

VEGETATIVE COMPATIBILITY AMONG *FUSARIUM* *OXYSPORUM* ISOLATES FROM BITTER GOURD AND BOTTLE GOURD IN THE PHILIPPINES

Christian Joseph R. Cumagun^{1*}, Zsachel C. Oribiana¹, Malve S. Tolentino¹
Cherry A. Relevante², Conrado H. Balatero²

¹Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños,
College, Laguna 4031 Philippines

²East West Seed Company, San Ildefonso, Bulacan 3008 Philippines

Received: February 15, 2008

Accepted: April 24, 2008

Abstract: Vegetative compatibility groups (VCGs) were studied in 57 *Fusarium oxysporum* isolates from *Momordica charantia* L. (bitter gourd) and *Lagenaria siceraria* (Mol.) Standley (bottle gourd) using nitrate-non-utilizing (*nit*) mutants. Out of these, 24 isolates that sectored frequently in chlorate medium were genetically unstable and not further used in the experiment. Only 32 isolates were used, among them 21 from *F. oxysporum* f. sp. *momordicae* and 11 from *F. oxysporum* f. sp. *lagenariae*. Sixty one *nit* mutants were generated from 21 isolates *F. oxysporum* f. sp. *momordicae* with their respective frequencies: *nit1* (31), *nit3* (11), *nitM* (19). Twenty five *nit* mutants were generated from 11 isolates of *F. oxysporum* f. sp. *lagenariae* with their respective frequencies: *nit1* (13), *nit3* (5), *nitM* (7). *F. oxysporum* f. sp. *momordicae* populations have higher frequency of reversion to wild type (39.4%) than *F. oxysporum* f. sp. *lagenariae* (27.3%). Non-reverted mutants were used in complementation tests. Four VCGs of *F. oxysporum* f. sp. *momordicae* were identified with the majority belonging to a single VCG. Five VCGs of *F. oxysporum* f. sp. *lagenariae* were identified. Low VCG diversity ratio ($VCG_{div} = 0.19$) was observed for *F. oxysporum* f. sp. *momordicae* whereas a higher value ($VCG_{div} = 0.45$) was obtained for *F. oxysporum* f. sp. *lagenariae*. *F. oxysporum* f. sp. *momordicae* and *F. oxysporum* f. sp. *lagenariae* isolates were not vegetatively compatible.

Key words: *Fusarium oxysporum* f. sp. *momordicae*, *Fusarium oxysporum* f. sp. *lagenariae*, bitter gourd, bottle gourd, vegetative compatibility groups

*Corresponding address:
christian_cumagun@yahoo.com

INTRODUCTION

Fusarium wilt is a devastating disease that occurs in all vegetable growing regions of the world (Nelson *et al.* 1981; Leslie and Summerell 2006). The disease is caused by *Fusarium oxysporum* Schlechtend.:Fr., a fungus that persists in the soil for long periods. As for a soil-borne pathogen, its control is difficult even when effective chemicals are available. Such pathogen behaviour makes resistant cultivars one of the most promising options for effective control of the disease.

No sexual stage of the pathogen has yet been discovered and parasexual recombination is the only mechanism by which reassortment of genetic material can occur (Kistler 1997). Although Fusarium wilt occurs annually in vegetable fields in the Philippines, little is known about its genetic diversity, origin and the distribution of races. Race identification and resistance breeding have been done in East–West Indonesia and Thailand. In East–West Philippines, Fusarium wilt is becoming a major limiting factor in bitter melon and bottle melon. No genetic diversity studies have been conducted with the Philippine isolates and thus important recommendations for the choice of resistant cultivars cannot be made. Therefore, this study is imperative for the amelioration of the industry.

