

Genotypic Identification for Some *Fusarium sambucinum* Strains Isolated from Wheat in Upper Egypt

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Abstract: Random amplified polymorphic DNA (RAPD) was used to study the genetic variations among strains from *Fusarium sambucinum* isolated from wheat in Upper Egypt. Using two different primer (V6 and M13), the used strains showed a high degree of similarities and a distinct RAPD patterns. The dendrogram that constructed based on M13 primers showed that, there is no correlation between isolation sources and clustering system for the studied strains.

Key words: Phylogenetic dendrogram • phylogenetic • PCR • RAPD

INTRODUCTION

The taxonomy of *Fusarium* spp. is confusing and various classification systems have been proposed [1]. Species identification by morphological traits is problematic because characteristics like mycelial pigmentation, formation, shape and size of conidia are unstable and highly dependent on composition of media and environmental conditions. Phenotypic variation is abundant and many expertise are required to distinguish between closely related species and to recognize variation within species [2].

Some species of fungi need more experiences during their identification by classical methods. Right now with this revolution in the molecular techniques by using polymerase chain reaction (PCR) techniques, those problematic strains did not need so much effort to do identify well. Random PCR approaches are being increasingly used to generate molecular markers, which are useful for taxonomy and for characterizing fungal populations. Random amplified polymorphic DNA (RAPD) assay have been used extensively to define fungal populations at species, intraspecific, race and strain levels.

The use of molecular markers based on the polymerase chain reaction for species identification and as diagnostic tool became very popular during the last decade [3]. Once the primers are designed and conditions

for a robust assay are optimized, PCR is very sensitive, rapid and relatively easy to handle assay. Welsh and McClelland [4] described RAPD-PCR technique for detecting genetic variation among different organisms. Genetic variability is assessed by employing short single primer of arbitrary nucleotide sequences. Specific sequence information of the organism under investigation is not required and amplification of genomic DNA is initiated at target sites which are distributed throughout the genome. Polymorphic fragments are the results of variation in the number of appropriate primer-matching sites of different DNAs. Nijs *et al.* [5] studied variation in random amplified polymorphic DNA patterns within *Fusarium* species from cereals from various parts of the Netherlands. Gherbawy [6] used RAPD technique to analyse different formae specialis of *Fusarium oxysporum*. Möller *et al.* [7] studied fungal populations of *F. moniliforme* and *F. subglutinans* using RAPD technique. Gherbawy *et al.* [8] used RAPD technique for identifying of *Fusarium subglutinans*, *F. proliferatum* and *F. verticillioides* strains isolated from maize in Austria. Pasquali *et al.* [9] characterized isolates of *Fusarium oxysporum* pathogenic on *Argyranthemum frutescens* L. using RAPD technique.

An objective of the present study was to determine possible phylogenetic relationships among 15 representative strains of the species *Fusarium sambucinum* isolated from wheat in upper Egypt.

Table 1: List of *Fusarium sambucinum* strains (isolated from wheat plants on two different types of media) used for RAPD-PCR analysis

Strains No.	<i>Fusarium sambucinum</i> strains	Sources of isolation	Media used for isolation
1	SVUML178	Rhizoplane	DCPA*
2	SVUML230	Rhizosphere	DCPA
3	SVUML255	Rhizosphere	DCPA
4	SVUML283	Rhizoplane	DCPA
5	SVUML300	Rhizoplane	DRBA**
6	SVUML303	Rhizoplane	DRBA
7	SVUML310	Soil	DCPA
8	SVUML320	Rhizoplane	DCPA
9	SVUML406	Soil	DCPA
10	SVUML420	Rhizoplane	DCPA
11	SVUML433	Soil	DRBA
12	SVUML440	Soil	DCPA
13	SVUML459	Soil	DCPA
14	SVUML491	Rhizoplane	DCPA
15	SVUML601	Rhizosphere	DCPA

DCPA* Dichloran chloramphenicol pepton agar

DRBA** Dichloran rose- bengal chloramphenicol agar

MATERIALS AND METHODS

Strains: Fifteen strains from *Fusarium sambucinum* were used in the present study. These strains were previously isolated from wheat plant in Upper Egypt by using dichloran chloramphenicol pepton agar (DCPA) and dichloran rose-bengal chloramphenicol agar (DRBA) media (Table 1).

