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Molecular identification of mango malformation pathogens in Egypt

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ABSTRACT

Diagnostic tests by molecular biology is made for studying the relations among *Fusarium* species for linking production of proteins, degree of relationship and occurrence of malformation. Determination of proteins for isolates causing-disease by SDS-PAGE explained there's specific band for each fungus and there are common bands among some isolates of fungi. Since, band with MW 30 KDa represented only in *F. proliferatum* and *F. oxysporum* and *F. subglutinans* respectively. This band considered as specific band for these isolates, which released high pathogenisity effect. RAPD-PCR markers were used to discriminate variations between *Fusarium* isolates and causing disease. There is specific band for each fungus which act as molecular marker for each fungus and there are some bands common among some isolates of pathogenic fungi. The dendrogram shows there is degree of relationship between *F. sterilihyphosum* and *F. proliferatum*; between *F. moniliforme* and *F. subglutinans*; between *F. oxysporum* and *F. chlamydospore;* the degree of relationship among *F. subglutinans, F. proliferatum* and *F. sterilihyphosum* and degree of relationship among *F. moniliforme, F. sterilihyphosum, F. proliferatum* and *F. subglutinans*

***Keywords*:** *Fusarium* Spp.; Mango Malformation

1. INTRODUCTION

Mango (*Mangifera indica* L.) is the most important fruit grown in tropical and subtropical region of the world. Mango is the most important fruit crop in Egypt. Mango malformation is one of the most destructive mango diseases [1]. Losses due to malformation have not been accurately assessed because yield loss is not a linear function of disease severity [2]. A number of *Fusarium* species has been reported to be associated with the malformation disease of mango [3]. Some *Fusarium* species especially those insection Liseola and their allied, identification process based solely on morphological characteristics are not always convincing and still incomplete and inconclusive. Therefore, molecular characterization can be used as additional criteria for species characterization and identification. Genetic diversity was examined among 74 *F. subglutinans*—like isolates from malformed mango in Brazil, Egypt, Florida (USA), India, Israel and South Africa. With nitrate-non-utilizing (nit) auxotrophic mutants, seven vegetative compatibility groups (VCGs) were identified. Three of the VCGs were found in a single country, and VCG diversity was greatest in Egypt and the USA where, respectively, four and three different VCGs were found. RAPD profiles generated with arbitrary decamer primers were variable among isolates in different VCGs, but were generally uniform for isolates within a VCG. In PCR assays, a 20-mer primer pair that was developed previously to identify *F. subglutinans* from maize (mating population (MP-E) of the Gibberella fujikuroi complex) also amplified a specific 448 bp fragment for isolates of *F. sacchari* from sugarcane (MP-B) and what was probably *F. circinatum* (pine, MP-H). With the exception of three isolates from Brazil, it did not amplify the fragment from *F. subglutinans*—like isolates from mango. A second pair of 20-mer primers was developed from a unique fragment in the RAPD assays. It amplified a specific 608 bp fragment for 51 of 54 isolates from mango (all but the three Brazilian isolates). It also amplified a smaller, 550 bp fragment from isolates of *F. nygamai* (MP-G), but did not amplify DNA of isolates of any other toxin of *Fusarium* that was tested [4].

A wild-type isolate of *F. subglutinans* causing mango malformation disease was transformed with the GUS (B glucuronidase) reporter and hygromycin resistance genes. Five stable transformants were isolated containing varying copy numbers at different integration sites. Specific

GUS activity was quantified for the transformants, whereas no activity was recorded for the wildtype isolate. The transformants and the wild-type isolate were inoculated into healthy mango floral and vegetative buds. Typical symptoms of misshapen shoots with short internodes, stub by leaves, and bunchy, malformed inflorescences were observed 6 to 8 weeks following inoculation. The presence of GUS—stained mycelium of the pathogen viewed microscopically within infected plant organs provided unequivocal evidence that *F. subglutinans* is indeed a causal agent of mango malformation disease [5].

