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RESEARCH ARTICLE / *ARTIGO*

Genetic diversity and PCR-based identification of potential fumonisin-producing *Fusarium verticillioides* isolates infecting corn in the Philippines

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ABSTRACT

Genetic diversity and identification of fumonisin-producing isolates of *Fusarium verticillioides* from two provinces in the Philippines were analyzed using molecular techniques. Using a Polymerase Chain Reaction (PCR)-based technique, 49 of the 54 isolates were identified as *F. verticillioides*, with an amplified product of 800 bp using VERT-1 and VERT-2 primers. Of these, VERTF-1/VERTF-2 primers detected 38 fumonisin-producing *F. verticillioides* isolates producing a single fragment of 400 bp. The other five isolates, which had previously been identified as *F. verticillioides* by TEF sequences, morphology and sexual crosses, were negative using this method. Using Universally Primed-PCR (UP-PCR) markers for *F. verticillioides*, no grouping was observed based on geographical origin and species, but intermediate (53.8%) to high (99.6%) bootstrap values and high genotypic diversity (H=0.99) were generated, suggesting that all isolates clearly belonged to *F. verticilliodes*. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis with Jaccard's coefficient showed that similarities among *F. verticillioides* isolates were intermediate at 71% similarity level.
Key words: *Zea mays*, ear rot, mycotoxin.

INTRODUCTION

Gibberella fujikuroi (Sawada) Ito in Ito and K. Kimura species complex (*Fusarium* section *Liseola*) is composed of an increasingly large number of morphological, biological and phylogenetic species (O'Donnell et al., 1998; Nirenberg & O'Donnell, 1998; Baird et al., 2008). Recent developments in molecular systematics revealed that this species complex includes at least 50 distinct species or phylogenetic lineages. Of these, 34 species have been formally described using morphological characters and 10 have been described based on sexual fertility (mating populations A-J) (Kvas et al., 2009). At present, 11 different mating populations have been recognized in the *G. fujikuroi* species complex (Lepoint et al., 2005; Leslie, 1991, 1999; Phan et al., 2004). *Fusarium verticillioides* (Sacc.) Nirenberg (synonym *F. moniliforme* J. Sheldon; teleomorph *G. moniliformis* Wineland; mating population A) is the major fungal pathogen of the *G. fujikuroi* species complex that plagues corn (*Zea mays* Linn.).

F. verticillioides is a diverse ascomycetous fungus frequently associated with corn worldwide (Glenn et al., 2001). This pathogen was first reported in the Philippines by Reinking in 1918 in corn ears (Cumagun, 2008) and is associated with root, stalk, and ear rots (Jardine & Leslie, 1999; Park et al., 2001). As described in previous massive surveys, *F. verticillioides* strains isolated from corn were genetically diverse members of mating population A, and can produce significant quantities of fumonisins. There were also considerable variations in the amount of fumonisins produced by strains from different geographic sources (Park et al., 2001).

Fusarium species are known for their ability to produce a remarkably wide range of secondary metabolites including mycotoxins that contaminate food and feed worldwide (Kvas et al., 2009). Among these mycotoxins, fumonisin B_1 (FB_1) is considered to be the most important due to its ability to induce leukoencephalomalacia in equines, porcine pulmonary edema, liver cancer in laboratory rats, and possibly esophageal cancer in humans (Glenn et al., 2001; Starr et al., 2006). Fumonisins are found to be phytotoxic and can indeed damage a wide variety of plants including corn. Three mating populations of *G. fujikuroi* species complex, designated as A, D, and E, are fumonisin-producers (Cumagun, 2008), but recent investigations revealed that mating populations C, F, G and I are also capable of producing this toxin (Kvas et al., 2009).

The detection of fumonisin producing fungal species by conventional approach is a laborious and time-consuming task. Molecular techniques are currently being established to address these drawbacks. Patiño et al. (2004), for instance, developed a polymerase chain reaction (PCR)

assay using specific primers to detect *F. verticillioides* as well as fumonisin-producing *F. verticillioides* isolated from various crops. In relation to genetic diversity studies, we used Universally Primed-PCR (UP-PCR) previously to analyze over 40 isolates of the biocontrol fungus *Trichoderma* in Philippine rice fields (Cumagun et al., 2000). The main advantage of this technique is that UP primers primarily target intergenic and more variable areas of the genome and are thus very suitable for detection of intraspecific variation in a fungal population (Bulat et al., 2000).

