

## OCCURRENCE OF THE SELF-FERTILE PHENOTYPE OF *Phytophthora infestans* IN EL-BEHERA GOVERNORATE, EGYPT

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### ABSTRACT

A survey for the self-fertile phenotype of *Phytophthora infestans* was conducted during the 1999-2001 growing seasons. Blighted potato were sampled from fields in different localities (El-Rahmania, Housh Eissa, Kafr El-Dawar, and Kafr El-Zayat) in El-Behera Governorate and the surrounding area where isolates of *P. infestans* were recovered. Out of 23 *P. infestans* isolates made in the first year (1999), only one isolate was self-fertile (SF). In the second year (2000), one more SF isolate was detected among 50 *P. infestans* isolates, while in the third year (2001) three isolates out of 172 were SF. That constituted 4.3%, 2%, and 1.7% for the three years of the study, respectively. Propagations made from single sporangia and single hyphal tips of the recovered SF isolates segregated A1, A2, and few SF colonies. This supported the view that self-fertility in *P. infestans* was a mixture of A1 and A2 mating types hyphae that interacted to form SF colonies and later segregated where new strains of *P. infestans* could be evolved. The low frequency of the SF isolates (2%) revealed in the present study with its high percentage of oospore abortion (15.3%), low viability (2.4%), and germination (1.8%) may indicate that the threat posed by the interaction between A1 and A2 mating type isolates is real. The potential for the time being could be undetectable. However, creation of new more vigorous strains via this mechanism can not be excluded.

**Keywords :** *Phytophthora infestans*, self-fertility, mating type, potato.

### INTRODUCTION

*Phytophthora infestans* was recorded as a heterothallic species (Tucker, 1931; Savage *et al.* 1968) containing two compatible types designated A1 and A2 mating types (Gallegly and Galindo, 1958). However, some isolates of *P. infestans* were noticed to have a different culture morphology as exhibited, on rye-A or V8 juice media, a waxy appearance with little aerial mycelium and few sporangia. Surprisingly, these cultures were packed full with oospores. Such phenotype in *Phytophthora* species was designated the self-fertile (SF) phenotype (Mortimer *et al.*, 1977). Recent studies revealed the existence of the SF phenotype of *P. infestans* in USA (Vartanian and Endo, 1985), Japan (Mosa *et al.*, 1989), Britain (Tantius *et al.*, 1986; Shattock *et al.*, 1990), Australia, Ireland, and Holland (Ko, 1994). Nature of the SF phenotype was studied in a number of *Phytophthora* species (Mortimer *et al.*, 1977; Niederhauser, 1991; Fyfe and Shaw, 1992) to establish whether self-fertility was a novel homokaryon genotype, a heterokaryon consisted of A1 and A2 genotypes, or a mixture of A1 and A2 mating types hyphae. Occurrence of the SF isolates in the field was considered a circumstantial evidence that mating does occur in the field which could be responsible for creating new strains of *P. infestans* that could

constitute a threat to potato cultivation in Egypt and all over the world (Shattock *et.al.*, 1990; Ko, 1994; Levin *et.al.*, 2001).

The present study, therefore, was conducted to investigate this issue in El-Behera Governorate and the surrounding area, where potato was intensively cultivated, through the following approaches :

Assessing the occurrence of the SF phenotype in the latest years to monitor the population dynamics of this phenotype.

Studying characteristics of the recovered SF isolates to reveal nature of self-fertility in the Egyptian isolates of *P.infestans*.

Evaluating the threat posed by the SF phenotype to potato cultivation in El-Behera Governorate and the surrounding area.

## **MATERIALS AND METHODS**

### **Isolation and identification of the sf isolates of *P. Infestans*:**

Blighted potato tubers and foliage were collected from fields in different localities (El-Rahmania, Housh Eissa, Kafr El-Dawar, and Kafr El-Zayat) in El-Behera Governorate and the surrounding area where potato was intensively cultivated. That was conducted for three successive growing seasons of 1999-2001. Isolation and identification of the SF isolates were conducted according to Fyfe and Shaw (1992) on rye- A medium (Caten and Jinks, 1968). Isolates were maintained on rye-A and if necessarily were stored for short periods under sterile mineral oil (Pittis and Shattock, 1994).

