J. Mycopathol. Res. 38(2) : 101–104 (2000) Printed in India

Gibberellic acid production by *Fusarium moniliforme*

M. SURULIRAJAN AND A. K. SARBHOY
Division of Plant Pathology, IAIR, Pusa, New Delhi 700 012

Four isolates of *Fusarium moniliforme* were isolated from various paddy field soils at IARI, New Delhi and Tamil Nadu. These isolates were designated as I, II, III and IV. The crude extract of gibberellin was extracted by appropriate procedure from these isolates. The extracts were tested on rice seedling growth and also compared with standard GA_3 through thin layer chromatography analysis. The isolate III showed 53.1% of rice seedling elongation over the control and the calculated R_f value was 0.42. In the persent investigation it was concluded that, isolate III was the potent producer of gibberellin.

Key word : *Fusarium moniliforme,* gibberellic acid

INTRODUCTION

The fungus *Fusarium moniliforme* was described by Sheldon in 1904. It is abundantly present in cultivated soils and most frequently isolated by Plant Pathologists from different ecosysterms and agroclimatic zones of India. Yabuta and Hayashi (1939) has isolated crystalline form of gibberellin from *Gibberella fujikuroi.* Among the 90 GAs known so far, GA_3 is considered to be the most important and has received the maximum attention because of it's variety of beneficial effects like internode elongation, overcoming of dwarfism and elimination of dormancy etc. Earlier experiments confirmed that *F. moniliforme* (*G. fujikuroi*) is the most potent fungi leading to the discovery of growth promoting substances and production of gibberellins. However, substantial work has been done in the study of biosynthesis of gibberellins using *G. fujikuroi.* Later on, Sanchez-Marroquin (1963) tested about 43 strains of *Fusarium* species and reported the gibberellic acid production level in *F. moniliforme.* In India, Thakur and Vyas (1983) screened several isolates of *Fusarium* and tested their growth promoting activity by qualitative and quantitative analysis. Recently, Qian *et al.* (1994) cultivated the fungus *F. moniliforme* under the solid state fermentation condition for the production of GA_3. The present study was undertaken to select the most potent strain for the production of crude GA_3. A high degree of uncertainty still clouds about the potential strains of *G. fujikuroi* for the production of gibberellic acid. The quantitative assessment is needed for gibberellic acid from different strains. Therefore keeping in view the relevance of gibberellic acid production from *Fusarium* species and an attempt has been made to screen the most potent strains of *F. moniliforme* for the production of crude gibberellin, which can be used for various metabolic activities.

MATERIALS AND METHODS

Screening of fungal isolates

Four isolates of fungi were tested for their activity to produce plant growth regulatory metabolites in their culture filtrates. These test fungi were isolated from various soil samples refer in the Table 1. Each of the test fungi was purified by single spore/hyphal tip isolation method.

Table 1 : Isolation of *Fusarium moniliforme* on different media 7 days after incubation at 25°C

S. No.	Fungi isolated	Different methods of isolation					
		From IARI soil			From TN soil		
		A	B	C	A	B	C
a. PDA							
	F. moniliforme-I	+	–	+	–	–	–
	F. moniliforme-III	–	+	+	–	–	–
	*F. semitectum**	+	–	+	+	+	+
	*Aspergillus niger**	+	+	+	+	+	+
	Trichoderma spp.*	+	+	+	+	+	+
b. CDA							
	F. moniliforme-I	+	–	+	–	–	–
	F. moniliforme-IV	–	–	–	–	+	+
	*F. semitectum**	–	–	–	–	–	+
	*Aspergillus niger**	+	+	+	+	+	–
	Trichoderma spp.*	+	+	–	+	+	+
c. PPA							
	F. moniliforme-II	–	+	+	–	–	–
	F. moniliforme-IV	–	–	–	+	–	+
	*F. semitectum**	+	+	–	+	–	+
	*Aspergillus niger**	–	–	–	–	–	–
	Trichoderma spp.*	–	–	–	–	–	–

