MOLECULAR CHARACTERIZATION AND PATHOLOGICAL VARIATION AMONG *BOTRYTIS* SPP. FROM FABA BEAN IN EGYPT

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Abstract

Variability of *Botrytis* species (26 isolates of *Botrytis fabae* and 3 isolates of *B. cinerea*) representing different geographical regions cultivated with faba bean in Egypt was studied. Phenotypic variation between isolates of the *fabae* sp. (sporulation less than 18.1 X 10^{6} / plate) compared to the *cinerea* sp. (sporulation more than 19.5 X 10^{6} / plate) was found. The *fabae* sp. produced small sized sclerotia in large number, while the *cinerea* sp. either did not produce sclerotia or produced large sized sclerotia but less in numbers than the *fabae* sp. The most virulent isolates were 28 and 29 of *fabae* sp that showed disease severity of 5.1 and 5.7, respectively. These isolates were obtained from Noubaria region. However, the less virulent isolates were of the *cinerea* sp. and were isolated from plants grown in Nubaria, Dakahlia and Kafr El-Sheikh regions. Other isolates showed intermediate reaction in their virulence. Primer pairs Bc 6 (r & f) amplified a single band at 90 bp for all *Botrytis fabae* isolates except isolate no.4 which was isolated from Nubaria that did not react positively. *Botrytis cinerea* produced polymerase chain reaction (PCR) products at 120 bp.On the other hand, primer pairs Bc 9 (r & f) amplified a single band of 90 bp for all *Botrytis fabae* isolates except isolates isolated from Nubaria region (isolates no. 4 and 7). *Botrytis cinerea* produces PCR product at the same size(90 bp) of *Botrytis fabae* isolates. Finally, primer BC6 was the best in differentiating between *Botrytis fabae* and *Botrytis cinerea* isolates.

INTRODUCTION

Faba bean is considered one of the most important food legumenous crops in Egypt. Chocolate leaf spot is considered the most destructive disease on faba bean, causing serious damage and great losses to the crop, especially in northern parts of the delta region, where low temperature and high relative humidity favour its spread and severity (Mohamed, 1982). Chocolate leaf spot is caused mainly by *Botrytis fabae* and can also be caused by *Botrytis cinerea* (Abou-Zeid 1978).

Botrytis species infects the plant at all stages of its development and has been isolated from every part of the plant. Its detection at early stage is difficult because it can be latented on host plant that make epidemiological studies difficult that much (Powelson, 1960).

The genus *Botrytis* contains 22 recognized species and one hybrid. This classification is largely based on morphological features and to a lesser extent on physiological properties, and host range. (Staats *et. al.*, 2005).

Botrytis cinerea Pers., is an important pathogen that attacks more than 200 host in the field, green houses and storage, without apparent specificity (Jarvis, 1980). Other species are considered more specialized with a narrow host range. They infect only one or few closely related species within the same plant genus, with the exception of *B. fabae* , which can infect species of *Vicia, Lens, Pisum , and Phaseolus,* all belonging to *Fabaceae* (Jarvis, 1977).

Field isolates of *Botrytis* spp. grown on synthetic media exhibit considerable variation in pathogenicty, phenotypic properties and or tolerance to fungicides which complicates the chemical control. Mutations and heterokaryosis are the main causes of variations. Nevertheless, the development of resistant strains has provided genetic markers and indicate the existence of distinct genotypic population of *Botrytis* spp.

Detection of genetic variations among microorganisms and identification based on the polymerase chain reaction (PCR) has been developed for several pathogenic fungi. Amplification of genomic DNA with specific pair of primers is useful for direct detection of plant pathogens in crude extracts using PCR (Rigotti *et. al.*, 2002).

The objectives of this work was to characterize the morphological, physiological, pathological differences and molecular variation among twenty nine isolates of *Botrytis* spp. isolated from different Egyptian geographical areas.

MATERIALS AND METHODS

Isolation and identification:

Diseased samples were collected from northern Egypt (Kafr El-Sheikh, Beheira, Noubaria, Dakahlia, Meunfia, Gharbia and Sharkia governorates).

