Tropical Agricultural Research and Extension 1 (1): 39-43, 1998.

Classification of *Fusarium oxysporum* strains associated with reddish-brown rot of asparagus on the basis of vegetative compatibility

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Accepted 11 June 1998

ABSTRACT

Nitrate reduction mutants (*nit*-mutants) were selected from 33 isolates of *Fusarium oxysporum* isolated from asparagus plantings showing reddish brown rot symptoms in Malaysia. These nitrate reduction mutants were used in complementation tests for vegetative compatibility. Nine vegetative compatibility groups (VCGs) were identified, but most (19) isolates fell into 4 VCGs that were found at more than one location. There was no strong interrelationship between VCG and location. However, seven isolates were identified as vegetatively self-incompatible based on their inability to form heterokaryons between complementary *nit*-mutants.

Keywords: Asparagus, *Fusarium oxysporum*, *nit*-mutants, vegetative compatibility groups.

INTRODUCTION

Asparagus (*Asparagus officinalis* L.), a newly introduced crop in Malaysia, was found to be susceptible to many indigenous pathogens. *Fusarium oxysporum* is one of several *Fusarium* spp. that causes reddish brown rot on asparagus. The disease is widespread and poses a serious threat to the asparagus plantations in many parts of Malaysia (Nik Norulaini and Salleh 1990; Salleh 1990).

Vegetative or heterokaryon compatibility has been widely documented among several plant-pathogenic fungi and has been proven to be a powerful tool in examining genetic diversity among their natural populations (Anagnostakis *et al.* 1986; Correll *et al.* 1987 ; Correll *et al.* 1986; Correll *et al.* 1988; Elmer and Stephens 1989; Jacobson and Gordon 1988; Larkin *et al.* 1988; Papa 1986; Ploetz and Correll 1988; Ploetz and Shokes 1986; Proffer and Hart 1988; Sidhu 1986). Fungal isolates that anastomose and form heterokaryons with one another are considered to be vegetatively compatible and are assigned to a single vegetative compatible group (VCG). Conversely, isolates that are incapable of anastomosing with one another and therefore failed to estabilish heterokaryons, are referred to as vegetatively incompatible.

Vegetative compatibility is mediated by multiple vegetative incompatibility loci (*vic*- or *het*- loci) in many fungi (Anagnostakis 1982, 1987; Brasier 1983; Croft and Jinks 1977; Leach and Yoder 1983; Perkins *et al.* 1982; Puhalla and Spieth 1983; 1985). In most cases, vegetative compitibility is homogenic; that is, two fungal isolates are vegetatively compatible only if the alleles at each of their corresponding *vic* loci are identical. In asexually reproducing fungi, vegetatively compatible isolates are much more likely to be genetically similar than vegetatively incompatible isolates.

Auxotrophic nitrate reduction (*nit*) mutants were first used by Puhalla (1985) to estabilish vegetative compatibility system in *F. oxysporum*. *Nit*-mutants can be readily recovered from resistant (fast growing) sectors on a chlorate medium, a method that has been extensively employed in several fungi (Correll *et al.*, 1988; Correll *et al.* 1987; Correll *et al.* 1986; Cove 1976; Elmer and Stephens 1989; Hooley and Shaw 1987; Klittich and Leslie 1988; Newton and Caten 1988; Papa 1986; Sidhu 1986).These mutants are usually recovered at high frequencies, and are easily generated without mutagen and subsequently grown on unsupplemented media. This technique has been used widely in vegetative compatibility studies with many fungal species (Bosland and Williams 1987; Correll *et al.* 1988; Correll *et al.* 1987; Jacobson and Gordon 1988; Katan and Katan 1988; Klittich and Leslie 1988; Larkin *et al.* 1988).

The objectives of this study were to examine populations of *F. oxysporum* isolated from asparagus showing reddish brown rot symptoms in fields of different areas throughout Malaysia for their VCG composition and to investigate the correlation between VCG and location.

Abbreviations: *nit*-mutants - nitrate reduction mutants, MM - minimal medium, MMC - minimal medium amended with 1.5% $KClO_3$, PSA - potato sucrose agar, VCGs - vegetative compatibility groups.

MATERIALS AND METHODS

Isolates and media

The 33 isolates of *F. oxysporum* used in the current study, including their host and geographic origin, are listed in Table 1. Single germinating conidium was isolated on water agar and transferred to potato sucrose agar (PSA). PSA was prepared as described by Booth (1971). All cultures were incubated as described by Salleh and Sulaiman (1984). Minimal medium (MM) (Puhalla and Spieth 1983) contains nitrate as the sole source of nitrogen and was used for complementation tests. Mutants were generated on MM amended with 1.5% KClO, (MMC) as described by Correll *et al.* (1987).