Our objectives were to sample *Fusarium* spp. isolates from two provinces in the Philippines and use the primers VERT-1 and VERT-2 to discriminate *F. verticillioides* from non-*F. verticillioides* isolates, to use the primers VERTF-1 and VERTF-2 to detect fumonisin-producing isolates, and to use UP-PCR markers to assess the degree of genetic variability and genetic distance/similarity among *F. verticillioides*.

MATERIALS AND METHODS

Fusarium isolates

Fifty-four isolates of *Fusarium verticillioides* collected from corn planted in Isabela, the top corn-producing province in the Philippines, and Laguna province, a small-scale corn-growing province, both under tropical conditions, were used in this study (Table 1). Six standard tester strains from the Fungal Genetics Stock Center (FGSC), Missouri, USA were also used as reference isolates. These are *F. verticillioides* (FGSC #7600, *MATA-2*; FGSC #7603, *MATA-1*), *F. proliferatum* (FGSC #7614, *MATD-1;* FGSC #7615, *MATD-2*), and *F. subglutinans* (FGSC #7616, *MATE-1*; FGSC #7617, *MATE-2*). All single-spored strains labeled with a Mycothéque de l'Université Catholique de Louvain (MUCL) prefix were stored for long-term preservation on PDA and Synthetic Nutrient Agar (SNA) slants in lyophilized condition in MUCL, Belgium (Table 1) and had been previously identified as *F. verticillioides* by TEF sequences (http://bccm.belspo.be/about/mucl.php). Those isolates with the University of the Philippines Los Baños (UPLB) prefix were identified as *F. verticillioides* based on morphology using the Fusarium laboratory manual by Leslie & Summerell (2006) and by sexual crosses (Cumagun, 2008) and stored in sterile dried filter paper at 0°C at the Postharvest Pathology, Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños.

DNA-based identification of fumonisin producing strains

The procedure of Cenis (1992) for rapid extraction of fungal DNA was followed. DNA was run in gel electrophoresis and quantified following the protocol of Ustaszewska (2004). DNA was amplified using two sets of primers (Table 2), VERT-1/VERT-2, to specifically detect *F. verticillioides*; and VERTF-1/VERTF-2 to discriminate fumonisin-producing *F. verticillioides* from strains that are unable to produce the toxin (Patiño et al., 2004). PCR was carried out in a MyCycler™ thermal cycler (Bio-Rad Laboratories, CA, USA) with the following settings: initial denaturation step at 94°C for 85 s, 25 cycles with DNA denaturation at 95°C for 35 s, primer annealing at 64°C for 30 s, and primer extension at 72°C for 2 min, and a final extension step at 72°C for 5 min. PCR products, along with 1000 base pair ladders (INtRON Biotechnology, Korea) as molecular size standards, were separated by electrophoresis in 2% (w/v) agarose gels for approximately 2 h. Fragment sizes of about 800 bp and 400 bp, for *F. verticillioides* strains and fumonisin-producing isolates, respectively, were observed. The experiment was performed twice.

UP- PCR analyses

The procedure of Cumagun et al. (2000) was carried out for UP-PCR amplification using a MyCycler™ thermal cycler (Bio-Rad Laboratories, CA, USA) with the following settings: first denaturation step at 94°C for 3 min, 29 cycles with DNA denaturation at 92°C for 50 s, primer annealing at 56°C for 70 s, and primer extension at 72°C for 60 s, and a final extension step at 72°C for 3 min. The UP-PCR primers tested are listed in Table 2. Data were recorded as the presence or absence of the amplified PCR products, coded as 1 and 0, respectively. A similarity matrix was constructed based on Jaccard coefficient using the SIMQUAL program in the Numerical Taxonomy and Multivariate Analysis (NTSYS-pc Version 2.1q; Exeter Software). The similarity coefficients were used to construct a dendrogram by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) using the Sequential Agglomerative Hierarchical Nested (SAHN) program in NTSYS-pc 2.1q (Zhong & Steffenson, 2001). To obtain a nonparametric estimate of confidence limits, bootstrap analysis was done with 700 replications using the Winboot computer program (Yap & Nelson, 1996). Genotypic diversity (H) among isolates was estimated from allele frequencies using the equation $H = \sum x_i^2$ where x_i is the frequency of the ith allele (Nei, 1973).

