Hormonal Regulation of Sexual Reproduction in *Phytophthora*

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Induction of oospore formation by the complementary strain occurred in both cases when A¹ and A² isolates of *Phytophthora parasitica* were separated by polycarbonate membranes and up to 3 mm of agarose. The number of oospores produced increased as separation distance decreased. The homothallic species *P. heveae* induced oospore formation in both the A¹ and A² isolates of *P. parasitica*. *Phytophthora megasperma* var. *sojae* stimulated oospore formation in the A² but not the A¹ isolate of *P. parasitica*, while *P. cactorum* and *P. katsurae* stimulated the A¹ but not the A² isolate. Sexual reproduction of homothallic species of *Phytophthora* may thus also be controlled by α hormones. Sixteen types of chemically regulated sexuality among the members of *Phytophthora*, differing in hormone production and responsiveness to hormones, are postulated.

INTRODUCTION

Evidence for hormonal regulation of sexuality in three species of heterothallic *Phytophthora* was obtained recently by using a polycarbonate membrane method (Ko, 1978). Both A¹ and A² types of *Phytophthora cinnamomi*, *P. parasitica* and *P. palmivora* formed oospores by selfing when they were paired with compatible types of the same or different species on opposite sides of polycarbonate membranes. These membranes were not penetrated by the test fungi. It was concluded that initiation of oospore formation by A¹ isolates of *Phytophthora* depends on a sex hormone α^2 secreted by A² isolates and that oospore formation by A² isolates depends on a hormone α^1 originating in A¹ isolates. I report here further evidence for the hormonal regulation of sexuality in both heterothallic and homothallic species of *Phytophthora*.

METHODS

Organisms. The heterothallic isolates of *Phytophthora* used were those described previously (Ko, 1978). The homothallic species were *P. heveae* from avocado (P1000) and rubber (P821) supplied by G. A. Zentmyer (Zentmyer *et al.*, 1978); *P. megasperma* var. *sojae* race 1 and race 3 supplied by J. L. Lockwood; *P. cactorum* supplied by D. L. McIntosh; and *P. katsurae* (Ko & Chang, 1979).

Mating. Culture blocks (15 × 10 × 3 mm) of both A¹ and A² isolates were cut from 6 d-old cultures grown on V-8 agarose (Ko, 1978). This medium consisted of distilled water, 10% (v/v) V-8 juice (Campbell Soup Co., Camden, N.J., U.S.A.), 0.02% (w/v) CaCO₃ and 0.8% (w/v) agarose (SeaKem HGT-P Agarose; Maine Colloids, Rockland, Maine, U.S.A.) which was used as a solidifying agent. V-8 juice was not centrifuged and freshly prepared medium was used for all experiments. Both centrifugation of V-8 juice and aging of the medium greatly reduced the number of oospores produced. A culture block of A² mating type of *P. parasitica* placed in the centre of a Petri dish was covered with permeable materials of different thickness and paired with an A¹ block on top. The permeable materials included (1) one layer of polycarbonate membrane (CPR, 0.2 μ m, 90 mm diam.; Nuclepore Corporation, Pleasanton, Calif., U.S.A.), (2) three layers of polycarbonate membrane, (3) one layer of Millipore filter (GSWP, 0.22 μ m, 90 mm diam.) and (4) two layers of polycarbonate membrane with a 0.8% water agarose block (15 × 10 × 3 mm) placed in between. The distances separating the two mating types were 10, 30, 135 and 3020 μ m, respectively. Both polycarbonate membranes

Table 1. *Induction of oospore formation by sex hormones originating in opposite mating type of P. parasitica at various distances*

Separation material*	Diffusion distance (μm)	Oospores (no. cm^{-2})	
		A ¹	A ²
1 Nuclepore membrane	10	32 423	1939
3 Nuclepore membranes	30	21 020	842
1 Millipore filter	135	842	318
2 Nuclepore membranes plus 1 agarose block	3020	86	334

* A culture block (15 × 10 × 3 mm) of *P. parasitica* A² placed in the centre of a Petri dish was covered with the permeable material and paired with a block of *P. parasitica* A¹ culture. Both isolates were 6 d-old.

Table 2. *Oospore formation by A¹ and A² isolates of P. parasitica induced by diffusible sex hormones originating in homothallic isolates of Phytophthora*

Hormone producer*	Oospore producer* (no. cm^{-2})	
	<i>P. parasitica</i> A ¹	<i>P. parasitica</i> A ²
<i>P. heveae</i> , avocado isolate	6735	918
<i>P. heveae</i> , rubber isolate	2819	255
<i>P. megasperma</i> var. <i>sojae</i> , race 1	0	727
<i>P. megasperma</i> var. <i>sojae</i> , race 3	0	1870
<i>P. cactorum</i>	232	0
<i>P. katsurae</i>	325	0

* Blocks of a 2 d-old culture of homothallic isolate (hormone producer) and a 6 d-old culture of heterothallic isolate (oospore producer) were separated by a polycarbonate membrane and incubated together in darkness at 24 °C for 6 d.

and Millipore filters were placed in between moistened filter papers and sterilized by autoclaving. The cultures were incubated in a moist chamber for 6 d at 24 °C in darkness.

For determining hormone production by homothallic *Phytophthora*, a culture block (15 × 10 × 3 mm) of 2 d-old *P. heveae*, *P. megasperma* var. *sojae*, *P. cactorum* or *P. katsurae* placed in the centre of a Petri dish was covered with a polycarbonate membrane and paired on the top with another block of 6 d-old *P. parasitica* A¹ or A². The cultures were incubated as described above.

