic soil microflora is an important trait for improvement of plant growth. The auxin stimulates both rapid (e.g., increases in cell elongation) and long-term (e.g., cell division and differentiation) responses in plants. It has also role in root initiation and better development of roots, thus increasing the absorptive surface of plant roots for uptake of water and nutrients. The hormone can be synthesized by the plants themselves but also by diverse soil microorganisms including bacteria (Idris et al., 2007; Shahab et al., 2009), fungi (Stein et al., 1985; Hasan, 2002) and algae (Finnie and Van Staden, 1985; Prasanna et al., 2010). Tryptophan is the key precursor for biosynthesis of IAA in plants and microorganisms, and application of exogenous tryptophan increases IAA production. Root exudates are the main sources of tryptophan in soil. Several biosynthetic pathways for IAA production exist, sometimes in parallel in the same organism (Davies, 1995). There are four intermediate pathways for production of IAA from tryptophan: (i) via formation of indole-3-pyruvic acid and indole-3- acetic aldehyde which was reported in majority of microorganisms such as bacteria (*Agrobacterium, Azospirillum, Pseudomonas*), cyanobacteria (*Nostoc*), yeast (*Sacchromyces uvarum*), phytopathogenic fungi (*Fusarium, Rhizoctonia, Colletotichum*); (ii) via tryptamine formation; (iii) via indole-3-acetamide formation as in *A. tumefaciens* and *Rhizobium*; and (iv) via acetonitrile formation. Tryptophan independent biosynthesis of IAA was found in *Azospirillum*

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(Costacurta and Vanderleyden, 1995), *Anabaena* (Prasanna et al., 2010).

The genus *Fusarium* represents one of the important groups of filamentous fungi belonging to *Deuteromycota* phylum, *Hyphomycetes* class, while their teleomorphs are mostly classified in the genus *Gibberella* of *Ascomycota* phylum. The members are abundance in soils as free-living saprophytes, pathogens, endophytes, and known to produce a range of mycotoxins that can adversely affect livestock and humans. *Fusarium* species produce three types of spores: banana-shaped 3-4 celled macroconidia, 1-2 celled microconidia and unicellular thick-walled chlamydospores. Since its establishment in 1809 by Link, the genus *Fusarium* has received much attention due to its high degree of variability. Leslie and Summerell (2006) reported more than 70 species under the genus. The pathogenic species of *Fusarium* are responsible for destructive diseases on cereal grains, vascular wilts or root rots on many important vegetable, ornamental and field crops. There are reports to control of *Fusarium* wilt by using non-pathogenic species of *Fusarium* (Larkin and Fravel, 1999). The non-pathogenic isolates with potential plant growth promoting attributes can be explored to increase crop productivity. This will eventually reduce the rampant use of chemical fertilizers in agricultural fields and minimize their detrimental effects on ecosystems. The objective of the present wok was to screen the efficiencies of five *Fusarium* soil isolates for production of IAA at different concentrations of tryptophan and their role in enhancement of germination of gram and cucumber seeds, and increase in root and shoot length of the seedlings.

II. MATERIALS AND METHODS

ISOLATION AND IDENTIFICATION OF THE FUNGI: The *Fusarium* spp. were isolated directly from the soil by dilution plate technique. Soil sample at a depth of 6 cm was collected from an arable land cultivated for various crops in Murshidabad district of West Bengal, India. One g of soil was mixed in 10 ml sterile water to prepare the crude soil suspension. From the crude suspension, $1/10^{th}$ and $1/100^{th}$ dilutions were prepared and were subsequently inoculated on potato sucrose agar (PSA) medium [composition (g/l): potato extract 200, sucrose 20, agar 20, pH 6] supplemented with pentachloronitrobenzene (0.1%) and chloramphenicol (0.01%) for selective growth of *Fusarium* spp. The plates were incubated at 28^0C for 5-7 days until visible sign of colony growth occurred. The fungal isolates were grown on Czapek Dox agar (CDA) medium for their subsequent characterizations. Reproductive structures of the isolates were also studied through microscopic observation.

GROWTH AND IAA ESTIMATION: Each of the fungal isolates were grown in potato dextrose (PD) broth supplemented with three different concentrations of tryptophan viz, 1000 ppm, 1500 ppm and 2000 ppm at 28^0C and in a control set having no tryptophan. After 14 days of incubation, concentration of IAA in the culture broth was estimated using Salkowski reagent. One ml of the supernatant was mixed with 2 ml of Salkowski reagent (2 ml of 0.5 M $FeCl_3$ + 98 ml 35% $HClO_4$) and the intensity of red colour developed after 30 minutes was measured at 530 nm. The concentration was determined using standard solutions of IAA. Concurrently, mycelial dry weights of the soil isolates were also determined to draw a correlation between growth and IAA production.

