

## Application of Molecular Methods for Characterization of *Botrytis fabae* and *Botrytis cinerea* of Faba Bean

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### Abstract

Application of the RAPD methods allowed to clearly characterization of isolates of *Botrytis fabae* and isolates of *B. cinerea*. Results from RAPD-PCR analysis indicate different groups. Clusters were related with groups based on conidial morphology, morphological characters of the isolates of *Botrytis* spp.

**Keywords:** *Vicia faba*; *Botrytis fabae*; *Botrytis cinerea*; identification; RAPD-PCR analysis; genetic finger print

### INTRODUCTION

*Botrytis fabae* Sard is a major cause of chocolate spot in faba bean (EL-HELALY 1938). *Botrytis cinerea* Pers.: Fr. also infects faba bean (NAGUIB 1948 and ABOU-ZEID *et al.* 1985). Chocolate spot causes severe epiphytotics, which are determined by weather conditions, inoculum density, and the age of plants. Epidemics generally occur on autumn-sown crops during late winter and spring and are exacerbated by damage to leaves and humid conditions. Also considered a limiting factor for faba bean production in the north and middle Egypt (MOHAMED 1982).

The traditional criteria used to differentiate these genera and their species are based on plant, host, symptoms, colony appearance, morphologic characterization of their conidia and telemorph (CORBIERE & BOUZNAD 1998).

The recent development of random amplified polymorphic DNA (RAPD) has allowed a rapid generation of reliable reproducible DNA fragment that showed of great use in identifying pathogenic variation in several fungal plant pathogens (JIMENEZ-GASCO *et al.* 1998).

The main objectives of this work were to study the possibility of using PCR assay to discriminate isolates of *Botrytis fabae* and isolates of *B. cinerea*.

### MATERIALS AND METHODS

**Fungal strains.** The *Botrytis* isolates used in this study obtained from various sources of diseased faba bean plants from wide range of geographical origins and Governorate over all Egypt. All cultures were derived from single spore. Data are listed in Table 1 (ABOU-ZEID 1999).

**DNA isolation and RAPD technique.** DNA was isolated from 50 mg tissue (*Botrytis* spp.) using Qiagen Kit for DNA extraction. The extracted DNA was dissolved in 100 µl of elution buffer and the concentration and purity of the obtained DNA was determined by using "Gene quantum" system-Pharmacia Biotech. The purity of the DNA for all samples were between 90–97%. The concentration was adjusted at 6 mg/µl for all samples using TE buffer pH 8.0.

**RAPD technique.** 30 mg/µl from the extracted DNA was used for amplification reaction the PCR. The PCR mixture contains tablet PCR beads (manufactured by Amessham Pharmacia Biotech) that contain all of the necessary reagents. The primer and DNA extract are add to the tablet. The sequences of RAPD analysis primer 2 and 3 are 6-d (CTAGACCCGT)-3 and 6-d (GTTTCGCTCC)-3.

The total volume was completed to 25 µl using sterile distilled water. The amplification program was

Table 1. Type, growth rate, and morphological characters of different isolates of *Botrytis* spp. isolated from faba bean plants

Isolate No.	Type	Geographic origin	Growth rate		Spores in $\text{ml} \times 10^5$	No. sclerotia/ $\text{cm}^2$	Size of sclerotia* (mm)	Year isolated
			PDA	FBLA				
1	<i>fabae</i>	Dkahlia	7.1	5.8	9.66	26.2	a	1998
2		Beni-Suief	8.1	5.1	2.0	16.7	a	1996
3		Nubaria	7.4	5.6	9.75	10.7	c	1995
4		Nubaria	7.5	6.7	6.50	11.3	b	1995
5		Etsa-Faium	8.3	6.6	50.16	23.0	b	1996
6		Nubaria	7.4	6.7	14.00	26.3	a	1998
7		Sakha	7.3	5.6	18.50	19.7	b	1998
8		Gemmeiza 1	8.4	5.3	7.75	15.7	b	1995
1	<i>cinerea</i>	Domiatte	8.6	6.4	10.00	0.0	–	1995
2		Mansora	9.0	8.7	17.25	0.0	–	1994
3		Behira	9.0	9.0	7.33	–	–	1996
4		Etai el-Barod	8.1	6.1	58.50	–	–	1999
5		Kafer et-Zyat	8.6	6.8	0.67	–	–	1999
6		Mahala	9.0	9.0	66.60	26.3	a	1998
7		Sakha 461	9.0	8.1	21.75	1.7	d	1995
8		Matbol	9.0	9.0	26.3	2.1	d	1996

\* a = 1 mm, b = 1.5–2 mm, c = 2–3 mm, d = 3–4 mm

carried out as follows using PCR unit II biometra and consisted of the following:

- a) Preheating at 95°C for 5 min
- b) Three-step cycle repeated 44 times:
  - Denaturation at 95°C for 1 min
  - Annealing at 36°C for 1 min
  - Extension at 72°C for 2 min
- c) Final extension at 72°C for 5 min
- d) Hold at 4°C.

Then add 7  $\mu\text{l}$  of 6X tracking buffer (manufactured by Qiagen Kit) to 25  $\mu\text{l}$  of the amplification product.

