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Gibberellin formation by some species of *Fusarium*

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Twenty eight isolates of *Fusarium* species were selected from various parts of India. These isolates were designated as *F. moniliforme* (FM), *F. moniliforme* var. *subglutinans* (FMS), *F. solani* (F. So)., *F. semitectum* (FS) and *Gibberella fujikuroi* (GF). These isolates were designated as FM-1 to F. So. -28. Gibberellins were extracted by standard procedure from these isolates. The extract of six isolates contained growth stimulating factors while others showed in traces. On the other hand, *F. moniliforme* gave quite satisfactory results. Chromatographic analysis revealed the presence of GA3 in their culture filtrates. The isolate FM-10 showed increase in second leaf length and seed yield 49.7% and 24.17% respectively of rice seedlings of CMS line Pusa 3A against control.

Key words : Gibberellic acid, *Fusarium moniliforme*, Thin layer chromatography

INTRODUCTION

The gibberellins are one of the major groups of growth promoting hormones which play an essential role in regulation of growth and development of angiospermic plants. *Fusarium moniliforme* is a serious pathogen associated with bakane diseases of rice. It is abundantly present in cultivated soils and most frequently isolated from different ecosystems and agroclimatic zones of India. Yabuta and Hayashi (1939) had isolated crystalline form a gibberellin from *Gibberella fujikuroi*. Among the 90 gibberellins know so far, GA3 is considered to be the most important and has received the maximum attention because of the several physiological effect like internode elongation, overcoming of dwarfism and elimination of dormancy, etc. Earlier experiments confirmed that *F. moniliforme* is the most potent fungus leading to the discovery of growth promoting substances and production of gibberellins. Sanchez-Morroquin (1963) tested 43 strains of Fusaria and reported maximum production of gibberellic acid in *F. moniliforme*. Graebe and Ropors (1978) reported that GA3 regulates the rate of growth and expansion of internodes of plants. In India, Kumar and Lonsane (1989) screened several isolates of *Fusarium* and tested their growth promoting activity by qualitative and quantitative analysis. Quian *et al.* (1994) cultivated the fungus *F. moniliforme* under the solid state fermentation for the production of GA3. The present study was undertaken to select the most potent strain for the production of GA3 and its role in CMS line Pusa 3A dwarf variety in anthesis to increase its yield potential as compared with other cultivars.

MATERIALS AND METHODS

Screening of fungal strains

Twenty eight species of *Fusarium* were tested for their activity to produce plant growth regulatory metabolites in their culture filtrate. Above fungi were isolated from various sources and soil samples from different parts of India. These were purified by single spores/hyphal tip isolation method. Each isolate was grown in Richard's broth containing sodium nitrate 10 g, sucrose 20 g, potassium di-hydrogen phosphate 5 g, magnesium sulphate 2.5 g, ferric chloride 20 mg, tryptophane 20 mg and distilled water 1000 ml. The test organism was inoculated aseptically with 8 mm disc taken from actively growing colony on PDA medium. These were inoculated in 250 ml conical flasks containing 50 ml of Richards broth medium and incubated for 10 days at 25°C in incubator Kuhner shaker. Cell

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free culture filtrates were obtained by aseptically filtering the content through Whatman's filter paper No. 41.

Extraction of crude gibberellins

Table 1 : Different species of *Fusarium* with their sererces, location and ability to produce gibberellius.

Fungus	Source	Location	Response
FM-1	*Oryza sativa*	ITCC Strain	+
FM-2	"	"	++
FM-3	"	"	++
FM-4	"	"	+
FM-5	"	"	+
FM-6	IARI Rice field soil	New Delhi	+
FM-7	Cyst nematode suspension	Rajasthan	+
FM-8	Wheat kernels	Punjab	+
FM-9	"	"	++
FM-10	Rice field, IARI	New Delhi	+
FMS-11	*Zea mays*	"	++
FMS-12	"	"	+
FMS-13	*Capsicum annum*	"	+++
FMS-14	"	"	+
FMS-15	Wheat kernels	Punjab	+
GF-16	"	"	+
GF-17	"	"	+++
GF-18	"	"	++
FO-19	"	"	+
FO-20	Katrain vegetable farm	H.P.	–
FO-21	"	"	–
FS-22	Rice field of Entomology, IARI	N.D.	++
FS-23	Wheat kernels	Punjab	+
FS-24	"	"	+
FS-25	Madras soil	Tamil Nadu	+
FSo-26	Rose field	New Delhi	+
FSo-27	Sagar University	M.P.	–
FSo-28	Katrain vegetable farm	H.P.	–