RESULTS

Genomic DNA of 54 isolates collected from corn planted in Isabela and Laguna were subjected to PCR assay using two sets of primers, VERT-1/VERT-2, to specifically detect *F. verticillioides*; and VERTF-1/VERTF-2 to discriminate fumonisin-producing *F. verticillioides* from strains that are unable to produce the toxin. Forty-nine of the 54 isolates exhibited an amplified product of approximately 800 bp using VERT-1 and VERT-2 primers (Table 2). Of the tester strains tested, only FGSC #7600 and FGSC #7603 were able to amplify with these primers. This result was expected since these tester strains were *MATA-2* and *MATA-1* of *F. verticillioides*, respectively. Mating population A of the *G. fujikuroi* species complex is the teleomorph of *F. verticillioides*, indicating an apparent

TABLE 1 - Origin, mating type and PCR amplification of *Fusarium* isolates using VERT-1/2 and VERTF-1/2 primers

Isolate code	Culture collection No[1]	Origin[2]	Species[3]	Primer[4] VERT1/2	VERTF1/2
-	FGSC7600	-	*F. verticillioides*	+	+
-	FGSC7603	-	*F. verticillioides*	+	+
-	FGSC7614	-	*F. proliferatum*	-	-
-	FGSC7615	-	*F. proliferatum*	-	-
-	FGSC7616	-	*F. subglutinans*	-	-
-	FGSC7617	-	*F. subglutinans*	-	-
HM2	MUCL51052	Los Baños, Laguna	*F. verticillioides*	+	+
HT4	MUCL51053	Los Baños, Laguna	*F. verticillioides*	+	+
IT4	MUCL51054	Los Baños, Laguna	*F. verticillioides*	+	-
BC1-cas	MUCL51055	Casibarag, Isabela	*F. verticillioides*	+	+
BC1-cat	MUCL51056	Catabayungan, Isabela	*F. verticillioides*	-	+
BC2-cas	MUCL51057	Casibarag, Isabela	*F. verticillioides*	+	+
BC2-cat	MUCL51058	Catabayungan, Isabela	*F. verticillioides*	-	-
BC3-cat	MUCL51059	Catabayungan, Isabela	*F. verticillioides*	+	-
BC4-cas	MUCL51060	Casibarag, Isabela	*F. verticillioides*	+	+
BC6-cas	MUCL51061	Casibarag, Isabela	*F. verticillioides*	+	+
IC6-cat	MUCL51062	Catabayungan, Isabela	*F. verticillioides*	+	-
IC7-cat	MUCL51063	Catabayungan, Isabela	*F. verticillioides*	-	-
MC3-cas	MUCL51064	Casibarag, Isabela	*F. verticillioides*	+	+
MC7-cas	MUCL51065	Casibarag, Isabela	*F. verticillioides*	-	-
SP1B	MUCL51066	San Pablo, Isabela	*F. verticillioides*	+	+
SP2M	MUCL51067	San Pablo, Isabela	*F. verticillioides*	+	+
SP3T	MUCL51068	San Pablo, Isabela	*F. verticillioides*	+	+
SP5M	MUCL51069	San Pablo, Isabela	*F. verticillioides*	+	+
SP6B	MUCL51070	San Pablo, Isabela	*F. verticillioides*	+	+
SP7M	MUCL51071	San Pablo, Isabela	*F. verticillioides*	+	+
ST1T	MUCL51072	Santa Maria, Isabela	*F. verticillioides*	+	+
ST2M	MUCL51073	Santa Maria, Isabela	*F. verticillioides*	+	+
ST4M	MUCL51074	Santa Maria, Isabela	*F. verticillioides*	+	+
IB2	MUCL51076	Los Baños, Laguna	*F. verticillioides*	+	-
3B	MUCL49897	Los Baños, Laguna	*F. verticillioides*	+	+
4C	MUCL49901	Los Baños, Laguna	*F. verticillioides*	+	+
IPB3	MUCL49902	Los Baños, Laguna	*F. verticillioides*	+	+
5B	MUCL49899	Los Baños, Laguna	*F. verticillioides*	+	+
C3	MUCL49896	Calamba, Laguna	*F. verticillioides*	+	+
IPB1	MUCL49885	Los Baños, Laguna	*F. verticillioides*	+	+
IRRI	MUCL49886	Los Baños, Laguna	*F. verticillioides*	+	-
D9	MUCL49888	Calamba, Laguna	*F. verticillioides*	+	+
D8	MUCL49889	Calamba, Laguna	*F. verticillioides*	+	+
2A	MUCL49890	Los Baños, Laguna	*F. verticillioides*	+	+
115	MUCL49891	Los Baños, Laguna	*F. verticillioides*	+	+
8.3	MUCL49894	Los Baños, Laguna	*F. verticillioides*	+	+
131	MUCL49895	Los Baños, Laguna	*F. verticillioides*	+	+
151	MUCL49900	Los Baños, Laguna	*F. verticillioides*	+	+
2.2	MUCL49903	Los Baños, Laguna	*F. verticillioides*	+	+
5.4	MUCL49904	Los Baños, Laguna	*F. verticillioides*	+	+
HM1	UPLB001	Los Baños, Laguna	*F. verticillioides*	+	-
HM2b	UPLB002	Los Baños, Laguna	*F. verticillioides*	+	+
HT1	UPLB003	Los Baños, Laguna	*F. verticillioides*	+	+
HT2	UPLB004	Los Baños, Laguna	*F. verticillioides*	-	-
HT3	UPLB005	Los Baños, Laguna	*F. verticillioides*	+	+
IB1	UPLB006	Los Baños, Laguna	*F. verticillioides*	+	-
IB2b	UPLB007	Los Baños, Laguna	*F. verticillioides*	+	-
IB3	UPLB008	Los Baños, Laguna	*F. verticillioides*	+	-
IM1	UPLB009	Los Baños, Laguna	*F. verticillioides*	+	-
IM2	UPLB010	Los Baños, Laguna	*F. verticillioides*	+	+
IM3	UPLB011	Los Baños, Laguna	*F. verticillioides*	+	-
IT1	UPLB012	Los Baños, Laguna	*F. verticillioides*	+	+
IT2	UPLB013	Los Baños, Laguna	*F. verticillioides*	+	-
IT3	UPLB014	Los Baños, Laguna	*F. verticillioides*	+	+