According to PCR—specific primer amplification, the pathogen was detected in 97% of seedling apical meristems, declining gradually to 5% colonization in roots. It was concluded that inoculum of the pathogen originates from infected panicles and affects seedlings from the meristem, with infections descending to lower stem sections and roots. Minor infections of roots may occur from inoculum originating from infected panicles, but the pathogen is not seed borne [6]. In order to characterize molecularly the etiological agent of mango floral and vegetative malformation in Brazil AFLPs, sequence analysis and assays were used. The AFLP patterns of the majority of isolates collected in Brazil were different from *Fusarium mangiferae* and *Fusarium sterilihyphosum*, two previously described *Fusarium* species associated with mango malformation. The cluster analysis of AFLP data using Dice coefficient produced a network where *Fusarium* spp. from Brazil were in one group apart from two other groups represented by isolates of *F. sterilihyphosum* from Brazil and South Africa, and by isolates of *F. mangiferae* from Egypt, India, South Africa and USA, respectively. *Fusarium* spp. from Brazil was compared with 24 species of the Gibberella fujikuroi complex (GFC) using AFLP data and showed to be a distinctive species. Sequence analyses of portions of amp; #946—tubulin and EF-1amp and #945; were used to elucidate the phylogenetic relationships between *Fusarium* spp. from Brazil and the species of the GFC. Maximum parsimony analyses grouped this *Fusarium* spp. in the American clade, but within a distinct subgroup which indicates a different species close related to *F. sterilihyphosum*. These species are not easily separated when only morphological characters are used, but can be distinguished through AFLP patterns, fertility and sequence analyses [3].

Thus, objective of the present study is to molecular characterization of *Fusarium* spp. to identify the mango malformation pathogens in Egypt

2. MATERIALS AND METHODS

Seven *Fusarium* specie *i.e. F. oxysporium*, *F. proliferatum*, *F. subglutinans*, *F. sterilihyphosium*, *F. monilifrme* and *F. Avenaceum* isolated from malformed mango blossom tissue were tested for their ability to cause malformation. Mango seedling cv. Sedekia (two years old) was inoculated with 10^5 colony forming units of *Fusarium* spp. as inoculated soil. Four replications of six seedlings each were evaluated. Sterilized water was used as a control. Transplanted seedlings were monitored for development of malformation. At the end of the experiment (120 days), all surviving seedlings were examined for apical disease symptoms. Data were recorded on symptoms manifestation as diseases incidence and severity. The isolates were cultured on PDA overlaid with four pieces of sterile-osmosis membrane for seven days under the standard growth conditions. Approximately 100 mg of mycelium were used for protein and DNA extractions

2.1. SDS-PAGE Analysis of Total Protein

Protein extraction: protein was extracted from *Fusarium* isolates according to Reuveni, *et al.* [7] with some modified. Harvest and rapidly wash the cell once with 0.1 M NaCl, then resuspended the cell pellet with lyses solution, which included of 100 mM Tris-HCL (PH 8.0), 5% (vol/vol) glycerol, 2 mM EDTA, 2% SDS, 5% sucrose. Then place the tube on ice for 3 min. and rapidly in water bath at 100˚C for 3 min. repeated that tree times. Examine the suspension by microscopy to accretion that breakage has occurred. If not, quickly freeze and boil the sample again. Centrifuge at high speed under cooling. Remove the supernatant to another tube.

2.1.1. Gel Preparation

Sodium dodocylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 12.5% acrylamide and 8% bis acrylamide running gel (65 mm × 70 mm) consisting of 0.375 M Tris-HCl (pH 8.8) and 0.1% SDS. Stacking gels (10 mm) were made using 4.5% acrylamide containing 8% bis-acrylamide in 0.125 M Tris-HCl (pH 6.8) and 0.1% SDS. The electrophoresis buffer contained 0.025 M Tris-HCl, 0.19 glycine and 0.1% SDS. The samples were homogenized in 0.12 M Tris-HCl (pH 6.8), 0.4% SDS, 10% β-merkaptoethanol, 0.02% bromophenol blue, and 20% glycerol. The samples were then heated for 3 min. in a boiling water bath before centrifugation. The gels were run under cooling at 90 V for the first 15 min, then 120 V for the next 0.5 h. and finally 150 V for the remaining 1.5 h.