Pathotypes can now be identified using a genetic marker combined with virulence. (Vakalounakis and Fragkiadakis 1999; Lori *et al.* 2004). This is the first step towards understanding the relationship between races within the formae speciales. Known as vegetative compatibility group (VCG) or heterokaryon compatibility (Leslie 1993), this technique is appropriate for developing countries with inadequate facilities for molecular work and can rapidly determine genetic groups of many fungal pathogens and their relation to pathogenicity. A VCG is defined as a group of isolates that are able to anastomose and form heterokaryons among one another, but not with isolates outside the group, thus being controlled by multiple incompatibility loci (Leslie 1993). The isolates of a given VCG typically possess very similar or identical multilocus haplotypes; therefore, VCGs can be good indicators of genetic relatedness (Kistler 1997). VCG analysis is utilized to differentiate formae speciales, races, and pathogens from non-pathogens (Correll *et al.* 1986). VCG may also correspond with races and formae speciales. Genetic diversity of *F. oxysporum* has been classified by VCGs in laboratories worldwide (Kistler *et al.* 1998). The objective of this study is to examine the genetic diversity of *F. oxysporum* populations in bitter melon and bottle melon in Batangas and Bulacan provinces of the Philippines using VCG analysis.

MATERIALS AND METHODS

Culture, growth rate and maintenance of *F. oxysporum*

F. oxysporum isolates from bitter melon and bottle melon (Table 1) at the Postharvest Pathology Laboratory, Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños were cultured in Spezieller Nährstoffarmer Agar (SNA) (Nirenberg 1976). Isolates of *F. oxysporum* from bitter melon and bottle melon were isolated in two provinces of the Philippines namely Batangas in 2005 and Bulacan in 2006. Each isolate was single-spored to ensure pure cultures (Burgess *et al.* 1994).

For long term storage, a piece of sterile filter paper was allowed to be colonized in PDA with one week old cultures of *F. oxysporum* at room temperature under fluores-

Table 1. *Fusarium oxysporum* isolates, their hosts, date of isolation, origin and pigmentation in PDA

Isolate Code	Host/habitat, host organ	Date of Isolation	Origin	Pigmentation in PDA
A ₁ 1	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₁ 2	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₁ 3	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₁ 4	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₁ 5	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₁ 6	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₁ 7	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₁ 10	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₁ 11	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₁ 14	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₁ 15	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₁ 18	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₁ 19	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₁ 20	bitter gourd, stem	Sept 15, 2005	Batangas	violet
A ₂ 3	bitter gourd, stem	Mar 07, 2006	Bulacan	violet
A ₂ 4	bitter gourd, stem	Mar 07, 2006	Bulacan	violet
A ₂ 5	bitter gourd, stem	Mar 07, 2006	Bulacan	violet
A ₂ 6	bitter gourd, stem	Mar 07, 2006	Bulacan	orange
A ₂ 7	bitter gourd, stem	Mar 07, 2006	Bulacan	violet
A ₂ 8	bitter gourd, stem	Mar 07, 2006	Bulacan	orange
A ₂ 9	bitter gourd, stem	Mar 07, 2006	Bulacan	violet
A ₂ 10	bitter gourd, stem	Mar 07, 2006	Bulacan	violet
A ₂ 11	bitter gourd, stem	Mar 07, 2006	Bulacan	violet
A ₂ 12	bitter gourd, stem	Mar 07, 2006	Bulacan	violet
A ₂ 14	bitter gourd, stem	Mar 07, 2006	Bulacan	violet
A ₂ 15	bitter gourd, stem	Mar 07, 2006	Bulacan	violet
A ₂ 16	bitter gourd, stem	Mar 07, 2006	Bulacan	orange
A ₂ 17	bitter gourd, stem	Mar 07, 2006	Bulacan	orange
A ₂ 18	bitter gourd, stem	Mar 07, 2006	Bulacan	violet
A ₂ 19	bitter gourd, stem	Mar 07, 2006	Bulacan	violet
A ₂ 20	bitter gourd, stem	Mar 07, 2006	Bulacan	orange
U ₁ 2	bottle gourd, stem	Sept 15, 2005	Batangas	orange
U ₁ 4	bottle gourd, stem	Sept 15, 2005	Batangas	violet
U ₁ 5	bottle gourd, stem	Sept 15, 2005	Batangas	violet
U ₁ 6	bottle gourd, stem	Sept 15, 2005	Batangas	violet
U ₁ 7	bottle gourd, stem	Sept 15, 2005	Batangas	white to pale violet
U ₁ 12	bottle gourd, stem	Sept 15, 2005	Batangas	white to pale violet
U ₁ 14	bottle gourd, stem	Sept 15, 2005	Batangas	violet
U ₁ 102	bottle gourd, soil	Sept 15, 2005	Batangas	violet
U ₁ 104	bottle gourd, soil	Sept 15, 2005	Batangas	violet
U ₁ 106	bottle gourd, soil	Sept 15, 2005	Batangas	orange
U ₁ 102	bottle gourd, soil	Sept 15, 2005	Batangas	violet
U ₁ 102	bottle gourd, soil	Sept 15, 2005	Batangas	orange
U ₁ 106	bottle gourd, soil	Sept 15, 2005	Batangas	yellow to orange
U ₂ 3	bottle gourd, stem	Mar 07, 2006	Bulacan	orange
U ₂ 4	bottle gourd, stem	Mar 07, 2006	Bulacan	violet
U ₂ 5	bottle gourd, stem	Mar 07, 2006	Bulacan	orange
U ₂ 7	bottle gourd, stem	Mar 07, 2006	Bulacan	violet
U ₂ 10	bottle gourd, stem	Mar 07, 2006	Bulacan	violet
U ₂ 12	bottle gourd, stem	Mar 07, 2006	Bulacan	violet
U ₂ 15	bottle gourd, stem	Mar 07, 2006	Bulacan	violet
U ₂ 106	bottle gourd, soil	Mar 07, 2006	Bulacan	orange
U ₂ 104	bottle gourd, soil	Mar 07, 2006	Bulacan	orange
U ₂ 106	bottle gourd, soil	Mar 07, 2006	Bulacan	orange
U ₂ 102	bottle gourd, soil	Mar 07, 2006	Bulacan	orange
U ₂ 102	bottle gourd, soil	Mar 07, 2006	Bulacan	orange
U ₂ 102	bottle gourd, soil	Mar 07, 2006	Bulacan	orange

