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Genetic Diversity of *Fusarium fujikuroi* Isolated from Bakanae Disease of Rice on the Basis of Vegetative Compatibility

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ABSTRACT

Fusarium fujikuroi which was originally studied in Japan is a pathogen of bakanae disease of rice. The disease was recorded in almost all countries where rice is grown commercially, including Malaysia and Indonesia. A total of 79 strains of *F. fujikuroi* were isolated from rice plants showing typical bakanae symptoms from major granary areas in Malaysia and Indonesia. They were identified using morphological characteristics for species delimitation. The objective of this study was to investigate genetic diversity of the *F. fujikuroi* strains by generating nitrate non-utilizing (*nit*) mutants, followed by phenotyping on diagnostic media, and pairing the mutants on minimal media (MM). About 96.2% of the strains were identified as heterokaryon self-compatible (HSC) based on their ability to form a stable heterokaryon while the remaining 3.8% of the strains were classified as heterokaryon self-incompatible (HSI) based on their inability to form a heterokaryon, even after repeated attempts. Those HSC strains that paired by producing robust growth were classified in the same vegetative compatibility group (VCG). The bakanae strains of *F. fujikuroi* were grouped into 26 VCGs; the largest group was VCG A01 which comprised of 23 strains. Out of 26 VCGs, 12 VCGs contained more than one strain member, and 14 VCGs were represented by a single strain and were not compatible with other strains. The ratio of VCGs to strains of *F. fujikuroi* in these samples was 0.29. The strains of *F. fujikuroi* that caused bakanae disease of rice in Malaysia and Indonesia are genetically diverse based on their multiple VCGs.

Keywords: Bakanae disease, *Fusarium fujikuroi*, heterokaryon, vegetative compatibility group

INTRODUCTION

Vegetative compatibility (VC) also known as heterokaryon compatibility, is another useful tool for identifying fungi and it also reveals the genetic diversity of several fungal genera, including *Fusarium* (Leslie, 1996). VC happens when hyphae of two strains are anastomosed and fused to form a stable heterokaryon and those strains are therefore classified in the same vegetative compatibility group (VCG) (Leslie, 1993), while those that cannot form such heterokaryons are vegetatively incompatible and are therefore grouped in different VCGs. The strains are vegetatively compatible if they have the same allele at each and every incompatible locus. Frequently, the strains in the same VCG have similarity in genetic characteristics; the strains usually share more traits than strains in different VCGs (Leslie, 1993).

Stable heterokaryons in *Fusarium* were generated, first by inducing complementary nitrate non-utilizing (*nit*) mutants, which practically used to force heterokaryosis and subsequently to identify VCGs (Sidhu, 1986; Klittich *et al.*, 1986; Sunder and Satyavir, 1998). Several classes of *nit* mutants e.g. chlorate resistant nitrate utilizing (*crn*), *nit1, nit3* and NitM can be distinguished based on differential growth on phenotyping media containing different nitrogenous compounds as the sole

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source of nitrogen such as hypoxanthine (HX), ammonium tartrate (NH_4), natrium nitrate ($NaNO_3$) and natrium nitrite ($NaNO_2$) (Correll *et al.*, 1987). Heterokayon formation is a complex process that depends on more than just the capability of the strains to complement each other physiologically. Strains that are unable to form heterokaryon, including even between mutants derived from the same strain have been identified as heterokaryon self-incompatible (HSI), but if stable heterokaryons are formed, the strains are determined as heterokaryon self-compatible (HSC). The process is accomplished by pairing distinct *nit* mutants from different strains in order to classify the strains into VCG.

Several researchers have used VC test to determine the genetic diversity of *F. graminearum* (McCallum *et al.*, 2004), *F. moniliforme* (Puhalla and Spieth, 1985,; Klittich *et al.*, 1986; Sunder and Satyavir, 1998), *F. proliferatum* (Elmer, 1991; Elmer *et al.*, 1999), *F. subglutinans* (Zheng and Ploetz, 2002) and *F. verticillioides* (Chulze *et al.*, 2000). The objectives of this study were to investigate the genetic diversity of *F. fujikuroi* strains that were isolated from bakanae disease of rice and to classify the strains into VCGs.

