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# Improving Detection Means for Strawberry Gray Mould Caused by *Botrytis cinerea* in Egypt

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***Botrytis cinerea*** is an economically important pathogen. Twenty seven *B. cinerea* isolates were characterized for their pathogenicity on strawberry fruits. A cheap and simple method was developed for the identification of *Botrytis cinerea* isolates, the cause of gray mould, using modified Kerssies' selective medium (mKERS). The other fungal species could not grow on this medium. Moreover, there was no relation between the aggressiveness of each tested isolate of *B. cinerea* and its radial growth level on mKERS. The results showed that the 27 isolates of *B. cinerea* grew well and produced a dark brown pigment on mKERS. These results indicate that this simple method could be used for the detection of gray mould. Additionally, genetic diversity was investigated using PCR assay. The Egyptian population structure of *B. cinerea* was investigated by detecting the existence of two transposable elements (TEs): *Flipper* and *Boty* in the genome. Among 12 isolates tested, 10 have only one transposable element, *Boty* element, whereas 2 have neither of these elements (*vacuma* type). This result is useful for identifying and classifying the Egyptian population of *B. cinerea*, especially for the isolates exhibited high virulence according to their genomic structure.

**Keywords**: *B. cinerea*, gray mould, detection, selective medium, strawberry and transposable elements.

*B. cinerea* (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel), the cause of gray mould, is a necrotrophic pathogen causing pre- and postharvest diseases in at least 235 plant species, whereas other *Botrytis* species are confined to a single host species (Elad *et al.*, 2007). Detection of latent or quiescent infections remains the most challenging and important problem because, in most cases, classical methods are too slow. Determination of fungal infections is important in many fields of research, particularly in determining levels of resistance in transgenic crops and the efficacy of biocontrol agents and new fungicides. Semi-selective and differential media are based on the selective inhibition of competing microbes, the encouragement of the target organism's growth and/or the expression of a characteristic property of *Botrytis*, (Kritzman and Netzer, 1978). These media have been developed for isolation of *Botrytis* species from soil and onion seed based on the development of dark pigments in the medium due to degradation of tannic acid and resistance to PCNB and maneb. These media were also used to monitor *Botrytis* populations in vineyards and greenhouses, and amended with fungicides to detect resistant populations (Elad *et al.*, 1992). For the detection of conidia of *B. cinerea* caught in spore traps, Kerssies (1990) used a very similar medium to which

fenarimol was added to inhibit growth from airborne conidia of *Penicillium* spp. However, this medium also shows the same browning halo with *B. aclada* and *B. allii* as *B. cinerea*. Several basal media amended with suitable fungicides have been used to monitor fungicide resistance within populations of *Botryotinia fuckeliana* (Baroffio *et al.*, 2003). The routine application of fungicides, irrespective of whether conditions are conducive to disease development, has become increasingly unacceptable. An alternative approach is to restrict fungicide application to periods conducive to disease development. A prerequisite for this approach is a disease forecasting system based on using classical methods. Moreover, the complexity and variability features of *B. cinerea* are one of the reasons that make control so difficult. Awareness of the existence of differentiated populations may make it possible to find more efficient methods of control. Molecular techniques have been used to study the genetic structure of this fungus. *Botrytis* spp. populations display a significant phenotypic variability in their level of aggressiveness (Choquer *et al.*, 2007). The genetic bases for this variability are not yet elucidated, but different nonexclusive hypotheses are under investigation. One of the genomic variability sources is the existing of transposable elements, TEs, (Dufresne *et al.*, 2007; Lopez-Berges *et al.*, 2009). Recent studies on populations of *B. cinerea* provided a new finding that this species is composed of two sympatric species, *transposa* and *vacuma* (Muñoz *et al.*, 2002; Muñoz *et al.*, 2010), and the *transposa* isolates did not differ in pathogenicity from the *vacuma* isolates (Martinez *et al.*, 2003). *Transposa* has the transposable elements *Boty* (Diolez *et al.*, 1995) and *Flipper* (Levis *et al.*, 1997), whereas these elements are absent in *vacuma*. More interestingly, some *vacuma* isolates are resistant naturally to many fungicides. Presently, it is unclear whether the two sympatric species are present in *B. cinerea* populations in Egypt. The objectives of the present study were to (i) develop a classical method needed for simple and efficient selectivity of *B. cinerea*, the cause of gray mould. (ii) characterize the genomic variation of the Egyptian isolates of *B. cinerea* in order to find a relation among their morphology, severity and genomic structure using TEs test.

