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Morphological and DNA Polymorphism Analyses of *Fusarium solani* Isolated from *Gyrinops versteegii* in the West Nusa Tenggara Forest

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Fusarium is the most common bio-induction agent in agarwood cultivation. Taxonomic study of *Fusarium* could reveal characteristics of *Fusarium* species that have high compatibility with agarwood, such as *Gyrinops versteegii*. DNA fingerprinting can be used in addition to the morphological data in a polyphasic approach for understanding the taxonomy of *Fusarium*. Thus, this study aims to infer the genetic diversity of selected *Fusarium solani* isolated from *G. versteegii* collected at four localities in West Nusa Tenggara Province using a polyphasic approach by comparing the dendrograms generated from morphological data and RAPD profiles. Four *F. solani* isolates (LU, LT, LB and BM) representing each of the collection locality were subjected to morphological observations and RAPD-PCR profiling. The fungal isolates were grown on three different media for morphological observations on the characteristics of colony, hyphae and conidia. All isolates produced colony with the largest diameter and highest conidia concentration on the bean sprout extract medium. Based on the RAPD profiles, isolate BM showed the highest number of polymorphic fragments among all isolates. Dendrograms generated from both morphological and molecular data were similar in showing that isolate BM was more dissimilar to other isolates. However, the dendrograms were slightly different in which isolate LT was more similar to isolate LB in the RAPD-based dendrogram, while it was more similar to isolate LU in the dendrogram generated from morphological characters. We concluded that molecular characterisation with RAPD analysis supports morphological characterisation of *F. solani* isolates in a polyphasic taxonomy.

Keywords: DNA polymorphism; *Fusarium solani*; *Gyrinops versteegii*; morphological character

I. INTRODUCTION

Gyrinops versteegii is one of the endemic agarwood producers from West Nusa Tenggara (Mulyaningsih & Issamu, 2008). This species could be found at its natural habitats in Senaru Forest, North Lombok Regency (Wangiyana & Malik, 2018). It is also found in the agarwood plantations in West Lombok (Wangiyana & Sami'un, 2018), indicating the recent increased interest in the cultivation of this species on the Lombok Island. The cultivation of agarwood requires support in the form of bio-induction technology development to make the cultivation process sustainable. Studies on the inoculating agents for bio-induction of agarwood are one of the most important foci in agarwood cultivation.

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Cultivation of *G. versteegii* using *Fusarium* as an inoculating agent is the most effective way based on the recommendation of Forest Research Development Agency (FORDA). *Fusarium* could induce resin formation in *G. versteegii* tissues, as a self-defence response from this agarwood-producing species. Sesquiterpenoids and phenylethylchromones are the main molecules responsible for the aromatic fragrance of the resin (Novriyanti *et al.*, 2011). *Gyrinops versteegii* trees with no inoculated *Fusarium* will not produce fragrance resin.

Since *Fusarium* has an important role in the cultivation of *G. versteegii*, the exploration of this fungal group is important. FORDA has a long-term project aimed to explore *Fusarium* from several islands in Indonesia (Santoso *et al.*, 2011). Exploration of *Fusarium* associated with *G. versteegii* from several regions in West Nusa Tenggara, followed by characterisation and diversity studies on the *Fusarium* isolates obtained from those exploration efforts would support FORDA in completing the project. Diversity study could offer a comprehensive understanding of the role of different *Fusarium* strains in agarwood resin formation. The findings from diversity study could also add to the database of *Fusarium* strains from *G. versteegii*.

Diversity study of *Fusarium* sp. has been carried out by morphological analysis by convention. This genus is defined based on the mycelial structure, as well as the size of macroconidia and microconidia (Seifert, 1996). However, morphological analysis alone could not provide sufficient data for proper classification in light of modern taxonomy. Morphological analysis should be supported with molecular data analysis as a robust approach in classification that is well known as polyphasic taxonomy (Uilenberg & Goff, 2006). Moreover, molecular characters are important in modern microorganism classification because they could distinguish microorganism taxa with very similar morphological characteristics (Prakash *et al.*, 2007). Thus, the aim of this study is to infer the genetic relatedness of *F. solani* using a polyphasic approach by comparing the dendrograms generated from morphological characters and DNA polymorphisms (molecular data).