cent light for at least one week. Afterwards, the colonized filter paper was transferred to sterile Petri dish for several days to be air dried. The colonized filter paper was then placed in eppendorf tubes for storage at 4°C (McCallum *et al.* 2001).

Selection, characterization and pairing of *nit* mutants

Mutants were generated for each isolate by placing 2 mm² mycelial growth from SNA to chlorate medium with 1.5 to 2% potassium chlorate as commonly used (Leslie and Summerell 2006). A pair of compatible mutants was generated for most of the isolates from bitter gourd and bottle gourd. The chlorate resistant and fast growing sectors which were observed every 2 days originated from the initially restricted colony and were transferred to minimal medium (MM) containing NaNO₃ as the sole nitrogen source with 4 days incubation (Puhalla 1985). Those that grew as thin expansive colonies with no aerial mycelium were considered *nit* mutants (Correll *et al.* 1987). These mutants were plated onto the following phenotyping media: Ammonium tartrate (NH₄) as positive control or with wild type growth, hypoxanthine (HX), and sodium nitrite (NaNO₂). The mutants were grown for 25 on each of the phenotyping media for 3–4 days. Mutants that were able to utilize both nitrite and hypoxanthine were considered *nit1*. Those that were able to utilize nitrite but not hypoxanthine were classified as *nitM* while those that could utilize hypoxanthine but not nitrite were considered as *nit3* (Klittich and Leslie 1988).

Pairing of mutants was done by placing mycelium from each *nit* mutant 1–3 cm apart on minimal medium. Pairings were incubated for 7–14 days (Correll *et al.* 1987) at 25°C (Elmer and Stephens 1989). Self compatibility was tested by pairing generated *nit1*, *nit3*, and *nitM* of the same isolate. All possible combinations of isolates were paired for each location. Selected isolates of *F. oxysporum* f. sp. *momordicae* were paired with *F. oxysporum* f. sp. *lagenariae* showing similar cultural characteristics (e.g. pigmentation). Complementation occurred when dense aerial mycelium in contact of two *nit* mutants was observed. Degree of complementation, weak or strong, was also recorded. Vegetatively compatible isolates were coded with same number. Isolates were grouped on a specific VCG code in which they belong.