Leaves of each sample were surface disinfested with 3 % sodium hypochlorite for two minutes, rinsed with sterilized distilled water, cut into small pieces, dried between sterilized filter papers and plated on Potato Dextrose Agar (PDA) supplemented with 0.035 g streptomycin/L. Incubation was made at 20° C under alternative light/dark (12h/12h), for 7 days (Rigotti *et. al.*, 2002). Purification was made by hyphal tip method on PDA plates and incubated at 20ºC for 12 days. Single sclerotia were picked up and plated on Faba Bean Leaf Agar (FBLA) medium {Leaf extract 259g, Sucrose 30g, NaCl 20g, Agar 20g} in Petri dishes. Cultures were then single spored before identification and kept on PDA slants for further studies. Isolates were identified to species level according to Ellis (1976).

Variation among isolates:

1- Morphological characters:

Isolates were compared on two media and observations on mycelial growth, sclerotial presence, number, and size of sclerotia, as well as spore production are recorded. The two media used were PDA and FBLA supplemented with 0.035 g streptomycin/L

Growth rate:

Growth rate of the obtained isolates was determined on both PDA and FBLA media. A 0.5 cm disc from each isolate was placed in the center of 9 cm diameter plate. The growth diameter of each isolate was determined after 7 days.

Spore Production:

Isolates were grown on FBLA medium and incubated at 20 ºC for 12 days. Ten ml of distilled water were poured in each Petri dish. Spores were separated using a small brush and filtered through cheese cloth. Spores were counted using a haemocytometer and expressed as a number of spores per plate.

Production of Sclerotia:

Isolates were grown on PDA medium at 20 ^{O}C for 21 days. Plates were examined for the presence, number and size of sclerotia. Sclerotia were calculated per cm^2 of agar surface of a Petri dish. Size of sclerotia was charactarized according to the following rates: Small: ≤ 1 – 1.5 mm, Medium ≥ 1.6 – 2.5 and Large: ≥ 2.6 – 3.0 mm.

Virulence test:

Isolates of *Botrytis* spp. were tested for their virulence on four Faba bean cultivars *i.e* Giza 40, Giza 3, Noubaria 1 and Sakha 1 using the detached leaf technique (Abou-Zeid, 1978). Plants of the tested cultivars were grown in clay pots, (25 cm in diam.) and kept in a plastic house. Leaflets were collected from 35 days old plants, arranged in sterilized trays on sterilized blotting paper moistened with sterile distilled water. Droplets of the spore suspension were placed on each of the leaflets; the trays were covered with polyethylene sheets to maintain high relative humidity. Spore suspensions were prepared from 12 days old cultures and adjusted to about15 $x10^4$ spores/ml. Each treatment was replicated 10 times and treatments were arranged in a completely randomized design. Data were recorded on the type of infection after 48 hrs using scale depending on the lesions area (Abou-Zeid, 1978).

Rapid DNA extraction and purification:

The purified fungi (*Botrytis fabae* isolates No. 1-20 and *Botrytis cinerea* isolate No. 24) were grown in 100 ml potato dextrose broth cultures under optimal conditions until complete colonization of the upper surface. Mycelium was harvested by filtration, washed using distilled water, lyophilized, submerged in liquid nitrogen and ground into a powder using a pestle and morter. Genomic DNA was extracted from dried

mycelium using fast prep system (BIO 101 Inc, CA). Harvested mycelium (20 mg) from each isolate were suspended in lysis buffer and transferred to eppendorf tubes containing homogenization matrix. Samples were immediately processed in the fast prep system twice for 30 sec. A binding matrix was added to the supernatant and after centrifugation; the pellet was resuspended and transferred to a spin column. The DNA was eluted from the column in 50 μl TE buffer. The eluates containing purified DNA were used for PCR reactions using the primers listed in Table 1.