II. MATERIALS AND METHODS

A. Isolation of Fusarium

Explorations of *Fusarium* from *G. versteegii* were conducted at several places in West Nusa Tenggara, Indonesia. The bark of *G. versteegii* stem was surface sterilised before the stem was cut into small pieces for fungal isolation. The isolation procedure was based on Nugraheni *et al.* (2015) with some modifications. A small piece of *G. versteegii* stem (1 mm × 1 mm) was placed on 2% (w/v) potato dextrose agar (PDA; Difco) and incubated for 5–7 d until visible growth of mycelia could be observed around the *G. versteegii* stem. Purification of the fungal isolate was conducted by repeated subculture on PDA. The isolates were screened for *Fusarium* based on their cultural characteristics and morphology of their vegetative and reproductive structures produced on different culture media according to different keys of identification. Rapid molecular characterisation was also conducted by PCR amplification using ITS and TEF 1α genes for preliminary screening (Nugraheni *et al.*, 2015). Isolates identified as *Fusarium solani* based on BLAST-n analysis with 98–99% gene sequence similarity were selected for subsequent analyses in this study.

B. Medium Preparation

PDA, bean sprout extract agar (Wangiyana *et al.*, 2018) and wood extract agar (Mudakir & Hastuti, 2015) were used to culture the selected *F. solani* isolates for morphological characterisation. PDA was prepared at a concentration of 2% (w/v), while the bean sprout extract

Table 1. Composition of bean sprout extract medium and wood extract media

Medium	Component	Concentration (g/L)
Bean sprout extract	Bean sprout	150
	Dextrose	15
	Agar	20
Wood extract	NaOH-treated sawdust	20
	Dextrose	15
	NH_4NO_3	2.5
	$Na_2HPO_4 \cdot 2H_2O$	1
	$MgSO_4 \cdot 7H_2O$	0.5
	$Fe(SO_4)_3 \cdot 5H_2O$	0.1
	$CO(NO_3)_2 \cdot 6H_2O$	0.005
	$CaCl_2 \cdot 2H_2O$	0.001
	KH_2PO_4	0.005
	$MnSO_4 \cdot 2H_2O$	0.001
	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.0001
	Agar	20

agar and wood extract agar were prepared by mixing the compounds listed in Table 1 in 1 L of distilled water, and sterilised in autoclave at 121°C, 1 atm for 15 min.

C. Morphological Characterisation

Morphological characterisation was based on observations of fungal colonies and fungal cells. All selected fungal isolates were cultured on PDA, bean sprout extract agar, and wood extract agar for 7 d at 27°C. Fungal colony morphology including colony diameter, pigmentation, and aerial hyphae formation were observed for characterisation.

Fungal cell morphology observations were conducted by fungal slide culture (Harris, 1986) with some modification. Sterile 1.5% water agar was poured into 9 cm Petri dishes and allowed to solidify. A sterile cover glass was placed on the water agar. One block of PDA about the size of 0.5 cm × 0.5 cm was aseptically placed on the cover glass. The PDA block was inoculated with fungal hyphae on 4 sides and a second sterile cover glass was placed on top of it. The slide culture was incubated at 27°C for 3 d or until adequate fungal hyphae growth had occurred. The top cover glass was lifted off with forceps and placed onto a microscope slide with one drop of lactoglycerol trypan blue. The hyphal structures and aerial hyphae were observed for fungal cell morphological characterisation. Macroconidia and microconidia observation was conducted in a similar manner by culturing the isolates on banana leaf agar (Nurbaya *et al.*, 2014).

