Genetic Evidence for Diploidy in Phytophthora megasperma var. sojae

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ABSTRACT

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Several *met* + *pur* heterokaryons constructed from a methionine- and a purine-requiring mutant of the homothallic fungus *Phytophthora megasperma* var. *sojae* produced only parental auxotrophic and recombinant prototrophic oospores when selfed. Oospore progeny obtained from selfing first-generation prototrophic recombinant progeny segregated into four classes as expected in a vegetatively diploid organism, but a 9:3:3:1 ratio was not obtained. Deviation from the classical ratio appeared to reflect inferior germination and colony establishment by the recombinant auxotrophic progeny.

Additional key words: heterokaryon auxotrophs, mutants, soybeans.

We have introduced auxotrophic and drug-resistant markers into *Phytophthora megasperma* Drech. var. *sojae* A. A. Hildb. and have constructed heterokaryons with various markers (5). Selfing of the appropriate heterokaryon to allow selection of recombinant prototrophic progeny and analysis of selfed progeny from these prototrophs should provide genetic segregation data pertinent to the ploidy level of *P. megasperma* var. *sojae*. We present data from such experiments in this report.

MATERIALS AND METHODS

Techniques for manipulation of the fungus and culture media composition were described previously (5, 6). In this paper, all first-generation oospore progeny obtained from selfed heterokaryons, whether from crossing of like or unlike nuclei, will be referred to as S_1 and all second-generation oospores obtained from selfed S_1 prototrophs will be referred to as S_2. Oospore progeny were tested for percent germination, colony establishment, nutritional requirements, and pathogenicity. The S_1 single-oospore progeny also were tested for ability to produce zoospores and the resultant zoospores were tested for colony establishment on cleared V8-juice agar (V8-JA) and growth on supplemented and minimal medium.

Oospores were obtained from the outer 1-cm of 3-wk-old V8-JA cultures and overlaid onto Bacto- water agar (WA) (Difco) plates either immediately after harvest or after a 48-hr water-soak treatment at 36 C (6). The overlaid plates then were viewed under a dissecting microscope every 2-3 days, and all newly germinated oospores were transferred individually to V8-JA for colony establishment; areas of the cultures in which germination had occurred but individual oospores could not be reliably picked were excised and discarded. Mycelial pieces of single-oospore cultures were tested for growth on methionine-, guanosine-, or methionine plus guanosine-supplemented and on minimal medium agar (MmA) (5). If growth occurred on MmA, the oospore was scored as a prototroph; if growth occurred only on supplemented medium, the oospore was scored as *met*, *pur*, or *met pur* depending on its requirement (5).

RESULTS

Single-oospore progeny from *met* + *pur* heterokaryons (from a *met* = methionine- and a *pur* = purine-requiring mutant) were either one of the two parental classes, or were recombinant prototrophic progeny that grew on MmA; no double auxotrophic progeny were recovered (Table 1). All of the S_1 prototrophic and *met* oospore progeny produced sporangia and motile zoospores, but S_1 *pur* oospore progeny did not. This is consistent with previously reported data (5); *met* strains produced normal sporangia and motile zoospores, but *pur* strains did not. Colony establishment by single zoospores from different S_1 prototrophic oospores varied (Table 2), but the overall average of 61% was comparable to the wild type (6). All zoospores from S_1 prototrophic oospores were prototrophic (Table 2) and all zoospores from S_1 *met* oospore progeny required methionine. All S_1 prototrophic oospores and their zoospore progeny were pathogenic and were identified as race 3 by inoculation of soybean plants (6).

Oospores from wild-type strains of *P. megasperma* var. *sojae* showed increased germination (two- to three-fold) when water-soaked at 36 C for 48 hr (6), but this treatment did not increase the germination of S_1 oospores from the *met* + *pur* heterokaryons or the germination of S_2 oospores from selfed S_1 prototrophs (Table 3). It also was observed that germinated S_1 oospores established colonies more frequently than germinated S_2 oospores (Table 3).

Second-generation (S_2) oospore progeny obtained from selfing S_1 prototrophic-oospore progeny disclosed segregation of the auxotrophic markers (Table 1), but the classic 9:3:3:1 ratio expected for two independently segregating genes in a diploid organism was not obtained. However, it was observed that the S_2 prototrophic oospores germinated more rapidly and established colonies more readily than the S_2 auxotrophic oospores (Table 4). Further, the S_1 and S_2 *met* oospores were more vigorous than the corresponding *pur* oospores in germination, colony establishment, and growth rate.