EFFECTS OF CULTURE FILTRATES ON SEED GERMINATION AND ROOT-SHOOT LENGTH: Fusarium isolates were grown in PD broth for 14 days. Culture supernatant of each isolates was separated from the mycelial biomass using Whatman no. 1 filter paper. Gram (*Cicer arietinum*) and cucumber (*Cucumis sativus*) seeds were surface sterilized using 0.1% $HgCl_2$, dipped in culture supernatant of each isolates and kept in dark at 4^0C for 24 hour. A control set was prepared using uninoculated PD broth. On the next day, these seeds were placed on pre-soaked blotting paper in separate petridishes and kept in well-illuminated place. After seven days, percentage of seed germination was calculated. In addition, root and shoot lengths of the seeds treated with each culture filtrates were also measured accordingly. The viability of seedlings was determined by calculating the vigour index [VI = Length of shoot + root X Germination%] of the seeds.

III. RESULTS AND DISCUSSION

ISOLATION AND CHARACTERIZATION OF THE FUSARIUM SPP.: A total of five *Fusarium* soil isolates, designated as SF2-6 were recovered from the soil sample studied. These were varied both in macroscopic and reproductive structures (Table 1). The colonies were white, circular with their diameter ranging from 42-83 mm (Fig.1). Isolate SF3 was found to produce faint pink pigment and isolate SF5 produced faint green pigment. All the isolates were fast growing, producing 1-2 celled microconidia amply with considerable degrees of curvature. Three-celled macroconidia and thick-walled intercalary chlamydospore were observed in isolate, SF4 (Fig.2).

Isolate no.	Colony morphology			Sporulation
	Diameter (mm)	Colour, Shape	Pigmentation	
SF-2	46	White, Circular	-	1-2 celled microconidia with minimum degree of curvature
SF-3	83	White, Circular, Compact	Faint pink	Microconidia with greater degree of curvature
SF-4	42	White, Circular, more compact	-	1-2 celled microconidia, 3-celled macroconidia ; thick-walled chlamydospore
SF-5	60	White, Circular,	Faint green	1-2 celled ovoid

		glittering		microconidia
SF-6	57	White, Circular, diffused	-	1-2 celled slightly elongated microconidia with prominent curvature

Table 1: Growth characteristics and sporulation of the Fusarium soil isolates on CDA medium after 7 days of incubation at 28^0C

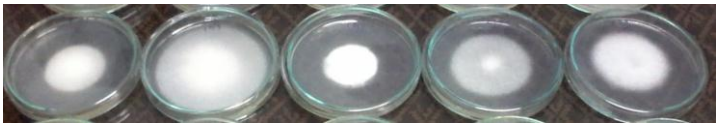

Figure 1: Colony characteristics of the Fusarium soil isolates on CDA medium after eight day of incubation (from left to right SF 2-6)

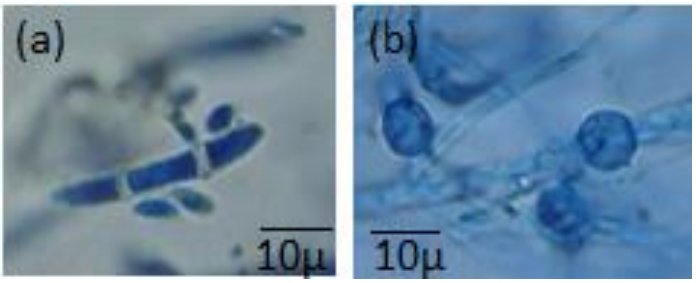

Figure 2: Macroconidia (a) and chlamydospore (b) of the Fusarium isolate, SF4

GROWTH AND IAA PRODUCTION: Growth of the *Fusarium* soil isolates was also satisfactory in PD broth after 14 days of incubations. Interestingly, in comparison to control, mycelial dry weights of all the isolates increased considerably with successive increase of tryptophan concentrations (Table 2). All the *Fusarium* soil isolates were found to possess IAA producing ability to a varied extent. All the isolates could produce IAA in minute amount (25-50µg/ml) in the absence of tryptophan except *Fusarium* SF2 which produced high amount of IAA (150µg/ml) even in the absence of precursor molecule (Table 3). Significantly, with the increase in tryptophan concentrations, all the *Fusarium* isolates were found to produce IAA in larger amount. In presence of tryptophan (2000 ppm), SF2 and SF4 produced high amount of IAA (350 µg/ml each) and SF3, SF5 and SF6 produced comparatively less IAA (275, 225 and 200 µg/ml, respectively). *Fusarium oxysporum* isolated from rhizosphere and rhizoplane of melochia (*Corchorus olitorius*), sesame (*Sesamum indicum*) and soyabean (*Glycine max*) produced IAA (100-140 µg/ml) when grown in 1% peptone and 1% glucose-Czapek's medium at 28°C for 7 days (Hasan, 2002). Since the *Fusarium* isolates SF2-6 produced IAA both in presence and absence of tryptophan, tryptophan-dependant as well as tryptophan –independent pathways of IAA biosynthesis may exist in these isolates as in *Azospirillum* (Costacurta and Vanderleyden, 1995) and *Anabaena* (Prasanna et al., 2010).

