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Genetical evidence for diploidy in Phytophthora

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SUMMARY

A spontaneous mutant of *Phytophthora drechsleri*, resistant to *p*-fluorophenylalanine and two induced mutants resistant to chloramphenicol have been selected and analysed. The pattern of inheritance of the mutant phenotypes was identical and conformed to that expected in a diploid organism.

1. INTRODUCTION

Phytophthora belongs to a group of non-septate, oogamous fungi, many of which cause blights and downy mildews of plants. Although Phytophthora and other members of the class Oömycetes were assumed to have haploid somatic nuclei, recent cytological and micro-chemical evidence leads to a questioning of this assumption (Sansome, 1965; Bryant & Howard, 1969). Genetical data are required to establish the position of meiosis in the life-cycle and to provide a basis for the investigation of the control of host specificity, incompatibility and other natural variation.

Attempts to analyse Phytophthora genetically have been frustrated by lack of suitable stable nuclear markers and the results have been inconclusive. Here we report briefly on the results of some simple matings designed to provide definitive genetical evidence of ploidy in *Phytophthora drechsleri*.

Shaw & Elliott (1968) chose drug resistance as a marker in *P. cactorum* because 'resistance mutations have often been found to be dominant or semi-dominant in other fungi and would be expected to express themselves in a heterozygote'. Developing this approach with the heterothallic species *P. drechsleri*, we have analysed the inheritance of resistance to *p*-fluorophenylalanine and chloramphenicol.

2. MATERIALS AND METHODS

Compatible isolates 6500 and 6503,† used by Galindo & Zentmyer (1967), were chosen as suitable wild-types because they produce hybrid oospores with a high percentage and rate of germination. A spontaneous mutant (6503 *FA 1*) resistant to *p*-fluorophenylalanine was selected by adding 10^8 zoospores of 6503 to liquid standard medium (Shaw & Elliott, 1968) containing 100 μg/ml *p*-fluorophenylalanine. Four mutants resistant to chloramphenicol were induced by treating 10^9 zoospores with *N*-methyl-*N*-nitroso-*N'*-nitroguanidine to give 75 % survival and selecting in liquid standard medium containing 100 μg/ml chloramphenicol. Two of these mutants, 6503 *C 1* and 6503 *C 2*, were analysed further. Details of the selection of mutants will be published elsewhere.

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Table 1 shows phenotypes of wild-type and mutant isolates on agars containing drugs. It can be seen that mutants are not cross-resistant. Crosses were made by placing 4 mm hyphal inocula of compatible isolates 1 cm apart on the surface of plates of oat-extract agar (Shaw & Elliott, 1968) and incubating in a light–dark regime for 14 days (Galindo & Zentmyer, 1967). Mature oospores were germinated on standard medium agar after treatment on water agar (Shaw, 1967).

3. RESULTS

Analysis of each mutant was carried out by crossing to the compatible wild-type, backcrossing selected progeny (F_1's) to each parent, and intercrossing compatible F_1's to give an F_2 generation (see Table 2). In backcrosses and inter-crosses an F_1 was selected which was compatible and highly fertile. Phenotypes of progeny are recorded in Table 2.

Linear growth was compared on medium with and without 100 μg/ml of the appropriate

Table 1. *The response of parental isolates to* p-*fluorophenylalanine and chloramphenicol*

	p-Fluorophenylalanine		Chloramphenicol	
Isolates	10 μg/ml	100 μg/ml	10 μg/ml	100 μg/ml
6500 *wt*	−	−	−	−
6503 *wt*	−	−	−	−
6503 *FA1*	+	+	−	−
6503 *C1*	−	−	+	+
6503 *C2*	−	−	+	+

+, Linear growth on standard medium agar uninhibited by presence of drug.
− Linear growth on standard medium agar inhibited by presence of drug.

Table 2. *Crosses between resistant and sensitive isolates and their progeny*

Mutant	Mating	Progeny, resistant/ sensitive	Expected* ratio	Germination of oospores (%)
6503 *FA1*	6503 *FA1* × 6500 *wt*	125:0	1:0	100
	F_1 × 6500 *wt*	68:57	1:1	100
	F_1 × 6503 *FA1*	65:0	1:0	100
	$F_1 \times F_1$	35:11	3:1	.
6503 *C1*	6503 *C1* × 6500 *wt*	66:0	1:0	51
	F_1 × 6500 *wt*	60:48	1:1	76
	F_1 × 6503 *C1*	59:0	1:0	47
	$F_1 \times F_1$	51:17	3:1	58
	F_2 (1) × 6500 *wt*	25:19	1:1	44
	F_2 (2) × 6500 *wt*	18:23	1:1	41
6503 *C2*	6503 *C2* × 6500 *wt*	71:0	1:0	97
	F_1 × 6500 *wt*	43:32	1:1	75
	F_1 × 6503 *C2*	60:0	1:0	60
	$F_1 \times F_1$	40:13	3:1	53

* Expected if parents are diploid and if resistant parents are homozygous.

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drug and in all cases progeny were either resistant (completely uninhibited) or sensitive (totally inhibited).

No phenotypes intermediate between resistant and sensitive were recorded in these progenies. Analysis of two test crosses of F_2's is included in Table 2.

4. DISCUSSION

The pattern of inheritance of drug resistance shown by all three mutants is similar: (1) no segregation in the F_1 generation; (2) segregation fitting a 1:1 ratio in backcrosses to wild-type; (3) no segregation in backcrosses to resistant parent; (4) segregation in F_2 fitting a 3:1 ratio. This pattern is consistent with diploidy of the somatic nuclei and determination of drug resistance by a single dominant allele. This interpretation is further supported by the results of two test crosses of resistant F_2's with wild-type. Both progenies showed a segregation fitting a 1:1 ratio, indicating that both F_2's were heterozygous. Our data indicate that all three mutants are homozygous for a dominant allele determining resistance. The origin of these homozygotes remains obscure, but we think the most likely explanation is that a heterozygote, formed by mutation of one allele, became homozygous by mitotic recombination. This possibility will be investigated.

Although we do not exclude the possibility that there are other interpretations, the simplest explanation of our data is entirely consistent with the somatic nuclei being diploid, and we propose to make this our working hypothesis.

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