Each fungal isolate was considered as an organism taxonomical unit (OTU). Morphological characters for each OTU were compiled into a binary matrix based on the presence and absence of characters denoted as "+" and "–" respectively. The Multivariate Statistical Package (MVSP version 3.1A) was used to construct a dendrogram based on the binary matrix of morphological characters with the unweighted pair group method with arithmetic mean (UPGMA) clustering method. The simple matching coefficient was used to derive the pairwise similarity matrix for dendrogram construction (Wangiyana, 2019a).

D. Genomic DNA Isolation

DNA extraction followed Younes *et al.* (2013) with modifications. Fungal isolates were cultured on potato dextrose broth for 3 d. Genomic DNA was extracted from 200 mg of fungal mycelia ground into powder using mortar and pestle in liquid nitrogen. The powdered mycelia were transferred into a 1.5 mL microtube and mixed with 200 µL lysis buffer containing 2% sodium dodecyl sulphate, 100 µg/mL proteinase K, 1 M Tris and 0.5 M EDTA, followed by incubation at 55°C for 15 min. The lysate was added with 500 µL of 2× CTAB buffer, homogenised on a vortex mixer and incubated at 65°C for 15 min. The mixture was then centrifuged at 12,000 rpm for 5 min, and supernatant was transferred into a clean 1.5 mL microtube, with 500 µL chloroform: isoamyl alcohol (24:1) added to the supernatant. The mixture was homogenised and centrifuged at 12,000 rpm for 5 min. DNA was precipitated overnight by adding 237 µL of isopropanol and 32 µL of 7.5 M ammonium acetate to 400 µL of supernatant. The DNA pellet was washed with 70% ethanol, air-dried and resuspended in TE buffer (10 mM Tris, 1 mM EDTA [pH 8]). Integrity of genomic DNA was assessed by electrophoresis on a 0.8% agarose gel with ethidium bromide staining, while the yield of DNA was measured from the readings of absorbance at 260 nm on a spectrophotometer.

E. RAPD-PCR

RAPD analysis was conducted using random OPA primers listed in Table 2. Primers were arbitrarily selected from the OPA series primers commonly used for the RAPD-PCR analysis of *Fusarium* species (Bahmani *et al.*, 2012). PCR was carried out in a total volume of 25 µL containing 12.5 µL 2× KAPA2G PCR Mix (Kapa Biosystems), 8.5 µL double distilled water, 2 µL primer (10 pmol/µL) and 2 µL template DNA (40 ng/µL). Amplification was run on a Bio-Rad thermocycler with the following profile: an initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 37°C for 1 min, extension at 72°C for 2 min, and a final extension at 72°C for 5 min.

Table 2. Sequences of OPA primers for PCR amplification

Primer	Sequence
OPA-01	5′- CAGGCCCTTC-3′
OPA-02	5′- TGCCGAGCTG-3′
OPA-04	5′-AATCGGGCTG- 3′
OPA-08	5′- GTGACGTAGG -3′
OPA-09	5′-GGGTAACGCC-3′
OPA-18	5′-AGGTGACCGT-3′

F. Amplified DNA Fragment Analysis

The amplified DNA fragments were separated by electrophoresis on 1.2% agarose gels and visualised with ethidium bromide staining. A 1kb plus DNA ladder (Invitrogen) was used to determine the molecular weight of the amplified DNA fragments. The banding patterns generated from all OPA primers visualised on UV-transilluminator were transformed into diagrammatic representations to simplify further analysis (Sembiring, 2012). The presence and absence of each band at specific position expressed by the primers was scored. Only the polymorphic loci were compiled into a binary matrix to construct the dendrogram. MVSP 3.1 was used to generate a dendrogram based on the binary matrix of DNA polymorphism using the same similarity coefficient and clustering method previously used to construct the dendrogram from the morphological characters.