DNA extraction: Fungal strains were cultured in 100 mL Erlenmeyer-flasks containing 20 mL Mandles Andreotti medium (per liter 10 g glucose; 2 g peptone; 2.8 g ammonium sulphate; 4 g KH_2PO_4 ; 10 g Na_2HPO_4 ; 10 mL of a simplified Czapek's conc.; 7 g MgSO_4 ; 0.05 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; 0.1 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 0.1 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; final pH adjusted to 5) for 5 days using a rotary shaker (30°C, 150 rpm). The mycelium was collected by filtration and ground to fine powder in liquid N_2 . Fifty milligram of the ground was transferred to a 1.5 mL Eppendorf tube and mixed with 0.7 mL 2 x CTAB buffer. Eppendorf tubes were incubated at 65°C for 30 min, then 0.7 mL of chloroform was added and mixed briefly. After centrifugation at 15,000 rpm for 30 min, the supernatant was transferred into a new tube mixed with 0.6 mL isopropanol and chilled to 20°C, followed by another centrifugation step for 5 min at maximum speed. The supernatant was discarded and the remaining pellet was twice washed with 1 mL of 70% ethanol, followed by drying under vacuum and thereafter dissolved in 1 mL TE (10 mM Tris, 1 mM EDTA, pH 7.5) buffer. DNA concentration were evaluated by agrose gel electrophoresis [10].

RAPD analysis: PCR conditions and separation of RAPD-PCR fragments were done according to the techniques of Messner *et al.* [11]. PCR's were carried out with the aid of primer V₆(5'DTGCAGBTGG; [12]) and M₁₃(GAGGGTGGCGGT-TCT; [13]). PCR amplifications were performed in 50 μL volumes containing 1-1.5 unit Taq DNA polymerase (Biotherm, Gene Craft, Germany) dNTP mix (0.2 mM each of dCTP, dGPT, dATP and dTTP); 20 mM Tris-HCl (pH 8.4); 50 mM MgCl_2 ; 0.5 mM primer and 15-20 ng of genomic DNA. Amplification was performed in a thermalcycler (Flexigene, Techne, Cambridge, UK) with the following temperature profiles: 98°C for 5 min to denature genomic DNA. There were 40 cycles at 98°C for 15 sec; annealing at 40°C for 90 sec and extension at 72°C for 100 sec, followed by an additional cycle at 72°C. the PCR product were resolved by electrophoresis on 1.4% agarose gel in 0.5 X Tris-Borate-EDTA (TBE) buffer, at 125 V for 2 h. Gels were stained with ethidium bromide and photographed under UV light using UVP BioImaging CDS 8000 system (UVP).

RAPD data analysis: Computer analysis of RAPD patterns were performed as described by Halmschlager *et al.* [14], in which the band pattern obtained from agarose gel electrophoresis was digitalized by hand to a two discrete-character-matrix (0 and 1 for absence and presence of RAPD-bands, respectively). The analysis data was based on the Nei and Lee Coefficient [15]. Dandograms were constructed by the unweighted paired group method of arithmetic average (UPGMA) based on Jaccard's similarity coefficient by using Phoretex ID software (version 5.2).

RESULTS AND DISCUSSION

Two different primers were used V₆ (5'dTGCAGCGTGG; [12]) and M₁₃ (5' dGAGGGTGGC GGTTCT; [13]) to analysis genetic variations among 15 strains of *Fusarium sambucinum*. The used primers (V₆ and M₁₃) in this work generated a considerable number of amplification products for comparison. Comparison of each profile for each of primers was based on the presence (1) versus absence (0) of RAPD amplimers that migrated to the same position in the gel. Bands of the same size obtained by the same primer were scored as identical, but only bands repeatable in at least two experiments with the same primer at different times were evaluated. All random primers resulted in robust RAPD fragment patterns (Fig. 1 and 2). All the used primers revealed a high degrees of similarities among