2.1.2. Sample Loading

A volume of 15 µl protein sample was applied to each well by micropipette. Control wells were loaded with standard protein marker.

2.1.3. Electrophoresis Conditions

Four liters of running buffer were poured into the running tank to be pre-cooled (4˚C). Eight hundred ml of running buffer was added in the upper tank just before

running so that the gels were completely covered. The electrodes were connected to power supply and adjust at 100 V until the bromophenol blue dye entered the resolving gel, and then increased to 250 V until the bromophenol blue dye reached the bottom of the resolving gel. The small triangle of one corner gel was marked so the orientation is not during staining.

2.1.4. Gel Staining and Destaining

After the completed of the run, gel was placed in staining solution consisting of (1 g Coomassie Brillint blue-R-250; 455 ml Methanol; 90 ml Acetic acid glacial and up to 1 L with D.D.W.), and detained with 200 ml destaining solution and agitated gently on shaker. The distaining solution was changed several times until the gel background was clear.

2.1.5. Gel Analysis

Gels were photographed using a Bio-Rad gel documentation system. Data analysis was obtained by Bio-Rad Quantity one software version 4.0.3.

2.1.6. Native Gel Preparation

The methods described by Stegemann *et al*. [8] 30% Acrylamide: 29.2 g Acrylamide, 0.8 g N,N-methylene bisacrylamide were dissolved 100 ml H_2O (dd.). 2% ammonium persulphate: 0.25 g ammonium persulphate was dissolved with 10 ml H_2O (dd.). This stock must be prepared immediately before use. Buffer solution: this Borate buffer (pH 8.9) was used for Isozymes analysis. The stock solution was composed of 605 g tris and 46 g boric acid dissolved in 5000 ml H_2O (dd.). Electrode buffer: (0.125 M, pH 8.9) was prepared by dilution of 300 ml of the stock solution with 2100 ml H_2O (dd.).

2.1.7. Gel Preparation

35 ml of 30% Acrylamide was added with 70 ml (0.125 M, pH 8.5) dilute buffer to get 8% Acrylamide, 33 mg sodium sulphate (dissolve completely) 66 ml TEMED (teteramethylenediamine) and 2.5 ml ammonium persulphate the gel solution was quickly poured immediately and 15 well combs were used, then gels were left for about 30 minutes for polymerization

2.2. Molecular Genetic Study (RAPD-PCR) A-DNA Extraction

DNA isolation was performed using the CTAB method of Doyle and Doyle [9]. 0.5 g fresh sample from *Sesamum indicum* was ground to powder in liquid nitrogen with a prechilled pestle and mortar, suspended in 1 ml preheated CTAB buffer, and incubated at 65˚C for 1 h with occasional shaking then centrifuge for 10 min at 1000 rpm. Transfer the supernatant to a new tube by wide pore, add 0.5 ml of (chloroform: isoamylol) 24:1 then centrifuge for 15 minutes at 14,000 rpm and the aqueous layer was transferred to a new sterilized tube (avoid protein surface). The ice cold isopropanol was added to precipitate the nucleic acid (RNA, DNA) then Incubate at −20˚C overnight and centrifugation was happened at 14,000 rpm for 20 minutes. The supernatant was discard and the pellet was washed carefully twice with cold 70% ethanol, dried at room temperature and resuspend in 100 µl of sterile deionized distilled water. DNA concentration was determined by electrophoresis of 5 µl of DNA with 2 µl of loading buffer and run at 100 V for approximately 30 minutes.