III. RESULTS AND DISCUSSION

A total of 43 fungal isolates from four regions in West Nusa Tenggara, including West Lombok, Central Lombok, North Lombok and Bima, were obtained after purification by repeated subculture. Four *F. solani* isolates representative of each collection locality were selected for further morphological and DNA polymorphism characterisation: isolate LB from West Lombok, isolate LT from Central Lombok, isolate LU from North Lombok and isolate BM from Bima. These isolates were chosen based on their ability to grow on all three tested growth media (PDA, bean sprout extract agar and wood extract agar), as well as the results from preliminary screening with gene sequences.

The growth of selected *F. solani* isolates on all three tested growth media was assessed from the colony diameter and conidia production (Figure 1). Compared to PDA which is well known as the common growth medium for fungi (Arti & Kalpana, 2016), the bean sprout extract performed better in supporting *F. solani* growth. The bean sprout extract was the best growth medium for *F. solani* since all isolates produced colony with the largest maximum diameter and the highest conidia concentration on this medium.

A. Morphological Analysis

All four selected *F. solani* isolates showed different colony and cell morphologies (Table 3). Different pigmentations among the *F. solani* isolates were only found on PDA. All isolates showed white pigmentation on bean sprout extract agar and wood extract agar. This result supported the statement that PDA is a useful medium for *F. solani* characterisation because the medium allows different strains of *F. solani* to grow with different pigmentations (Pradeep *et al.*, 2013).

A total of 42 morphological characters of *F. solani* isolates (Table 3) were analysed with numerical phenetic analysis to assess the similarity among the fungal isolates. Numerical phenetic analysis requires a lot of characters for similarity analysis and dendrogram construction. A morphology-based dendrogram represents an ordered arrangement of isolates based on the degree of morphological similarity among isolates. The higher similarity index between isolates means the isolates have higher similarity in their morphological characters.

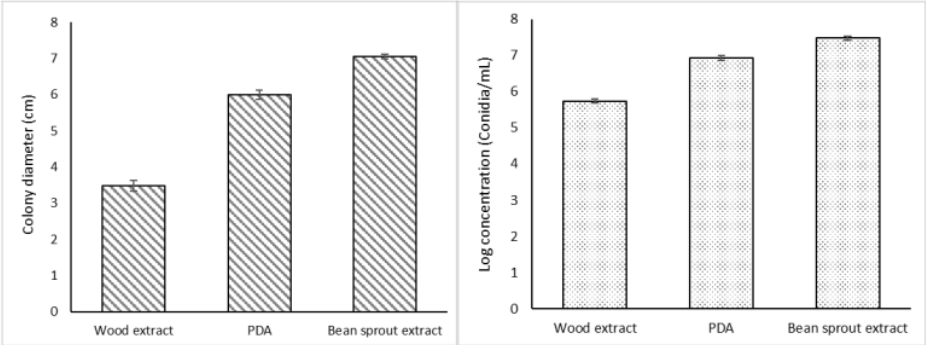

Figure 1. Growth of selected isolates (LU, LT, LB and BM) on different growth media expressed as colony diameter (left) and conidia concentration (right) for 7-day-old cultures. Data represent average of all isolates in triplicate ± standard error.

Table 3. Morphological characterisation of *Fusarium solani* isolates from North Lombok (LU), Central Lombok (LT), West Lombok (LB), and Bima (BM)