[1]Isolates deposited at MUCL (Mycothèque de l'Université catholique de Louvain), UPLB (University of the Philippines Los Baños).
[2]FCGC tester isolates are kindly provided by K McKluskey (University of Missouri, USA)
[3]Identification by EF-1α sequences (http://bccm.belspo.be/about/mucl.php), sexual crosses (Cumagun, 2008) and morphology.
[4]+, fumonisin producer; - , non fumonisin producer

specificity of this primer combination. Furthermore, no amplified product was generated on *F. proliferatum* and *F. subglutinans* tester strains.

Using VERTF-1 and VERTF-2 primers, 38 isolates or 70% of the total isolates exhibited a single fragment of approximately 400 bp. Tester strains FGSC #7600 (*MATA-2*) and FGSC #7603 (*MATA-1*) also gave similar amplified products which corresponded with the results using VERT-1/VERT-2 primers. In both VERT-1/VERT-2 and VERTF-1/VERTF-2 primers, only isolates BC2-cat, IC7-cat, MC7-cas, and HT2 showed no amplified product, even though BC2-cat, IC7-cat and MC7-cas were identified as *F. verticillioides* mating type A by sexual crosses and based on sequence of EF-1α gene (data not shown). All tester strains except FGSC #7600 (*MATA-2*) and FGSC #7603 (*MATA-1*) showed no amplification using these two primer combinations.