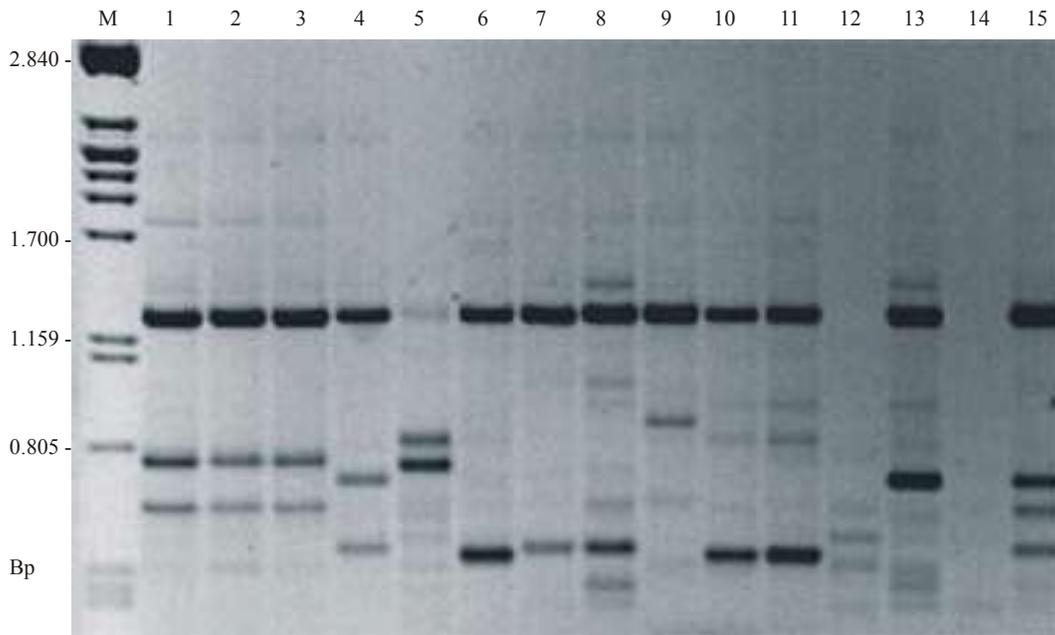


Fig. 1: DNA banding patterns from random amplified polymorphic DNA analysis of *Fusarium sambucinum* isolates primed by V6 (5'dTGCAGCGTGG; [12]). Lane M is a 100 Kb DNA

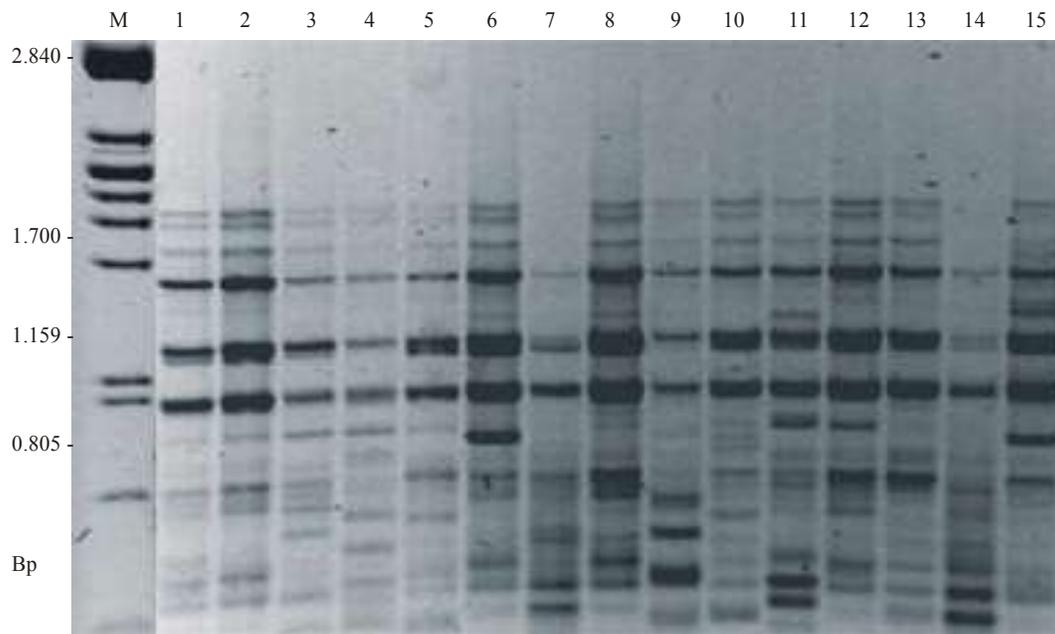


Fig. 2: DNA banding patterns from random amplified polymorphic DNA analysis of *Fusarium sambucinum* isolates primed by M13 (5' dGAGGGTGGCGTTCT; [13]). Lane M is a 100 Kb DNA

Fusarium sambucinum strains (Fig. 1 and 2). Most amplification products were reproducible.