## Materials and Methods

*Plant materials:*

Strawberry fruits, "var. Festival", were obtained from Modern Agricultural Company (PICO) and Agro-farms Company. The collected fruits were surface-sterilized with ethanol (95%) for 30 sec and then rinsed with distilled water. The fruits were placed separately on filter paper, and then wetted with distilled water in small sterile packets. The packets were incubated at the room temperature (approximately 20°C) for 2-5 days.

*Isolation and identification of the causal pathogen:*

Samples of strawberry fruits, showing symptoms of gray mould were collected from different governorates (Esmailiya, Sharkiya, Kaliobiya and Behira) in Egypt. These samples were cut into small pieces of 0.5 cm. long, and then surface sterilized by dipping in 0.5% sodium hypochlorite ($NaOCl_3$) solution for 30 sec and washed with sterile distilled water, then dried well between two sterilized filter papers.

These sample pieces were transferred to Petri dishes (9 cm) containing water agar medium. All Petri dishes were incubated at 24°C for 4-7 days and observed daily. Developed fungal growth has been purified, using the hyphal tip technique, on water agar medium. These purified cultures were recultured on potato dextrose agar (PDA) medium. Fungal isolates, obtained from different locations, were identified on the basis of their morphological features according to keys described by Elad *et al.* (2007) and maintained on PDA medium at 5°C.

*Preparation of B. cinerea spores:*

An experiment was conducted to compare the ability of 15 isolates of *B. cinerea* (Bc1, Bc2, Bc3, Bc4, Bc5, Bc6, Bc7, Bc8, Bc9, Bc10, Bc11, Bc12, Bc13, Bc14 and Bc15) to cause gray mould. For each isolate of *B. cinerea*, one single spore was grown on PDA plate amended with 100 mg $L^{-1}$ streptomycin (SPDA). All isolates were incubated at 24°C for two weeks receiving 12 h in fluorescent light and 12 h in dark to obtain sufficient fungal growth. The plates were flooded with sterilized distilled water supplemented with 0.01% Tween 80. Each plate surface was scraped gently with a sterile loop to release the spores. The resulting spore suspension was adjusted to approximately 1 to $2\times10^6$ conidia per ml.

*Pathogenicity test:*

Three replications, each of ten fruits per treatment, were inoculated with 10 µl of the conidial suspension of *B. cinerea*, and then covered with sterilized plastic containers at 20°C with high relative humidity for 3 days. Controls were prepared using the same suspensions but without *B. cinerea*. After 5 days, each infected fruit was used for re-isolation of *B. cinerea* and conserved on PDA and paraffin oil.

*Disease assessment:*

Infection with gray mould was determined as percentage of infected fruits.

*Preparation of selective media:*

Selective media were prepared in two ways. The first one, called Kerssies medium (KERS), was prepared as detailed in previous publications (Kritzman and Netzer 1978; Kerssies, 1990). The other medium is called modified Kerssies medium (mKERS) and contained: glucose, 20g; NaNO3, 1g; KH2PO4, 1.2g; MgSO4.7H2O, 0.2g; KCl, 0.15g; chloramphenicol, 0.05g; pentachloronitrobenzene 0.02g; tannic acid, 5g; CuSO4, 2.2g; Cabrio Top, 0.1g; agar, 25g. This medium, with an unadjusted pH of approx. 5.4, was autoclaved at 121°C for 20 min. Various fungal species (*B. cinerea*, *Fusarium oxysporum*, *Alternaria alternata*, *Rhizoctonia solani*, *Aspergillus niger* and *Penicillium expansum*) were obtained from the collection of the Plant Pathol. Dept., Fac. of Agric., Ain Shams Univ. and grown on PDA medium as a control. For each fungus, mycelial plug-inoculated plates were incubated at 24 °C until mycelia had reached the edge of the plates (4-5 days). These different fungi were then transferred to KERS and mKERS media. The mycelium growth and the brown halo formation of either *B. cinerea* or other fungal species were also noted. This procedure was repeated twice.

*Morphological test of B. cinerea isolates on mKERS:*

Each isolate (Bc1, Bc2, Bc3, Bc4, Bc5, Bc6, Bc7, Bc8, Bc9, Bc10, Bc12, Bc31, Bc32, Bc33, Bc71, Bc72, Bc73, Bc74, Bc75, Bc82, Bc83, Bc101, Bc102, Bc103,

Bc104, Bc105 and/or Bc106) was grown on mKERS at 24°C. After 21 days, their growth variation on mKERS was observed. Subcultured isolates were then transferred to PDA and stored under paraffin oil to preserve isolates for further analyses.