2.3. B-RAPD Analysis

RAPD was performed as described by Williams *et al.* [10] with minor modifications. Briefly, PCR amplification was performed in 25 µl reaction mix (**Tables 1-3**) containing 20.40 ng genomic DNA, 0.5 unit Taq polymerase (Sigma), 0.2 mM each of dATP, dCTP, dGTP and dTTP, 5 Pico mole random primer and appropriate amplification buffer. The reaction was assembled on ice, overlaid with a drop of mineral oil. Amplification was performed for 45 cycles (**Table 2**) using Biometera Uno thermal cycler, as follows: One cycle at 95˚C for 3 minutes and then 44 cycles at 92˚C for 2 minutes, 37˚C for 1 minute and 72˚C for 2 minutes (for denaturation, annealing and extension, respectively). Reaction was finally incubated at 72˚C for 10 minutes and further incubated on 4˚C .Five primers were used for RAPD analysis based on their ability to amplify *Amaransis* genome and producing reproducible amplification patterns (**Table 4**).

2.4. C-Agarose Electrophoresis

The amplification products were analyzed by electrophoresis in 2% agarose in TAE buffer stained with 0.2 µg/ml ethidium bromide and photographed under UV light. The buffer was added to the agarose then heated in a microwave tell melting, cooling to 60˚C then the ethia microwave tell melting, cooling to 60˚C then the ethidium bromide was added. Sample was prepared by using

Table 1. Components of RAPD-PCR mixture.

Reagent	Concentration	Volume
d NTP_S	0.2 mM	2.5 µl
PCR buffer	10×	5 µl
Ampli Taq polymerase (RTS Taq DNA polymerase).	2 Units	0.25 µl
$MgCl_2$		1.5 µl
Primer	5 p mole	3 µl
Distilled sterile water	-	9.75 µl
Total genomic DNA	20.40 ng	3 µl
Total volume	-	**25 µl**

Table 2. PCR program (temperature profile).

Order	Action	Temperature	Duration	No. of cycles
1	1stDenaturation	95˚C	3 minutes	1 cycle
2	Denaturation	92˚C	2 minutes	
3	Annealing	37˚C	1 minutes	44 cycles
4	Extension	72˚C	2 minutes	
5	Last extension	72˚C	10 minutes	1 cycle
6	Incubation	4˚C		

Table 3. Composition of stocks.

Stock	Composition
CTAB	1.4 M Nacl, 0.2% β-mercaptoethanol, 100 mM Tris-HCl and 20 mM EDTA
50× Tris-Acetate Buffer	242 g Tris-base, 57.1 ml Glacial acetic acid and 100 ml EDTA (0.5 M, pH 8.0)
Loading buffer	0.25 g bromophenol blue and 100 ml Glycerol (30%)
Ethidium bromide	**0.2 µg/ml ethidium**

Table 4. Name and sequences of the selected random primers used in RAPD-PCR analysis and make amplification.

Primer code		Nucleotide sequences (5 - 3)
1-	**A1**	CAGGCCCTTC
2-	**A3**	AGTCAGCCAC
3-	**A4**	AATCGGGCTG
4-	**B1**	GTTTCGCTCC
5-	**B4**	GGACTGGAGT
6-	**G2**	GGCACTGAGG
7-	**Z1**	TCTGTGCCAC
8-	**G3**	GAGCCCTCCA
9-	**Z3**	CAGCACCGCA
10-	**A2**	TGCCGAGCTG

Name of primers that did not make amplification G2, B4, B1, A1, A4.

10 µl PCR-product and 2 µl loading buffer. One marker was used, 100 bp DNA ladder (Axygen).

3. RESULTS AND DISCUSSION

Seven fungi viz. *F. subglutinans*, *F. oxyspoum, F. sterilihyphosum*, *F. proliferatum*, *F. moniliforme*, *F.avena* and *F. chlamydspore* isolated from mango malformed tissue were tested using susceptible Sadekia cultivar as inoculated soil (**Table 5**). Data pertaining to artificial inoculations revealed that effort to produce disease by soil inoculation with spores suspension. Four *Fusarium subglutinans* proved to be the dominant fungus with 100% sample's infection in inoculated soil. Fungi *F. oxysporum*, *F. sterilihyphosum* and *F. proliferatum* showed moderate infection in induced typical malformation symptoms in inoculated mango seedling and were re-isolated . Other *Fusarium* spp. give grown and root rots symptoms.