DNA Amplification:

The reaction mixture (25 µl) contains 12.5 µl of the master mix (2x), 2µl from each primer, 2 µl DNA and 6.5 µl of sterilized distilled water. DNA templates were amplified according to Fournier *et. al.,* (2002) using Biometra thermocycler lid temperature 105 ^{O}C with the following program; 1 denaturation step at 94 ^{O}C for 3 min, 30 cycles of 94 ^{O}C for 30 s, annealing temperature of 50 ^{O}C for 30 s. and 72 ^{O}C for 30 s for extention. The thermocycler was then held at 4 ^{O} C. PCR products were separated on a 1.5 % agarose gel in TE buffer (1x) and visualized by UV illumination after staining with ethidium bromide.

Table 1. Primers used for DNA amplification and sequencing

Primer Name		Primer sequences (5¯- 3¯)
BC1	F	AGGGAGGGTATGAGTGTGTA
	R	TTGAGGAGGTGGAAGTTGTA
BC3	F	GGATGAATCAGTTGTTTGTG
	R	CACCTAGGTATTTCCTGGTA
BC6	F	ACTAGATTCGAGATTCAGTT
	R	AAGGTGGTATGAGCGGTTTA
BC9	F	CTCGTCATAACCACGCAGAT
	R	GCAAGGTCTCGATGTCGATC

RESULTS

Identification and distribution of *Botrytis* isolates:

Twenty nine isolates of *Botrytis* spp. collected from different faba bean growing areas in Egypt were identified to the species level. The identification of the obtained isolates proved that 26 isolates were *Botrytis fabae,* while the other three isolates were *Botrytis cinerea.* Also, both species of *Botrytis* were found in all faba bean growing governorates (Table 2).

Effect of different media on mycelial radial growth:

Two solid media were used to study the differences between these isolates according to their cultural characteristics at 20°C. PDA medium was better for mycelial growth

of all *Botrytis* isolates than FBLA. Growth of most isolates covered the agar surface of the Petri dishes within 5 days.

Spore production:

Data in Table 2 showed that isolates were differed in the number of spore production per Petri dish on FBLA. Most of the *fabae* isolates produced high numbers of spores (more than 19.5 X 10^{6} per dish) on FBLA media when compared to the *cinerea* isolates (less than 18.1 X 10^{6} per dish).

The low spore production (isolates No. 9, 12 16, 27 and 8), which ranged between 6.5 X 10^{6} and 8 X 10^{6}/petri dish were produced by the *fabae* isolates. The highest spore production (isolates No. 24, 25 and 26), ranging between 19.5 – 20.0 X 10^{6}/ petri dish, were produced by the *cinerea* isolates.

Number and size of sclerotia:

Data in Table 2 showed that, isolates No. 24, 25 and 26 produced few number of sclerotia on PDA medium, while the others produced sclerotia of different numbers and size which varied widely among the isolates. Isolates No. 23, 20, 17, 4 and 2 produced the highest number of sclerotia (19 – 26 sclerotia / cm^2 per plate) while isolates No. 29, 22, 13 ,12 and 8 produced the least number of sclerotia (3.3 – 8.0 sclerotia/ cm^2 per plate). Moreover, on PDA medium, some isolates produced small sclerotia while others produced large ones.*B. fabae* isolates produced small size of sclerotia in large number , while *B. cinerea* isolates failed to produce small sclerotia but produced large sclerotia in few in numbers.

Virulence of different isolates:

Results in Table 3 showed clear differences between the isolates in their aggressiveness on the four tested cultivars. Isolates of *B. fabae* were more virulent than those of the *B. s cinerea*. The most virulent *fabae* isolates were No. 28 and 29 resulting in an average disease severity of 5.1 and 5.7, respectively. These isolates were isolated from plants grown in Nubaria area. The least virulent, on the four cultivars, were isolates No. 24, 25 and 26 resulting in an average disease severity of 1.7, 1.7. and 1.6, respectively. These *cinerea* isolates were obtained from plants grown in Noubaria, Dakahlia and Kafr El-Sheikh governorates. Other isolates were intermediate in this respect.

Table 2. The morphological features of different isolates of *Botrytis* spp. Grown on PDA and FBLA media.