### **Characteristics of the SF field isolates of *P. infestans*:**

#### **- Stability of self-fertility during subculturing:**

Culture plugs on rye-A (5mm in diameter), taken from the advancing margin of 7-day-old cultures of the tested SF isolates, were transferred to plates of fresh rye-A at one month intervals for one year. Five replicate plates were conducted for each SF isolate. Inoculated plates were incubated at 18<sup>0</sup>C in darkness and monitored for any segregation.

#### **Yield of oospores:**

Plates of rye-A were inoculated with culture plugs of the SF field isolates tested, in the same manner described above and incubated at 18<sup>0</sup>C in darkness. Three weeks later, an oospore suspension of each of the five replicate plates of each tested SF isolate was obtained by blending a culture block (2 cm in diameter) containing oospores with 20ml sterile distilled water according to Pittis and Shattock (1994). A 0.01ml of the obtained oospore suspension was taken where oospores were counted under 10x light microscope. Five counts were conducted for each oospore suspension and mean total yield of oospores was calculated.

#### **Percentage of oospore abortion:**

Oospores had disorganized contents were considered aborted (Rutherford and Ward, 1985). The same slides tested above for yield of oospores were reinvestigated under 40x light microscope for the existence of



aborted oospores. Five counts were conducted for each oospore suspension and mean percentage of oospore abortion was calculated for each SF isolate.

**Percentage of oospore viability:**

This test was conducted according to Pittis and Shattock (1994). A 5ml of each of the previously obtained oospore suspensions was mixed with equal volume of 2M NaCl solution. Oospores responded to saline solution and plasmolysed were considered viable. A 0.01 ml of oospore suspension in saline solution was investigated under 40x light microscope for the plasmolysed oospores. Five counts were conducted for each oospore suspension and mean percentage of oospore viability was calculated.

**- Percentage of oospore germination:**

A 10 ml of each of the previously obtained oospore suspension was filtered through 20 µm-pore nylon filters. Collected oospores were treated according to Pittis and Shattock (1994) and resuspended in 5 ml sterile deionized water. A 0.5 ml of the resultant oospore suspension was spread on a thin-layer plate of rye-A and incubated at 18°C under blue filter with background of continuous cool-white fluorescent light (Ko, 1994). Number of germinated oospores was assessed weekly by investigating plates under dissecting microscope. Mean percentage of oospore germination was calculated six weeks after plating.

**Propagations from single sporangia and single hyphal tips:**

Trials were made to obtain single zoospore progeny to analyse the SF isolates, however, viable zoospores were lacked. Consequently, analysis of self-fertility was confined to single sporangia and single hyphal tips according to Fyfe and Show (1992) as follows:

Three SF isolates representing the three years of the study were tested for propagation analysis. Seven-day-old cultures of the SF isolates were flooded with sterile distilled water (10ml per plate). A 0.5 ml of the sporangial suspension was plated on a thin-layer-plate of rye-A, five plates for each SF isolate. Germination of sporangia was monitored daily under dissecting microscope. Fifty germinating sporangia of each SF isolate were individually transferred to fresh rye-A plates and incubated at 18°C in darkness.

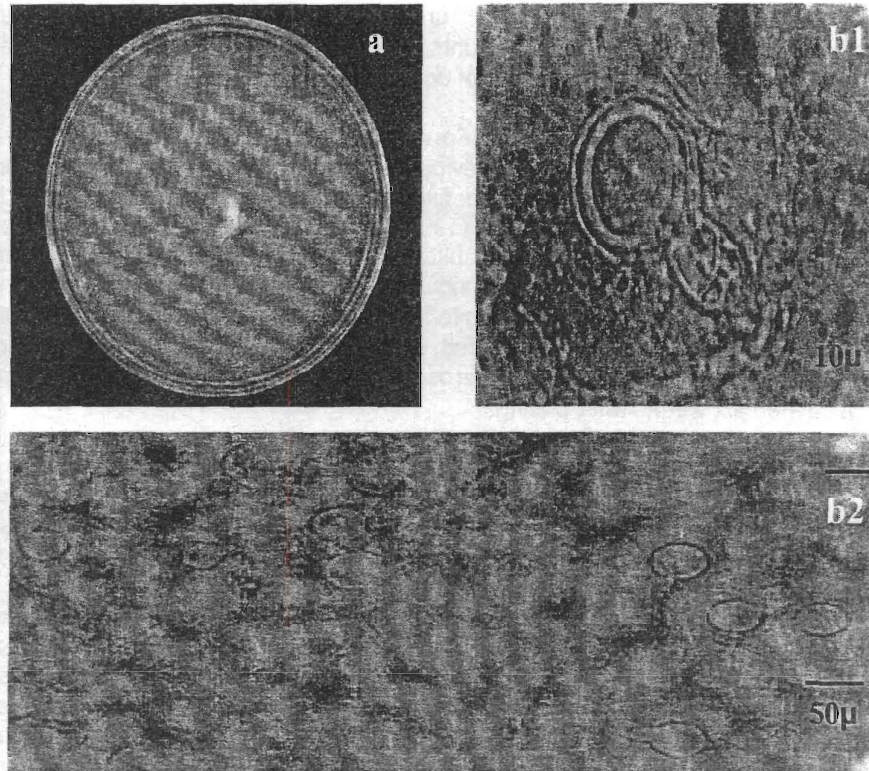
For hyphal tip propagation analysis, thin-layer plates of clarified rye-A (Caten and Jinks, 1968) were inoculated with culture plugs of the tested SF isolates and incubated at 18°C until the hyphae had grown to 2-3 cm. Hyphal tips (50 for each SF isolate) were excised on agar block, with sterile scalpel under dissecting microscope, and individually transferred to fresh rye-A plates and incubated at 18°C in darkness.