*Preparation of fungal cultures for molecular test:*

Twelve isolates of *B. Cinerea, i.e.* Bc1, Bc2, Bc3, Bc4, Bc5, Bc6, Bc7, Bc8, Bc9, Bc10, Bc11 and Bc12, were used to study the genetic variation by using primers specific to transposable elements, *Flipper* and *Boty*. Each isolate was used to inoculate 100 ml of potato dextrose broth medium in flasks (250 ml) and incubated at 24°C for seven days. The mycelial mats were harvested by filtration through Whattman No.1 filter paper under vacuum, washed three times with deionized water and blotted to dry.

*DNA extraction and Detection of transposable elements:*

Genomic DNA was extracted according to the method described by Moller *et al.* (1992). Mycelia were collected from 10-day-old *B. cinerea* cultures grown at 24°C in the dark. The yield and integrity of the DNA were checked by agarose gel electrophoresis and the concentration of DNA suspension was adjusted to 50 ng/µl.

In order to evaluate genetic diversity within *B. cinerea* isolates obtained from different governorates in Egypt, *Boty*, a long-terminal repeats (LTRs) (Diolez *et al.*, 1995), and *Flipper*, a mobile Fot1-like transposable element (Levis *et al.*, 1997), have been identified from *B. cinerea*. The PCR primer pair F300 (5'-GCA CAA AAC CTA CAG AAG A-3') and F1550 (5'-ATT CGTTTC TTG GAC TGT A-3') has been developed to detect the *Flipper* element successfully (Levis *et al.*, 1997). This primer pair amplified a 1.250-bp product representing the *Flipper* element (Gen Bank accession No. U74294). To detect the element *Boty*, a pair of primers *Boty* F4 (5'-CAG CTG CAG TAT ACT GGG GGA-3') and *Boty* R4 (5'-GGTGCT CAA AGT GTT ACG GGA G-3') was designed based on the DNA sequence of the *Boty* element (Gen Bank accession No. X81790). The PCR reaction was performed in a 50-µl volume containing 50 ng of fungal DNA, 0.2 µM of each primer, 0.2 mM of each dNTP, 2.0 mM MgCl2, 1× *Taq* polymerase buffer and 2 U of *Taq* polymerase. The PCR was performed using the following parameters: an initial preheat for 3 min at 95°C, followed by 40 cycles of denaturation at 94°C for 40 s, annealing at 60°C for the primer pair F300 and F1550 or 68°C for the primer pair *Boty* F4 and *Boty* R4, for 40s, extension at 72°C for 1 min, and terminated with a final extension at 72°C for 10 min. PCR amplicons were size verified on 1.5% agarose gels in Tris-acetate-EDTA (TAE) buffer. Each isolate was amplified two times with each primer pair.

## Results

*Identification and morphological characterization of Botrytis cinerea:*

Fifteen isolates of *B. cinerea* were recovered from eight strawberry orchards located at four governorates (Esmailiya, Sharkiya, Kaliobiya and Behira). These isolates were classified according to their cultural characterization (Table 1).

*Pathogenicity test:*

Data presented in (Table 1) show that the value of infection percentage of strawberry fruits was the highest (88%) for Bc7 isolate, obtained from Kaliobiya. Whereas, the lowest (63%) was due to infection by Bc10 isolate, obtained from the same governorate. This indicates that aggressiveness variation of the tested isolates exist in the same governorate.

**Table 1. Morphological characterization of *B. cinerea* obtained from different locations and their infection percentage**

| Location | Isolate | Morphological characteristic | | Infection (%) |
|---|---|---|---|---|
| | | Production of conidia | Production of sclerotia | |
| Behira | Bc1 | + | +f | 71 |
| | Bc2 | + | +f | 67 |
| | Bc3 | + | + | 80 |
| | Bc4 | + | +f | 80 |
| Kaliobiya | Bc5 | + | + | * |
| | Bc6 | + | + | 83 |
| | Bc7 | + | - | 88 |
| | Bc8 | + | + | 83 |
| | Bc9 | + | + | * |
| | Bc10 | + | + | 63 |
| Sharkiya | Bc11 | + | + | * |
| | Bc12 | + | +f | * |
| | Bc13 | + | +f | * |
| Esmailiya | Bc14 | + | + | * |
| | Bc15 | + | + | * |

* Isolates not tested by artificial inoculation.
f: Indicates the production of few sclerotia.
+: Presence of conidia/or sclerotia.
-: Absence of conidia/or sclerotia.