Isolate No.	Origin	Species	Mycelial growth after 7 days (mm)		No. of spores / mean Petri dish X 10^6 days on FLBA media	No. of Sclerotia/cm^2 After 12 days on PDA media	Size of Sclerotia
			PDA	FBLA			
1	Sharkia	*fabae*	85	76	13.5	12	M
2	Noubaria	"	84	75	16.25	20	S
3	Kafr El-Sheikh	"	85	75	17.5	5.7	S
4	Kafr El-Sheikh	"	85	72	15.4	19.7	L
5	Dakahlia	"	80	71	9.1	8	M
6	Dakahlia	"	85	80	10.5	15	M
7	Dakahlia	"	72	70	9.8	10.7	L
8	Gharbia	"	81	72	8.0	16.3	S
9	Beheira	"	77	68	6.5	7.7	L
10	Noubaria	"	84	77	8.9	19	L
11	Noubaria	"	85	80	9.7	13.7	M
12	Gharbia	"	85	81	7.5	18.3	S
13	Kafr El-Sheikh	"	84	83	12.0	16.7	S
14	Beheira	"	71	65	11.9	16	L
15	Beheira	"	75	64	10.7	12	M
16	Monufia	"	75	68	7.6	3.3	L
17	Monufia	"	75	70	18.1	26.7	S
18	Sharkia	"	82	77	11	12.7	M
19	Sharkia	"	85	78	13.2	15	L
20	Sharkia		85	76	12.3	19.3	M
21	Beheira	"	85	71	8.8	10.3	M
22	Kafr El-Sheikh	"	76	70	15.4	17.3	S
23	Kafr El-Sheikh	"	74	69	18	21	S
24	Noubaria	*cinerea*	88	75	20.2	--	--
25	Dakahlia	"	90	70	19.9	--	--
26	Kafr El-Sheikh	"	90	81	19.5	--	--
27	Noubaria	*fabae*	90	78	7.8	7.8	M
28	Noubaria	"	85	77	17.2	19	M
29	Noubaria	"	87	81	16.8	16.7	M

L = large
M = medium
S = small

Table 3. Disease severity of *Botrytis* spp. isolates on four faba bean cultivars using detached leaf technique.

Isolate NO.	Average disease severity on cutivars				Mean
	Giza 40	Giza 3	Noubaria 1	Sakha 1	
1	5.5	4.4	3.1	3.8	4.2
2	6.8	4.5	3.5	3.2	4.5
3	5.2	3.0	2.8	3.0	3.5
4	5.0	3.5	3.0	3.1	3.7
5	4.5	4.0	3.5	3.3	3.8
6	4.4	4.0	3.3	3.0	3.7
7	4.8	3.1	2.2	2.5	3.2
8	2.8	2.5	2.0	2.5	3.5
9	4.5	3.1	3.0	3.2	3.5
10	5.0	4.3	4.0	4.0	4.3
11	5.8	3.8	3.0	3.5	4.0
12	3.8	2.6	2.5	3.0	3.0
13	5.8	3.5	4.0	4.2	4.4
14	5.5	3.1	2.8	3.0	3.6
15	5.5	3.3	3.0	3.5	3.8
16	3.2	3.0	2.9	3.4	3.1
17	3.4	3.6	3.0	3.1	3.3
18	3.5	3.0	2.6	3.0	3.0
19	3.1	3.0	2.0	3.0	2.8
20	5.2	4.3	4.0	4.0	4.4
21	4.0	3.7	3.0	3.9	3.4
22	4.8	4.1	3.5	3.5	4.0
23	4.6	4.0	3.6	3.1	3.8
24	2.0	1.8	1.3	1.5	1.7
25	2.2	1.5	1.2	1.8	1.7
26	2.1	1.5	1.2	1.6	1.6
27	5.1	4.0	4.0	4.0	4.3
28	6.3	5.1	4.5	4.6	5.1
29	7.5	6.0	4.3	4.8	5.7
Mean	4.6	3.5	3.0	3.2	3.6

L.S.D at	1%	5%
For Isolates (I)	0.34	0.44
For Cultivar (C)	0.96	1.10
For I x C	0.64	0.88

Extraction of DNA using Fast Prep Protocol:

The results indicate that the Fast Prep system is a rapid and efficient method for extracting genomic DNA from *Botrytis* isolates where it showed good results in DNA yield for using in DNA amplification protocol.