Pathogenicity test

Each isolate was tested for pathogenicity on greenhouse by inoculating them on asparagus (cv. MW 500). Three-month-old healthy plants in 15 cm diameter polythene bags, containing clay soil, cowdung and compost in the proportion of 3:2:1 (v/v) respectively, were used. Stems of healthy plants at 10 cm above the soil surface were injured by plunging a sterile needle (0.05 cm diameter) to a depth of 2 mm after surface sterilization by swabbing with cotten wool moistened with 95% ethyl alcohol. Mycelial mat from 7-day-old MM test cultures were then placed on the injured sites and covered with damp sterilised cotton swabs and sealed with adhesive tape. MM blocks placed on injured stems of healthy plant sealed with wet sterilised cotton swabs and covered with adhesive tape were used as controls. The pathogenicity test for each isolate was replicated on five plants. Plants were incubated in the greenhouse with day and night temperature 25-30 °C and 18-25 °C respectively. The plants were fertilised once a week with a low dose (2 g) of compound fertilizer (N:P:K, 15:15:15). Fourteen days after inoculation, plants were observed qualitatively for symptoms and the test pathogens were reisolated from the inoculated plants following the method of Salleh and Sulaiman (1984).

Generation of *nit*-mutants

A mycelial transfer (2 mm^3 PSA block) of each isolate was put in the center of a petridish containing MMC. The plates were incubated for 14 days under conditions described above. After 5-14 days, rapidly expanding sectors that grew away from the restricted growth were transferred to MM, and those that grew as thin expansive colonies with no aerial mycelium were considered *nit*-mutants.

***Nit*-mutant phenotyping**

The physiological phenotypes of *nit*-mutants recovered were later distinguished by their growth on MM amended with different compounds as the sole nitrogen source (Correll *et al.* 1987; Sapumohotti and Salleh 1993). The plates were incubated as described above.

Vegetative compatibility tests

Isolates were placed in VCGs by complementation tests for heterokaryon formation using *nit*-mutants (Correll *et al.* 1987; Klittich and Leslie 1988). The formation of heterokaryons was recognised as a line of dense aerial mycelium where two complementary *nit*-mutants grow together on MM.

Three complementary *nit*-mutants coming from each phenotypic class were recovered from each isolate. These *nit*-mutants were used as heterokaryon tester for that isolate in subsequent inter-isolate pairings. Pairings were made by placing mycelia from each *nit*-mutant 1-3 cm apart on MM and incubated as described above for 7-14 days, and then scored for complementation. When testers from two different isolates successfully formed a heterokaryon they were placed in the same VCG. *Nit*-mutants recovered from the heterokaryon self-incompatible isolates (Table 1) were tested for complementation more rigorously. At least 450 *nit*-mutants from each self-incompatible isolate (mutants from each phentypic class were available) were recovered and intra-isolate pairings were made in all combinations.

To preclude the possibility that paired isolates were not anastomosing but simply cross-feeding extracellularly, pairings between *nit*-mutants yielding prototrophic growth at the line of mycelial contact were retested by interposing a strip of sterilised cellophane between the two complementary mutants. Prototrophic growth occuring between complementary mutants separated by cellophane would be indicative of cross-feeding, as the hyphae could not make physical contact to anastomose.

RESULTS AND DISCUSSION

All isolates of *F. oxysporum* tested were found to be pathogenic to asparagus (cv. MW 500) causing reddish brown rot symptoms which were similar but milder to those observed in the field.

Out of 33 isolates of *F. oxysporum* collected and used in complementation tests, 26 isolates fell into 9 VCGs indicating its genetic diversity (Table 1). Among them three VCGs were represented by a single isolate. Although this isolate in each VCG produced complementary *nit*-mutants, it failed to pair with any other isolates. Each VCG was unique in the sense that member of a group were not compatible with members of any other groups. In no case was an isolate found to belong to more than one VCG.

In the present study, isolate within the same VCG were not necessarily restricted to the same region. These results were similar to those reported in *Aspergillus flavus* (Papa 1986) and *F. proliferatum* (Elmer 1991). Most of the isolates obtained from different sites in the same location (Lorong Pandan) in Melaka were in VCG FOA1012USM (Table 1).

In this study, incompatibility between some isolates in the same VCG were observed. That is, two isolates might be vegitatively incompatible even though each one is compatible with a third isolate. This has been observed previously in *F. oxysporum*

(Gordon and Okamoto 1991) and in *Cryphonectria parasitica* (Kuhlman and Bhattacharyya 1984).

Although intra-isolate pairings were made between *nit*-mutants recovered from seven isolates of *F .oxysporum*, no complementation occurred. Therefore, these isolates were designated as heterokaryon self-incompatible (Table 1). This phenomenon had been reported previously for *F. oxysporum* (Correll *et al.* 1987; Jacobson and Gordon 1988) and *F. moniliforme* (Klittich and Leslie 1988) and a few other fungal species (Brooker *et al.* 1991; Correll *et al.* 1988; Hyakumachi and Ui 1987). The mechanism of vegetative self-incompatibility is unknown. However, Correll *et al.* (1989) showed that it is a heritable trait in *Giberella fujikuroi*, the teleomorph of *F. Moniliforme*.