Mating types of the colonies developed, through hyphal tips and single sporangia propagations, were determined against E14 *P.infestans* tester as described by Hanson and Shattock (1998). The E14 *P.infestans* tester was kindly supplied by D.S. Shaw, University College of North Wales, U.K.

## RESULTS

### The self-fertile field isolates recovered:

Only five SF field isolates of *P. infestans* were detected over the three years of the study during the 1999-2001 growing seasons. SF colonies exhibited waxy appearance, lacked of aerial hyphae (Fig. 1a) and cultures were packed full with oospores (Fig. 1b).



**Fig. 1: Colony morphology (a) and oospores (b1&b2) of self-fertile isolates of *P. infestans* (isolate, 5/2001) recovered from El-Behera Governorate.**

One isolate out of 23 *P. infestans* isolates obtained during the first year of the study (1999) was self-fertile. Also, in the second year (2000) one more isolate out of 50 isolates recovered was SF while in the third year (2001), three isolates were SF out of 172 *P. infestans* isolates obtained. That constituted 4.3% 2.0%, and 1.7% for the three years of the study, respectively. Four SF isolates out of the five made were recovered from blighted tubers while only one isolate was isolated from blighted foliage (Table1).



**Table (1):** Number of SF isolates recovered from different localities in El-Behera Governorate and the surrounding area during the 1999-2001 growing seasons.

Year of isolation	Organ <sup>a</sup>		Total	%
	Tubers	Foliage		
1999	1/11 <sup>b</sup>	0/12	1/23	4.3
2000	1/23	0/27	1/50	2.0
2001	2/124	1/48	3/172	1.7
Total	4/158	1/87	5/245	2.0 <sup>c</sup>

<sup>a</sup> Potato organ from which isolates recovered

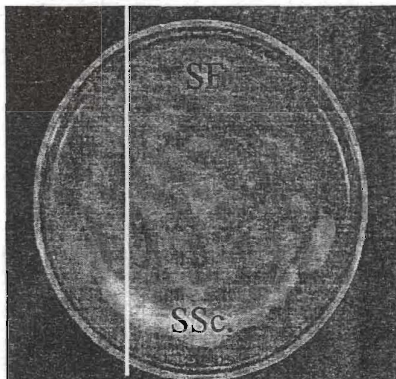
<sup>b</sup> Number of SF isolates / Number of total isolates recovered.

<sup>c</sup> Mean percentage of SF isolates recovered.

**Characteristics of the SF field isolates of *P. infestans*:**

Self-fertile cultures of the recovered isolates were stable (i.e. self-fertility did not disappear) when inocula plugs were transferred to plates of fresh rye-A. The five SF field isolates tested remained fertile over the whole period of the study. However, after several transfers all SF isolates segregated self-sterile sectors of either A1 or A2 mating type (Fig. 2).

The tested SF field isolates of *P. infestans* considerably varied in yield of oospores produced *in vitro*. Yield of oospores produced in 1-cm diameter of rye-A disc ranged between 554 and 3195 with bigger yield for cultures of the 1999/2000 seasons. Percentage of aborted oospores (Fig. 3) ranged between 11.6% and 19.2% with a tendency was parallel to the yield of oospores. Percentage of viable oospores ranged between 1.4% and 3.7% with lower figures in the 1999/2000 isolates. Percentage of oospore germination was 0.6%-2.7% with a trend approximately similar to percentage of oospore viability (Table 2).



