Polyploidy and Induced Gametangial Formation in British Isolates of *Phytophthora infestans*

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SUMMARY

Gametangial (sex organ) formation in cultures of a British isolate of *Phytophthora infestans*, mating type A¹, was induced by simultaneous inoculation with both A¹ and A² isolates of *P. palmivora*. Inoculation with the A² mating type only was ineffective. Three isolates of *P. infestans* were examined: two were polyploid, of which one was probably tetraploid; the third had both diploid and polyploid nuclei.

Phytophthora infestans also produced gametangia when inoculated simultaneously with both A¹ and A² types of *P. cambivora* and *P. cinnamomi*. The ecological significance of these findings is discussed.

INTRODUCTION

Since *Phytophthora infestans* is heterothallic and only the A¹ mating type is present in temperate regions, it has been widely assumed that it does not reproduce sexually in these regions. However, it has long been known that the mating type reaction in *Phytophthora* is non-specific, A¹ types of one species often reacting with A² types of a different species (Stamps, 1953; Haasis & Nelson, 1963; Ribeiro, Erwin & Zentmyer, 1975). In such reactions where both parents produce both oogonia and antheridia, there may be reciprocal hybrid gametangial combinations and also a certain proportion of selfed gametangial combinations. The latter might be expected to produce some viable oospores even if the hybrid combinations fail to do so.

Some preliminary matings of British isolates of *P. infestans* with A² mating types of other species were largely ineffective. It was thought that these isolates might be able to react with A² types stimulated to undergo sexual reproduction by another compatible A¹ type. To interpret mating reactions in a triple inoculation it is essential to choose compatible A¹ and A² types with easily recognizable gametangia. Such 'marked' gametangia are provided by the 'L' and 'S' chromosome types of the *P. palmivora* complex (Sansome, Brasier & Griffin, 1975). Recent observations on isolates obtained from cocoa in West Africa, attributed to *P. palmivora*, have indicated the presence of two types which have very distinctive chromosome complements (Sansome *et al.*, 1975): the 'S' (small chromosome) type, having a basic complement of about nine small chromosomes; and the 'L' (large chromosome) type, having a basic complement of five much larger chromosomes. The 'L' type has very distinctive nuclei at metaphase since, besides having larger and fewer chromosomes than is usual in *Phytophthora*, the chromosomes form a characteristic narrow spindle. Four or possibly six chromosomes are associated in a ring or chain at metaphase. Because of the distinctive appearance of the 'L' type nuclei it was possible to investigate the

Table 1. *Origin of Phytophthora species*

Organism	Isolate no.	Mating type	Origin
<i>Phytophthora palmivora</i> 'L'	P132	A ¹	Nigeria; Sansome <i>et al.</i> , 1975
<i>P. palmivora</i> 'L'	P184	A ²	Cameroun; Sansome <i>et al.</i> , 1975
<i>P. palmivora</i> 'S'	P131	A ²	Nigeria; Sansome <i>et al.</i> , 1975
<i>P. infestans</i>	B25	A ¹	UK; Shattock & Shaw, 1975
<i>P. infestans</i>	PYF2	A ¹	UK; Dr R. Shattock, University College of North Wales
<i>P. infestans</i>	Treb A	A ¹	UK; Dr R. Shattock
<i>P. infestans</i>	63B	A ¹	Mexico; Sansome & Brasier, 1973
<i>P. infestans</i>	445	A ²	Mexico; Sansome & Brasier, 1973
<i>P. cambivora</i>	IMI77374	A ²	France
<i>P. capsici</i>	504	A ¹	Mexico; Timmer <i>et al.</i> , 1970
<i>P. capsici</i>	505S	A ²	Mexico; Timmer <i>et al.</i> , 1970
<i>P. cinnamomi</i>	IMI197715	A ²	UK
<i>P. cryptogea</i>	IMI45168	A ¹	New Zealand
<i>P. cryptogea</i>	IMI132646	A ²	Egypt
<i>P. drechsleri</i>	6500	A ¹	Mexico; Galindo & Zentmyer, 1967
<i>P. drechsleri</i>	6503	A ²	Mexico; Galindo & Zentmyer, 1967
<i>P. erythroseptica</i>	—	—	UK; Professor J. Webster, University of Exeter
<i>P. nicotianae</i> var. <i>parasitica</i>	IMI35087		UK

effect of 'L' A¹ × 'L' A² *P. palmivora* cultures on *P. infestans*. The first *P. infestans* culture so tested was found to be polyploid and therefore distinctive; thus it was possible to test the reaction of this isolate with other species.

METHODS

The isolates used in these experiments are described in Table 1.

The mating reaction tests were carried out on carrot agar (Brasier, 1975*a*). A large inoculum (about 1 to 1.5 cm diam.) of an actively growing culture of *P. infestans* on rye agar (Caten & Jinks, 1968) was placed on the carrot agar together with an A² type of another species. For triple inoculations, inocula of *P. infestans* and of the A¹ and A² types of the other species were placed on the agar approximately equidistant from each other.