Note : (+) isolated ; (–) not isolated ; * discarded
A — Direct method B — Warcup method C — Serial dilution method
IARI — Indian Agricultural Research Institue, New Delhi.
TN — Tamil Nadu
PDA = Potato dextrose agar
CDA = Czapek's-Dox agar
PPA = Peptone-PCNB agar

Composition of screening medium for the gibberellin production

For the secretion of plant growth regulatory fungal metabolites, each isolate was grown in Richard's broth enriched with 20 mg tryptophan per liter. The test organism was inoculated aseptically with 8 mm disc of fungal culture from petridishes in 250 ml conical flask containing 50 ml of broth medium. Inoculated broth was kept for 10 days at 28°C in incubator shaker. Cell free culture filtrates were obtained by aseptically filtering the contents through bacteriological filters.

Rice seedling growth test

The culture filtrates of four isolates of *Fusarium* were collected for investigating their effect on elongation of second leaf of rice seedlings as per the guidelines set by Murakami (1970) on second leaf length of rice seedlings and Thakur and Vyas (1983) on oat coleoptile. For this purpose, dwarf rice variety was obtained from Genetics Division, IARI, New Delhi. The seeds were first surface sterilized with 0.2% $HgCl_2$ solution for 5 minutes and then washed 8 to 10 times with sterile water. The surface sterilized seeds were sown aseptically in 500 ml 'Borosil' conical flasks. Each flask contained 100 ml of 2% plain agar. Five ml of cell free culture filtrate of the test fungus was added after solidification of the sterilised agar medium in every flask. Experiments were done in triplicates. The control sets contained 5 ml of the uninoculated broth instead of fungal filtrate. The flasks were then incubated at 28±1°C for 7 days under fluorescent light.

Extraction of crude gibberellins (Thakur and Vyas, 1983)

The culture filtrate was acidified to pH 2.5 and extracted with ethyl acetate. The aqueous phase was re-extracted with 1% sodium hydrogen carbonate solution and again with ethyl acetate. The extract was evaporated at room temperature to dryness. Thin layer chromatography (TLC) was performed for the identification of extracted meterials. The samples were placed on silica gen G coated glass plates. Gibberellin like compounds were separated by using ethyl acetate-chloroform-acetic acid in the ratio of 15 : 5 : 1.. Spots were developed by spraying a mixture of concentrated sulphuric acid in 5% ethanol for the location of gibberellin. Through thin layer chromatography analysis, gibberellin like compounds were qualitatively analysed with the help of standard GA_3. Finally R_f values were calculated.

RESULTS AND DISCUSSION

Gibberellin like substances are elaborated from culture filtrates which are able to promote plant growth markedly. The filtrate was tested for growth promoting substance. Isolates III was found to produce maximum growth promoting effect on rice second leaf elongation followed by isolate IV. Data were recorded and presented in the form of percentage in Table 2. On the other hand culture

Table 2 : Effect of culture filtrate of *Fusarium moniliforme* on the second leaf length of rice seedlings

Fungi tested	Mean length (cm)	Elongation over control (cm)	Percentage in growth
Isolate - I	2.3	–0.9	–28.1
Isolate - II	3.4	0.2	6.25
Isolate - III	4.9	1.7	53.1
Isolate - IV	3.5	0.3	9.37
Control	3.2		

Table 3 : Chromatographic analysis of gibberellin like substances secreted by four isolates of *Fusarium moniliforme*

Fungi tested	R_f value	Gibberelline positive Samples
Standard GA_3	0.46	+
Isolate - I	–	–
Isolate - II	–	–
Isolate - III	0.42	+
Isolate - IV	0.39	+