The RAPD data from M13 primer were used to construct a dendrogram. The dendrogram showing the relationship among the studied strains. This dendrogram

showed that there is little correlation between some clusters of *Fusarium sambucinum* and isolation media, for example strains SVUML 255 (from rhizosphere and isolated on DCPA), SVUML 230 (from rhizosphere and isolated on DCPA) and SVUML 178 (from rhizoplane and

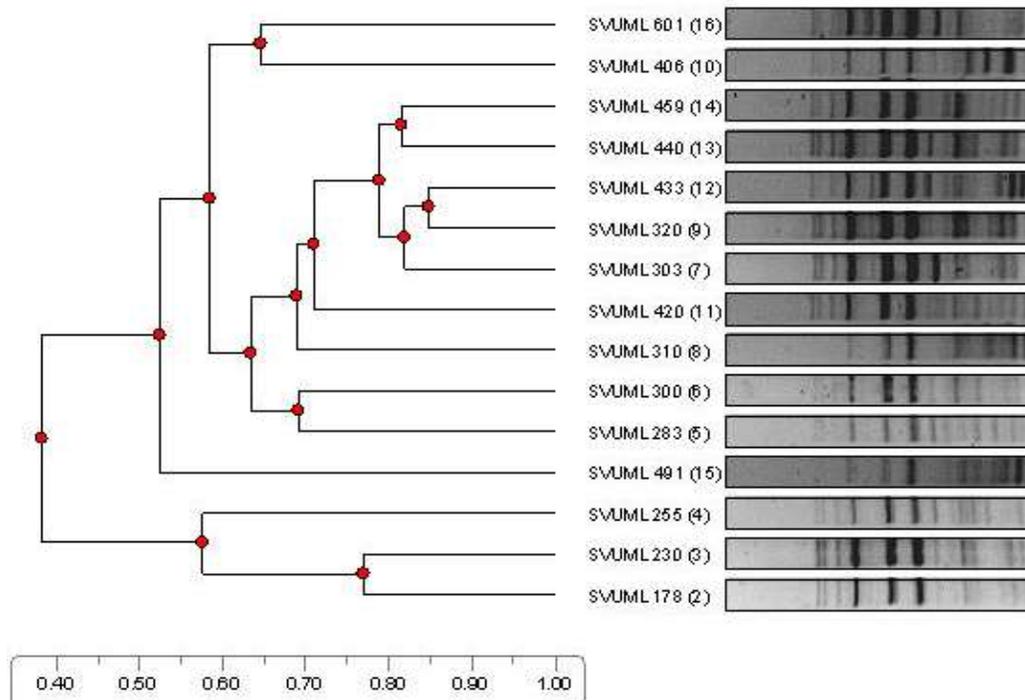


Fig. 3: Dendrogram showing relationships among 15 strains of *Fusarium sambucinum*. Genetic distances were obtained by random amplified polymorphic DNA analysis using M13 primer

isolated on DCPA) were clustered together in one group Fig. 3. In the other hand strains SVUML 300 (from rhizolane on DRBC) and SVUML 283 (from rhizoplane DCBA) clustered together. *Fusarium sambucinum* dendrogram indicated a little correlation between some clusters of *Fusarium sambucinum* and isolation media. DNA polymorphisms generated by the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) were used to analyse 41 isolates investigated in the European *Fusarium sambucinum* Project (EFSP) by Hering and Nirenberg [16]. They were employing ten arbitrary (10-mer) oligonucleotides and simple repeat sequences (M13, (GACA)₄) as single primers, informative banding patterns typical for identifying European populations of *Fusarium sambucinum* Fuckel s. str., *F. torulosum* (Berk. and Curt.) Nirenberg and *F. venenatum* Nirenberg were obtained by them. Sixty seven authentic isolates, representing six species from *Fusarium* section *Fusarium* (= section *Discolor*) were subjected to random amplified polymorphic DNA (RAPD) analysis and polymerase chain reaction using species-specific primers by Wendy and Christianson [17]. They obtained remarkably uniform RAPD banding patterns intraspecifically, irrespective of the geographical origin of the isolates or the host/substratum from which they were

isolated. Their molecular and morphological data support the identification of the Quorn strain as *F. venenatum* Nirenberg (= *F. sambucinum* Fuckelsensu lato).

The present study has shown that there is considerable genotypic variability among the Egyptian strains of *Fusarium sambucinum* obtained from different isolation source on different types of media.

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