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PATHOGENICITY AND OPHIOBOLIN-PRODUCING ABILITY OF DIFFERENT ISOLATES OF *HELMINTHOSPORIUM ORYZAE*

BY

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More than 50 isolates of *H. oryzae* were evaluated for correlation between pathogenicity and ophiobolin-producing ability. The virulent, but not the avirulent isolates showed toxicity of culture filtrates in seedling root growth bioassay. Quantitative estimation of the relative yield of ophiobolin by vitulent and avirulent isolates indicated that the isolate Kal-H 3 produced the highest titre of ophiobolin in the culture filtrates. The moderately virulent isolates Kal-H 19 and Cut-H produced relatively less amount of ophiobolin and the avirulent isolate AV-H produced low levels of ophiobolin in the culture filtrates. The data suggest a role of ophiobolin in disease.

INTRODUCTION

Helminthosporium oryzae Breda de Haan, the causal organism of brown spot of rice is known to produce ophiobolin in culture filtrates (Orsenigo, 1957). Roles of ophiobolin in the development of brown spot symptoms (Nakamura and Oku, 1960), permeability (Chattopadhyay and Samaddar, 1976) and other physiological changes (Chattopadhyay and Samaddar, 1980 a, b) comparable to infected tissues have been reported. In this paper we report correlation between pathogenicity and ophiobolin-producing ability of isolates of the pathogen.

MATERIALS AND METHODS

Seeds of rice *(Oryza sativa* L.*)* cultivar IR-8 were obtained from the Economic Botanist, Regional Rice Research Station, Chinsurah, West Bengal. Seedlings for bioassay and larger plants for inoculation experiments were grown following the methods of Chattopadhyay and Samaddar (1976).

Local isolates were obtained from naturally infected rice plants from different fields of the district Nadia. The authentic isolate Cut-H was obtained form the Director, Central Rice Research Institute, Orissa. The avirulent isolate AV-H was obtained from Dr. A. K. Sinha, Department of Plant Pathology, B. C K. V., West Bengal. The isolates were maintained on potato-dextrose agar (PDA) slopes at 20° C with monthly subculturing. Virulency of pathogenic isolates

was maintained by inoculating IR-8 rice plants in the greenhouse and re-isolating the pathogens at an interval of 3 months. All isolates produced abundant conidia when grown in PD broth at 28°C in the dark.

Two-week-old greenhouse grown potted plants were inoculated by spraying with conidial suspension (10^6/ml) using a glass atomizer. Plants after inoculating were kept in a humidity chamber at 25° C with 12hr. photoperiod of natural light conditions.

Ophiobolin was isolated from culture filtrates following the method of Orsenigo (1957). The crystalline preparation had an absorption maxima at 225nm. The purity of the preparation was confirmed by co-chromatography with authentic sample of ophiobolin supplied by Dr. L. Canonica, Unversity of Milan, Italy, following the method of Canonica et. al. (1966).

Toxicity of culture filtrates or ophiobolin preparations was determined by rice seedling root growth bioassay (Chattopadhyay and Samaddar, 1976).

RESULTS

Pathogenicity and toxin-producing ability of isolates. The pathogenicity and toxicity of the culture filtrates of 50 local isolates were compared with that of Cut-H and AV-H under laboratory conditions. Results (Table 1) indicated that AV-H produced a few chlorotic flecks on the leaves, whereas Cut-H and all local isolates were virulent and produced typical brown spot symptoms within 48 to 60 hours of inoculation. The isolate Kal-H3 and Kal-H19 appeared to be more virulent than the isolate Cut-H. The virulence of other local isolates was comparable to Cut-H (28/50), Kal-H19 (19/50) or Kal-H3 (3/50).

Production of toxin by Kal-H3, Kal-H19, Cut-H and AV-H was tested by bioassy of the 14-day-old culture filtrates using rice seedlings. Significant inhibition of seedling root growth occurred in 15 fold dilution of the culture filtrates of virulent isolates, but culture filtrates of AV-H showed little or no toxicity.

TABLE 1. Comparative pathogenicity and toxin-producing ability of isolates of *Helminthosprium oryzae*

Isolate	No. of spots per leaf	Mean dimension of spots	Toxicity of culture filtrates
Cut-H	8±4	3.5 mm x 2 mm	+
Kal-H 3	15±3	4 mm x 2 mm	++
Kal-H 19	10±2	4 mm x 2 mm	+
AV-H	1=2 minute flecks		—

a Inoculum density used was 1 x 10^4 conidia/ml in each case and the number of spot developed on the second leaf from the top were counted.

TABLE 2 Relaive yield of ophiobolin by virulent and avirulent isolates of *Helminthosporium oryzae*

Isolate	Nature	Ophiobolin yield (mg/l[a])
Kal-H 3	Virulent	15.5±3
Kal-H 19	Virulent	13.0±4
Cut-H	Moderately virulent	10.0±3
AV-H	Avirulent	1.5±1

a Ophiobolin was isolated and crystalised following the method of Orsenigo (1957).

To test the pathogenic stability of the isolates 20 monoconidial isolates of each of Kal-H3, Kal-H19, Cut-H and AV-H were isolated and examined for pathogenicity in the greenhouse and toxin-producing ability in culture. Isolates derived from Kal-H3, Kal-H19 and Cut-H showed virulency comparable to that of their respective parent strains. Isolates derived from AV-H were all non-pathogenic. All virulent isolates produced toxin in culture filtrates, but not AV—H.

Ophiobolin yield potential of the isolates. The relative quantitative yield of ophiobolin (mg/ml) of the culture filtrates was determined. Results (Table 2) showed that the ophiobolin-producing ability of the isolates was correlated with the degree of virulence of the isolates in general. The virulent isolate Kal-H3 produced 8 to 10 times more ophiobolin than AV-H.

DISCUSSION

Results obtained in this investigation indicated some degree of correlation between ophiobolin-producing ability and pathogenicity of isolates of *H. oryzae*. The isolate Cut-H and all local isolates were pathogenic to IR-8 plants and produced ophiobolin in culture. Monoconidial isolates obtained from the pathogenic isolates were pathogenic and ophiobolin producers, whereas monoconidial isolates from AV-H were nonpathogenic and produced little or no toxin in culture. The isolate Cut-H and Kal-H 19 were less virulent than Kal-H3 and produced comparatively less amount of ophiobolin in culture. This conclusion is based on quantitative estimation of the titre of ophiobolin.

It was of interest to note that the concentration of ophiobolin accumulated in the culture filtrates of pathogenic isolates was more than the minimum titre required for complete inhibition of root growth in rice seedling bioassay. Ophiobolin at a concentration of 10 μg/ml is known to cause necrotic spots on leaves (Nakamura and Oku, 1960) and inhibition of roots in seedling assay

(Chattopadhyay. 1976). Accumulation of considerable amount of ophiobolin in diseased tissue (Oku. 1967) and biosynthesis of ophiobolin by pathogenic isolates of *H. oryzae* (Canonica et.al., 1967) have been reported.

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