Specificity of the (PCR) to detect *Botrytis* spp.

The Bc 6 and Bc 9 primer pairs were used in PCR including 2 *ul* of total genomic DNA of *B. fabae* or *B. cinerea*.

The results in Fig.1 & 2 show that, the pairs of Bc 6 primer amplified a single band of 90 bp for all *B. fabae* isolates except for isolate no.4 which was isolated from Nubaria that did not amplify any PCR product while *B. cinerea* (isolate no.24) produced PCR product at 120 bp .

On the other hand, the results presented in Figs.3 and 4., reveal that the pairs of Bc 9 primer amplified a single band of 90 bp for all *Botrytis* isolates except isolated from Noubaria region (isolates no. 4 and 7). *B. cinerea* (isolate no.24) produce PCR product at the same size of *B. fabae* isolates (90 bp).

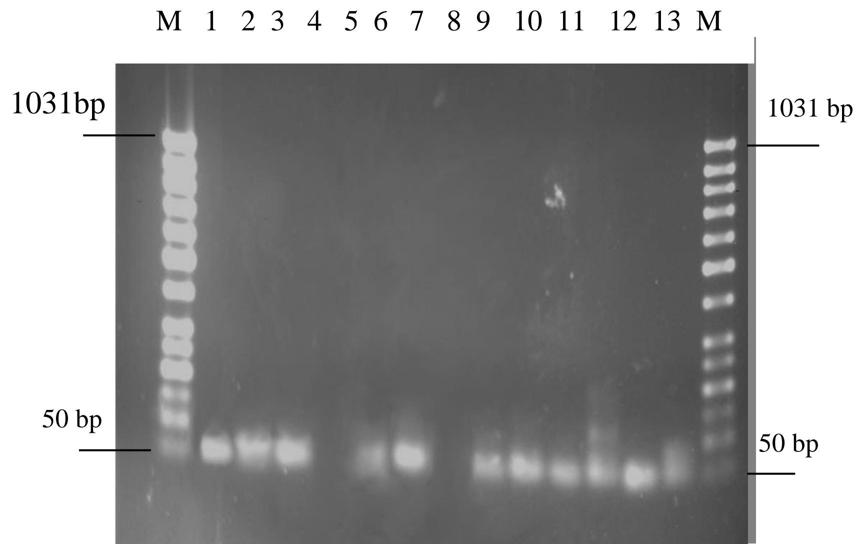

Fig.1. Electrophoretic patterns for amplified fragments generated from 13 isolates of *B. fabae* with primer BC6 (R&F) on 1.5 agarose gel. Lane (M): 50 bp marker. . Lanes (1-13) *B. fabae isolates No. 1-13.*

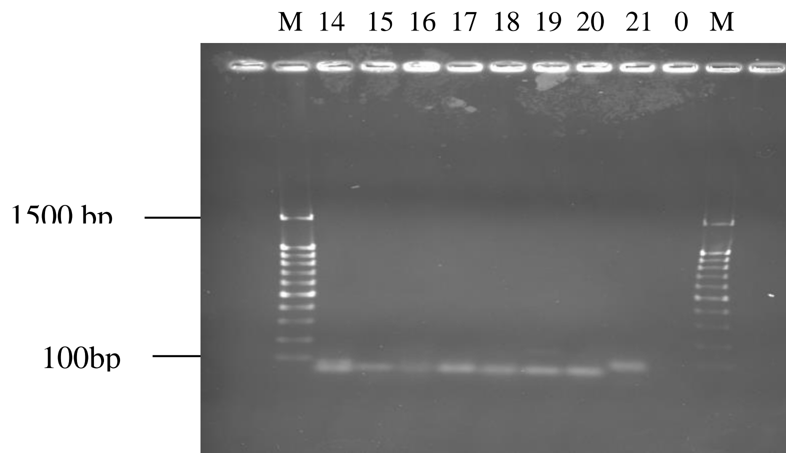

Fig.2. Electrophoretic patterns for amplified fragments generated from 8 isolates of *Botrytis* with primer BC6 r&f on 1.5 agarose gel. Lane (M) :100 bp marker. Lanes (14-20): *B. fabae* isolates No.14-20. *Lane (21) B. cinerea* isolate No. 24*,* Lane :0, negative control,