**Amplification product analysis.** Two method were used for electrophoresis technique:

1. The amplified DNA for all samples were electrophorated (15  $\mu\text{l}$ ) (using electrophoresis unit WIDE mini- sub-cell GT Bio-RAD on 1% agarose containing ethidium bromide at concentration 0.5  $\mu\text{g}/\text{ml}$ ).

2. The PCR products was separated and determined using polyacrylamide gel electrophoresis slabs (according to PIETER *et al.* 1995).

Using sub electrophoresis unit Hoefer SE600 series Pharmacia (8  $\mu\text{l}$ ) well form, each sample was loaded. The running was done at 2 melamper/sample constant

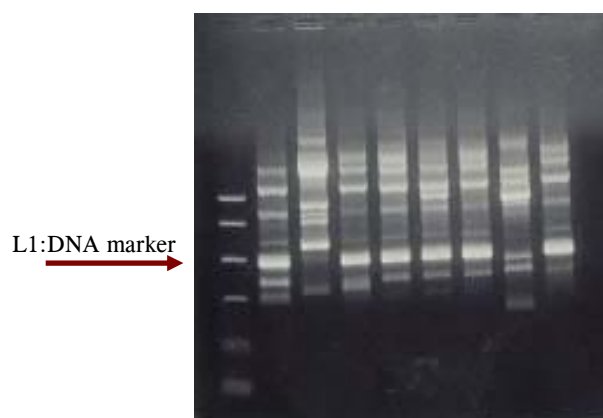
current. The running took about 2 hours, the amplified pattern was developed using silver stain (according to CAIRNS & MURRAY 1994).

Restriction fragment were compared in all pair wise combinations and the matrix was displayed as a dendogram using the unweighted pair-group method using arithmetic average (UPGMA).

**Gel analysis.** The polyacrylamide gel was scanned using gel documentation system (AAB advanced American Biotechnology 1166 E.Valencia Dr.Unit 6C, Fullerton CA 92631). The different molecular weights of bands were determined against PCR marker Promega G316A.

## RESULTS

Clusters analysis of similarity coefficients based on RAPD profiles revealed discrete clusters of isolates possibly representing different lineages, among isolates of *Botrytis* spp. from and faba bean. These corresponded to differences in morphological characteristics and were distinguished from isolates representing species of *Botrytis* spp.



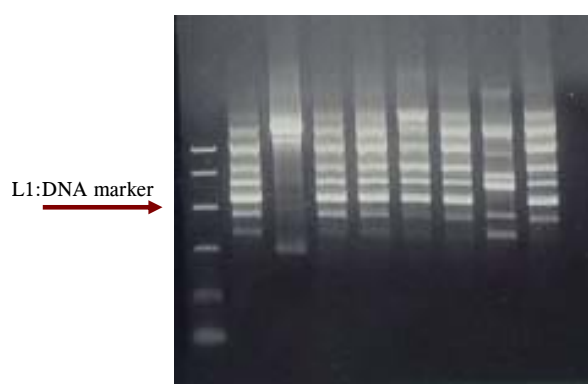
L1:DNA marker, from L2–L9: *Botrytis fabae* samples isolated from faba bean

Figure 1. Electrophoresis analysis of RAPD for isolates of *Botrytis fabae* using primer No. 3

DNA from 8 *Botrytis fabae* isolates were amplified using RAPD analysis primer 2 and primer 3, which identified that characterized *Botrytis fabae* isolates RAPD amplifications analysis observed that, from the eight faba bean isolates, it could be subdivided into different clusters. When using primer 3; isolate No. 4 can grouped with isolate No. 6 with 99% similarity, and this results agree with their characters as shown in Table 1, and could be sub grouped with isolate No. 8 with 95% similarity (Figures 1 and 2).

When using primer 2, it could divide the isolates into different clusters. However, isolate No. 1, 2, 5, 7, could be divided as separate isolate, also No. 6 and isolate No. 8 the similarity between isolates could be due to the origin of the isolates also to their morphology (Figures 3 and 4).

In the case of isolates of *Botrytis cinerea*, when using primer 2, UPGMA analysis separated the isolates of



L1:DNA marker, from L2–L9: *Botrytis fabae* samples isolated from faba bean

Figure 3. Electrophoresis analysis of RAPD for isolates of *Botrytis fabae* using primer No. 2

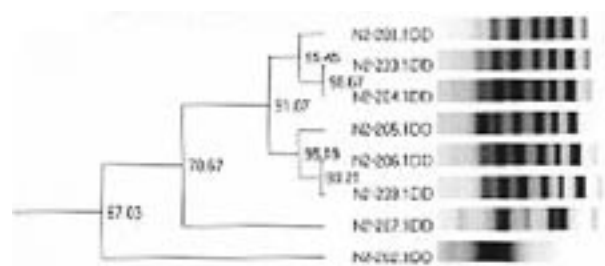


Figure 2. UPGMA cluster analysis of RAPD obtained from selected *Botrytis fabae* using primer No. 3. Similarity values are indicated and final linkage for the subclusters are marked

faba bean into different distinct of clusters. All isolates were not grouped together each isolate forming a separate cluster unless between isolate 7 and 8 which grouped together and showing 98% similarity, and was grouped into subcluster showing 95% similarity with isolate 6 (Figures 5 and 6).

