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Vegetative compatibility and self-incompatibility within isolates of *Fusarium udum* Butl.

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Six isolates of *Fusarium udum* Butl.obtained from different parts of India were studied for vegetative compatibility by using nitrate non-utilizing (*nit*) mutants as a preliminary tests towards understanding the relationship among isolates within the species. *Nit* mutants were recovered from isolates cultured on potato dextrose agar containing 1..5%$KClO_3$(PDC) and vegetative compatibility was assessed through the complementation test by pairing *nit* mutants on Czapek·s minimal medium (MM) containing $NaNO_3$ as the sole nitrogen source. Mutants those not forming heterokaryon among each other were considered to be self-incompatible. Among six isolates five produced *nit* mutants and categorised into three groups on the basis of pairing pattern. Three isolates were considered within the same vegetative compatibility group (VCG) and remainings were vegetatively self-incompatible or with intermediate reaction as demonstrated in a limited scale here.

Key Words : Pigeonpea, anastomosis,vascular wilt pathogen

INTRODUCTION

Yellow disease of pigeonpea [*Cajanus cajan* (L.) Millsp.]caused by the vascular wilt pathogen *Fusarium udum* Butl. is a major production constraint in pigeonpea growing region of India particularly in Bihar, Uttar Pradesh and some parts of West Bengal (Murshidabad and Malda districts.). Strains of *Fusarium udum* are often highly host specific. Variability among the isolates of *F. udum* in respect of their cultural and pathogenic reaction against host cultivars and the existence of physiological races within the species have been reported by several workers (Reddy and Basu Chaudhary, 1985,Rajendra and Patil, 1992 ; 1993;Shit and Sen Gupta, 1980; Das and Sen Gupta ,1998.) But there is no standard criterion or a set of differential host cultivar for separating *F. udum* isolates into definite groups based on their cultural and pathogenic characters. Besides, these pathogenicity and virulence tests are difficult, time consuming and often inconclusive. As a result , alternate methods to expedite the identification of pathogenic isolates those cause vascular wilt disease would be advantageous.

Recently, grouping of *Fusarium* spp. on the basis of vegetative compatibility (heterokaryon formation)by nitrate non-utilizing *(nit)* mutants have been done by several workers (Puhalla, 1985; Correll *et al.,* 1986; 1987; Elias and Schneider, 1986; Jacobsons and Gordon, 1988; 1990). All isolates which are vegetatively compatible are said to belong to the same vegetative compatibility group (VCG). Vegetative compatibility which is the result of hyphal fusion (anastomosis) between two fungal strain, has been shown to be under the control of many gene loci (Anagnostakis, 1997; Puhalla and Spieth, 1983). Only strains with the same allele at each of these loci are compatible. This suggests that strains within a VCG are genetically similar. There is no such report of work on vegetative compatibility of *Fusarium* spp. particularly *F. udum* from India.

This study reports a preliminary work on vegetative compatibility groups (VCG) within *F. udum* as a first step towards understanding the relationship among

MATERIALS AND METHODS

Vegetative compatibility group (VCG) were determined through the complementation of nitrate non-utilizing *(nit)* mutants as a visual indicator of heterokaryon formation (Puhalla, 1985).

Six strains of *F. udum* Butl. (F_1 - F_6) isolated from wilt affeeted pigeonpea stem pieces obtained from different parts of pigeonpea growing areas in India were used (Das and Sen Gupta, 1998).

Potato dextrose agar containing 1.5% $KClO_3$ (PDC) was used initially to generate *nit* mutants (Correll *et. al.*, 1986). The concentration of $KClO_3$ increased to 3-5% when needed for isolates that were not restricted with 1.5% $KClO_3$. A mycelial transfer (6 mm disc) of each isolate was put in the centre of a petridish (10 cm in diameter) containing PDC. The plates were incubated at 28°C and examined periodically for the appearance of fast-growing sectors from the initially restricted colony. Growth of wild-type strains is restricted on chlorate (Puhalla, 1985). Chlorate-resistant sectors were screened on Czapek's minimal medium (MM) containing sodium nitrate ($NaNO_3$) as the sole source and those that grew as thin expansive colonies with no aerial mycelium were considered *nit* mutants.

Vegetatively compatible *nit* mutants may complement one another by forming a heterokaryon on MM (Puhalla, 1985). *ie,* dense aerial mycelial growth develops where mycelia of the two thin *nit* mutant colonies come in contact, anastomose, and form a heterokaryon. Pairings were made by placing mycelia (6 mm diameter disc) from each *nit* mutants 1-3 cm apart on MM. Pairings were incubated at 28° C for 7-14 days and then scored for complementation. *Nit* mutants were generated from each isolate until two complementary *nit* mutants were found that formed a vigorous heterokaryon when paired on MM. These *nit* mutants were used as heterokaryon tester *nit* mutants for that isolate in subsequent inter-isolate pairings. When testers from two differents isolates successfully formed a heterokaryon they were placed in the same VCG. Certain isolates that were not vegetatively compatible within themselves or other isolates were not placed into a VCG (Correll *et. al.*, 1986 ; Puhalla, 1984).

RESULTS AND DISCUSSION

Of the total six test isolates (F_1 - F_6) studied, five yielded *nit* mutants, isolate F_4 did not produced *nit* mutants. After pairing among different *nit* mutants, the isolates were categorized into three groups on the basis of the pairing patterns (reaction tyes are elaborated below the table). Results based on the observations of three replications are presented in the Table 1.

Table 1. Reaction patterns of the pairings among different *nit* mutants.

Pairings of *nit* mutants	Reaction type *
nit 1x *nit* 2	1
nit 1x *nit* 3	2
nit 1x *nit* 5	0
nit 1x *nit* 6	2
nit 2x *nit* 3	0
nit 2x *nit* 5	1
nit 2x *nit* 6	0
nit 3x *nit* 5	0
nit 3x *nit* 6	2
nit 5x *nit* 6	2

* Reaction patterns : 0= no reaction, 1= little or no aerial mycelium and 2= abundant aerial mycelium at point of contact. In all cases isolate no. and *nit* mutant no. obtained from the isolate remain the same.

From the Table 1, it is evident that in four combinations namely nit 1 x nit 3, nit 1 x nit 6, nit 3 x nit 6, and nit 5 x nit 6 reaction patterns 2 were observed.In two combinations by nit 1xnit 2 and nit 2x nit 5 reaction pattern 1 were demonstrated while in other combinations reaction pattern 0 were observed. For this, isolate F_1, F_3 and F_6 may be considered within the same VCG, while the remainings were vegetatively self-incompatible or with intermediate reaction. Virulence has been, and undoubtedly will continue to be a very usefull trait for the characterization of diversity among different strains of *Fusarium.* Vegetative compatibility, however, is another usefull tool for indentifying diversity among strains of different species of *Fusarium.* Vegetative of heterokaryon incompatibility is wide spread in many fungi (Anagnostakis, 1982 ; Correll, 1986 ; Elmer and Stephens, 1986 ; Gordon *et al.*, 1986 ; Puhalla and Spieth, 1985) including *F. udum* as demonstrated in a

limited scale of studies here. Further detailed studies on this aspect are necessary.

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