*Characterization of the morphology of B. cinerea isolates on mKERS medium:*

The mycelial growth of *B. cinerea* isolates was fast developed on modified KERS medium (mKERS) and surrounded by a dark brown halo clearly visible after 2-3 days (Table 2 and Fig. 1), while the unmodified KERS medium showed restricted radial growth with a faint brown halo surrounding the mycelial growth of *B. cinerea*, compared to the mycelial growth of *Fusarium oxysporum* (Fig. 1). In addition, after 21 days, all other fungi gave similar faint brown haloes surrounding their restricted growth on both KERS and mKERS media (Table 3). This result means that the mKERS medium has greater selectivity resulting in an ability to detect the presence of *B. cinerea*. Strawberry fruits inoculated with isolates: Bc1, Bc2, Bc3, Bc4, Bc6, Bc7, Bc8 and Bc10 showed aggressiveness

variation after pathogenicity test (Table 1). New other *B. cinerea* isolates were obtained from strawberry fruits inoculated with the previous isolates (Bc1 to Bc10). 27 isolates of *B. cinerea* were tested on mKERS in order to show the efficacy of this medium to detect isolates differently resistant to Cabrio Top. These isolates showed a great variation in their mycelial growth on mKERS after 21 days (Table 4 and Fig. 2).

**Table 2. Characterization of the mycelium growth of *B. cinerea* isolates on both KERS and mKERS media during one week**

| Tested media | Incubation period (day) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| KERS | 0 | 0 | 0 | 0 | + | ++ | +++ |
| mKERS | 0 | + | ++ | +++ | +++ | ++++ | ++++ |

+, ++, +++ and ++++ indicate, from left to right, increasing of the fungal mycelial growth on KERS/ mKERS media within one week.

**Table 3. Fungal mycelial growth on KERS and mKERS media, 7 and 21 days after incubation**

| Tested fungus | Incubation period | | | |
|---|---|---|---|---|
| | 7 days | | 21 days | |
| | Medium | | | |
| | KERS | mKERS | KERS | mKERS |
| *Botrytis cinerea* | ++ | ++++ | ++/+++ | ++++ |
| *Fusarium oxysporum* | - | - | - | -/+ |
| *Alternaria alternata* | - | - | + | ++ |
| *Rhizoctonia solani* | - | - | - | - |
| *Aspergillus niger* | - | - | ++ | + |
| *Penicillium expansum* | - | - | - | - |

+, ++, +++, ++++ and +++++ indicate the degree of mycelial growth surrounded with the brown halo.

**Table 4. Radial mycelial growth variation among 27 isolates of *B. cinerea* on mKERS medium after 21 days incubation at 24°C**

| | Radial mycelial growth degree* | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Tested isolate | Bc6<br>Bc10<br>Bc8<br>Bc4 | Bc7<br>Bc3<br>Bc1<br>Bc2 | Bc101- Bc31- Bc 104- - Bc74- Bc71- Bc 72- Bc75 - Bc82<br>Bc32 – Bc102 | Bc103- Bc33<br>Bc12 – Bc83<br>Bc106 – Bc73<br>Bc5 –B105- Bc9 |

* 1, 2, 3 and 4 indicate the arrangement of 27 isolates according to their radial growth degree on mKERS medium.

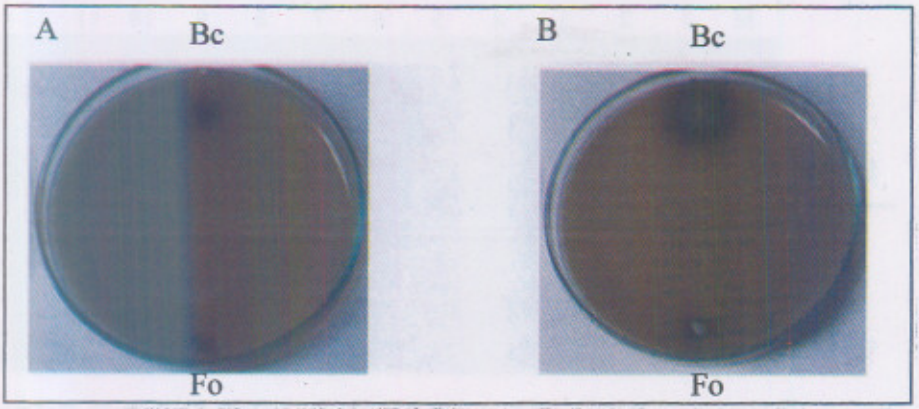


**Fig. 1. Characterization of the phenotype of *B. cinerea* (Bc) and *Fusarium oxysporum* (Fo) on KERS (A) and mKERS (B) media, 7 days after incubation.**