TABLE 1. The classes of first- and second-generation oospore progeny from *met* + *pur* heterokaryons[a] of *Phytophthora megasperma* var. *sojae* are those expected in a vegetatively diploid organism

Classes of oospore progeny	First generation (S_1)[b]		Second generation (S_2)[c]	
	Number of oospores	Percentage of total	Number of oospores	Percentage of total
Prototrophs	403	74.5	925	85
met	136	25.1	118	11
pur	2	0.4	25	2
met pur	0	0	23	2

[a]Heterokaryons were constructed from a *met* = methionine- and a *pur* = purine-requiring mutant of *P. megasperma* var. *sojae*.

[b]Cumulative data from two experiments; S_1 oospores were obtained from four different *met* + *pur* heterokaryons.

[c]Cumulative data from six experiments; S_2 oospores were obtained from four different S_1 prototrophic oospore cultures.

DISCUSSION

The observation of a large monokaryotic, prototrophic class in the S_1 generation, coupled with segregation of the auxotrophic markers in the S_2, supports the conclusion that *P. megasperma* var. *sojae* is vegetatively diploid. Since no S_1 double auxotrophic progeny were recovered

TABLE 2. Colony establishment and nutritional type of zoospore progeny from prototrophic first generation (S_1) oospores from four different *met* + *pur* heterokaryons[a] of *Phytophthora megasperma* var. *sojae*[b]

Source of zoospores		Zoospore progeny		
Heterokaryon	Number of S_1 prototrophic oospore progeny tested	Colonies established (%)	Prototrophs	Auxotrophs
1	5	42	124	0
2	9	56	252	0
3	2	30	19	0
4	26	63	695	0
Total number of isolates	42	1,811	1,090	0

[a]Heterokaryons were constructed from a *met* = methionine- and a *pur* = purine-requiring mutant of *P. megasperma* var. *sojae*.

[b]Single germinated zoospores were tested for colony establishment on V8-JA; approximately one-half (1,090) of the zoospores that established colonies (1,811) were tested for nutritional requirements.

TABLE 3. Effect of water-soaking[a] on the germination and colony establishment of first generation (S_1) oospores from *met* + *pur* heterokaryons[b] and second generation (S_2) oospores from S_1 prototrophic oospores of *Phytophthora megasperma* var. *sojae*

Oospore generation	Experiment number	Oospore germination (%)		Colonies established (%)	
		Treated	Nontreated	Treated	Nontreated
S_1	1	9	7	82	90
	2	19	18	93	93
S_2	1	13	9	90	80
	2	5	2	79	89
	3	12	7	60	59
	4	3	2	65	60
	5	2	3	77	70
	6	4	4	81	80

[a]Treated oospores were soaked in sterile distilled water for 48 hr at 36 C. Treated and nontreated oospores were overlaied on water-agar and single germinated oospores were tested for colony establishment on V8-JA.

[b]Heterokaryons were constructed from a *met* = methionine- and a *pur* = purine-requiring mutant of *P. megasperma* var. *sojae*.

TABLE 4. Percentage colony establishment and nutritional classes of second-generation oospores of *Phytophthora megasperma* var. *sojae* that germinated at various times after plating[a]

Time required to germinate (days)	No. of germinated oospores tested	Colonies established (%)	Nutritional classes of oospores (%)[b]			
			Prototroph	*met*	*pur*	*met pur*
0-8	629	81	93	4	1	2
8-14	548	70	83	11	3	3
14-24	279	70	66	27	6	1

[a]Second generation oospores were from selfed first generation prototrophic oospores of *met* + *pur* heterokaryons of *Phytophthora megasperma* var. *sojae*. Oospore colonies were established on V8-JA and then tested for growth on supplemented and minimal media.
[b]Abbreviations: *met* = methionine-requiring oospores, *pur* = purine-requiring oospores, and *met* + *pur* = oospores requiring both methionine and purine.

and since S_1 prototrophs all segregated for the auxotrophic markers, this establishes that the parent auxotrophic nuclei were homozygous at the *met* and *pur* loci. Our genetic evidence therefore supports previous cytological and genetical work with *Phytophthora* spp. (1, 2, 3, 4, 7, 8, 10) and, in particular, the cytological research of Sansome and Brasier (9) with *P. megasperma* var. *sojae*. The germination data in Table 4 suggest that the observed skewing of the expected 9:3:3:1 S_2 segregation ratio in favor of prototrophic progeny was caused by the inferior germination (and/or viability) of the auxotrophic recombinants, especially *pur* and *met pur*. However, if polysomy occurs in this fungus as suggested by Sansome and Brasier (9), it also may have influenced the segregation ratio.

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