The mated cultures were examined microscopically for sex organs when the colonies met. After sexual reproduction was observed in the intraspecific combination, particular attention was given to the *P. infestans* inoculum and its immediate surroundings. When sex organs were observed in or near the *P. infestans* inoculum (usually 2 or more days after vigorous mating in the intraspecific region), they were fixed and examined cytologically.

Samples of agar containing oogonia were sliced and treated with iced water for 1 to 2 h. After removal of excess water, the samples were fixed for 30 to 45 min in ethanol/glacial acetic acid (3:1, v/v), transferred to ethanol/diethyl ether (1:1, v/v) for 2 days to remove fatty substances, then placed in ethanol/acetic acid (3:1, v/v) or ethanol for not less than 1 h and thoroughly washed in water. The material was stained or stored in 70% (v/v) ethanol. Aceto-orcein squash preparations were made as described previously (Sansome, 1976), after treatment for 10 min in 1% (w/v) citric acid.

RESULTS AND DISCUSSION

Induction of sex organ formation in P. infestans by mated cultures of other Phytophthora species

No oogonia were produced when B25 (*P. infestans* A¹) was paired with P184 (*P. palmivora* 'L' A²) and only once in many tests were a few oogonia produced when B25 was paired with P131 (*P. palmivora* 'S' A²). However, when strain B25 was inoculated with P184 × P132 (*P. palmivora* 'L' A² × 'L' A¹) or with P131 × P132 (*P. palmivora* 'S' A² × 'L' A¹), oogonia were freely produced in the region of the *P. infestans* inoculum a few days after they had appeared at the junction of the *P. palmivora* A¹ × A² colonies – usually about 6 days after inoculation. These oogonia, which could be identified cytologically because their nuclei were at late prophase or metaphase, were found to be predominantly of a new type, distinct from either the 'L' or 'S' *P. palmivora* types. They had larger chromosomes than the 'S' type and more chromosomes than either the 'S' or 'L' types. Presumably they were *P. infestans* oogonia. Multivalents in the form of rings or chains were often present. In most cases when the oogonial nuclei were at metaphase, the antheridial nuclei had already divided and could not therefore be identified. However, Fig. 1 shows metaphase in a *P. palmivora* 'L' oogonium and Fig. 2 shows metaphase in a *P. infestans* oogonium and in the attached 'L' type antheridium. The number of chromosomes in the nuclei of the *P. infestans* oogonium is approximately twice that previously observed in a cross between two isolates of *P. infestans* from Mexico, (445) A² × (63B) A¹ (Sansome & Brasier, 1973). In the latter cross, metaphases were observed with an association of up to six chromosomes and about six pairs of chromosomes. Figure 3 shows metaphase in an oogonium from the 445 × 63B cross; comparison with Fig. 2 indicates that the oogonial nuclei from a British isolate of *P. infestans* have approximately twice as many chromosomes.

Polyploidy in British isolates of P. infestans

The discovery of polyploidy in *P. infestans* B25 raises the question as to how widespread this phenomenon is in temperate regions. Since strain B25 had been maintained in culture for some time, two new strains, PYF2 and Treb A, were tested with mated *P. palmivora* cultures. Strain PYF2 produced polyploid oogonia (Fig. 2) in the combination PYF2 × *P. palmivora* 'L' A¹ × *P. palmivora* 'L' A². Strain Treb A produced polyploid oogonia, diploid oogonia and some oogonia with both diploid and polyploid nuclei and was therefore a diploid-polyploid heterokaryon. These findings suggest that *P. infestans* may exist in nature in the tetraploid condition and this may indeed be the prevalent condition in temperate isolates. An extensive examination of the ploidy of such isolates is urgently needed. It may be that the tetraploid is better adapted to cooler conditions and that it has resulted from the selection of the auto-tetraploid nuclei which must occasionally arise in regions of rapid division. Alternatively, the tetraploid could be an allopolyploid which arose from the doubling of a hybrid between *P. infestans* and some other species. The fact that many pathogenic races of *P. infestans* have been discovered (Malcolmson, 1969) may be due to polyploidy in *P. infestans*.

Reactions of P. infestans B25 with other species

Since *P. infestans* B25 could be distinguished cytologically by its high chromosome number, it was possible to identify it in combinations involving species other than *P. palmivora*. Tests were made with a number of species including some found in temperate zones.

Bar marker represents 1 μm .

Fig. 1. Metaphase I in a *P. palmivora* 'L' oögonium (from Treb A $\times$ P132 $\times$ P184).

Fig. 2. Metaphase I in a *P. infestans* oögonium (British) (from PYF2 $\times$ P132 $\times$ P184).

Fig. 3. Metaphase I in a *P. infestans* oögonium (Mexican) (from 63B $\times$ 445).

Fig. 4. Metaphase I in a *P. nicotianae* var. *parasitica* oögonium (from *P. nicotianae* var. *parasitica* $\times$ B25).