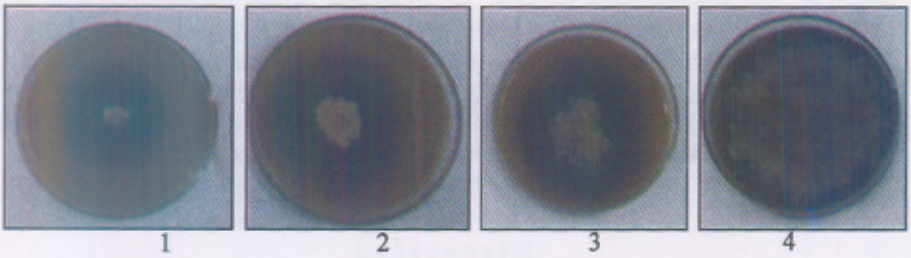


**Fig. 2. Radial mycelial growth variation of different isolates of *B. cinerea* grown on mKERS after 21 days at 24°C. 1, 2, 3 and 4 show the mycelial growth degree of 27 isolates on mKERS medium and the brown halo surrounding their growth.**

*Molecular analysis using TEs specific primers.*

Molecular fingerprinting of twelve isolates of *B. cinerea,* using TEs test, was used to mark the genetic diversity and study its relation to their capability to infect strawberry fruits. In fact, it was previously published that *B. cinerea* group II has been described to mostly exhibit the *transposa* genotype (contains both *Boty* and *Flipper*) and occasionally the *vacuma* genotype (contains neither of them). In order to determine whether the Egyptian populations exhibit the *transposa* genotype and the *vacuma* genotype, the presence of both transposable elements in 12 isolates was tested by PCR method. The results documented that indeed the vast majority of isolates (10, corresponding to 83.3%) contained only *Boty* element (Fig. 3).

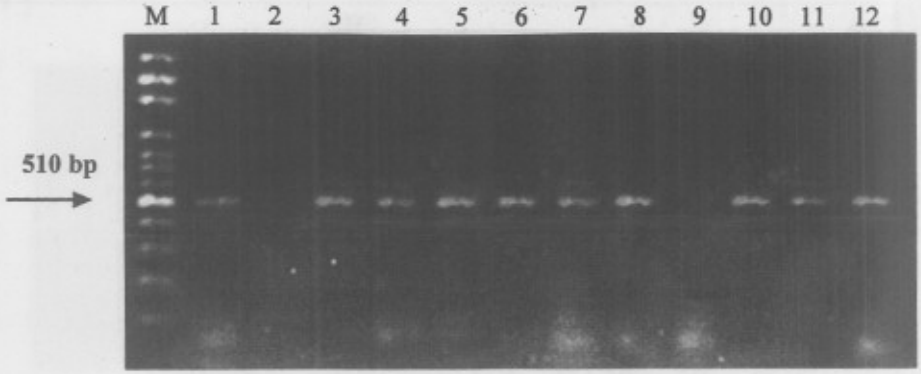


**Fig. 3. Polymerase chain reaction (PCR) amplicons of *B. cinerea* isolates (1 to 12: Bc1, Bc2, Bc3, Bc4, Bc5, Bc6, Bc7, Bc8, Bc9, Bc10, Bc11 and Bc12, respectively) obtained by using specific primer pair to the *Boty* element. M: 100 bp DNA Ladder.**

However, two isolates were *vacuma, i.e.* contained neither transposable elements (Bc2 and Bc9). Two primers were used for this purpose. The first primer, specific to *Flipper* element showed no amplification product (data not shown), whereas the second primer, specific to *Boty* element, showed amplification product in 10 isolates (Bc1, Bc3, Bc4, Bc5, Bc6, Bc7, Bc8, Bc10, Bc11 and Bc12) from 12 (Fig. 3). These isolates were obtained from different governorates and exhibited different morphological and pathological characterization (Tables 1 & 4).

## Discussion

The first part of the results revealed phenotypic variation either on PDA medium or on mKERS medium. Two main morphological types of *B. cinerea* isolates have been shown on PDA medium, mycelial type with few or without sclerotia and sclerotial type with many sclerotia. Such morphological types were reported previously (Goto *et al.*, 1980; Martinez *et al.*, 2003; Mirzaei *et al.*, 2009). These phenotypic variations could be caused by mutation and heterokaryosis (Coley-Smith *et al.*, 1980; Elad *et al.*, 2007). The use of mKERS medium has monitored high selectivity to the mycelium growth of *B. cinerea* surrounded with dark brown pigments. This coloration is due to the fungal production of laccases (benzenediol: oxygen oxidoreductases) which have been found in many fungal species like *B. cinerea* (Dubernet *et al.*, 1977; Gigi *et al.*, 1981). Laccase enzyme is induced by many substrates like tannic acid which is degraded producing the brown pigment (Schouten *et al.*, 2002). In fact, several selective media have been developed by other research groups for the isolation of *Botrytis* spp. In addition, previous studies have determined that the duration needed to trigger latent infections is over than 14 days using Kritzman's agar (Kritzman, 1983). In this study, the mycelium growth