MATERIALS AND METHODS

Fusarium Strains

A total of 79 strains of *F. fujikuroi* were isolated from bakanae-infected rice from major granary areas in Malaysia and Indonesia. All strains were purified through sub-culturing of single conidia and identified by using morphological characteristics following Burgess *et al.* (1994) before starting the *nit* mutant's generation.

Generation of Chlorate Resistant Sectors (CRSs) and Nit Mutants

Pure cultures of the strains were placed on a complete medium (CM; Correll *et al.*, 1987) for generation of actively growing colonies with a dense fungal growth. Preliminary studies on concentrations of chlorate in media i.e. minimal medium (MM) and potatoes dextrose agar (PDA) amended with 1.5%, 2.0%, 2.5% and 3.0% of $KClO_3$, hence designated as MMC and PDC respectively, were done earlier. The results indicated that 2.5% of $KClO_3$ concentration was selected as the optimum concentration for generation of CRSs. Plates of MMC and PDC containing 2.5% of $KClO_3$ were inoculated with 2 mm^2 mycelial discs taken from an actively growing colony and incubated under standard growth conditions (Salleh and Sulaiman, 1984). The colonies began to produce CRSs that appeared like sectors or fans after 7 days. The individual sectors from each colony was transferred to slant agar of MM containing $NaNO_3$ as the sole of nitrogen and incubated as above (Puhalla, 1985). Sectors that produced thin growth on MM were selected and considered to be *nit* mutants as they could not reduce nitrate present in the medium and subsequently kept at 4°C for phenotyping. Those that grew densely as wild type or reverted cultures were discarded.

Phenotyping of Nit Mutants

The physiological phenotyping of *nit* mutants were interpreted by their growth on MM that was modified by replacing $NaNO_3$ with $NaNO_2$ (0.5 gL^{-1}), hypoxanthine (0.2 gL^{-1}) or ammonium tartrate (1.0 gL^{-1}). The plates were incubated in complete darkness and colony growth was scored after 4 days of incubation. The colony growth of *nit* mutants on these nitrogen sources was recorded for identification of mutants as *nit1, nit3* or NitM following Klittich and Leslie (1988) (*Fig. 1*; Table 1). The mutants that grew vigorously on MM were either reverted, wild-type, mixed or *crn* and these cultures were discarded.

Pairing of Complementary Nit Mutants

Pairings of complementary *nit* mutants were made on MM following a procedure described by Leslie (1993). All inoculated plates were incubated at room temperature in complete darkness and the pairing results were recorded after 7 - 21 days. The strain was classified either HSC that formed heterokaryon (robust growth) or HSI when the *nit* mutants were unable to form heterokaryon (only thin growth at intersection of the colonies) at the line of contact between the mutants. Only *nit* mutants of HSC strains obtained from different parents were paired between each other. Two complementing strains were termed vegetatively compatible and designed as members of the same VCGs, whereas, strains that did not complement each other were termed vegetatively incompatible and assigned in different VCGs. VCGs of all *F. fujikuroi* strains were identified by pairing all possible combinations of *nit* mutants from each strain.