VCG Diversity Ratio

VCG diversity ratio was computed using the following formula (McCallum *et al.* 2001):

$$\text{VCG}_{\text{div}} = \frac{\text{No. of VCGs}}{\text{No. of isolates}}$$

Determination of frequency of reversion

Frequency of reversion was computed using the following formula:

$$\% \text{ revision to wild type of isolate} = \frac{\text{No. of reverted } nit \text{ mutants}}{\text{total generated } nit \text{ mutants}} \times 100$$

RESULTS

Selection and characterization of *nit* mutants

Among 31 isolates from Batangas and Bulacan, chlorate – resistant sectors were obtained among 22 isolates (70.97%) of *F. oxysporum* f. sp. *momordicae* while 11 isolates (42.31%) of *F. oxysporum* f. sp. *lagenariae* generated *nit* mutants among 26 isolates. The *nit* mutants from chlorate-resistant sectors were not able to utilize nitrate as a sole nitrogen source and continuously grew as thin expansive colonies without aerial mycelium growth on MM. The number of heterokaryotic sectors containing the three mutants could indicate that three mutation events had occurred. However, a few chlorate resistant sectors of some isolates were able to utilize nitrate. These are called chlorate resistant mutant (*crn*) or revertant which were not included in the complementation test.

The *nit* mutant phenotypes of *F. oxysporum* were characterized on the basis of their colony morphology on media with one of four different nitrogen sources. Among 61 *nit* mutants of *F. oxysporum* f. sp. *momordicae* from Batangas and Bulacan, *nit* mutants recovered with the highest frequency were *nit1* (51%) followed by *nitM* (31%) and *nit3* (18%) Among 25 *nit* mutants of *F. oxysporum* f. sp. *lagenariae*, the frequencies of occurrence are as follows: *nit1* (52%), *nitM* (28%), and *nit3* (20%) (Table 2).

Table 2. Nitrate non-utilizing (*nit*) mutants recovered and their frequency of reversion in *Fusarium oxysporum* f. sp. *momordicae* and *Fusarium oxysporum* f. sp. *lagenariae* from Batangas and Bulacan provinces, Philippines

Formae speciales	Location	Mutants			Total generated <i>nit</i> mutants	% reversion to wild type ¹
		<i>nit1</i>	<i>nit3</i>	<i>nitM</i>		
<i>momordicae</i>	Batangas	15	7	8	30	20.0
	Bulacan	16	4	11	31	19.4
	Total	31	11	19	61	39.4
<i>lagenariae</i>	Batangas	8	1	2	11	27.3
	Bulacan	5	4	5	14	0
	Total	13	5	7	25	27.3

¹(No. of reverted *nit* mutants/ total generated *nit* mutants) x 100

Complementation of *nit* mutants and VCG determination

Formation of dense aerial growth of mycelia in contact between two different *nit* mutants was an indicator of complementation by heterokaryon formation. Complementary pairs varied in the speed and vigor of heterokaryon formation. A *nitM* paired with *nit1* or *nit3* complemented rapidly and produced robust heterokaryon after 5–7 days (Fig. 1A). However, pairing of *nit3* with *nit1* mutants showed weak and slow reaction after 2 weeks (Fig. 1B). No complementation in some pairings of *nit1* and *nit3* was noted.

F. oxysporum f. sp. *momordicae* isolates were grouped into four VCGs (Fig. 2). Six isolates from Bulacan were compatible with 10 isolates from Batangas and this constitute the largest VCG1. VCG2 has three isolates whereas VCG3 and VCG4 each comprise only of one isolate. Eleven isolates *F. oxysporum* f. sp. *lagenariae* fell into five