(+) present
(–) absent

filtrates of isolate I induced growth inhibiting responses. Culture filtrate form isolate II showed minimum growth promoting effects. Chromatographic analysis of culture filtratres of *F. moniliforme* isolate III and IV revealed the presence of crude gibberellic acid. Fluorescent spots were detected corresponding to standard GA_3 (R_f value 0.46) in the culture filtrate of isolate III (R_f value 0.42). These results confirmed that the gibberellin are elaborated by isolate III and IV (Table 3). Thakur and Vyas (1983) performed the same experiments for pea seedlings but they used Czapek's as basal medium. The rice second leaf length elongation was measured after seven days of incubation. The present investigation revealed that the presence of gibberellin showed growth promoting effects on rice seedlings in their second leaf growth. These results are in accordance with Murakami (1970) and Thakur and Vyas (1983). Isolate III recorded the maximum increase in leaf length which might be due to the ability of the culture to excrete more GA_3. Based on rice seedling

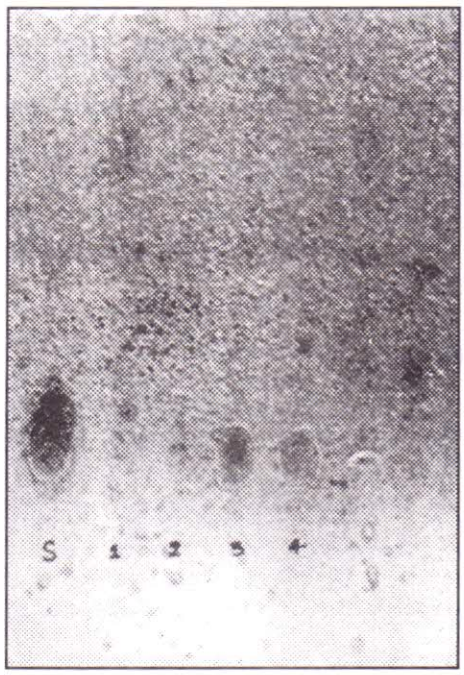

Fig. 1 : Thin layer chromatography analysis of gibberellin like substances secreted by four isolates of *Fusarium moniliforme* in Richard's medium.
S : Standard GA_3 ; 1 : Isolate – I ; 2 : Isolate – II ; 3 : Isolate – III ; 4 : Isolate – IV ;

growth test and TLC analysis, the present studies clearly identified the potential isolate III for the production of gibberellin. The isolate III has accessioned vide No : 4916 in the Indian Type Culture Collection, New Delhi-12.

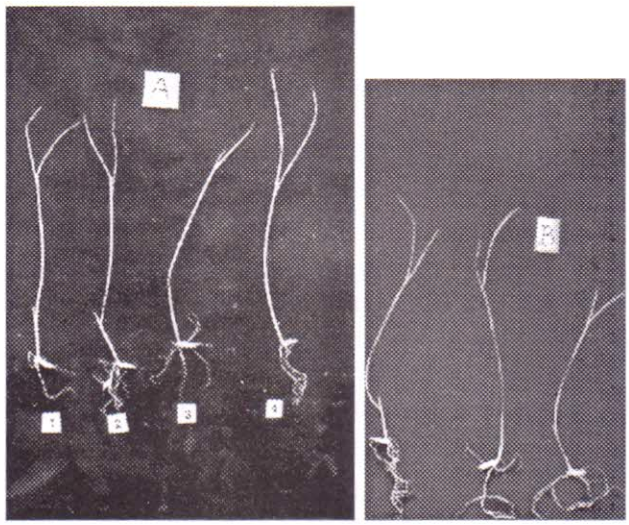

Fig. 2 : Effect of culture filtrate of *Fusarium moniliforme* on second leaf length of rice seedlings.
A : Treatment ; 1 : Isolate – I ; 2 : Isolate – II ; 3 : Isolate – IV ; 4 : Isolate – III ; B. Control.

ACKNOWLEDGEMENT

The authors are very grateful to Dr. J. P. Verma. FNA, FNASc., Head, Division of Plant Pathology, IARI New Delhi for prdoviding necessary facilities for this study and are thankful to Dr. D. K. Agarwal Senior Scientist and Mr. Shahid Research Accociate for their timely help during the course of present investigation.

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(Accepted for publication July 24 2000)