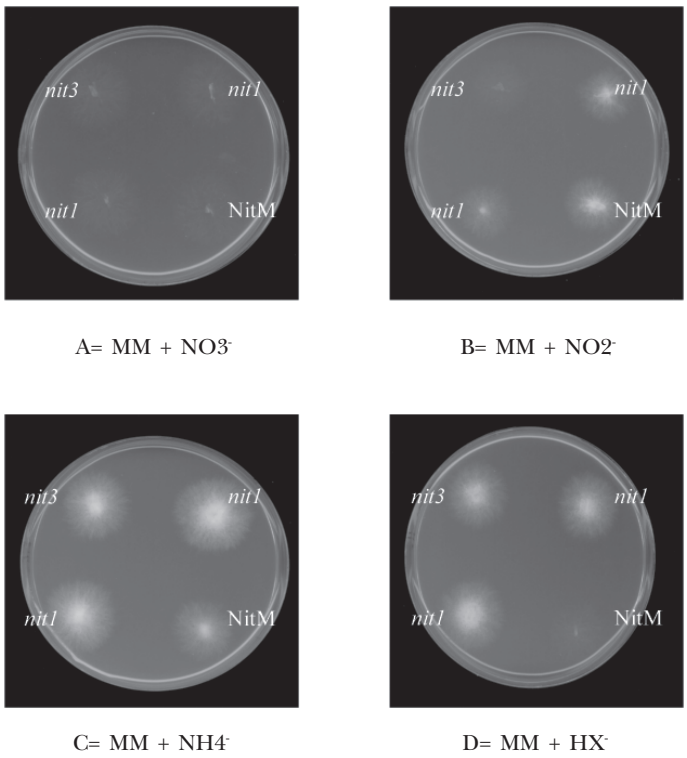


Fig. 1: Growth of three nitrate non-utilizing (nit) mutant phenotypes of F. fujikuroi *strain B3132P on media with one of four different nitrogen sources*

TABLE 1
Phenotyping of *nit* mutants based on colony growth on media with different nitrogen sources

Mutation[a]	Mutant type	Colony growth on MM with different nitrogen sources[b]			
		NO_3^-	NO_2^-	NH_4^-	HX^-
None	*crn*	+	+	+	+
Locus of nitrate reductase	*nit1*	-	+	+	+
Locus of pathway-specific regulator protein	*nit3*	-	-	+	+
Locus of molybdenum co-factor	NitM	-	+	+	-

[a]Carried out by Garrett and Amy (1978) and Marzluf (1981) based on analysis of mutants of *Aspergillus nidulans* and *Neurospora crassa*
[b]Colony growth on BM with different sources of nitrogen
+ = dense, fuzzy growth (wild-type); - = thin, transparent growth without aerial mycelium

RESULTS AND DISCUSSION

Generation of Chlorate-resistant Sectors (CRSs) and Nit Mutants

Spontaneous CRSs were recovered from all wild-type strains of *F. fujikuroi* when cultured on two media i.e. MMC and PDC containing chlorate as a toxic analogue of nitrate. Most of CRSs grew as thin expansive colonies with no aerial mycelium (*nit* mutants) because they were unable to utilize nitrate present in the media. The result revealed that 2.5% $KClO_3$ was the optimum concentration for *nit* mutant's generation. Majority of the *F. fujikuroi* strains and other species tested were insufficiently inhibited when lower or higher

concentrations of $KClO_3$ were applied. All the *nit* mutants had wild-type morphology on media containing an ammonium salt and produced thin sparse growth on nitrate medium. The *nit* mutants that produced wild-type growth on media containing both nitrite and hypoxanthine were classified as *nit1*; whereas, those with wild-type growth on medium with hypoxanthine or nitrite were identified as *nit3* or NitM, respectively.

Chlorate has been very practical for studying nitrate assimilation in *Fusarium* species (Correll *et al.*, 1986, 1987). Therefore, chlorate was used to induce CRSs for compatibility test. The number of CRSs obtained depended on the strains and amount of chlorate in the medium (Liu and Sundheim, 1996). Thus, the concentration of chlorate in the medium is an important variable. Therefore, a preliminary study on the effect of $KClO_3$ concentration on generation of *nit* mutants was conducted. Under normal growth condition, most fungi have the ability to utilize nitrate as a source of nitrogen by the internal reduction of chlorate into the ammonium form via nitrate and nitrite reductase (Garraway and Evan, 1984). However, the strains were unstable when grown on media containing chlorate and the colonies were unable to reduce chlorate to chlorite. This type of unstable growth on chlorate medium was previously observed in *Fusarium* spp. such as *F. oxysporum* (Correll *et al.*, 1987), *F. moniliforme* (Klittich and Leslie, 1988), *F. poae* (Lui and Sundheim, 1996) and *F. proliferatum* (Elmer *et al.*, 1999).