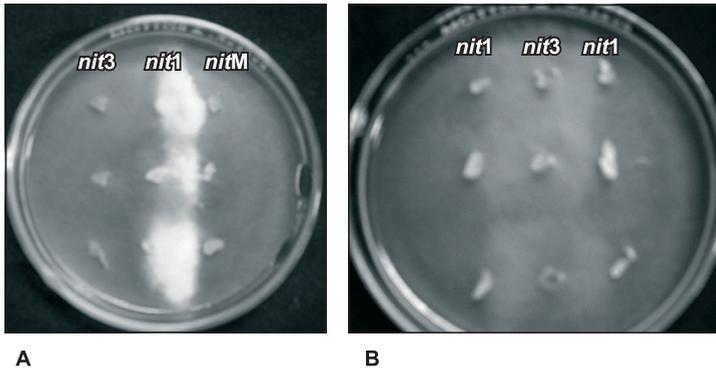


Fig. 1A. Robust heterokaryon formation in contact between *nit1* and *nitM* isolates of *Fusarium oxysporum* f. sp. *lagenariae*. 1B. Thin growth in contact between *nit1* and *nit3* isolates of *Fusarium oxysporum* f. sp. *momordicae*

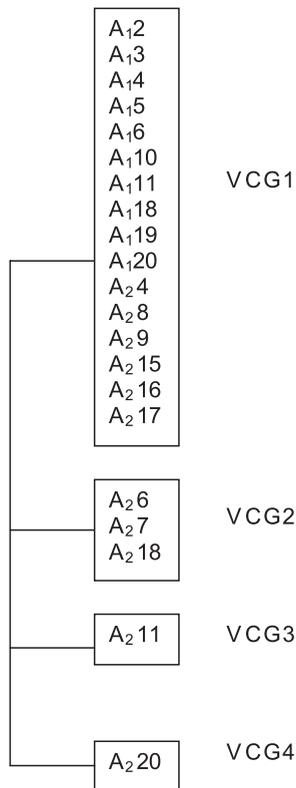


Fig. 2. Vegetative compatibility groups of *Fusarium oxysporum* f. sp. *momordicae*. A₁ isolates (Batangas) and A₂ (Bulacan)

VCGs (Fig. 3). A single isolate belongs to VCG3 and VCG5. No isolates from either of the two locations belong to the same VCG. Two isolates belonging to VCG2 are compatible with the first two isolates in VCG1; thus they are considered bridge isolates. Bridge isolates are isolates with overlapping VCGs.

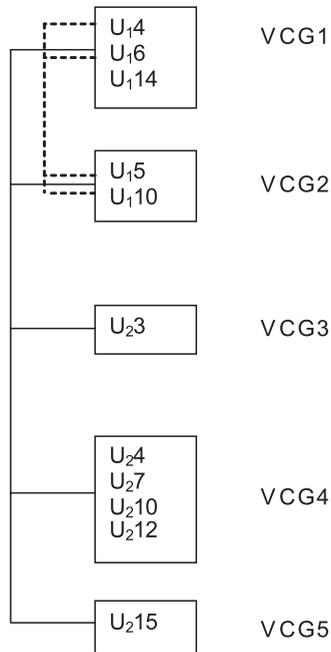


Fig. 3. Vegetative compatibility groups of *Fusarium oxysporum* f. sp. *lagenariae*. U₁ isolates (Batangas) and U₂ (Bulacan). Bridging isolates are connected with broken lines

DISCUSSION

A total of 24 out of 57 isolates which sector frequently and spontaneously on chlorate medium showed genetic instability and were not further used in the experiment. It was more difficult to generate chlorate-resistant mutants in isolates from bottle gourd, especially those that were isolated from soil, than in bitter melon. Klittich and Leslie (1988) investigated whether the chlorate medium induces or selects for preexisting mutations. They found that instability could be associated with a transposable element. High mutation frequencies could be due to transposon movement in a number of eukaryotic organisms. Environmental stress is also a factor of mutation since frequent sectoring was observed on toxic chlorate medium. Variation in susceptibility of loci to mutation could be attributed to the physical size of the gene, with larger genes having a larger target. Another reason is that some loci may include sequences which are mutational "hot spots", making them more susceptible to mutation (Klittich and Leslie 1988).