**Fig. 2:** Segregation of SF isolate of *P. infestans* to self-sterile sector (SSc.) of A1 mating type (isolate, 3/2001).



**Fig. 3:** Aborted oospores, showed disorganized contents, formed in SF colonies of *P. infestans* (isolate, 5/2001).

**Table (2): Characteristics of SF field isolates of *P.infestans* recovered during 1999/2001 growing seasons from different localities in El-Behera Governorate and the surrounding area.**

Isolate Code No.	Location	Yield of oospores	% Oospore abortion	% Oospore viability	% Oospore germination
1/1999	El-Rahmania	2011 <sup>a</sup> ± 143 <sup>b</sup>	17.3 ± 2.9	1.4 ± 0.4	1.6 ± 0.2
2/2000	Kafr El-Zayat	3195 ± 317	19.2 ± 3.7	2.3 ± 0.9	2.1 ± 0.9
3/2001	El-Rahmania	1901 ± 168	11.6 ± 1.1	3.7 ± 1.1	2.7 ± 0.8
4/2001	Kafr El-Zayat	554 ± 82	13.7 ± 1.8	2.1 ± 0.7	0.6 ± 0.3
5/2001	Housh Eissa	1932 ± 171	14.7 ± 3.1	2.9 ± 0.9	2.3 ± 0.4
Mean		1918	15.3	2.4	1.8

Assessment based on five replicates.

<sup>a</sup> Mean number of oospores produced in 1-cm diameter of rye-A disc.

<sup>b</sup> Standard Deviation.

#### Propagations from single sporangia and single hyphal tips:

Cultures derived from single sporangia of the three SF field isolates analysed were mostly self-sterile A1s. One isolate yielded approximately equal frequencies of A1 and A2 mating type colonies. Progenies of the three isolates analysed yielded SF colonies but at low frequencies (Table 3). Single hyphal tips of the analysed SF isolates segregated mostly A1s or A2s in a manner similar to the single sporangia segregation. Self-fertile colonies also occurred but at low frequencies. (Table 3).

**Table (3): Segregation of the analysed SF field isolates of *P.infestans*.**

Isolate	Method of propagation							
	Single sporangia				Single hyphal tips			
	A1	A2	SF	%*	A1	A2	SF	%*
1/1999	49	0	1	2	37	12	1	2
2/2000	25	24	1	2	26	22	2	4
5/2001	34	13	2	4	39	9	2	4
Mean	36	12.3	1.3	2.6	34	14.3	1.6	3.3

\* Number of SF colonies / Total number of colonies segregated.

## DISCUSSION

Results obtained in the present study confirmed the occurrence of the SF phenotype of *P.infestans* in Egypt. SF isolates were recovered over the three years of the study (1999,2000,2001) from blighted potato samples collected from fields in El-Behera Governorate and the surrounding area. Frequencies of SF isolates were as low as 4.3%, 2%, and 1.7% for the three successive years of the study, respectively. Occurrence of SF phenotype in Egypt is not unexpected as it has been recorded in different part in Europe (Tantius *et.al.*, 1986; Shattock *et.al.*, 1990; Ko, 1994) from which Egypt routinely imports potato seeds for summer plantation. It had been also recorded in USA, Japan, Australia, and Mexico at similar low frequencies (Vartanian and Endo, 1985; Mosa *et.al.*, 1989; Niederhauser,1991; Ko,1994).



Much controversy has been conducted concerning nature of the SF isolates of *Phytophthora* species. Niederhauser (1991), suggested a homokaryotic nature in his Mexican *P. infestans*. SF isolates as self-fertility transmitted through the uninucleate zoospores. However, Mortimer *et.al.* (1977) indicated a heterokaryotic nature for self-fertility in *P. drechsleri* as hyphal tips and zoospore propagations segregated A1 and A2 mating types colonies. Fyfe and Shaw (1992) suggested that SF phenotype was a mixture of A1 and A2 mating types hyphae as SF cultures were synthesized *in vitro*, by co-cultivation of A1 and A2 mating types. Consequently, Ko (1994) designated the SF phenotype as the A1A2 type.