Nit mutants that have been generated are usually unable to reduce chlorate to chloride because of a lesion at one or more loci that control reductase, thus rendering them as a chlorate-resistance (Correll *et al.*, 1986). Previously, some researchers successfully recovered *nit* mutants from some *Fusarium* species associated with those in section Liseola i.e. *F. moniliforme* (Puhalla and Spieth, 1985; Klittich *et al.*, 1986; Sunder and Satyavir, 1998), *F. proliferatum* (Elmer, 1991; Elmer *et al.*, 1999), *F. subglutinans* (Zheng and Ploetz, 2002) and *F. verticillioides* (Chulze *et al.*, 2000). *Nit* mutants have also been recovered from other *Fusarium* species namely *F. poae* (Lui and Sundheim, 1996), *F. solani* (Hawthorne and George, 1996), *F. graminearum* (McCallum *et al.*, 2004) and *F. oxysporum* (Puhalla, 1984; Fernandez *et al.*, 1994; Vakalounakis and Fragkiadakis, 1999; Katan, 1999; Katan and Katan, 1999; Mes *et al.*, 1999) and other fungus, including *Colletotrichum acutatum* (Freeman *et al.*, 2000), *Neurospora crassa* (Marzluf, 1981), *Aspergillus flavus* (Papa, 1986) and *Verticillium albo-atrum* (Gordon *et al.*, 1986). In this study, complementary *nit* mutants recovered from each strain were categorized into one of the several phenotypic classes by their relative growth on phenotyping media containing different nitrogen sources. These classes of *nit* mutants presumably reflect mutations at a nitrate reductase structural locus (*nit1*); a nitrate assimilation pathway-specific regulatory locus (*nit3*), and the loci (at least five) that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity (NitM) (Klittich *et al.*, 1986). Some chlorate-resistant mutants, however, reverted to wild-type growth (dense growth); these mutants were classified as *crn* mutants.

Complementation Test

The complementation occurred more rapidly and subsequent growth of the resulting heterokaryon was more robust in the pairings between NitM and *nit1* or NitM and *nit3* mutants than those between *nit1* and *nit*3 mutants, which usually formed weak heterokaryons. Results from this study showed that seventy-six strains (96.2%) were classified as HSC (*Fig. 2A*; Table 2). Majority of the strains from the same location were classified in the same group of VCG; e.g. most strains from Kuala Selangor were grouped in VCG A01, all strains from Melaka were in VCG A07 and all Indonesian strains were in VCG A03 as well as VCG A05.

Complementation did not occur between phenotypically distinct *nit* mutants from three strains i.e. A3059P, B3111P and D0673P, where no heterokaryon was formed as an indicator. The lack of complementation between phenotypically distinct *nit* mutants recovered from those strains, even after repeated attempts lead to the designation of these strains as HSI *(Fig. 2B)*. This lack of complementation observed due to a different inability to anastomose among the strains. HSI have been observed in several strains of *Fusarium* species such as *F. moniliforme* (Sidhu, 1986; Correll *et al.*, 1987, 1989), *F. oxysporum* (Jacobson and Gordon, 1988; Vakalounakis and Fragkiadakis, 1999), *F. solani* (Hawthorne and George, 1996), *F. proliferatum* (Al-Amodi, 2006) and *F. graminearum* (McCallum *et al.*, 2004). HSI has also been observed in other

filamentous Ascomycetes including *Neurospora crassa* and *Podospora anserine* (Saupe, 2000) and *Aspergillus sp.* (Papa, 1986). Correll *et al.* (1987) suggested that HSI might be due to anomaly of the *nit* mutants themselves, in the lack of anastomosis. In addition, the lack of complementation has also been caused by a double mutation in some of the *nit* mutants (Papa, 1986). On the other hand, Correll *et al.* (1989) reported that low frequency of hyphal fusions per mm^2 on MM after they were paired may also caused HSI. HSI strains averaged 0.2 and 1.1 hyphal fusions per mm^2 compared with 6.9 and 8.1 fusions per mm^2 for HSC, which formed heterokaryon normally. Correll *et al.* (1989) also reported that when HSI strains branched less frequently, then there would be fewer anastomosed cell formation.