The frequency of reversion depends on parental cultures (Sidhu 1985). Higher frequency of reversion was found in *F. oxysporum* from bitter gourd than in bottle gourd. Locationwise, more *nit* mutants reverted in Batangas than in Bulacan isolates. *F. oxysporum* f. sp. *lagenariae* isolates produced stable *nit* mutants. An isolate is considered self-compatible when pairing of two different *nit* mutants from the same isolate forms heterokaryon. Most isolates used in this study are self-compatible (data not shown). The *nit1* mutants from different isolates usually exemplified weak or negative complementation. According to Fincham (1966), the pattern of *nit1* complementation follows complementation between mutants within the same gene (intragenic) instead of complementation between different genes (intergenic). On the other hand, complementation reaction between *nitM* mutants was fast and robust. It is known that within these mutants, intergenic complementation occurred (Fincham 1966). For *nit3* mutants, a pair of isolates (A₂18 and A₂8) from Bulacan showed weak compatibility which contradicts the findings of Correll *et al.* (1987).

The use of vegetative compatibility to infer inter-isolate relationships is based on the assumption that all vegetatively compatible isolates are clonally related. These include: first, sexual reproduction is either rare or entirely absent and therefore does not have a significant influence on population structure, and second, considerable attrition has occurred since the last occurrence of outcrossing, making the existence of isolates with the same VCG genotype in otherwise different genetic backgrounds very unlikely. However, in a period of time, the occurrence of somatic mutations would lead to genetic differentiation among vegetatively compatible isolates that would not, therefore, constitute true genetic clones. Thus, isolates grouped in one VCG belong to the same clonal lineage (Puhalla 1985).

Isolates belonging to the same vegetative compatibility group (VCG) have similar or identical multilocus haplotypes and belong to the same clonal lineage (Kistler 1997). In our experiments, low genetic diversity was observed in the population of *F. oxysporum*. *F. oxysporum* f. sp. *momordicae* population has a VCG_{div} ratio of 0.19. *F. oxysporum* f. sp. *lagenariae* populations has a VCG_{div} ratio of 0.45. VCG was not correlated with radial growth rates of *F. oxysporum* in both hosts and locations (data not shown). In contrast, Correll *et al.* (1986) found that colony size and vegetative compatibility of *F. oxysporum* f. sp. *apii* was correlated. Small colony type paired with *F. oxysporum* f. sp. *apii* race 2 tester strain whereas large colony types were vegetatively incompatible to the tester strain. When bitter gourd and bottle gourd isolates were paired in NH₄ tartrate medium from same location, no compatibility was observed. The negative complementation implied host specificity of each formae speciales.

Within formae speciales, it is possible that more than one race may occur within a single VCG or isolates of a single race may belong to several different VCGs, and/or mutation could have occurred which increases diversity of the *F. oxysporum* (Correll 1991). Since race determination is not part of this study, it is not known whether the isolates belonging to a single VCG are of different races. However, Gerlagh and Blok (1988) classified *F. oxysporum* f. sp. *momordicae* as race mo and *F. oxysporum* f. sp. *lagenariae* as race la. In this case, *F. oxysporum* f. sp. *momordicae* and *F. oxysporum* f. sp. *lagenariae* fits the second model of VCG diversity as described by Correll (1991). In this model, though the isolates belong to single race, they could still be grouped into different VCGs.

F. oxysporum infecting members of family *Cucurbitaceae* like cucumber and melon showed also low genetic diversity. Isolates of *F. oxysporum* f. sp. *cucumerinum* from cucumber in China were assigned to VCG 0183, four new VCGs from 0184 to 0187, and a single member VCG included in the artificial VCG 018 – (Vakalounakis *et al.* 2004). Schreuder *et al.* (2000) found that *F. oxysporum* f. sp. *melonis* isolates from South Africa belonged to single VCG 0134 which indicates a high degree of genetic homogeneity among the isolates' population. *F. oxysporum* f. sp. *cubense* with *Musa* as its host showed the most diverse genetic population having 24 VCGs. *F. oxysporum* f. sp. *lagenariae* coded as 041 belonged to a single VCG (Katan 1999). However, *F. oxysporum* f. sp. *momordicae*, which was discovered in Taiwan in June 1981 (Sun and Huang 1983), has unknown number of VCGs yet. To our knowledge, this is the first VCG analysis of *F. oxysporum* f. sp. *momordicae* populations. It is presumed that most *F. oxysporum* isolates of bitter gourd belonged to VCG1 owing to the majority of isolates that belong to this group. Being strictly asexual reproducing fungi, *F. oxysporum* exhibit a large population of predominant clones that are well adapted under prevailing conditions. (Gagkaeva *et al.* 2002). It is inferred that majority of the bitter gourd isolates belong to a single VCG. However, this finding is based only on a few isolates. It is also speculated that more VCGs are present in bottle gourd isolates. Bridging isolates were found.