No.	Character	Isolate			
		LU	LT	LB	BM
1	Front pigmentation pure white	-	-	-	-
2	Front pigmentation white mixed with other colours	+	+	+	+
3	Back pigmentation orange	+	-	-	-
4	Back pigmentation white	-	+	-	-
5	Back pigmentation peach	-	-	+	+
6	Smooth colony texture	+	+	+	+
7	Compact colony texture	-	-	-	-
7	Concentric circle	+	+	+	-
8	Concave elevation	+	+	-	+
9	Convex elevation	-	-	+	-
10	Cotton-like aerial mycelia	+	+	+	+
11	Spiral hyphae without branching	+	+	+	-
12	Spiral hyphae with branching	-	-	-	+
13	Septate hyphae	+	+	+	+
14	Macroconidia length 20.5–25.5 µm	-	-	+	-
15	Macroconidia length 25.6–30.5 µm	-	-	-	+
16	Macroconidia length 30.6–35.5 µm	+	+	-	-
17	Macroconidia width 2.55–2.75 µm	-	-	-	+
18	Macroconidia width 2.76–2.95 µm	-	+	-	-
19	Macroconidia width 2.96–3.15 µm	+	-	+	-
20	Macroconidia straight	+	+	-	-
21	Macroconidia curved dorsiventrally	-	-	+	+
22	Macroconidia with hooked apical cell	+	+	-	-
23	Macroconidia with papillate apical cell	-	-	-	+
24	Macroconidia with blunt apical cell	-	-	+	-
25	Macroconidia with foot-shaped basal cell	+	-	-	-
26	Macroconidia with elongated foot-shaped basal cell	-	+	-	-
27	Macroconidia with grooved basal cell	-	-	+	+
28	Macroconidia septa ≤ 5	-	+	+	-
29	Macroconidia septa > 5	+	-	-	+
30	Microconidia length 3.00–3.30 µm	+	-	-	-
31	Microconidia length 3.31–3.60 µm	-			+
32	Microconidia length 3.61–3.80 µm	-	+	+	-
33	Microconidia width 1.00–1.20 µm	+	-	-	-
34	Microconidia width 1.21–1.40 µm	-	+	-	+
35	Microconidia width 1.41–1.60 µm	-	-	+	-
36	Oval-shaped microconidia	+	+	+	+
37	Microconidia on monophialide	+	+	+	+
38	Only 1 septum in microconidia	+	+	+	+
39	Chlamydospore	+	-	+	-
40	Chlamydospore present singly	-	-	+	-
41	Chlamydospore present in pairs	+	-	-	-
42	Chlamydospore in intercalary position	+	-	+	-

+ = presence of character, - = absence of character

Based on Figure 2, the highest similarity was found between isolates LU and LT, while the lowest similarity was between isolate BM and the cluster comprised of isolates LU and LT. Isolate BM was least similar to all other isolates.

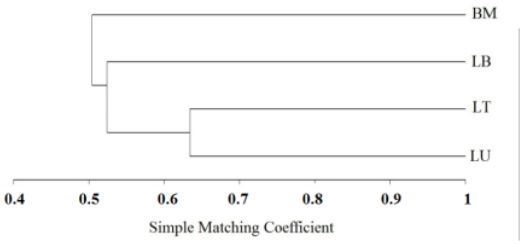

Figure 2. Dendrogram based on morphological characters of *Fusarium solani* isolates from West Lombok (LB), Central Lombok (LT), North Lombok (LU) and Bima (BM).

The taxonomy of genus *Fusarium* was significantly developed by Booth (1971) who introduced the use of the morphology of conidia-producing cells, especially those that produced macroconidia. A manual with identification keys and illustrations for members of this genus provided by Nelson *et al.* (1983) had been widely used for studying *Fusarium*. The key characters used for species identification of *Fusarium* included the characteristics of hyphae, macroconidia, and microconidia (Moretti, 2009). Morphological identification of *Fusarium* species based on those characters had been adopted in many studies, including research that focused on identification of *Fusarium* from agarwood (Budi *et al*., 2010). However, to our knowledge, none of the previous studies on *Fusarium* taxonomy had integrated morphological data with numerical phenetic analysis to create dendrogram which could better describe the morphological variations among *Fusarium* isolates. We suggest that constructing dendrogram based on the morphological characters as in this study could be an alternative approach for studying the taxonomy of *F. solani*.

B. DNA Polymorphism Analysis

RAPD analysis with random OPA primers showed different banding patterns of the fungal isolates. The bands at particular positions represent RAPD loci that can be monomorphic or polymorphic on the genomic DNA of isolates (Figures 3). A locus is monomorphic if the band is present in all sample isolates. A polymorphic locus is a band that does not present in all isolates. In other words, a band that is absent in at least one isolate is considered as polymorphic. Polymorphic loci specific to a particular isolate represent the unique characters that can be used to differentiate the isolates (Younes *et al.*, 2013).