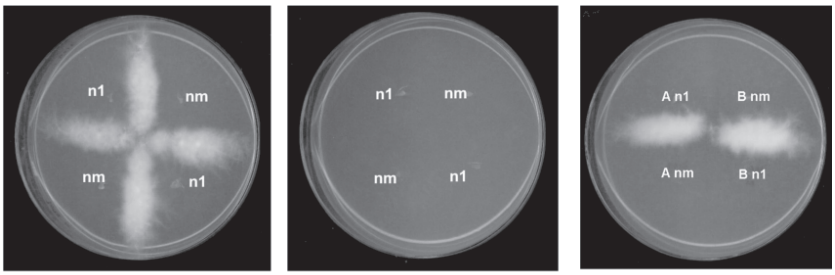
From the results, twenty-six distinct VCGs were identified among 76 strains of *F. fujikuroi* (Table 2), the ratio of VCGs to strains was 0.29. In comparison, Hsieh *et al.* (1977) reported 58 strains of *F. moniliforme,* belong to MP-C recovered from bakanae-infected rice were assigned to 22 VCGs (ratio; 0.37). In addition, Puhalla and Spieth (1985) recorded 12 VCGs of MP-C strains from China and Taiwan, whereas, Sunder and Satyavir (1998) recorded 10 VCGs for 28 strains with a ratio of 0.36. These phenomena took place, probably due to effects of the small numbers and sources of *Fusarium* strains. The HSC strains were classified in different VCGs when the mutants of the strains were unable to form heterokaryons during the compatibility test (*Fig. 2C*). The multiple VCGs of *F. fujikuroi* associated with bakanae disease of rice in Malaysia and Indonesia indicated the existence of substantial genetic diversity.

TABLE 2
Geographical location and VCGs of the 76 strains of *F. fujikuroi* isolated from rice in Malaysia and Indonesia, classified as HSC

VCGs	No.	Strain	Geographic locations	*Nit* mutants[1]		
				nit1	*nit3*	NitM
VCG A01	1	B2449P	Tanjong Karang, Selangor, Malaysia	+	-	+
	2	B2453P	Tanjong Karang, Selangor, Malaysia	+	-	+
	3	B3092P	Kuala Selangor, Selangor, Malaysia	+	-	+
	4	B3099P	Kuala Selangor, Selangor, Malaysia	+	+	+
	5	B3101P	Kuala Selangor, Selangor, Malaysia	+	+	-
	6	B3104P	Kuala Selangor, Selangor, Malaysia	+	+	-
	7	B3106P	Kuala Selangor, Selangor, Malaysia	+	+	+
	8	B3114P	Kuala Selangor, Selangor, Malaysia	+	+	-
	9	B3115P	Kuala Selangor, Selangor, Malaysia	+	+	+
	10	B3116P	Kuala Selangor, Selangor, Malaysia	+	+	-
	11	B3117P	Kuala Selangor, Selangor, Malaysia	+	+	-
	12	B3119P	Kuala Selangor, Selangor, Malaysia	+	+	+
	13	B3120P	Kuala Selangor, Selangor, Malaysia	+	-	+
	14	B3121P	Kuala Selangor, Selangor, Malaysia	+	+	+
	15	B3122P	Kuala Selangor, Selangor, Malaysia	+	-	+
	16	B3123P	Kuala Selangor, Selangor, Malaysia	+	-	+
	17	B3129P	Sungai Besar, Selangor, Malaysia	+	+	+
	18	B3131P	Sungai Besar, Selangor, Malaysia	+	-	+
	19	B3132P	Sungai Besar, Selangor, Malaysia	+	+	+
	20	B3133P	Sungai Besar, Selangor, Malaysia	+	+	-
	21	B3136P	Sungai Besar, Selangor, Malaysia	+	+	+
	22	B3139P	Sungai Besar, Selangor, Malaysia	+	+	-
	23	B3143P	Sungai Besar, Selangor, Malaysia	+	+	+
VCG A02	24	A3066P	Seberang Perak, Perak, Malaysia	+	+	+
	25	B3093P	Kuala Selangor, Selangor, Malaysia	+	+	+
	26	B3094P	Kuala Selangor, Selangor, Malaysia	+	-	+
	27	B3097P	Kuala Selangor, Selangor, Malaysia	+	-	+

Table 2 Cont.