Instability of two isolates to form heterokaryons does not necessarily mean that they are unrelated genetically; in other fungi a single gene difference can prevent heterokaryosis (Anagnostakis 1977). Due to heterokaryon incompatibility, therefore, parasexuality is obviously not important in genetic recombination between VCGs.

Elmer and Stephans (1989) reported that a single nonpathogenic strain of *F. oxysporum* isolated from the same asparagus field was sharing the same VCG with *F. oxysporum* f. sp. *asparagi*, a pathogen causing crown and root rot of asparagus in the USA. Additional studies are needed to confirm this possibility with the isolates of *F. oxysporum* causing reddish brown rot on asparagus in Malaysia. Such nonpathogenic strains may be so closely related to virulant strains that they may be useful in biological control by competing with virulent strains in the root and rhizosphere.

Armstrong and Armstrong (1969) first described the virulence of *F. oxysporum* f. sp. *apii* race 1 isolate on asparagus crowns and stated that common genes for pathogenicity probably exist in many forme speciales. Because, in the present study, the reciprocal pathogenicity tests of *F. oxysporum* strains on other crops were not done, it is not known if these strains could infect multiple hosts.

The importance of vegetative self-incompatibility in nature is unclear, but its presence and frequency should be taken into account in description of VCGs within the species. If heterokaryon self-incompatibility is widespread in *F. oxysporum*, then other measures of genetic diversity, such as isozyme patterns (Bosland and Williams 1987) and DNA restriction fragment length polymorphisms (Taylor 1986; Klister *et al.* 1987) could be employed to determine relationships among isolates.

ACKNOWLEDGEMENTS

The author is grateful to Professor Baharuddin Salleh, School of Biological Sciences, Universiti Sains Malaysia, Malaysia for his advice and constructive criticism throughout this investigation. This study was supported partly by the R and D Grant No. 123/ 3103/ 2104 of the Government of Malaysia.

Table 1. Source of *F. oxysporum* isolates and their vegetative compatibility groups

Strain	Source	Geographical area	VCG
P1690A	J 200	Sungai Ara, Penang	FOA1011USM
P1733A	MW 500	Sungai Ara, Penang	FOA1011USM
C1777A	MW 500	Cameron Highlands, Pahang	FOA1011USM
C1680A	MW 500	Cameron Highlands, Pahang	FOA1011USM
C1697A	MW 500	Cameron Highlands, Pahang	FOA1011USM
B1785A	Jainang	Klang, Selangor	FOA1011USM
P1663A	J 200	Sungai Ara, Penang	FOA1012USM
M1851A	MW 500	Lorong Pandan, Melaka	FOA1012USM
M1852A	MW 500	Lorong Pandan, Melaka	FOA1012USM
M1854A	UC 800	Lorong Pandan, Melaka	FOA1012USM
M1855A	MW500	Lorong Pandan	FOA1012UFM
S1857A	MW 500	Kundasang, Sabah	FOA1012USM
C1669A	MW 500	Cameron Highlands, Pahang	FOA1013USM
C1681A	MW 500	Cameron Highlands, Pahang	FOA1013USM
M1853A	UC 800	Lorong Pandan, Melaka	FOA1013USM
U1806A	MW 500	Birau, Brunei	FOA1013USM
C1676A	MW 500	Cameron Highlands, Pahang	FOA1014USM
B1771A	Debris	Klang, Selangor	FOA1014USM
B1791A	Debris	Klang, Selangor	FOA1014USM
B1754A	Soil	Klang, Selangor	FOA1015USM
B1790A	Soil	Klang, Selangor	FOA1015USM
P1533A	Soil	Sungai Ara, Penang	FOA1016USM
C1696A	Soil	Cameron Highlands, Pahang	FOA1016USM
M1796A	Soil	Lorong Pandan, Melaka	FOA1017USM
U1841A	MW 500	Birau, Brunei	FOA1018USM
U1843A	MW 500	Birau, Brunei	FOA1019USM
M1793A	Soil	Lorong Pandan, Melaka	FOA VCG?*
B1802A	Debris	Klang, Selangor	FOA VCG?*
B1805A	Debris	Klang, Selangor	FOA VCG?*
B1849A	Schwehinger Mersterschab	Klang, Selangor	FOA VCG?*
U1811A	MW 500	Birau, Brunei	FOA VCG?*
U1842A	MW 500	Birau, Brunei	FOA VCG?*
U1847A	MW 500	Birau, Brunei	FOA VCG?*

*These strains were heterokaryon self-incompatible.

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