About six UP-PCR primers used individually or in combination (Cumagun et al. 2000) and two primer pairs (Bulat et al., 2000; Yli-Mattila et al., 2004) were routinely utilized in UP-PCR, but due to the lack of amplified products only three primers which generated a sufficient number of bands for all isolates were considered (Table 2). All *F. verticillioides* isolates, including six tester strains, belonged to three different mating types (*MATA-1/2*, *MATD-1/2* and *MATE-1/2*) and were tested to determine intraspecific genetic variability using AA2M2, AA2M2-AS15INV and L45 primers. A total of 117 UP-PCR bands were recorded across all *F. verticillioides* isolates using the three aforementioned primers with 109 polymorphic bands (Figure 1, Table 3). UPGMA cluster analysis with Jaccard's coefficient showed that similarities among isolates within *F. verticillioides* were intermediate to high ranging from 53.8 to 99.6% at the 71% similarity level (Figure 2). Geographical groupings were not observed in the dendrogram. This indicates that UP primers were able to resolve inter- and intraspecific variability within and among *Fusarium* species. This was

TABLE 2 - Name of primers, length, their DNA sequence and reference

Name of Primer	Primer Length	Sequence	Reference
VERT-1	(21 mer)	5'-GTCAGAATCCATGCCAGAACG-3'	Patiño et al., 2004
VERT-2	(20 mer)	5'-CACCCGCAGCAATCCATCAG-3'	Patiño et al., 2004
VERTF-1	(20 mer)	5'-GCGGGAATTCAAAAGTGGCC-3'	Patiño et al., 2004
VERTF-2	(20 mer)	5'-GAGGGCGCGAAACGGATCGG-3'	Patiño et al., 2004
3-2	(16 mer)	5'-TAAGGGCGGTGCCAGT-3'	Cumagun et al., 2000
AA2M2	(16 mer)	5'-CTGCGACCCAGAGCGG-3'	Cumagun et al., 2000
AS4	(16 mer)	5'-TGTGGGCGCTCGACAC-3'	Cumagun et al., 2000
AS15	(17 mer)	5'-GGCTAAGCGGTCGTTAC-3'	Cumagun et al., 2000
HE45	(16 mer)	5'-GTAAAACGAGGCCAGT-3'	Cumagun et al., 2000
L45	(17 mer)	5'-GTAAAACGACGGCCAGT-3'	Cumagun et al., 2000
AA2M2 /HE45	(16 mer) /	5'-CTGCGACCCAGAGCGG-3' /	Yli-Mattila et al., 2004
	(16 mer)	5'-GTAAAACGAGGCCAGT-3'	Yli-Mattila et al., 2004
AA2M2 /AS15INV	(16 mer)/	5'-CTGCGACCCAGAGCGG-3' /	Bulat et al., 2000
	(17 mer)	5'-CATTGCTGGCGAATCGG-3'	Bulat et al., 2000

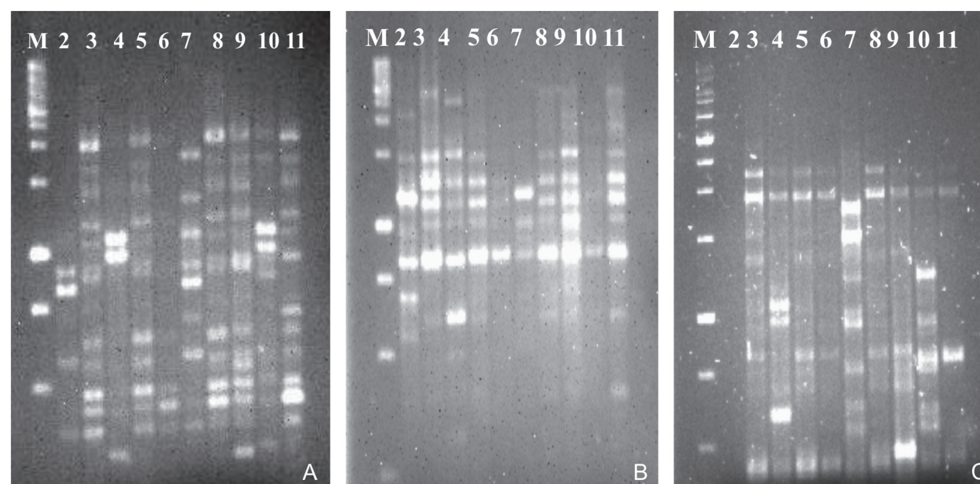

FIGURE 1 - DNA fingerprints of several *F. verticillioides* isolates showing amplified products using **A.** AA2M2, **B.** L45 and **C.** AA2M2-ASI5inv primers. M: 1000bp DNA ladders, Lane 2: 2.2, Lane 3: 5.4, Lane 4: 8.3, Lane 5: 115, Lane 6: 131, Lane 7: 151, Lane 8: 2A, Lane 9: 3B, Lane 10: 4C, Lane 11: 5