RAPD-PCR with different OPA primers produced DNA fragments that differed both in the number of loci and the amplification pattern among isolates. All tested OPA primers also produced different number of polymorphic fragments and isolate-specific polymorphic fragments (Figure 3 & Table 4). The primer OPA-02 produced the highest number of loci with 10 out of the 14 loci were polymorphic, while primer OPA-08 produced the lowest number of loci, but all six of them were polymorphic, with two specific to isolate BM (4027 bp and 563 bp). The primer OPA-02 produced the highest number of scored polymorphic fragments, with a total of 22 fragments at 10 polymorphic loci scored in all tested isolates. Three of the 22 polymorphic fragments were specific for isolate BM (693 bp, 579 bp, and 373 bp). Only one of the loci produced by the primer OPA-09 was monomorphic, the rest were polymorphic with one of them specific to isolate LU (1000 bp) and three of them specific to isolate BM (850 bp, 740 bp, and 527 bp). The primer OPA-04 produced only five polymorphic loci, but all of them were isolate-specific. The primer also produced the highest number of polymorphic fragments specific to a particular isolate, with four loci for isolate BM (1459 bp, 977 bp, 598 bp and 560 bp) and one for isolate LB (2400 bp) among all others primers. On the other hand, primer OPA-18 produced five polymorphic loci but only one of them was specific to isolate BM (600 bp). Out of seven loci generated by primer OPA-01, four of them were polymorphic but none of the loci were specific to any of the *F. solani* isolates (Figure 3). The number of fragments, especially that of polymorphic fragments specific to certain strain or species, is one of the most important parameters in RAPD analysis. This parameter could determine the interspecific and intraspecific relationship based on the genetic diversity analysed using RAPD (Dwivedi *et al.*, 2018). Thus, OPA-02 and OPA-04 are ideal random primers for RAPD analysis to discriminate the *F. solani* isolates from *G. versteegii*.