3.1. Molecular Characterization of *Fusarium* Isolates

Molecular characterization of the eight *Fusarium* isolates was carried out using sodium dodocyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

Table 5. Comparative virulence of selected *Fusarium* isolates on inoculated mango cv. Sedekia seedlings.

Tested isolates	Infested soil with spore suspension	
	Disease incidence %	Disease severity
F. subglutinans	100.0 a	4.0 a
F. oxyspoum	50.0 b	1.3 c
F. sterilihyphosum	50.0 b	2.3 b
F. solani	0.0 c	0.0 d
F. avenaceum	0.0 c	0.0 d
F. chlamydspore	0.0 c	0.0 d

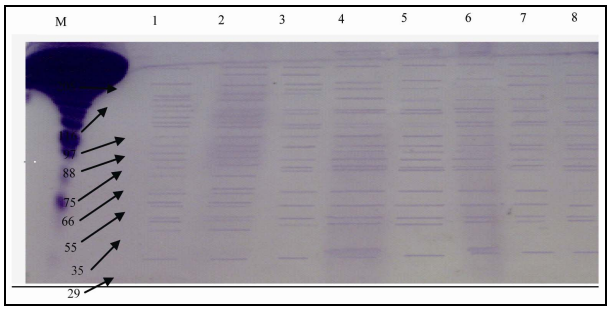

Figure 1. SDS-PAGE of total proteins extracted from eight *Fusarium* isolates (1 = *F. proliferatum*, 2 = *F. oxysporum*, 3 = *F. solani*, 4 = *F. chlamydsporium*, 5 = *F. moniliforme*, 6 = *F. sterilihyphosum*, 7 = *F. avenaceum*, 8 = *F. subglutinans*, M refers to protein stander).

3.2. SDS-PAGE Analysis of Total Protein

Total proteins were separated by SDS-PAGE. **Figure 1** shows the electrophoretic pattern of *Fusarium* isolates. The maximum number of the bands was twenty-six, as shown in **Table 6**. The molecular weight of the bands obtained with SDS-PAGE ranged from 14 to 215 KDa. Most band are considered as common band exept bands with molecular weight (MW) 215 and 210 KDa which represented only in three isolates , *F. chlamydsporium*, *F. moniliforme and F. sterilihyphosum* respectively. As they appeared only in two isolates *F.proliferatum* and *F. oxysporum* respectively bands with MW 100, 88, 50, 30 and 20. These bands are not found in the rest of isolates and considered as potential marker associated with theses isolates and their pathogenisty. While band with MW 30 KDa represented only in *F. proliferatum*, *F. oxysporum* and *F. subglutinans* respectively. This band considered as specific band for these isolates, which released high pathogenisity effect.

Table 6. SDS-PAGE protein banding pattern extracted from eight *Fusarium* isolates.

No.	MW	1	2	3	4	5	6	7	8
1	215	–	–	–	+	+	+	–	–
2	210	–	–	–	+	+	+	–	–
3	205	+	+	+	+	+	+	+	+
4	200	–	+	+	–	–	–	–	–
5	116	+	+	+	+	+	+	+	+
6	115	–	–	+	+	+	+	+	+
7	110	+	+	+	–	–	–	–	–
8	100	+	+	–	–	–	–	–	–
9	97	+	+	+	+	+	+	+	+
10	88	+	+	–	–	–	–	–	–
11	80	+	+	+	+	+	+	+	+
12	75	+	+	+	+	+	+	+	+
13	66	+	+	+	+	+	+	+	+
14	60	+	+	–	+	+	+	+	+
15	55	+	+	+	+	+	+	+	+
16	50	+	+	–	–	–	–	–	–
17	45	–	–	+	+	+	+	+	+
18	40	–	–	+	+	+	+	+	+
19	35	+	+	–	–	–	–	–	+
20	30	+	+	–	–	–	–	–	–
21	29	+	+	+	+	+	+	+	+
22	26	+	+	+	+	+	+	+	+
23	25	–	–	+	+	+	+	+	+
24	20	+	+	–	–	–	–	–	–
25	16	–	–	–	+	–	+	–	–
26	14	+	+	+	+	+	+	+	+

MW = Molecular weight; + = Presence of band.; – = Absence of band. From 1 = *F. proliferatum*, 2 = *F. oxysporum*, 3 = *F. solani*, 4 = *F. chlamydsporium*, 5 = *F. moniliforme*, 6 = *F. sterilihyphosum*, 7 = *F. avenaceum*, 8 = *F. subglutinans*, respectively.