of 27 isolates of *B. cinerea* became clearer and surrounded by dark brown pigments on mKERS medium within 3 days after incubation. The mycelium growth degree of these isolates was found to be uncorrelated with their severity variation on strawberry fruits. Moreover, the tested fungal species have showed neither brown pigment nor mycelium growth on mKERS medium during 3 weeks after incubation. Therefore, mKERS medium is more efficient than the others previously reported (Edwards and Seddon, 2001; Gielen *et al.*, 2003). This medium is developed selective which contains Cabrio Top, a fungicide showed its effect against the growth of many fungal species. It is clear that any fungal species could be returned resistant to this fungicide if this one will be used repeatedly. Indeed, the mKERS medium proved advantageous over previous media because it had greater selectivity for only *B. cinerea*. Therefore, mKERS medium could be suitable for further studies in order to isolate and identify *B. cinerea* from directly unsterilized plant materials which have latent infection.

Concerning PCR analysis, using two specific primers to TEs (*Flipper* and *Boty* elements), the results revealed the presence or absence of *Boty* element among different isolates. In fact, the transposable elements, TEs, play a major role in the genetic variability in all organisms such as fungi (Dufresne *et al.*, 2007; Lopez-Berges *et al.*, 2009) due to their movement in the genome producing numerous mutations which affect gene expression. *B. cinerea* was believed to consist of two sympatric species, of which one (=*transposa*) contains both *Flipper* and *Boty* and the other (=*vacuma*) contains neither of them (Giraud *et al.*, 1997). In fact, the study of the existence of TEs in the genome of *B. cinerea* is very important as previous studies have showed that *vacuma* and *transposa* were also found to significantly differ in phenotype such as conidial size, growth rates, and resistance against vinclozolin and diethofencarb (Giraud *et al.*, 1999). However, previous studies provided the evidence for two different sibling species of *B. cinerea* which they named group I and group II (Fournier *et al.*, 2005). In comparison to the *vacuma* and *transposa* concept, *B. cinerea* group I indeed contained only *vacuma* isolates. The results from the present study show that the *B. cinerea* populations from different regions in Egypt exhibited an unusual transposable elements composition; as the majority of isolates (>83%) contain only *Boty* element. In fact, the presence of only *Flipper* element in *B. cinerea* has so far not been detected in isolates from California (Ma and Michailides, 2007) and Chile (Muñoz *et al.*, 2002), and seems to be strongly underrepresented in samples from England, France, and Tunisia (Albertini *et al.*, 2002; Ben Ahmed and Hamada, 2005). In contrast, more recent study on *B. cinerea* isolates from Croatia (Milicevic *et al.*, 2006) reported that 26% of isolates contain only *Flipper*, whereas 41% contain *transposa*. In addition, almost all analyzed isolates collected from Mendoza region of Argentina are *transpose* type (Munoz *et al.*, 2010). The higher number of isolates containing only *Boty* element raises the question of how these strains evolved, *i.e.* from *transposa* strains by loss of *Flipper* element, or from *vacuma* strains by gain of *Boty* element. This investigation is in contrast with previous studies (Munoz *et al.*, 2002). The occurrence of *Boty* isolates in the Egyptian population may be explained by.crosses between *vacuma* and *transposa*, or by the existence of an invading population of the *vacuma* group. Because of the low number of the tested isolates, the authors cannot

rely on these two possibilities. Generally, efficient molecular methods are considering expensive and not easy to perform routinely for diagnostic process. This is why the classical methods which have been explained above must be used in parallel with molecular tests. However, further analysis should be done on a large number of isolates collected from different locations and hosts in Egypt, in order to confirm the existence or not of *Flipper* and *Boty* elements in their genomes. These results will make a huge impact on further research as this study will enable the disease to be diagnosed cheaply, easily before normal expression of symptoms, pre- or post harvest, leading to better management of gray mould.

## References

Albertini, C.; Thebaud, G.; Fournier, E. and Leroux, P. 2002. Eburicol 14α demethylase gene (CYP51) polymorphism and speciation in *Botrytis cinerea*. *Mycol. Res.*, **106**:1171-1178.

Baroffio, C.A.; Siegfried, W. and Hilber, U.W. 2003. Long-term monitoring for resistance of *Botryotinia fuckeliana* to anilinopyrimidine, phenylpyrrole and hydroxyanilide fungicides in Switzerland. *Plant Dis.*, **87**: 662-666.