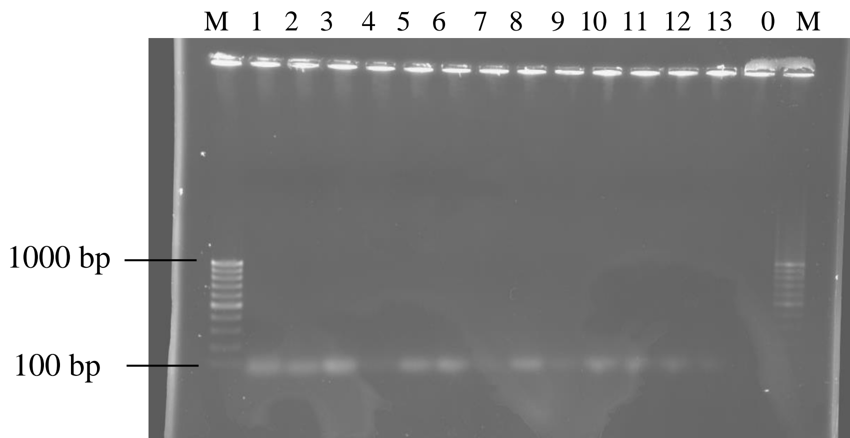

Fig.3. Electrophoretic patterns for amplified fragments generated from 13 isolates of *Botrytis fabae* with primer BC9 r&f on 1.5 agarose gel. Lane (M) =100 bp marker Fermentas, lanes (1-13): *Botrytis fabae* isolates No.1-13 and lane 0: negative control

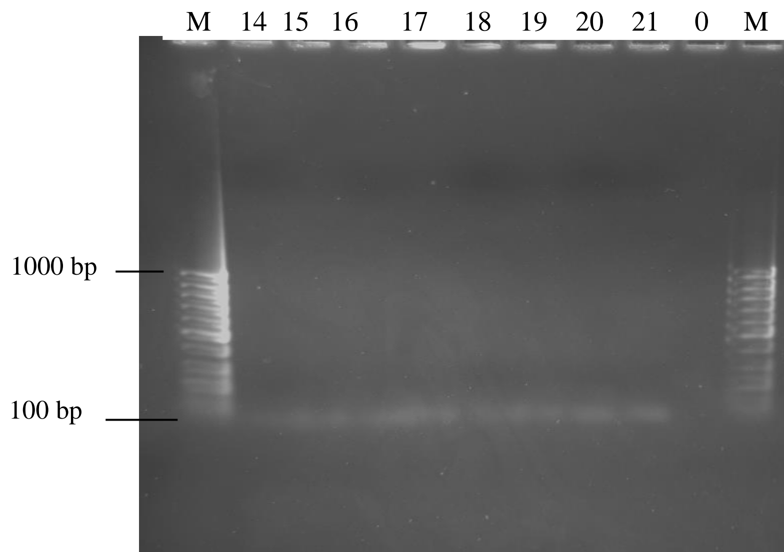

Fig.4. Electrophoretic patterns for amplified fragments generated from 8 isolates of *Botrytis* isolates with primer BC9 r & f on 1.5 agarose gel. Lane (M):100 bp marker. lanes (14-20): *B. fabae* isolates No.14-20, lane 21 *B. cinerea* isolate No. 24 and lane 0: negative control

DISCUSSION

Isolates of *Botrytis* spp. from different governorates in Egypt were compared for their morphological, physiological and molecular characters. Growing the 29 isolates of *Botrytis* spp. on two different media, PDA and FBLA, showed differences between their growth rate, spore production, number and size of sclerotia. Isolates of *B. fabae* (26 isolates) were the lower in growth rate, spore production and produced small sclerotia in high number, while isolates of *B. cinerea* (3 isolates) were faster in growth , with higher spore production but no sclerotia were produced under the experimental conditions.

