OCCURRENCE OF THE SELF-FERTILE PHENOTYPE OF _Phytophthora infestans_ IN EL-BEHERA GOVERNORATE, EGYPT
El-Korany A. E.
Dept. of Agric. Bot., Fac. of Agric, Damanhour, Alex. Univ.

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 Elissa, 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., 1988) containing two compatible types designated A1 and A2 mating types (Gallegly and Galindo, 1952). 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; Shattuck 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; Fyle 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 (Shatlock 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 Elissa, 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 Shatlock, 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°C in darkness and monitored for any segregation.

Yield of oosporcs:

- 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°C in darkness. Three weeks later, an oosporc 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 Shatlock (1994). A 0.01ml of the obtained oosporc suspension was taken where oospores were counted under 10x light microscope. Five counts were conducted for each oosporc suspension and mean total yield of oospores was calculated.

Percentage of oosporc 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 Shatlock (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 plasmolyzed were considered viable. A 0.01 ml of oospore suspension in saline solution was investigated under 40x light microscope for the plasmolyzed 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 Shatlock (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 Shatlock (1996). 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).

![Image of colony morphology and oospores](image)

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.

<table>
<thead>
<tr>
<th>Year of isolation</th>
<th>Organ ¹</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>Tubers</td>
<td>1/11</td>
<td>1/23</td>
</tr>
<tr>
<td></td>
<td>Foliage</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>Tubers</td>
<td>1/23</td>
<td>1/50</td>
</tr>
<tr>
<td></td>
<td>Foliage</td>
<td>0/27</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>Tubers</td>
<td>2/124</td>
<td>3/172</td>
</tr>
<tr>
<td></td>
<td>Foliage</td>
<td>1/48</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>4/158</td>
<td>5/245</td>
</tr>
</tbody>
</table>

¹ Potato organ from which isolates recovered 
² Number of SF isolates / Number of total isolates recovered. 
³ 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-Beheira Governorate and the surrounding area.

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

Assessment based on five replicates.

* Mean number of oospores produced in 1-cm diameter of rye-A disc.
* 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*.

<table>
<thead>
<tr>
<th>Isolate Code No.</th>
<th>Single sporangia</th>
<th>Single hyphal tips</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method of propagation</td>
<td>A1</td>
</tr>
<tr>
<td>1/1999</td>
<td>SF</td>
<td>37</td>
</tr>
<tr>
<td>2/2000</td>
<td>SF</td>
<td>26</td>
</tr>
<tr>
<td>5/2001</td>
<td>SF</td>
<td>39</td>
</tr>
<tr>
<td>Mean</td>
<td>SF</td>
<td>34</td>
</tr>
</tbody>
</table>

* 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-Beheira 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 parts in Europe (Tanilus et al., 1986; Shatlock 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 (Vantanian and Endo, 1985; Mosa et al, 1989; Niederhauser, 1991; Ko, 1994).

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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 heteroplastic 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.

REFERENCES


تواقيد طب طب الزاحي ميم التيفوتورا إنفستنر بمحافظة البحيرة بمصر
أحمد السيد الكراني
قسم النباتات إلزاعية - كلية الزراعية بمصر - جامعة الإسكندرية

قامت دراسة بعمل حمض لعديد تواجد الطائر الحصب ذاتيا ومن القطرة إيفاكيورا إنفستنر بمحافظة البحيرة والمنطقة المحافظة بها في الفترة من 1999 إلى 2001. وأسفرت هذه الرسالة عن تأكيدين وجود إنشاء هذا الطائر بمناطق زراعة البطاطس بمحافظة البحيرة والمنطقة المحافظة إلا أن نسبة تواجدها كانت منخفضة حيث كشف عن عزلة واحدة في العام الأول من الدراسة (1999) بين 22 عزلة من القطرة إيفاكيورا إنفستنر تم الحصول عليها. وكذا استمرت الدراسة في العام الثاني (2000) عن الكشف عن عزلة واحدة فقط بين 50 عزلة، بينما في العام الثالث (2001) تم الكشف عن ثلاثة عزلات من هذا الطائر بين 172 عزلة من القطرة إيفاكيورا إنفستنر تم الحصول عليها، وقد أظهرت هذه النسبة 3.6%، 2.17% بين عنصر طبق القطرة إيفاكيورا إنفستنر بمحافظة البحيرة والمنطقة المحافظة، وذلك في الاتجاه الثلاثة من الدراسة على التوالي، وفي دراسة استهدفت الكشف عن طبيعة هذا الطائر الحصب ذاتيا، تم توظيف أطبار جزيرة مدرسة وزراعتها على البيئة المناسبة (بيكتروراي A)، فكشفت الدراسة أن هذا الطائر هو نتاج خطأ بين هياكل الطيور A2A،11 A من القطرة إيفاكيورا إنفستنر وتفاعلهم لعملياته المفيدة في هذا الطائر الحصب ذاتيا، والذي تحدث عبر تغيير بطيء صيانة جدوى من القطرة إيفاكيورا إنفستنر، أثر كواء على إحداث مرض القطرة المفيدة في البيئة، مما قد يشكل خطرا على زراعة هذا المحصول بالمنطقة. وظهرت الدراسة أن نسبة تواجد هذا الطائر بمحافظة البحيرة امتصحة (2.4%)، وكذا كانت نسبة الجراح البنية المحصنة بها عالية (10.3%)، سالفة الكيرستنر المحصنة (2.4) وسببية إيباتها المنخفضة (1.8%) مما يشير إلى أنه في الوقت الحالي قد لا يكون حظر ملحوظ إلا أنه قلب ويثر دراسة كافية لبحثه عليه قبل أن يسفر براءة بشكل فعال وكبير بالنظام. وتحت دراستها لقياسها لمنع حدوثها.