Ben Ahmed, D. and Hamada, W. 2005. Genetic diversity of some Tunisian *Botrytis cinerea* isolates using molecular markers. *Phytopathol. Mediterr.*, **44**: 300-306.

Choquer, M.; Fournier, E.; Kunz, C.; Levis, C.; Pradier, J.M.; Simon, A. and Viaud, M. 2007. *Botrytis cinerea* virulence factors: new insights into a necrotrophic and polyphageous pathogen. *FEMS Microbiol Letters*, **277**: 1-10.

Coley-Smith, J.R.; Verhoeff, K. and Jarvis, W.R. 1980. *The Biology of Botrytis*. Academic Press, London. 318pp.

Diolez, A.; Marches, F.; Fortini, D. and Brygoo, Y. 1995. *Boty*, a long-terminal-repeat retroelement in the phytopathogenic fungus *Botrytis cinerea*. *Appl. Environ. Microbiol.*, **61**: 103-108.

Dubernet, M.P.; Ribereau-Gayon, H.R.; Lerner, E.; Harel and Mayer, A.M. 1977. Purification and properties of laccase from *Botrytis cinerea*. *Phytochemistry*, **16**: 191-193.

Dufresne, M.; Hua-Van, A.; Abd El Wahab, H.; Ben M'Barek, S.; Vasnier, C.; Teysset, L.; Kema, G.H.J. and Daboussi, M.J. 2007. Transposition of a fungal MITE through the action of a Tc1-like transposase. *Genetics*, **175**: 441-45.

Edwards, S.G. and Seddon, B. 2001. Selective media for the specific isolation and enumeration of *Botrytis cinerea*. *Letters in Appl. Microbiol.*, **32**: 63-66.

Elad, Y.; Yunis, H. and Katan, T. 1992. Multiple resistance to benzimidazoles, dicarboximides and diethofencarb in field isolates of *Botrytis cinerea* in Israel. *Plant Pathol.*, **41**: 41-46.

Elad, Y.; Williamson, B.; Tudzynski, P. and Delen, N. 2007. *Botrytis* spp. and diseases they cause in agricultural systems - an introduction. *Botrytis: Biology, Pathology and Control*. Netherlands, Springer, 1-8.

Fournier, E.; Giraud, T.; Albertini, C. and Brygoo, Y. 2005. Partition of the *Botrytis cinerea* complex in France using multiple gene genealogies. *Mycologia*, **97**: 1251-1267.

Gielen, S.; Aerts, R. and Seels, B. 2003. Development of a selective medium for the determination of the spore concentrations of *Botrytis cinerea* in the air. *Commun Agric. Appl. Biol. Sci.*, **68**: 685-693.

Gigi, O.; Marbach, I. and Mayer, A.M. 1981. Properties of gallic acid-induced extracellular laccase of *B. cinerea*. *Phytochemistry*, **20**: 1211-1213.

Giraud, T.; Fortini, D.; Levis, C.; Leroux, P. and Brygoo, Y. 1997. RFLP markers show genetic recombination in *Botryotinia fuckeliana* (*B. cinerea*) and transposable elements reveal two sympatric species. *Mol. Biol. Evol.*, **14**: 1177-1185.

Giraud, T.; Fortini, D.; Levis, C.; Lamarque, C.; Leroux, P.; LoBuqlio, K. and Brygoo, Y. 1999. Two siblings species of the *Botrytis cinerea* complex, *transposa* and *vacuma*, are found in sympatry on numerous host plants. *Phytopathology*, **89**: 967-973.

Goto, S.; Terabayashi, T. and Yokotsuka, I. 1980. Identification, cultural properties and pathogenicity of grey mould of grape *Botrytis cinerea*. *J. Agric. Chem. Soc. Jpn.*, **54**: 117–121.

Kerssies, A. 1990. A selective medium for *Botrytis cinerea* to be used in a spore-trap. *Netherlands J. Plant Pathol.*, **96**: 247-250.

Kritzman, G. and Netzer, D. 1978. A selective medium for isolation and identification of *Botrytis* spp. from soil and onion seed. *Phytoparasitica*, **6**: 3-7.

Kritzman, G. 1983. Identification of latent *Botrytis allii* Munn in onion bulbs. *Crop Protec.*, **2**: 243-246.

Levis, C.; Fortini, D. and Brygoo, Y. 1997. *Flipper*, a mobile Fot1-like transposable element in *B. cinerea*. *Mol. Gen. Gene.*, **254**: 674-680.