Testing the virulence of these isolates on the detached leaflets of faba bean cultivars indicated that isolates of *B. fabae* were more virulent and aggressive than those of *B. cinerea*. Moreover, results showed differences in virulence among isolates of *B. fabae* as isolates from Nubaria were more virulent than those from other governorates. Similar results were previously observed by several investigators as Abou-Zeid (1978) mentioned that chocolate spot disease of faba bean can be caused by either *B. cinerea* or *B. fabae.* The spots caused by *B. cinerea* are inconspicuous compared with those of *B. fabae*, only epidermal cells being affected by *B. cinerea*,

whereas *B. fabae* always causes necrosis of the mesophyll (Harrison, 1983). Moreover, Hassanein *et. al.*, 1990 mentioned that isolates of *B. fabae* obtained from Nubaria, were in general, more virulent than those isolated from Kafr El- Sheikh. This could be due to that *B. fabae* was exposed to some environmental factors of Nubaria that might enhanced their virulence genes.

PCR assays used to diagnose and identify microorganisms have shown to be a powerful tool for the isolation of molecular markers and to design specific primers for PCR-based diagnosis in phytopathology (Pryor and Gilbertson 2001).

Conventional diagnosis of infected plants with *B. fabae* and *B. cinerea* is depending on culturing the pathogen from latent-infected plant tissues on agar plates. However, the specificity and sensitivity of PCR now allow the rapid detection of small amounts of target DNA (Rigotti *et. al.*, 2002).

Twenty isolates of *B. fabae* and one isolate of *B. cinerea* were selected to study the genetic variation based on PCR amplification. Four primer pairs *i.e.* Bc1 R & F, Bc3 R & F, Bc 6 R & F and Bc9 R & F were tested. Bc 6 and Bc9 R & F primer pairs successfully amplified fragment of expected size of 90 bp and 120 bp respectively for most *B. fabae* and *B. cinerea* isolates except isolate No. 4 (*B. fabae*) isolated from Noubaria region which did not produce any product with Bc 6 and Bc 9 primer pairs. The obtained PCR product appeared with most isolates of *Botytis* spp. was in agreement with the results of Fournier *et. al.*, (2002)

Bc 9 can differentiate *B. cinerea* from *B. fabae,* where *B. cinerea* produce PCR product at 110 bp. These data confirm the high genetic diversity in the *Botrytis* spp*.* complex and open opportunities to study the variation in the population dynamics of genus Botrytis (Regotti *et. al.*, 2002 and 2006). Also, having the successful DNA amplification giving a single unique band when using the tested primers is a good means for detecting the incidence and the proper cause of the chocolate spot disease on faba bean.

REFERENCES

1. Abou-Zeid, N. M. 1978. Contribution A la connaissance de la resistance naturelle de *Vicia faba* L. contre les *Botrytis.* Thesis Docteur Ing*.* ENSAR, Univ. de Rennes 1, France.
2. Ellis, M.B. 1976. More Dematiaceous Hyphomycetes, CMI, kew, Surrey,England.
3. Fournier, E., T. Giraud, A. Loiseau, D. Vautriin, A. Estoup, M. Solignac, J. M. Cornuet and Y. Brygoo. 2002. Characterization of nine polymorphic microsatellite

loci in the fungus *Botrytis cinerea* (Ascomycota). Molecular Ecology Notes, 2: 253 – 255.