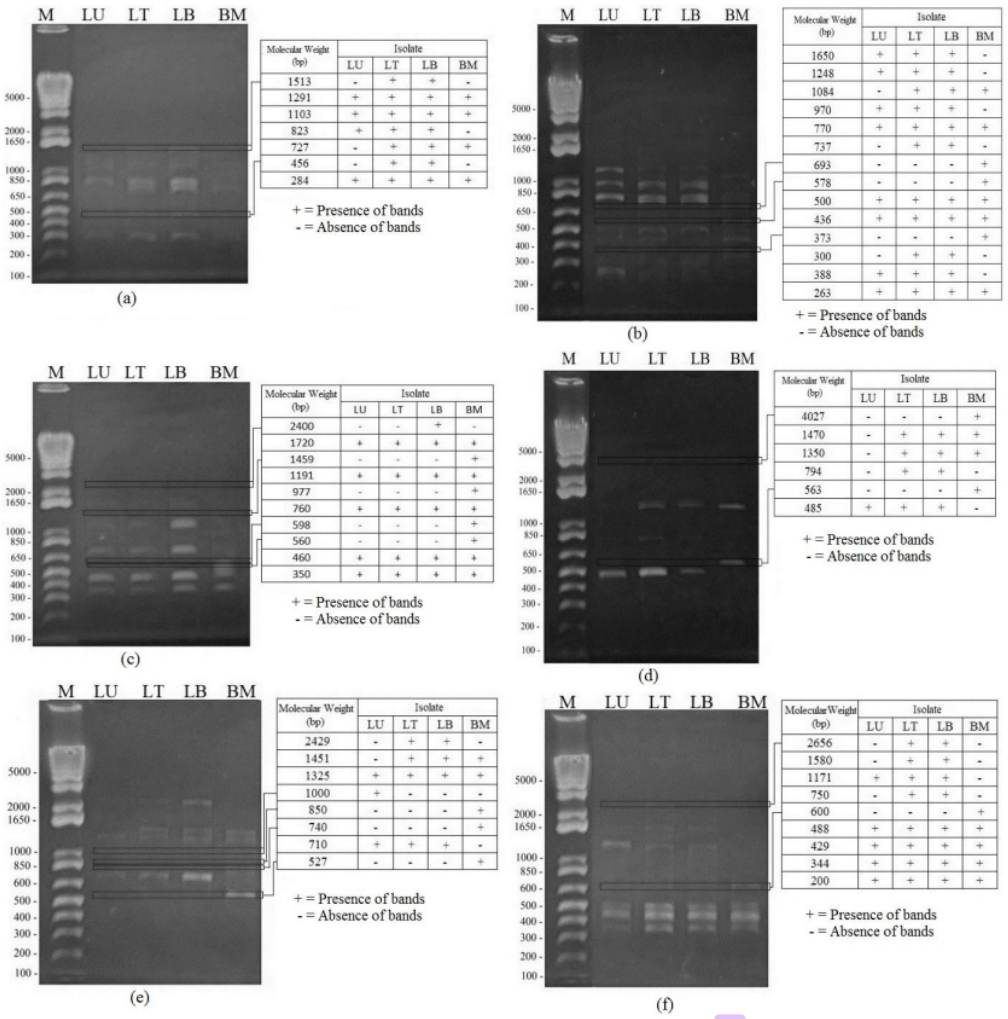

Figure 3. RAPD profiles and scoring of the presence or absence of loci obtained with primers (a) OPA-01, (b) OPA-02, (c) OPA-04, (d) OPA-08, (e) OPA-09, and (f) OPA-18 for selected isolates from North Lombok (LU), Central Lombok (LT), West Lombok (LB), and Bima (BM) against the 1kb ladder (M).

The four *F. solani* isolates from *G. versteegii* were somewhat similar by sharing a number of polymorphic bands generated at the same position using all OPA primers tested. The isolates were also distinguished from one another based on the polymorphic fragments specific to each isolate (Figure 3 & Table 4). Isolate BM showed the highest number of unique polymorphic fragments among all isolates, with a total of 13 unique polymorphic fragments specific to this isolate produced by all tested primers except for the primer OPA-01. Both isolates LB and LU produced an isolate-specific polymorphic fragment using primers OPA-04 and OPA-09, respectively. On the other hand, none of the primers produced any polymorphic fragment specific to isolate LT. The results showed that isolate BM was most unique among all four *F. solani* isolates obtained from *G. versteegii*.

RAPD is the most common technique for detecting the genetic relatedness and diversity of *Fusarium* species at interspecific level (Bonde *et al.*, 2013). Based on the RAPD dendrogram in Figure 4, *F. solani* isolates LB and LT had the highest simple matching coefficient, meaning the two isolates shared the greatest similarity in their DNA

Table 4. Analysis of the RAPD fragments generated using random primers for each *Fusarium solani* isolate from North Lombok (LU), Central Lombok (LT), West Lombok (LB), and Bima (BM).

Primer	OPA-01	OPA-02	OPA-04	OPA-08	OPA-09	OPA-18	Total
Total no. loci	7	14	10	6	8	9	54
No. monomorphic loci	3	4	5	0	1	4	17
No. polymorphic loci	4	10	5	6	7	5	37
No. isolate-specific loci	0	3	5	2	4	1	15
Isolate LU	0	0	0	0	1	0	1
Isolate LT	0	0	0	0	0	0	0
Isolate LB	0	0	1	0	0	0	1
Isolate BM	0	3	4	2	3	1	13
Total no. scored fragments	22	38	25	13	16	26	140
No. scored monomorphic fragments	12	16	20	0	4	16	68
Isolate LU	3	4	5	0	1	4	17
Isolate LT	3	4	5	0	1	4	17
Isolate LB	3	4	5	0	1	4	17
Isolate BM	3	4	5	0	1	4	17
No. scored polymorphic fragments	10	22	5	13	12	10	72
Isolate LU	1	4	0	1	2	1	9
Isolate LT	4	7	0	4	3	4	22
Isolate LB	4	7	1	4	3	4	23
Isolate BM	1	4	4	4	4	1	18