TABLE 3 - Estimates of genotypic diversity of a population of *Fusarium verticillioides* using two pairs of UP-PCR primers

Primer	Total number of bands	Number of polymorphic bands	Number of private alleles	Number of haplotypes	H[1]
AA2M2/L45/AS15INV	117	109	8	58	0.998185
AA2M2/L45	85	81	4	79	0.999513

[1]Calculated as described by Nei (1973).

supported by the bootstrap values, signifying relatively intermediate to robust groupings. The number of private alleles ranged from 4 to 8, while genotypic diversity was high (H= 0.99) for both UP primers (Table 3).

DISCUSSION

The isolates infecting corn collected from two provinces in the Philippines were identified as *F. verticillioides* using specific primers VERT1/VERT2. The populations were very diverse, as evidenced by high genotypic diversity. We found that majority of the isolates were fumonisin producers based on their positive amplifications with specific primers VERTF1/VERTF2. No grouping with respect to geographical origin was observed. Comparing our results with two independent fumonisin detection studies of Indian *Fusarium* showed very similar results. For instance, Sreenivasa et al. (2008) used the VERTF-1/and VERTF-2 primer pair to analyze 83 *F. verticillioides* isolates for their potential to produce fumonisins. Sixty-four isolates were positive for fumonisin production with the expected 400 bp amplicon. Fuuthermore, Nakaya et al. (2010) used the same primers, which amplified a single fragment in fumonisin-producing strains of *F. verticillioides,* whereas no amplification product was detected from other isolates that were unable to produce detectable levels of fumonisins. It is absolutely essential to discriminate fumonisin-producing from non-fumonisin-producing fungal strains. Doing so will provide additional tools that can lead to the understanding of toxin biosynthesis on fumonisin-producers, and to gain precise knowledge of the potential toxigenicity of various fungal populations (Patiño et al., 2004).

Primer VERT-1 is located within the 28S ribosomal DNA (rDNA), 256 base pairs (bp) upstream of the intergenic spacer (IGS) region, and VERT-2 is located at position +501, using the IGS sequence of *F. verticillioides* isolate A0999 as reference. On the other hand, primers VERTF-1 and VERTF-2 are located at positions +642 and +1014, respectively, in the IGS sequence of isolate A0999 (Patiño et al., 2004). Patiño et al. (2004) failed to observe any amplified product when DNA from strains other than *F. verticillioides* was used, which is consistent with the results of this study.

Isolate BC1-cat showed no amplified product with VERT-1/VERT-2, but exhibited a positive amplification with VERTF-1/VERTF-2 primers. This isolate is probably related to a certain mating population of *G. fujikuroi* species complex, other than mating population A, which also has the ability to produce fumonisins (Cumagun, 2008; Kvas et al., 2009). The identity of four other isolates, BC2-cat, IC7-cat, MC7-cas and HT2, which showed no amplification on both primer pairs, is not in agreement with the results based on sexual crosses and on sequence of EF-1α gene. It is in this regard that current studies are aimed at increasing the detection sensitivity of this assay (Patino et al., 2004). To improve the detection assay, Sreenivasa et al. (2008), for example, designed a new reversed primer VERT-R (5'- CGA CTC ACG GCC AGG AAA CC -3') based on an intergenic spacer sequence (IGS) combined with VERTF-1 developed by Patino et al. (2004).