	28	B3098P	Kuala Selangor, Selangor, Malaysia	+	-	+
	29	B3109P	Kuala Selangor, Selangor, Malaysia	+	+	+
	30	B3110P	Kuala Selangor, Selangor, Malaysia	+	-	+
	31	D3076P	Tumpat, Kelantan, Malaysia	+	+	+
VCG A03	32	I3206P	Padang, Sumatra, Indonesia	+	+	+
	33	I3209P	Padang, Sumatra, Indonesia	+	-	+
	34	I3211P	Padang, Sumatra, Indonesia	+	-	+
	35	I3213P	Padang, Sumatra, Indonesia	+	+	+
	36	I3215P	Padang, Sumatra, Indonesia	+	+	-
VCG A04	37	B3138P	Sungai Besar, Selangor, Malaysia	+	+	-
	38	B3140P	Sungai Besar, Selangor, Malaysia	+	+	+
	39	B3141P	Sungai Besar, Selangor, Malaysia	+	+	+
	40	B3142P	Sungai Besar, Selangor, Malaysia	+	-	+
VCG A05	41	I3207P	Padang, Sumatra, Indonesia	+	+	+
	42	I3208P	Padang, Sumatra, Indonesia	+	+	+
	43	I3214P	Padang, Sumatra, Indonesia	+	+	-
	44	I3216P	Padang, Sumatra, Indonesia	+	+	-
VCG A06	45	K0661P	Kampung Paya, Kedah, Malaysia	+	+	+
	45	K0686P	Kampung Paya, Kedah, Malaysia	+	+	+
	47	K3219P	Pendang, Kedah, Malaysia	+	+	+
	48	K3220P	Pendang, Kedah, Malaysia	+	+	-
VCG A07	49	M3234P	Merlimau, Melaka, Malaysia	+	-	+
	50	M3235P	Merlimau, Melaka, Malaysia	+	+	+
	51	M3236P	Merlimau, Melaka, Malaysia	+	+	+
	52	M3237P	Merlimau, Melaka, Malaysia	+	+	+
VCG A08	53	B3102P	Kuala Selangor, Selangor, Malaysia	+	+	+
	54	B3105P	Kuala Selangor, Selangor, Malaysia	+	-	+
VCG A09	55	B3127P	Sungai Besar, Selangor, Malaysia	+	+	+
	56	B3128P	Sungai Besar, Selangor, Malaysia	+	+	+
VCG A10	57	C3090P	Rompin, Pahang, Malaysia	+	+	+
	58	C3091P	Rompin, Pahang, Malaysia	+	+	+
VCG A11	59	P0654P	Seberang Perai, Penang, Malaysia	+	+	+
	60	P0655P	Seberang Perai, Penang, Malaysia	+	+	+
VCG A12	61	T3067P	Jabi, Terengganu, Malaysia	+	+	+
	62	T3068P	Jabi, Terengganu, Malaysia	+	+	+
VCG A13	63	A3052P	Seberang Perak, Perak, Malaysia	+	+	-
VCG A14	64	A3060P	Seberang Perak, Perak, Malaysia	+	-	+
VCG A15	65	B3100P	Kuala Selangor, Selangor, Malaysia	+	+	+
VCG A16	66	B3103P	Kuala Selangor, Selangor, Malaysia	+	+	+
VCG A17	67	B3107P	Kuala Selangor, Selangor, Malaysia	+	+	+
VCG A18	68	B3112P	Kuala Selangor, Selangor, Malaysia	+	-	+
VCG A19	69	B3113P	Kuala Selangor, Selangor, Malaysia	+	+	+
VCG A20	70	B3118P	Kuala Selangor, Selangor, Malaysia	+	+	+
VCG A21	71	B3130P	Sungai Besar, Selangor, Malaysia	+	+	+
VCG A22	72	B3134P	Sungai Besar, Selangor, Malaysia	+	+	-
VCG A23	73	D0674P	Peringat, Kelantan, Malaysia	-	+	+
VCG A24	74	I3422P	Samarinda, Kalimantan, Indonesia	+	-	+
VCG A25	75	K0688P	Kampung Paya, Kedah, Malaysia	+	-	+
VCG A26	76	R0621P	Cuping, Perlis, Malaysia	+	-	+