However, for primer 3, UPGMA analysis separated the isolates of faba bean into different distinct of clusters isolate 7 and 8 which grouped together and showing 97% similarity as in primer 2, and was grouped into subcluster showing 95% similarity with isolate 3, also between isolates No. 4 and No. 6 and showing 98% similarity and was grouped into subcluster showing 95% similarity with isolate 1 (Figures 7 and 8).

## DISCUSSION

The aim of this work was to determine whether isolates could be characterized by RAPD analysis and to provide a methodology for the diagnosis of the isolate type.

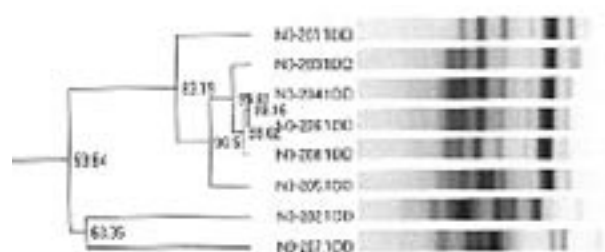
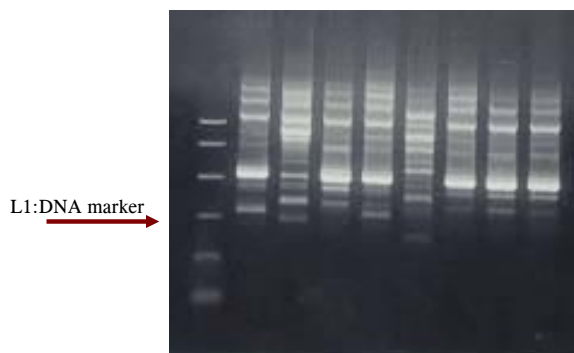
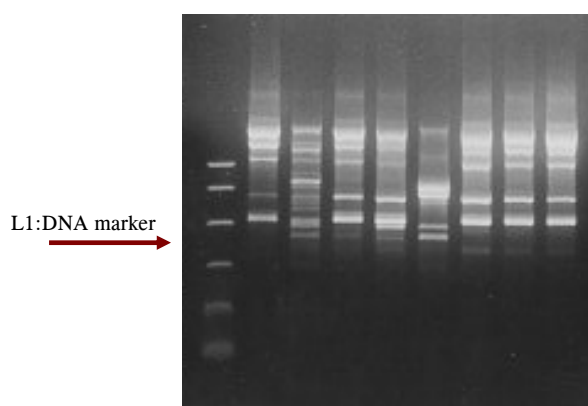


Figure 4. UPGMA cluster analysis of RAPD obtained from selected *Botrytis fabae* using primer No. 2. Similarity values are indicated and final linkage for the subclusters are marked



L1:DNA marker, from L2-L9: *Botrytis fabae* samples isolated from faba bean

Figure 5. Electrophoresis analysis of RAPD for isolates of *Botrytis cinerea* using primer No. 2



L1:DNA marker, from L2-L9: *Botrytis fabae* samples isolated from faba bean

Figure 7. Electrophoresis analysis of RAPD for isolates of *Botrytis cinerea* using primer No. 3

Results showed that amplified DNA bands can be used reproducibly to assign a *Botrytis* spp. isolates to different groups with the result of their morphological characters (ABOU-ZEID 1999). However, this molecular method is very helpful to characterize isolates of *Botrytis* spp. and may complement traditional characters for the better knowledge of the *Botrytis* and be very useful to precise or re-define species.

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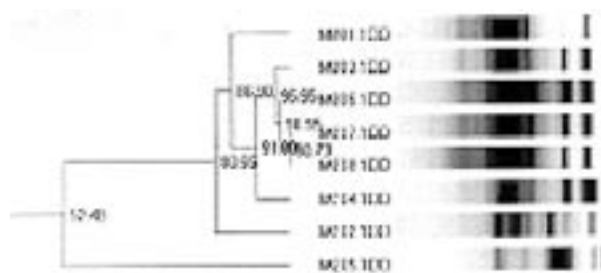


Figure 6. UPGMA cluster analysis of RAPD obtained from selected *Botrytis cinerea* using primer No. 2. Similarity values are indicated and final linkage for the subclusters are marked

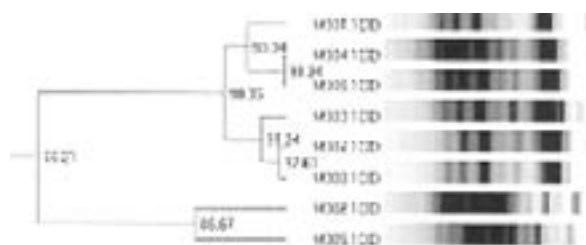


Figure 8. UPGMA cluster analysis of RAPD obtained from selected *Botrytis cinerea* using primer No. 3. Similarity values are indicated and final linkage for the subclusters are marked

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