Isolate no.	Mycelial dry weight (g)			
	Control	Tryptophan concentration		
		1000 ppm	1500 ppm	2000 ppm
SF-2	0.102	0.140	0.157	0.177
SF-3	0.109	0.121	0.141	0.188
SF-4	0.109	0.110	0.124	0.148
SF-5	0.147	0.173	0.178	0.180
SF-6	0.135	0.143	0.149	0.157

Table 2: Mycelial dry weight of the Fusarium soil isolates at different tryptophan concentrations

Isolate no.	IAA production (µg/ml)			
	Control	Tryptophan concentration		
		1000 ppm	1500 ppm	2000 ppm
SF-2	150	175	250	350
SF-3	50	125	175	275
SF-4	50	175	325	350
SF-5	25	125	150	225
SF-6	25	150	175	200

Table 3: IAA production of the Fusarium soil isolates at different tryptophan concentrations

EFFECTS OF CULTURE FILTRATES ON SEED GERMINATION AND ROOT-SHOOT LENGTH: The culture filtrates of the *Fusarium* soil isolates were found to be stimulatory on the germination of both gram and cucumber seeds. Percentage of seed germination remained steady or increased to a certain extent in both the seeds as compared to control. In control, the percentages of germination for gram and cucumber seeds were 80 and 70, respectively. But, cent percent germination was seen in gram seeds treated with each of the culture filtrates of *Fusarium* isolates SF2-4, whereas percentage of germination for cucumber seed was found 75 and 80, respectively treated with culture filtrates of *Fusarium* SF2-4 (Table 4). Root and shoot lengths of the seeds were also found to improve largely on account of growth promoters (IAA) in *Fusarium* culture filtrates. There was significant increase in root lengths of both gram and cucumber seeds when inoculated in culture filtrates of *Fusarium* SF2-4 in comparison to control. In control, root lengths was 23mm for gram seeds but near 1.5 fold increases in the root length was observed in gram seeds when plunged in culture filtrates of *Fusarium* SF2-4 with root lengths of 34mm, 32mm and 36mm respectively. Root lengths of cucumber seeds treated with *Fusarium* SF2-4 culture filtrates were found 102mm, 100mm and 101mm respectively in compare to control with root length (91mm). Likewise, shoot lengths were also increased to a very reasonable extent in both gram and cucumber seeds when matched with control. In control, shoot lengths were 6mm and 80mm for gram and cucumber seeds respectively, but highest shoot lengths with 10mm and 110mm were noticed in gram and cucumber seeds respectively treated with culture filtrate of *Fusarium* SF4. *Fusarium* SF2 was also found to increase shoot lengths of both gram and cucumber seeds to a satisfactory extent with a length of 9mm and 108mm respectively. Seed viability was also enhanced for the treatment of culture filtrates, SF2 and SF4 (Fig.3). This result may help to explain the beneficial effects of fungi to host plants and the role of microbial synthesis of the auxin to

improve crop productivity as well as soil fertility as reported earlier (Asghar *et al.* 2002; Patten and Glick, 2002).

Isolate no.	Gram				Cucumber			
	% of germination	Root length (mm)	Shoot length (mm)	VigourIndex (mm)	% of germination	Root length (mm)	Shoot length (mm)	VigourIndex (mm)
Control	80	23	06	23.2	70	91	80	119.7
SF-2	100	34	09	43	75	102	108	157.5
SF-3	100	32	06	38	70	100	86	130.2
SF-4	100	36	10	46	80	101	110	168.8
SF-5	80	27	07	27.2	70	92	84	123.2
SF-6	80	24	06	24	70	90	82	120.4

Table 4: Effects of Fusarium culture filtrates on seed germination and root-shoot lengths of gram and cucumber seedlings

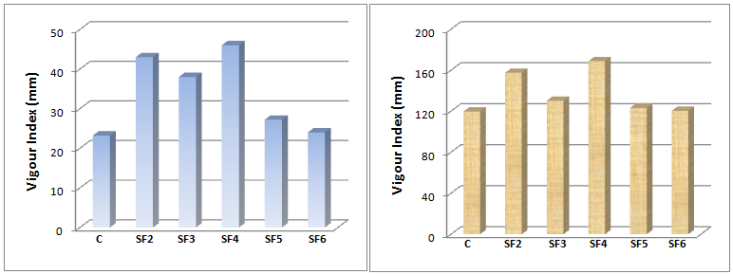

Figure 3: Effect of culture filtrates on viability of seeds of gram (left) and cucumber (right) after overnight imbibitions

IV. CONCLUSION

From our study, it can be concluded that with the increase in tryptophan concentration, both mycelial growth and IAA production increased considerably in all the five *Fusarium* soil isolates. Thus, there is a significant correlation between growth and IAA production of the *Fusarium* spp. isolated from an agricultural soil of Murshidabad district in West Bengal, India. Higher tryptophan concentrations may be attributed to higher mycelial biomass of the soil isolates and hence higher IAA productions. This is clearly evident in the increased germination ability of gram and cucumber seeds with improved root and shoots lengths. These IAA producing trait make the *Fusarium* soil isolates potential candidates for their ability to augment plant growth in agricultural fields.

V. ACKNOWLEDGEMENTS

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