Where : FM : *Fusarium moniliforme* ; FMS : *Fusarium moniliforme* var. *subglutinans* ; GF : *Gibberella fujikuroi* ; FS : *Fusarium solani* ; FSo : *Fusarium semitectum* ; +++ : Excelent ; ++ : Good ; + : Poor ; – : Nil

Free gibberellins were extracted from fungus culture filtrate as per techniques of Moore (1967). The culture filtrate was acidified to pH 2.5 by N/10 hydrochloric acid aseptically and extracted twice with equal volume of ethyl acetate. The ethyl acetate fraction was pooled and poured in a 500 ml round bottom flask. The flask was attached to a rotatory evaporator, at 40°C till it was evaporated to dryness. Dissolve the residue in 5 ml of distilled water containing Tween 20. It was later used for bioassay and chromatographic analysis. The residue was dissolved in 1-2 ml of ethanol with a sterilised pipette. Thin layer chromatography was used for identification of extracted materials. Gibberellins like compounds were separated with benzene-n-butanol-acetic acid (30 : 15 : 5). Commercial gibberellic acid (GA3) was used as a standard RF value for standard gibberellic acid (GA3) and extracted culture filtrates were calculated by the following formula.

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

For each sample two plates of silica gel were spotted. The plates were kept in the solvent reagent benzene-n butanol-acetic acid (30 : 15 : 5). Spots were developed by ethanol-conc. H_2SO_4 (95 : 5). The sprayed plates were then dried for 15 min. at 110°C in an electric oven. Through TLC, gibberellin like compounds were qualitatively analysed. Gibberellins fluoresce in the ultra violet light. Rf values were calculated as described above. The corresponding spots of unsprayed TLC plates were then eluted and used for bioassay.

Rice seedling growth test

The culture filtrates of three isolates of *Fusarium* were taken for investigating their effect on elongation of second leaf of rice seedlings as per the guidelines set by Murakami (1970) and Thakur and Vyas (1983) on oat coleoptiles. For this purpose, dwarf rice CMS line Pusa 3A was obtained from Genetics division of IARI, New Delhi. The seeds were first surface sterilised with 0.2% $HgCl_2$ solution for 5 min and then washed 8 to 10 times with sterile distilled water. The surface sterilized 10 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 sterilized agar medium in every flask. Experiments were conducted in triplicates. The control flask contained 5 ml of the uninoculated broth instead of fungal filtrate. The flasks were then incubated at 28 ± 1°C for 10 days under incubator.

RESULTS AND DISCUSSIONS

Twentyeight isolates of *Fusarium* collected from different sources and locations were grown in Richard's broth and tested for their ability to produce gibberellins like substances. The data are

given in Table 1 which revealed that 24 isolates produce gibberellin like substances. Of these FMS-13 and GF-17 produce maximum amount of gibberellins like substances.

Chromatographic analysis of culture filtrates of *F. moniliforme* isolate FM-9 and FM-10 revealed the presence of crude gibberellic acid. Fluorescent spots were detected (Fig. 2) corresponding to standard GA3 (Rf value 0.46) in the culture filtrate of isolate FM-10 (Rfvalue 0.46). These results confirmed that the gibberellins are elaborated by isolate FM-10 and FM-9 (Table 2).