A B C

Fig. 2: Compatible interactions. A) HSC strain B) HSI strain; C) Two incompatible strains (A and B) were grouped in the different VCGs. n1 = nit1, nm = NitM

REFERENCES

AL-AMODI, M.O.S. (2006). Morphological, pathogenic, genetic and molecular variabilities of *Fusarium* spp., the pathogens of asparagus crown and root rot in Malaysia and Brunei Darussalam (PhD thesis, Universiti Sains Malaysia, Penang, Malaysia).

BURGESS, L.W., SUMMERELL, B.A., BULLOCK, S., GOTT, K.P. and BACKHOUSE, D. (1994). *Laboratory Manual for Fusarium Research.* University of Sydney. 133 pp.

CHULZE, S.N., RAMIREZ, M.L., TORRES, A. and LESLIE, J.F. (2000). Genetic variation in *Fusarium* Section Liseola from no-till maize in Argentina. *Applied and Environmental Microbiology*, 66, 5312 - 5315.

CORRELL, J.C., KLITTICH, C.J.R. and LESLIE, J.F. (1987). Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology, 77,* 1640 - 1646.

CORRELL, JC., KLITTICH, C.J.R. and LESLIE, J.F. (1989). Heterokayon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). *Mycological Research, 93,* 21 - 27.

CORRELL, J.C., PUHALLA, J.E. and SCHNEIDER, R.W. (1986). Vegetative compatibility groups among nonpathogenic root colonizing strains of *Fusarium oxysporum. Canadian Journal Botany, 64,* 2358 - 2361.

ELMER, W.H. (1991). Vegetative compatibility groups in *Fusarium proliferatum* from asparagus and comparisons of virulence, growth rates, and colonization of asparagus residues among groups. *Phytopathology, 81,* 852 - 857.

ELMER, W.H., SUMMERELL, B.A., BURGESS, L.W. and NIGH, E.L.JR. (1999). Vegetative compatibility groups in *Fusarium proliferatum* from asparagus in Australia. *Mycologia, 9,* 650 - 654.

FERNANDEZ, D., ASSIGBETSE, K., DUBOIS, M.P. and GEIGER, J.P. (1994). Molecular characterization of races and vegetative compatibility groups in *Fusarium oxysporum* f. sp. *vasinfectum. Applied and Environmental Microbiology, 60,* 4039 - 4046.

FREEMAN, S., SHABI, E. and KATAN, T. (2000). Characterization of *Colletotrichum acutatum* causing anthracnose of anemone (*Anemone coronaria* L.). *Applied and Enviromental Microbiology, 66,* 5267 - 5272.

GARRAWAY, M.O. and EVANS, R.C. (1984). *Fungal Nutrition and Physiology.* New York: John Wiley & Sons.

GARRET, R.H. and AMY, N.K. (1978). Nitrate assimilation in fungi. *Advances in Microbial Physiology, 18,* 1 - 65.

GORDON, T.R., CORRELL, J.C. and MCCAIN, A.H. (1986). Host specificity and vegetative compatibility in *Verticillium albo-atrum. Phytopathology, 76,* 1111 (Abstract).

HAWTHORNE, B.T. and GEORGE, J.R. (1996). Use of nitrate non-utilizing mutants to study vegetative incompatibility in *Fusarium solani* (*Nectria heamatococca*) especially members of mating populations I, V and VI. *Mycological Research, 100,* 1075 - 1081.

HSIEH, W.H., SMITH, S.N. and SNYDER, W.C. (1977). Mating groups in *Fusarium moniliforme*. *Phytopathology, 67,* 1041 - 1043.

JACOBSON, D.J. and GORDON, T.R. (1988). Vegetative compatibility and self-incompatibility within *Fusarium oxysporum f. sp. melonis*. *Phytopathology, 78,* 668 - 672.