4. Harrison, J. G. 1983. Distinguishing between lesions caused by *B. cinerea* and *fabae* on field bean leaves. Transactions of the British Mycological Soc., 81:663-664.
5. Hassanein, A. M., N. M. Abou-Zeid, and H. A. Mohamed. 1990. Correlation between morphological and pathological characters of *Botrytis* spp., The incident of chocolate spot. Agri. Res. Rev., 68(3):423-432.
6. Jarvis, W. R. 1977*.* *Botryotinia* and *Botrytis* species : Taxonomy, Physiology and Pathogenicity , Monograph No.15, Research Branch , Canada Department of Agriculture, Ontario, Canada, 195 pp.
7. Jarvis, W. R. 1980. Taxonomy. In: The Biology of *Botrytis* (J.R. Coley-Smith, Verhoeff, W. R. Jarvis, ed.), Academic Press Inc., London, UK, New York, NY, USA, 1 – 18.
8. Mohamed, H. A. 1982. Major disease problems of faba beans (*Vicia fabae*) in Egypt. In G. Hawtin and C. Webb,ebs, faba bean improvement. Martinus Nijhoff publishers The Hague, Netherlands 213-225.
9. Powelson, R. L. 1960. Initiation of strawberry fruit rot caused by *Botrytis cinerea*, Phytopathology 50: 491 – 494.
10. Pryor, B. M. and R. L. Gilbertson. 2001. A PCR-based assay for detection of *Alternaria radicina* on carrot seed. *Plant Dis*. 85: 18 – 23.
11. Rigotti, S., K. Gindro, H. Richter and O. Viret. 2002. Characterization of molecular markers for specific and sensitive detection of *Botrytis cinerea* Pers.:Fr. in strawberry (*Fragaria xananassa* Duch.) using PCR. *FEMS Microbiology Letters* 209: 169 – 174.
12. Rigotti, S., O. Viret and K. Gindro. 2006. Two new primers highly specific for the detection of *Botrytis cinerea* Pers.:Fr. Phytopathol. Mediterr. , 45:253 – 260.
13. Staats, M., P. Van Baarlen and J. A. L. Van Kan. 2005. Molecular phylogeny of the plant pathogenic genus *Botrytis* and the evaluation of host specificity. Molecular Biology Evalution*,* 22: 333 – 346.
14. Wilson, A. R. 1937. The chocolate spot disease of beans (*V. faba* L) caused by *cinerea* Pers. Ann. Appl. Biol., 24:258-288.

الخصائص الجزيئية والاختلافات المرضية لمسببات مرض التبقع البنى فى الفول البلدى فى مصر

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تم عزل 29 عزلة من فطر البوتريتس من مناطق جغرافية مختلفة بجمهورية مصر العربية والمزروعة بالفول البلدى منهم 26 عزلة بوتريتس فابى و3 عزلات بوتريتس سينيريا وقد تم عزلهم لدراسة الاختلافات بين العزلات من حيث الصفات المزرعية و القدرة المرضية و الحامض النووى.اتضح من الدراسة أن فطر البوتريتس فابى ينتج أعداد جراثيم أكثر من 19.5×10^6 / طبق وأجسام حجرية صغيرة بينما البوتريتس سينيريا ينتج أعداد من الجراثيم أقل من 18.1×10^6 / طبق وأجسام حجرية كبيرة.كما اتضح من الدراسة أن أقوى العزلات كانت بوتريتس فابى رقم28 ،29 المعزولة من منطقة النوبارية من نباتات الفول المزروعة بتلك المنطقة وكانت شدة الاصابة 5.1، 5.7 على التوالى. وكانت أقل العزلات شراسة بوتريتس سينيريا رقم 24، 25 ،26 المعزولة من مناطق النوبارية ، الدقهلية ، كفر الشيخ من نباتات الفول المزروعة بتلك المناطق وكانت شدة الاصابة 1.7 ، 1.7 ، 1.6 على التوالى. تم استخدام 4 بادئات عشوائية (BC1, BC3, BC6, BC9) للوصول إلى مدى الاختلافات الجينية بين العزلات المختبرة حيث كانت البادئات BC9& BC6 لها القدرة على التفريق بين العزلات ولكن كان البادىء BC6 له القدرة على التفريق بين عزلات فطر بوتريتس فابى وعزلات فطر بوتريتس سينيريا.