The present study, however, showed that propagations made from single sporangia and single hyphal tips of the analysed SF field isolates segregated A1, A2 and few SF colonies. This indicated that self-fertility in the Egyptian isolates of *P. infestans* was not equivalent to homothallism where single protoplasmic units, *i.e.* single sporangia and single hyphal tips or even single nuclei would transmit the self-fertility. Instead, a heterokaryotic or a heteroplasmic condition has been identified. This supported Fyfe and Shaw (1992) view that SF phenotype was, indeed, a mixture of A1 and A2 mating type hyphae which interacted as described by Shaw (1987) to form SF colonies that later segregated to generate new strains of *P. infestans*. Such new strains could be more vigorous and could constitute a threat to potato cultivation (Shattock *et.al.*, 1990; Fyfe and Shaw, 1992; Ko, 1994; Drenth *et.al.*, 1995). However, the low frequency of the SF *P. infestans* isolates occurred (2%) in the present study with its high percentage of oospore abortion (15.3%), low viability (2.4%), and germination (1.8%) may indicate that the threat is real but the potential for the time being could be undetectable. However, creation of new more aggressive strains of *P. infestans* via this mechanism could not be excluded (Hanson and Shattock, 1998; Levin *et.al.*, 2001). More studies are required to understand mechanisms controlling such oospore formation to avoid a sudden outbreak of unexpected epidemics.

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## تواجد أطراز الخصب ذاتياً من الفيتوفثورا إنفستانز بمحافظة البحيرة بمصر أحمد السيد الكوراني قسم النبات الزراعي - كلية الزراعة بدمنهور - جامعة الاسكندرية

قامت دراسة بعمل حصر لمدي تواجد الطراز الخصب ذاتياً من الفطر فيتوفثورا إنفستانز بمحافظة البحيرة والمنطقة المحيطة بها في الفترة من ١٩٩٩ الي ٢٠٠١ وأسفرت الدراسة عن تأكيد تواجد وانتشار هذا الطراز بمناطق زراعة البطاطس بمحافظة البحيرة والمنطقة المحيطة إلا أن نسبة تواجده كانت منخفضة حيث كشف عن عزلة واحدة في العام الأول من الدراسة (١٩٩٩) بين ٢٣ عزلة من الفطر فيتوفثورا إنفستانز تم الحصول عليها، وكذا أسفرت الدراسة في العام التالي (٢٠٠٠) عن الكشف عن عزلة واحدة فقط بين ٥٠ عزلة، بينما في العام الثالث (٢٠٠١) تم الكشف عن ثلاثة عزلات من هذا الطراز بين ١٧٢ عزلة من الفطر فيتوفثورا إنفستانز تم الحصول عليها وعليه فقد شكل هذا الطراز نسبة ٤,٣%، ٢,٧%، ١,٧% بين عشيرة فطر الفيتوفثورا إنفستانز بمحافظة البحيرة والمنطقة المحيطة، وذلك في الاعوام الثلاثة من الدراسة علي التوالي. وفي دراسة استهدفت الكشف عن طبيعة هذا الطراز الخصب ذاتياً تم التقاط أكياس جرثومية مفردة وكذا أطراف لهيئات مفردة من عزلات هذا الطراز وزراعتها علي البيئة المناسبة (بيئة الراي A) فكشفت ان دراسة أن هذا الطراز هو نتيجة خلط بين هيئات الطرازين A2,A1 من الفيتوفثورا إنفستانز وتفاعلهما لتعطي هذا الطراز الخصب ذاتياً والذي تحدث إنعزالات به ليعطي سلالات جديدة من الفطر فيتوفثورا إنفستانز ربما أكثر قدرة علي إحداث مرض اللفحة المتأخرة في البطاطس مما قد يشكل خطراً علي زراعة هذا المحصول بالمنطقة هذا وظهرت الدراسة أن نسبة تواجد هذا الطراز بمحافظة البحيرة منخفضة (٢%)، وكذا كانت نسبة الجراثيم البيضية المجهضة بها عالية (١٥,٣%) إضافة الي حيويتها المنخفضة (٢,٤%) ونسبة إنباتها المنخفضة (١,٨%) مما تشير الي أنه في الوقت الحالي قد لا يكون الخطر ملموس إلا أنه قائم وتجب دراسة كيفية السيطرة عليه قبل ان يتفجر رياء بشكل فجائي وكبير في ظروف يجب دراستها للتنبؤ بها لمنع حدوثها.