Low genetic diversity among *F. oxysporum* from bitter gourd could be attributed to clonal reproduction, parasexual recombination and limited gene flow (McDonald and Linde 2002). Thus, it is recommended that vertical or race-specific resistance be used in managing *Fusarium* wilt of bitter gourd and bottle gourd. Some of limitations of the study are the following: (1) few replicates are not enough to obtain chlorate resistant (*crn*) mutants in chlorate medium to generate *nit* mutants, (2) thus, limited number of isolates was used in complementation test due to difficulty of generating *nit* mutants especially from bottle gourd isolates, and (3) pairing of *nit1* and *nit3* provides ambiguous results because of its slow and weak complementation.

ACKNOWLEDGEMENTS

This work was supported by the East–West Seed Company, Philippines.

REFERENCES

- Burgess L.W., Summerell B.A., Bullock S., Gott K.P., Backhouse D. 1994. Laboratory manual for *Fusarium* research. 3rd Edition. University of Sydney and Royal Botanic Gardens, Australia.
- Correll J.C. 1991. The relationship between formae speciales, races, and vegetative compatibility groups in *Fusarium oxysporum*. *Phytopathology* 81: 1061–1067.
- Correll J.C., Klittich C.J.R., Leslie J.F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility test. *Phytopathology* 77: 1640–1646.
- Correll J.C., Puhalla J.E., Schneider R.W. 1986. Identification of *Fusarium oxysporum* f. sp. *apii* on the basis of colony size, virulence, and vegetative compatibility. *Phytopathology* 76: 396–400.
- Elmer W.H., Stephens C. 1989. Classification of *Fusarium oxysporum* f. sp. *asparagi* into vegetatively compatible groups. *Phytopathology* 79: 88–93.
- Fincham J.R.S. 1966. Genetic Complementation. Benjamin, New York, 143 pp.