Fig. 5. Metaphases from a squashed out *P. infestans* oögonium (from *P. nicotianae* var. *parasitica* $\times$ B25).

Fig. 6. Metaphase I in part of a *P. cambivora* oögonium (from *P. cambivora* A² $\times$ B25).

Fig. 7. Metaphase I in part of a *P. infestans* oögonium (from *P. cambivora* A² $\times$ B25).

The homothallic *P. erythroseptica*, tested because it was found on potato (Pitt, 1975), was ineffective in inducing sexual reproduction in *P. infestans* B25. However, a homothallic strain of *P. nicotianae* var. *parasitica*, which had $2n = \sim 18$ chromosomes, induced the production of oogonia and antheridia in *P. infestans* B25. Figure 4 shows metaphase in a *P. nicotianae* var. *parasitica* oogonium and in the attached *P. infestans* antheridium. The nuclei in the antheridium are much larger than those of the oogonium. The oogonia produced in this combination are sparse and, possibly because of this, the B25 oogonia have particularly thick, heavily staining walls which obscure the nuclei. Figure 5 shows the squashed out contents and part of the wall of such an oogonium. The nuclei are readily distinguishable from those of *P. nicotianae* var. *parasitica* seen in the oogonium in Fig. 4 since they have about twice as many chromosomes. *Phytophthora nicotianae* var. *parasitica* has heterothallic as well as homothallic strains and therefore the factors required for sexual reproduction in this species may be different from those of a truly homothallic species such as *P. erythroseptica*. *Phytophthora cambivora* A², which had $2n = \sim 18$ chromosomes, was tested because it is widely distributed (Brasier, 1975*b*) and because it has characteristic projections on the oogonial wall which could possibly serve as an additional distinguishing feature. It reacted slowly with *P. infestans* B25, gametangial formation starting in the region of the B25 inoculum after about 11 days. Samples were fixed between 11 and 16 days after inoculation. The production of oogonia was sparse but a number of oogonia at late prophase and metaphase were observed. Figure 6 shows part of a *P. cambivora* oogonium and Fig. 7, part of a squashed *P. infestans* oogonium, both at metaphase. The *P. cambivora* oogonia were usually larger than those of *P. infestans* and the nuclei were sparser and less readily stained. The oogonial wall projections characteristic of *P. cambivora* were not always visible in the early stages. This character may be variable in expression or it may be influenced by the type of attached antheridium. In later stages, the *P. cambivora* oogonia had less deeply staining walls with projections and a narrower layer of periplasm than the *P. infestans* oogonia. Mated cultures of *P. drechsleri* (6500 × 6503) and of *P. capsici* (504 × 505S) induced gametangial production in *P. infestans* B25 although *P. drechsleri* A² alone did not. The interspecific combinations, *P. cinnamomi* A² × *P. cryptogea* A¹ and *P. cambivora* A² × *P. cryptogea* A¹, were also effective. It seems that the reaction of the British *P. infestans* with an A² mating type of another species may be initiated or enhanced by the presence of another A¹ type although mated cultures of *P. cryptogea* A¹ × A² were ineffective.

Ecological significance

The fact that British isolates of *P. infestans* may undergo sexual reproduction in the presence of A² mating types of such species as *P. cambivora* and *P. cinnamomi*, which are of widespread occurrence in the A² form (Brasier, 1975*b*; Zentmyer, 1976), may have important ecological implications. Selfing is readily induced in A² mating types of the two latter species by *Trichoderma viride*. The sexual reaction of *P. infestans* with a *P. cambivora* or *P. cinnamomi* A² type might be greatly increased in the presence of *Trichoderma viride* or other micro-organisms with a similar effect. Since the oogonial and attached antheridial nuclei were often at different stages, it was rarely possible to distinguish between hybrid and selfed gametangial combinations. However, a number of hybrid gametangial combinations were observed (Figs 2 and 4). No selfed combination was identified and it seems probable that hybrids are more frequent than selfed gametangial combinations. It is, therefore, likely that most oospores resulting from the reaction of *P. infestans* with another species would be hybrid and possibly non-viable. However, a certain proportion of selfed oospores would be expected to occur and some of these might survive in nature. Boccas & Zentmyer

(1976) found that a cross between *P. cinnamomi* and *P. parasitica* resulted in the formation of oospores but only 5 % were well-formed and only 5 % of these germinated to give single oospore progeny. Some of the progeny resembled the parental types and were assumed to be the result of selfing. Others were assumed to be the result of segregation in selfed oospores. In the case of tetraploids, such as the *P. infestans* isolates studied here, occasional unfertilized oospores might develop giving rise to diploid cultures. If these are somewhat unbalanced, somatic doubling would give a diploid-tetraploid heterokaryon such as occurs in the Treb A isolate.

If viable oospores are sometimes formed by *P. infestans* in nature by reaction with other *Phytophthora* species, they would enable the organism to survive unfavourable conditions. They would also lead to a greater degree of segregation than ordinary somatic reproduction.

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