Conversely, 12 isolates which exhibited an 800 bp fragment using VERT-1/VERT-2 showed no amplified products with VERTF-1/VERTF-2 primers. These isolates were considered non-fumonisin-producing strains of *F. verticillioides*. Certain deletions, transposon insertions, or mutations could probably exist in some of the genes of these isolates, which resulted in the deficiency or absence of fumonisin production (Baird et al., 2008). Another group of isolates (2.2, 115, 151, 2A, C3, and D8), which have undetectable levels of FB_1 based on high performance liquid chromatography (HPLC) analysis (Cumagun et al., 2009), exhibited an amplification using VERTF-1/VERTF-2 primers (Table 1). This is paralleled by another study, where *F. verticillioides* strains from Nepal that had been reported to be non- or low-fumonisin-producers (Desjardins & Plattner, 2000), showed amplification with VERTF-1/VERTF-2 primers (Patiño et al., 2004). Patiño et al. (2004) found that 54 *F. verticilioides* tested with VERTF1 and VERTF2 differentiated fumonisin-producing *F. verticillioides* strains from those non-fumonisin producing strains, confirming that the IGS region is a good choice for finding specific sequences to differentiate closely related species or strains at the intraspecific level.

PCR-based technique, as a diagnostic tool, is an effective and quick way to specifically detect *F. verticillioides* and its fumonisin-producing strains. Here, two sets of designed primers derived from the IGS region of a particular organism were utilized. Aside from the convenience of using species-specific primers, this assay had greater sensitivity compared to conventional cultural approaches (Grimm & Geisen, 1998) and avoided labor- and time-consuming tasks, allowing more accurate and sound results in a relatively short period of time. Generally, a