3.3. Molecular Genetic Study (RAPD-PCR)

In the present study RAPD-PCR markers were used to discriminate variations between *Fusarium* isolates. RAPD would be the markers of choice, since it offers the advantages of being technically undemanding, use no radioactivity or polyacrylamide. Furthermore, RAPD-markers tend to reside in regions with many repeated sequences and their fore in non coding regions, which are more susceptible to mutations. Consequently, they usually reveal more polymorphism compared with isozymes or RFLPs, which are mostly representative of conserved genome regions [10]

3.4. RAPD-PCR Using G3 Primer

The results of RAPD analysis using primer G3 are illustrated in **Figure 2** and **Table 7** for all *Fusarium* isolates. The maximum number of bands by this primer was nine. The molecular weight of the PCR products generated by this primer ranged from 90 to 950 base paires (bp). Only band with MW 600 bp could be considered as common band. Bands with MW 950, 870 and 350 bp are considered as specific bands or positive markers of *Fusarium* isolates *i.e. F. oxysporum*, *F.subglutinans*. While the band with MW 90 bp could be considered as specific marker for *F. moniliforme*. The disappearance of the band with MW 250 bp could considered as negative marker to *F. chlamydsporium.*

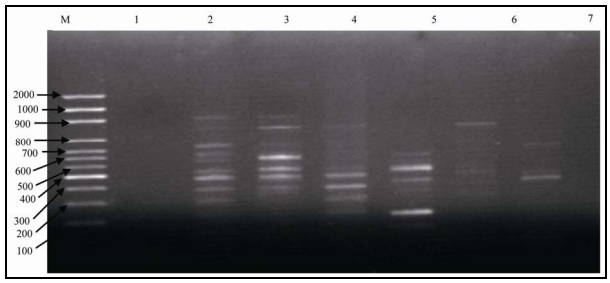

Figure 2. DNA polymorphesim based on RAPD-PCR analysis of the seven *Fusarium* isolates against the primer G3 (1 = *F. proliferatum*, 2 = *F. oxysporum*, 3 = *F. subglutinans*, 4 = *F. chlamydsporium*, 5 = *F. moniliforme*, 6 = *F. sterilihyphosum*, 7 = *F. avenaceum*, M refers to ladder DNA stander marker).

Table 7. RAPD profiles of the *Fusarium* isolates using primer G3.

No.	MW	1	2	3	4	5	6	7
1	950	–	+	+	–	–	–	–
2	870	–	+	+	+	–	–	–
3	750	–	+	+	+	–	–	–
4	600	–	+	+	+	+	+	+
5	550	–	+	+	–	+	–	–
6	350	–	+	+	–	–	–	–
7	250	–	+	+	–	+	+	+
8	150	–	+	+	+	+	–	–
9	90	–	–	–	–	+	–	–

MW = Molecular weight; + = Presence of band.; – =Absence of band. From 1 to 7 = *F. proliferatum*, *F. oxysporum*, *F. subglutinans*, *F. chlamydsporium*, *F. moniliforme*, *F. sterilihyphosum*, *F. avenaceum* respectively.

3.5. RAPD-PCR Using Z3 Primer

The results of RAPD analysis using primer Z3 are illustrated in **Figure 3** and **Table 8** for all *Fusarium* isolates. The maximum number of bands by this primer was eight. The molecular weight of the PCR products generated by this primer ranged from 100 to 800 base paires (bp). Only band with MW 500 bp could be considered as common band. Bands with MW 800 and 700 bp are considered as specific bands or positive markers of *Fusarium* isolates *i.e. F. oxysporum* and *F. subglutinans*. While the band with MW 100 bp could be considered as specific marker for *F. proliferatum* and *F. moniliforme*. The disappearance of the band with MW 200 and 300 bp could consider as negative marker to *F. avenaceum*.