Table 2 : Chromatographic analysis of gibberellin like substances secreted by six isolates of *F moniliforme.*

Fungi tested	Rf value	Gibberellin positive samples.
FM-2	—	—
FM-3	0.46	+
FM-5	0.46	+
FM-7	0.46	++
FM-9	0.46	++
FM-10	0.46	++
Standard GA3	0.46	+++

Gibberellin like substances are elaborated from culture filtrates of different isolates of *Fusarium moniliforme* which are able to promote plant growth markedly. The filtrate was tested for growth promoting substances. Isolate FM-10 was found to pruduce maximum growth promoting effect on rice second leaf elongation followed by isolate FM-9. (Table 3 and Fig. 1). On the other hand, culture filtrates of isolate FM-2 induce growth inhibitory responses. Culture filtrate from isolate FM-2 showed minimum growth promoting response. Thakur and Vyas (1983) performed the same experiments for oat seedlings but they used Czapek's as basal medium. The rice second leaf length elongation was measured after 10 days of incubation. The above investigation revealed that the presence of gibberellin showed growth promoting effects on rice seedling at their second stage of the leaf growth. These results are in accordance with Murakami (1970) and Thakur and Vyas (1983).Isolate FM-10 recorded the maximum increase in leaf length which might be due to the ability of the culture to produce more GA3. Based on rice seedling growth test and TLC analysis, in the present studies we have selected most potential isolate FM-10 for the production of GA3 amongst the 28 isolates of *Fusarium moniliforme*. This will be further exploited for the production of GA3 at pilot scale level.

Table 3 : Effect of culture filtrates of three isolates on the second leaf length of Pusa Basmati-5 rice seedlings.

Fungi tested	Mean length	Elongation over control	Percent increase in the second leaf sheath over control
FM-10	4.58	2.28	49.78
FM-9	3.76	1.46	38.82
FM-2	3.15	0.85	26.98
Standard GA3	4.70	2.40	51.06
Control	2.3	–	–

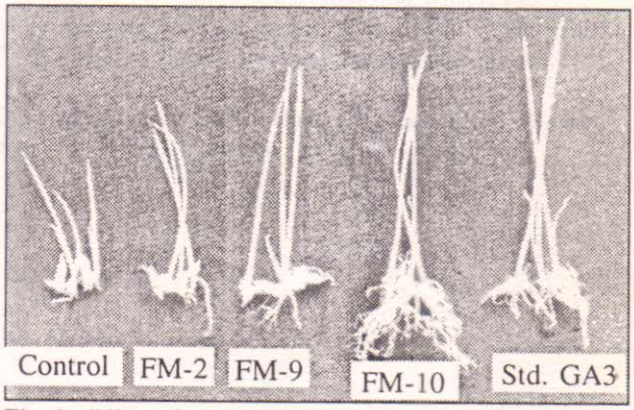

Fig. 1 : Effect of culture filtrate of three isolates on the second leaf length of CMS line of Pusa-5A rice seedlings.

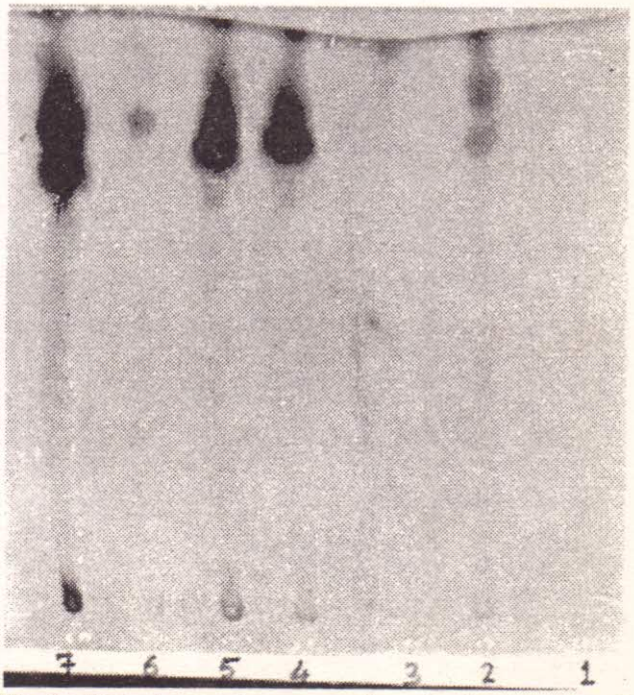

Fig. 2 : Thin layer chromatography of six isolates of *F. moniliforme* and standard GA3 (Sigma make)

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