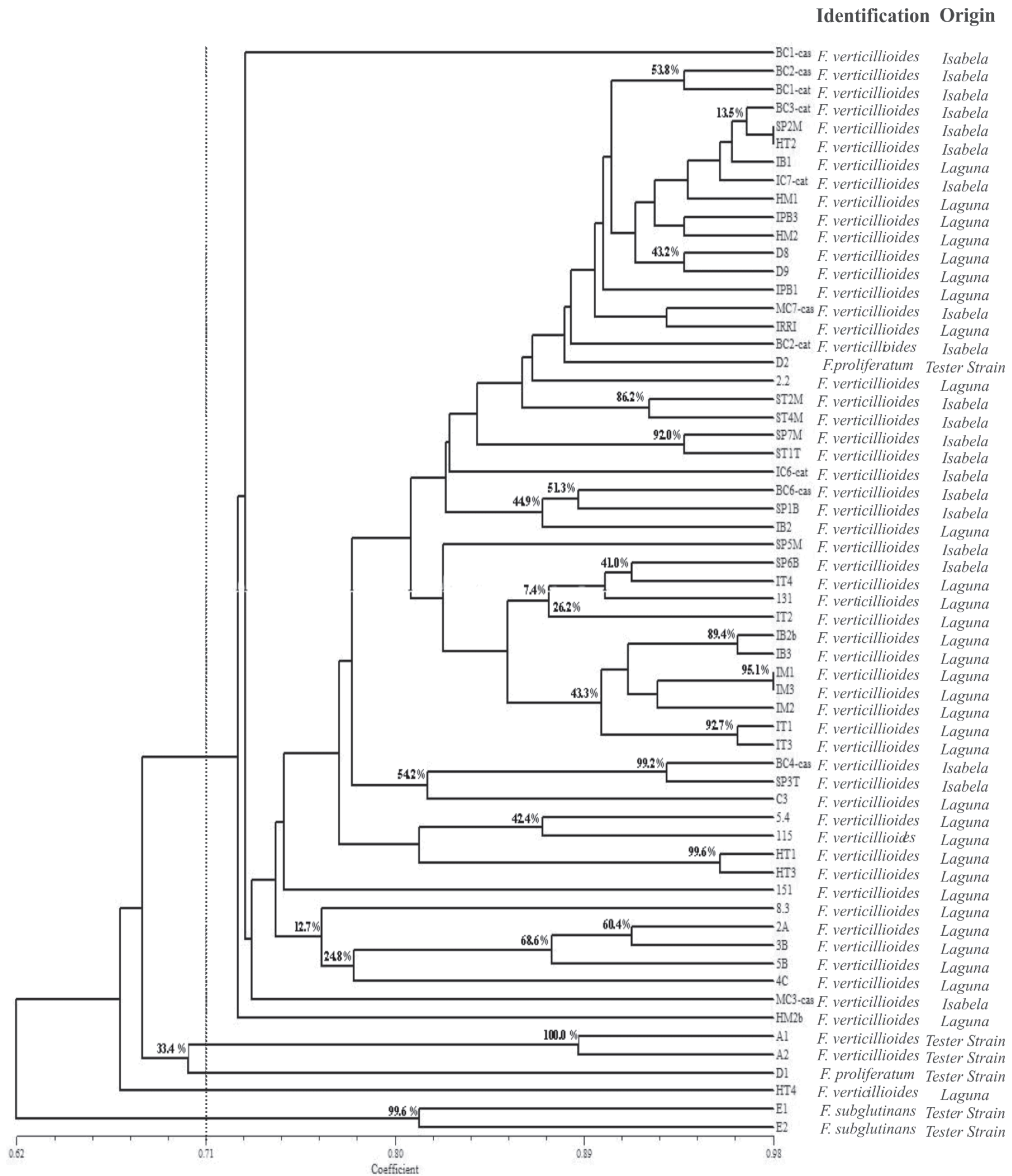

FIGURE 2 - Unweighted pair group method with arithmetic mean (UPGMA) cluster analysis of *Fusarium verticillioides* isolates using AA2M2, AA2M2-AS15INV and L45 primers. Bootstrap *P* values were indicated at the corresponding node for each cluster.

better knowledge of the relationship between the taxonomy and mycotoxigenic potential is fundamental, as exemplified in this particular study.

To obtain a nonparametric estimate of confidence limits, bootstrap analysis was done with 700 replications, as reflected on the nodes of each cluster (Figure 2). Felsenstein (1985) suggested that only groups with bootstrap *P* values of 95% or greater be considered significant. In accordance with this rule, only five small clusters were truly robust, with a *P* value ranging from 95.1 to 100% (Figure 2). Bootstrap values below 50% imply that the positions of these genotypes might change if other primers were used or other genotypes were involved in the analysis (Cesoniene et al., 2007). With this, nine additional clusters (*P*>50%) were considered to be intermediately reliable (Figure 2). Moreover, FGSC #7616 and #7617 tester strains, which identified as *MATE-1* and *MATE-2* (*P*=99.6%), respectively, were separated from the groups of *MATA-1* and *MATA-2* (*P*=100%), as well as *MATD-1* and *MATD-2*. The number of private alleles ranged from 4 to 8, while genotypic diversity was high (H= 0.99) for both UP primers (Table 3), suggesting a significant genetic exchange has occurred among the *F. verticillioides* population in corn.

It was observed that some isolates of *F. verticillioides* sourced from the two localities were distributed randomly throughout the dendrogram (Figure 2). In some studies, *F. verticillioides* isolates from seven geographical locations in Africa and Asia formed a single cluster in a maximum-parsimony tree based on single-gene sequences from *G. fujikuroi* species complex (Wulff et al., 2010). Amoah et al. (1995) and Park et al. (2001) also noted the association of *F. moniliforme* (*F. verticillioides*) strains sourced from several regions of Ghana and Korea, respectively, using RAPD markers. In addition to that, about 43 isolates of *F. moniliforme* (*F. verticillioides*) from five regions of Israel and from the USA were divided into only two major clusters utilizing six RAPD primers. It has been predicted that this might be the result of gene flow between locations in Israel or from a common gene pool of *Fusarium* isolates that became established in the different locations (Huang et al., 1997). The same principle has also been considered to explain why the random distribution of *F. verticillioides* isolates from two different locations is observed.

Genetic variation observed within and among populations of *F. verticillioides,* using the UP-PCR experiment, could indicate the speed at which a pathogen evolves (Huang et al., 1997; Cumagun, 2007). The amplification products generated in this experiment represent primer-directed but random samples of the genome. The polymorphisms observed were the results from the variation in the number of appropriate primer-matching sites of different DNAs. This information about the genetic variability in natural pathogen populations is essential to develop efficient breeding strategies and might eventually be used to predict the efficacy of control measures, such as the use of resistant cultivars or fungicide application (Huang et al., 1997; Cumagun, 2007). Overall, our study has yielded useful information regarding the mycotoxin risks in corn due to *F. verticillioides* in the Philippines.

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