3.6. RAPD-PCR Using A2 Primer

The results of RAPD analysis using primer A2 are illus-

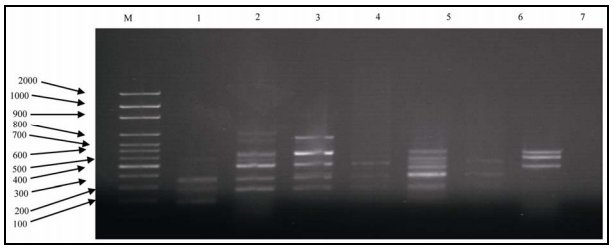

Figure 3. DNA polymorphesim based on RAPD-PCR analysis of the seven *Fusarium* isolates against the primer Z3 (1 = *F. proliferatum*, 2 = *F. oxysporum*, 3 = *F. subglutinans*, 4 = *F. chlamydsporium*, 5 = *F. moniliforme*, 6 = *F. sterilihyphosum*, 7 = *F. avenaceum*, M refers to ladder DNA stander marker).

Table 8. RAPD profiles of the *Fusarium* isolates using primer Z3.

No.	MW	1	2	3	4	5	6	7
1	800	–	+	+	–	–	–	–
2	700	–	+	+	–	–	–	–
3	600	–	+	+	–	+	–	+
4	500	+	+	+	+	+	+	+
5	400	–	+	+	+	+	+	+
6	300	+	+	+	+	+	+	–
7	200	+	+	+	+	+	+	–
8	100	+	–	–	–	+	–	–

MW = Molecular weight; + = Presence of band.; – =Absence of band. From 1 to 7 = *F. proliferatum*, 2 = *F. oxysporum*, 3 = *F. subglutinans*, 4 = *F. chlamydsporium*, 5 = *F.moniliforme*, 6 = *F.sterilihyphosum*, 7 = *F. avenaceum*, respectively.

trated in **Figure 4** and **Table 9** for all *Fusarium* isolates. The maximum number of bands by this primer was nine. The molecular weight of three PCR products generated by this primer ranged from 100 to 900 base paires (bp). Band with MW 800 bp is considered as specific bands or positive marker of *F. subglutinans*. While the band with MW 600 and 400 bp could be considered as specific markers for *F. oxysporum*. Bands with MW 700 and 200 bp are considered as specific bands or positive marker of *F. moniliforme* and *F. proliferatum*. The disappearance of the band with MW 250 bp could considered as negative marker to *F. chlamydsporium*.

3.7. The Relationship between *Fusarium* Isolates

The dendrogram shows in **Figure 5**, there is degree of relationship between *F. sterilihyphosum* and *F. proliferatum*; between *F. moniliforme* and *F. subglutinans*; between *F. oxysporum* and *F. chlamydospore*; the degree of relationship among, *F. proliferatum* and *F. sterilihyphosum* and degree of relationship among F. moniliforme, *F. sterilihyphosum*, *F. proliferatum* and *F. subglutinans*. These species are not easily separated when only

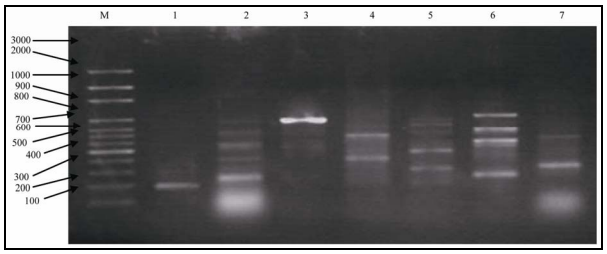

Figure 4. DNA polymorphesim based on RAPD-PCR analysis of the seven *Fusarium* isolates against the primer A2 (1 = *F. proliferatum*, 2 = *F. oxysporum*, 3 = *F. subglutinans*, 4 = *F. chlamydsporium*, 5 = *F. moniliforme*, 6 = *F. sterilihyphosum*, 7 = *F. avenaceum*, M refers to ladder DNA stander marker).