profiles. Isolate LU was clustered with isolate LU was clustered with isolates LB and LT with a similarity index higher than 0.7, while isolate BM was grouped with other isolates with the lowest similarity index. Isolate BM had the most dissimilar DNA profile compared to its counterparts from other localities. Preliminary analysis using the ITS and TEF 1α genes sequences identified all four isolates LB, LT, LU and BM as *F. solani*. However, genomic DNA analysis using RAPD revealed unique DNA profiles for each isolate, with that of isolate BM found to be most dissimilar to the other isolates. The results were not unexpected, as *F. solani* is known to be a species complex with high genetic diversity. Members of this species were clustered into a large group known as *Fusarium solani* species complex (FSSC) (Zhang *et al*., 2006) that could be divided into several formae speciales associated with specific host plants (Šišić *et al.*, 2018). A previous study on *F. solani* suggested that isolates of this species had an average similarity index below 70% for RAPD profile, which indicated high genetic diversity in the isolates of *F. solani* (Younes *et al.*, 2013). Similarly, our study also revealed high DNA variation among the tested *F. solani* isolates.

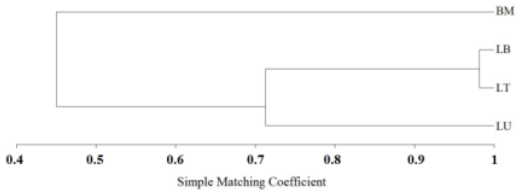

Figure 4. Dendrogram based on RAPD analysis of *Fusarium solani* isolates from West Lombok (LB), Central Lombok (LT), North Lombok (LU) and Bima (BM).

The RAPD-based dendrogram (Figure 4) was slightly different from that generated from morphological data (Figure 2) in terms of dendrogram topology, similarity coefficient and clustering of taxa. Higher similarity coefficients among the isolates LB, LT and LU were observed in the RAPD-based dendrogram. Isolate LT was directly paired with isolate LB in the RAPD-based dendrogram while isolate LT was directly paired with isolate LU in the dendrogram based on morphological characters. However, isolate BM was the last OTU to be clustered with other samples, indicating the isolate to be least similar among the four tested isolates, in both dendrograms generated based on the morphological and

RAPD data. It is important to consider both morphological and molecular data (e.g. RAPD analysis) for identification and characterisation of *Fusarium* (Zakaria *et al*., 2009). Different types of data could be analysed together to provide complementary information for inferring the taxonomic relationship among the fungal isolates with increased discriminating power (Wangiyana, 2019b). Thus, the comparison of dendrograms based on morphological characteristic and RAPD would be useful for genetic diversity study (Martinello *et al*., 2001).

Limitation in this study mainly concerns the considerably small sample size and random primers used which would affect the cluster analysis and thus the topology of dendrogram. Cluster analysis using the RAPD-PCR data was highly dependent on the polymorphic fragments, therefore robust screening for amplification conditions and random primers that result in reproducible banding patterns and a higher number of polymorphic loci is important (Bahmani *et. al.*, 2012; Younes *et al*., 2013). Further study using RAPD analysis on *F. solani* from *G. versteegii* with increased sample size by including more sampling sites and samples within a locality would reveal useful loci that can differentiate the *F. solani* populations and provide more insights into the diversity of *F. solani* in West Nusa Tenggara.

IV. CONCLUSION

We concluded that molecular characterisation by RAPD analysis and morphological characterisation could complement each other to study the underlying diversity of isolates considered as *F. solani* in a polyphasic approach.

V. ACKNOWLEDGEMENTS

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