Lopez-Berges, M.S.; Di Pietro, A.; Daboussi, M.J.; Abdel Wahab, H.; Vasnier, C.; Roncero, I.G.; Dufresne, M. and Hera, C. 2009. Identification of virulence genes in *Fusarium oxysporum* f.sp. *lycopersici* by large-scale transposing tagging. *Mole. Plant Pathol.*, **10**: 95-107.

Ma, Z. and Michailides, T. J. 2007. Approaches for eliminating PCR inhibitors and designing PCR primers for the detection of phytopathogenic fungi. *Crop Protec.*, **26**: 145-161.

Martinez, F.; Blancard, D.; Lecomte, P.; Levis, C.; Dubos, B. and Fermaud, M. 2003. Phenotypic differences between *vacuma* and *transposa* types of *Botrytis cinerea*. *Eur. J. Plant Pathol.*, **109**: 479-488.

Milicevic, T.; Topolovec-Pintaric, S.; Cvjetkovic, B.; Ivic, D. and Duralija, B. 2006. Sympatric populations of *Botrytis cinerea* on strawberries based on the content of transposable elements and their connection with resistance to botryticides. *Acta Hort.*, **708**: 115-118.

Mirzaei, S.; Goltapeh, I.; Shams-Bakhsh, M.; Safaie, N. and Chaichi, M. 2009. Genetic and phenotypic diversity among *Botrytis cinerea* isolates in Iran. *J. Phytopathol.*, **157**: 474-482.

Moller, E.; Bahnweg, G.; Sandermann, J.R.H. and Geiger, H. 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies and infected plant tissues. *Nucleic Acids Res.*, **20**: 6115-6116.

Muñoz, G.; Hinrichsen, P.; Brygoo, Y. and Giraud, T. 2002. Genetic characterization of *Botrytis cinerea* populations in Chile. *Mycological Res.*, **106**: 594-601.

Muñoz, C.; Gómez Talquenca, S.; Oriolani, E. and Combina, M. 2010. Genetic characterization of grapevine-infecting *Botrytis cinerea* isolates from Argentina. *Rev. Iberoam Micol.*, **27**: 66-70.

Schouten, A.; Wagemakers, L.; Stefanato, F.L.; Van der Kaaij, R.M. and Van Kan, J.A.L. 2002. Resveratrol acts as a natural profungicide and induces self-intoxication by a specific laccase. *Mole. Microbiol.*, **43**: 883-894.

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# تحسين وسائل الكشف عن مرض العفن الرمادى فى الفراولة المتسبب عن فطر *Botrytis cinerea* فى مصر

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يُسبب فطر *Botrytis cinerea* أمراضا للعديد من المحاصيل الهامة اقتصاديا. تم اختبار القدرة المرضية لسبعة وعشرين عزلة من فطر *Botrytis cinerea* ، عزلت من أماكن مختلفة بمصر، على ثمار الفراولة. ثم تم تطوير طريقة بسيطة وغير مكلفة لتعريف عزلات هذا الفطر الممرض، المسبب لمرض العفن الرمادى فى الفراولة، وذلك باستخدام بيئة صناعية متخصصة، وهى بيئة Kerssies المعدلة (mKERS). فضلا عن ذلك، لم تظهر الأنواع الفطرية الأخرى المختبرة أى نموعلى هذه البيئة. كما أوضحت النتائج أنه لا توجد علاقة بين النسبة المئوية للإصابة الناتجة من كل عزلة مختبرة من فطر *B. cinerea* ودرجة النمو الميسليومى لهذه العزلات على بيئة mKERS. كذلك أظهرت سبعة وعشرون عزلة نمواً واضحاً على هذه البيئة محاطة بهالة ذات لون بنى داكن. هذه النتائج تشير إلى إمكانية استخدام هذه الطريقة المبسطة لعزل وتعريف فطر *B. cinerea* المسبب لمرض العفن الرمادى فى الفراولة.

تم اختبار التباين الوراثى على اثنى عشر عزلة، باستخدام تقنية بلمرة الحمض النووى (PCR)عن طريق الكشف عن وجود اثنين من العناصر المتنقلة بالجينوم: *Flipper* و *Boty*. أظهرت النتائج وجود العنصر *Boty* فى عشرة عزلات، بينما لم يظهر وجود أى من هذين العنصرين فى عزلتين فقط، والتى بالتالى تتبع المجموعة التقسيمية *vacuma type*.

هذه النتائج تُعتبر هامة من ناحية تعريف وتقسيم عزلات فطر *B. cinerea* المصرية، طبقا لتركيبها الجينومى، خاصة بالنسبة للعزلات عالية القدرة المرضية.