- Gagkaeva T., Levitin M., Yli-Matilla 2002. Vegetative compatibility and incompatibility of utilizing and non-utilizing mutants in *Fusarium avenaceum*. J. Appl. Genet. 43A: 45–54.
- Gerlach M., Blok W.J. 1988. *Fusarium oxysporum* f. sp. *cucurbitacearum* n. f. embracing all formae speciales of *F. oxysporum* attacking cucurbitaceous crops. Neth. J. Plant Pathol. 94: 17–31.
- Katan T. 1999. Current status of vegetative compatibility groups in *Fusarium oxysporum*. Phytoparasitica 27: 51–64.
- Kistler H.C. 1997. Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. Phytopathology 87: 474–479.
- Kistler H.C. 1998. Systematic numbering of vegetative compatibility groups in the plant pathogenic fungus *Fusarium oxysporum*. Phytopathology 88: 30–32.
- Klittich C.J.R., Leslie J.F. 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics 188: 417–423.
- Leslie J.F. 1993. Fungal vegetative compatibility. Annu. Rev. Phytopathol. 31: 127–151.
- Leslie J.F., Summerell B.A. 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing, Iowa, USA, 388 pp.
- Lori G., Edel-Hermann V., Gautheron N., Alabouvette C. 2004. Genetic diversity of pathogenic and non-pathogenic populations of *Fusarium oxysporum* isolated from carnation fields in Argentina. Phytopathology 94: 661–668.
- McCallum B.D., Tekauz A., Gilbert J. 2001. Vegetative compatibility among *Fusarium graminearum* (*Gibberella zeae*) isolates from barley spikes in southern Manitoba. Can. J. Plant Pathol. 23: 83–87.
- McDonald B.A., Linde C. 2002. The population genetics of plant pathogens and breeding strategies for durable resistance. Euphytica 124: 163–180.
- Nelson P.E., Toussoun T.A., Cook R.J. (eds). 1981. *Fusarium: Diseases, Biology and Taxonomy*, Pennsylvania State University Press, University Park, Pennsylvania, 457 pp.
- Nirenberg H.I. 1976. Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion *Liseola*. Mitt. Biol. BundAnst. Ld. -u. Forstw. H. 169: 1–117.
- Puhalla J.E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63: 179–183.
- Schreuder W., Lamprecht S.C., Holz G. 2000. Race determination and vegetative compatibility grouping of *Fusarium oxysporum* f. sp. *melonis* from South Africa. Plant. Dis. 84: 231–234.
- Sidhu G.S. 1985. Genetics of *Gibberella fujikuroi*. VIII. Vegetative compatibility groups. Can. J. Bot. 64: 117–121.
- Sun S.K., Huang J.W. 1983. A new *Fusarium* wilt of bitter melon in Taiwan. Plant Dis. 67: 226–227.
- Vakalounakis D.J., Fragkiadakis G.A. 1999. Genetic diversity of *Fusarium oxysporum* isolates from cucumber: Differentiation by pathogenicity, vegetative compatibility, and RAPD fingerprinting. Phytopathology 89: 161–168.
- Vakalounakis D.J., Wang Z., Fragkiadakis G.A., Skaracis G.N., Li D-B. 2004. Characterization of *Fusarium oxysporum* isolates obtained from cucumber in China by pathogenicity, VCG, and RAPD. Plant Dis. 89: 645–649.

POLISH SUMMARY

ZGODNOŚĆ WEGETATYWNA WŚRÓD IZOLATÓW *FUSARIUM OXYSPORUM* Z *MOMORDICA CHARANTIA* I *LAGENARIA SICERARIA* NA FILIPINACH

Badano grupy zgodności wegetatywnej (VCG) wśród 57 izolatów *Fusarium oxysporum* z *Momordica charantia* L. i *Lagenaria siceraria* (Mol.) Standey wykorzystując mutanty nie zużywające azotanów (*nit*). Z nich 24 izolaty tworzące często sektory w pożywce chloranowej były genetycznie niestabilne, więc nie uwzględniono ich w dalszych badaniach.

Do badań użyto 32 izolaty, z nich 21 izolatów zaliczonych do *F. oxysporum* f. sp. *lagenariae*. Z 21 izolatów *F. oxysporum* f. sp. *momordicae* uzyskano 61 mutantów *nit* należących do grup: *nit1* (31 izolatów), *nit3* (11 izolatów) i *nitM* (19 izolatów). Z 11 izolatów *F. oxysporum* f. sp. *lagenariae* uzyskano 25 mutantów *nit* w tym *nit1* (13 izolatów), *nit3* (5 izolatów) i *nitM* (7 izolatów). Populacje *Fusarium oxysporum* f. sp. *momordicae* wykazują większą częstotliwość rewersji do typu dzikiego (39,4%) niż *F. oxysporum* f. sp. *lagenariae* (27,3). Mutanty *nit* wykazujące stabilność użyto do testów zgodności. Cztery grupy zgodności wegetatywnej zidentyfikowano u badanych izolatów *F. oxysporum* f. sp. *momordicae*, przy czym większość z nich należała do jednej grupy VCG. W przypadku *F. oxysporum* f. sp. *lagenariae* stwierdzono 5 grup VCG oraz niską proporcję różnorodności ($VCG_{div} = 0,19$) u izolatów *F. oxysporum* f. sp. *momordicae*, podczas gdy ta proporcja była wyższa ($VCG_{div} = 0,45$) u izolatów *F. oxysporum* f. sp. *lagenariae*. Nie stwierdzono zgodności wegetatywnej pomiędzy izolatami *F. oxysporum* f. sp. *lagenariae* i *F. oxysporum* f. sp. *momordicae*.