Table 9. RAPD profiles of the *Fusarium* isolates using primer A2.

No.	MW	1	2	3	4	5	6	7
1	900	–	–	–	–	–	+	–
2	800	–	–	+	–	–	–	–
3	700	+	–	–	–	+	–	–
4	600	–	+	–	–	–	–	–
5	500	–	+	–	+	–	–	+
6	400	–	+	–	–	–	–	–
7	300	+	+	–	–	–	+	–
8	200	+	–	–	–	+	–	–
9	100	–	+	–	–	–	–	+

MW = Molecular weight; + = Presence of band.; – =Absence of band. From 1 to 7 = *F. proliferatum*, 2 = *F. oxysporum*, 3 = *F. subglutinans*, 4 = *F. chlamydsporium*, 5 = *F. moniliforme*, 6 = *F. sterilihyphosum*, 7 = *F. avenaceum*, respectively.

morphological characters are used. Therefore, molecular characterization can be used as additional criteria for species characterization and identification. In order to identify and characterize molecularly the etiological agent of mango floral and vegetative malformation, SDS-PAGE of total proteins and RAPD-PCR assays were used. The RAPD-PCR technique has been used successfully by the Tree Pathology. SDS-PAGE of total proteins of portions and RAPD-PCR were used to elucidate the phylogenetic relationships between *Fusarium* species [4,11,12]. In many ways, molecular approaches are more easier and can provide results that are less ambiguous and the most important criteria is the same observations can be made between different researchers, as compared to morphological approach.

RAPD banding patterns showed similarity and variations between the seven *Fusarium* species isolated from mango infected plants. Since, relationship between *F. sterilihyphosum* and *F. proliferatum*; between *F. moniliforme* and *F. subglutinans*; between F.oxysporum and *F. chlamydospore*; the degree of relationship among, *F.*

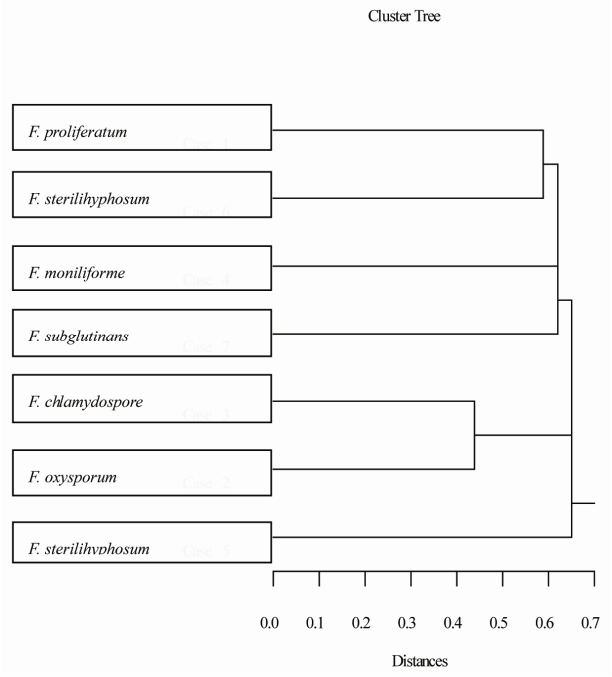

Figure 5. The dendrogram that release the relation ship between the seven *Fusarium* isolates.

proliferatum and *F. sterilihyphosum* and degree of relationship among F. moniliforme, *F. sterilihyphosum*, *F. proliferatum* and *F. subglutinans* were found. In addition, these bands are not found in the rest of isolates and considered as potential marker associated with theses isolates and their pathogenisty.

Results of RAPD analysis can be used to differentiate isolates of pathogenic *Fusarium* species of mango malformation namely *F. subglutinans*, *F. sterilihyphosum*, *F. oxysporum* and *F. proliferatum*. The Polymorphism of RAPD profiles have also been observed in various isolates of *Fusarium* species such as *F. moniliforme*, *F. subglutinans*, *F. oxysporum* and *F. proliferatum* [13,14].

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