KATAN, T. (1999). Current status of vegetative compatibility groups in *Fusarium oxysporum*. *Phytoparasitica, 27,* 51 - 64.

KATAN, T. and KATAN, J. (1999). Vegetative compatibility grouping in *Fusarium oxysporum* f. sp. *radicis-lycopersici* from the UK, the Netherlands, Belgium and France. *Plant Pathology, 48,* 541 - 549.

KLITTICH, C.J.R. and LESLIE, J.F. (1988). Nitrate reduction mutants of *Fusarium moniliforme (Gibberella fujikuroi)*. *Genetics, 118,* 417 - 423.

KLITTICH, C.J.R., LESLIE, J.F. and WILSON, J.D. (1986). Spontaneous chlorate-resistant mutants of *Gibberella fujikuroi (Fusarium moniliforme)*. *Phytopathology, 76,* 1142 (Abstract).

LESLIE, J.F. (1993). Fungal vegetative compatibility. *Annual Review Phytopathology, 31,* 127 - 150.

LESLIE, J.F. (1996). Fungal vegetative compatibility - promises and prospects. *Phytoparasitica, 24,* 3 - 6.

LIU, W. and SUNDHEIM, L. (1996). Nitrate nonutilizing mutants and vegetative compatibility groups in *Fusarium poae*. *Fungal Genetic and Biology, 20,* 12 - 17.

MARZLUF, G.A. (1981). Regulation of nitrogen metabolism and gene expression in fungi. *Microbiological Review, 45,* 437 - 461.

MCCALLUM, B.D., TEKAUZ, A. and GILBERT, J. (2004). Barrage zone formation between vegetatively incompatible *Fusarium graminearum (Gibberella zeae)* isolates. *Phytopathology, 94,* 432 - 437.

MES, J.J., WESTSTEIJN, E.A., HERLAAR, F., LAMBALK, J.J.M., WIJBRANDI, J., HARING, M.M. and CORNELISSEN, B.J.C. (1999). Biological and molecular characterization of *Fusarium oxysporum* f. sp. lycopersici divides race 1 isolates into separate groups. *Phytopathology, 89,* 156 - 160.

PAPA, K.E. (1986). Heterokaryon incompatibility in *Aspergillus flavus*. *Mycologia, 78,* 98 - 101.

PUHALLA, J.E. (1984). A visual indicator of heterokaryosis in *Fusarium oxysporum* from celery. *Canadian Journal Botany, 62,* 540 - 545.

PUHALLA, J.E. (1985). Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Canadian Journal Botany, 63,* 179 - 183.

PUHALLA, J.E. and SPIETH, P.T. (1985). A comparison of heterokaryosis and vegetative incompatibility among varieties of *Gibberella fujikuroi (Fusarium moniliforme)*. *Experimental Mycology, 9,* 39 - 47.

SALLEH, B. and SULAIMAN, B. (1984). Fusaria associated with naturally diseased plants in Penang. *Journal of Plant Protection in the Tropics, 1,* 47 - 53.

SAUPE, S.J. (2000). Molecular genetics of heterokaryon incompatibility in filamentous ascomycetes. *Microbiology and Molecular Biology Review, 64,* 489 - 502.

SIDHU, G.S. (1986). *Genetic of Gibberella* fujikuroi. III. Vegetative compatibility groups. *Canadian Journal Botany, 64,* 117 - 121.

SUNDER, S. and SATYAVIR, S. (1998). Vegetative compatibility, biosynthesis of GA_3 and virulence of *Fusarium moniliforme* isolates from bakanae disease of rice. *Plant Pathology, 47,* 767 - 772.

VAKALOUNAKIS, D.J. and FRAGKIADAKIS, G.A. (1999). Genetic diversity of *Fusarium oxysporum* isolates from cucumber: differentiation by pathogenicity, vegetative compatibility and RAPD fingerprinting. *Phytopathology, 89,* 161 - 168.

ZHENG, Q. and PLOETZ, R. (2002). Genetic diversity in the mango malformation pathogen and development of a PCR assay. *Plant Pathology, 51,* 208 - 216.