In each test the number of oospores produced in 10 microscopic fields per block was counted using a 10 × or 40 × objective. Two replicates per treatment were used and all the experiments were repeated at least three times.

RESULTS

The number of oospores produced by A¹ and A² isolates of *P. parasitica* increased as the thickness of separating permeable materials decreased (Table 1). Even when they were separated by more than 3 mm, 86 and 334 oospores cm^{-2} were produced by A¹ and A² isolates, respectively. This indicated that the amount of α hormone reaching the complementary isolates was inversely correlated with diffusion distance. The test fungi did not penetrate polycarbonate membranes or Millipore filters during the incubation period. When only the top or bottom layer was inoculated with a single isolate of the fungi, the opposite layer remained sterile after 6 d incubation. Sterility was tested by incubating the non-inoculated layers on nutrient agar for 6 d at 24 °C. When observed under a microscope after incubation, no micro-organisms were found in agarose blocks placed between polycarbonate membranes to separate the mating isolates.

All the homothallic isolates of *Phytophthora* tested were capable of inducing oospore formation of either A¹ or A² or both isolates of *P. parasitica* (Table 2). Both A¹ and A² isolates of *P. parasitica* formed oospores when they were paired with the avocado or rubber isolate of *P. heveae* on opposite sides of the polycarbonate membranes. *Phytophthora*

Table 3. Possible types of chemically regulated sexuality among members of the genus *Phytophthora*

Sexuality type	Production of hormones*		Responsiveness to hormones†	
	Hormone α^1	Hormone α^2	Hormone α^1	Hormone α^2
Group I: Cross-induction ('heterothallic')				
1	+	—	—	+
2	+	—	—	—
3	—	—	—	+
4	—	+	+	—
5	—	+	—	—
6	—	—	+	—
7	+	+	—	—
8	—	—	+	+
Group II: Self-induction ('homothallic')				
9	+	—	+	—
10	—	+	—	+
11	+	—	+	+
12	—	+	+	+
13	+	+	+	—
14	+	+	—	+
15	+	+	+	+
Group III: Neuter				
16	—	—	—	—

* +, Productive; —, non-productive. † +, Responsive; —, non-responsive.

megasperma var. *sojae*, races 1 and 3 induced oospore formation in the A^2 but not in the A^1 isolate of *P. parasitica*, while only the A^1 isolate of *P. parasitica* was induced to form oospores by *P. cactorum* and *P. katsurae*. When the homothallic isolates of *Phytophthora* were paired with uninoculated blocks separated by polycarbonate membranes, the blocks remained sterile after 6 d incubation.

DISCUSSION

Induction of oospore formation in *P. parasitica* by the opposite mating type at a distance of more than 3 mm strongly supports the previous report of regulation of sexual reproduction in *Phytophthora* by diffusible substances, designated α hormones (Ko, 1978). The ability of *P. heveae*, *P. megasperma* var. *sojae*, *P. cactorum* and *P. katsurae* to induce oospore formation in A^1 or A^2 (or both) isolates of *P. parasitica* indicates that homothallic species of *Phytophthora* are also capable of producing α hormones. Brasier (1972) also showed that sexual reproduction of both A^1 and A^2 isolates of *P. palmivora* was stimulated by *P. heveae*, although the test fungi were capable of penetrating the cellophane membranes used to separate them. Timmer *et al.* (1970) reported that a very small percentage of their single-oospore cultures of *P. capsici*, which produced a few oospores in single culture, produced abundant oospores when mated with A^1 or A^2 isolate of the same species. A similar phenomenon was observed in some single-zoospore cultures of the same fungus by Kamjaipai & Ui (1978). It is therefore suggested that sexual reproduction of homothallic *Phytophthora* may also be controlled by α hormones. Hormonal regulation of sexual reproduction in homothallic as well as heterothallic species of *Achlya* has been reported by Raper (1940, 1950) and Barksdale & Lasure (1973).

This and a previous paper (Ko, 1978) suggest that sexual reproduction in *Phytophthora* requires two distinct and independent processes, i.e. hormone production and recognition

of, or response to, hormones. Different isolates of the same mating type of *Phytophthora* vary in the amount of α hormones produced (Ko, 1978). Responsiveness to a certain hormone may be determined by the presence of an active receptor of that particular hormone, as in the case of other related organisms (Kochert, 1978). Mortimer *et al.* (1977) showed that single-zoospore cultures derived from the A² type of *P. drechsleri* occasionally gave A¹ type. It is therefore possible that non-responsiveness to a certain hormone may be due to the presence of an inactive receptor or to the absence of a receptor to that hormone.

Based on hormone production and responsiveness to hormones, 16 possible types, divided into three groups, of chemically regulated sexuality among the members of *Phytophthora* are postulated (Table 3). Members of group I (cross-induction) do not produce oospores in single culture. They can either stimulate others to produce oospores or produce oospores themselves when stimulated by others. Their sexual reproduction requires cross-induction; therefore, it is comparable to those commonly called 'heterothallic'. The A¹ compatibility type or mating type of 'heterothallic' *Phytophthora* may belong to type 1, 2 or 3, while the A² type may belong to type 4, 5 or 6. The A¹ and A² isolates of *P. cinnamomi*, *P. parasitica* and *P. palmivora* can stimulate the A² and A¹ isolates, respectively, to form oospores. Their oospore formation can also be stimulated by the opposite mating type (Ko, 1978). Thus they belong to types 1 and 4, respectively. Members of group II (self-induction) are capable of producing oospores in single culture and are therefore comparable to those commonly called 'homothallic'. Based on this and Brasier's report (1972), *P. heveae* may belong to type 13, 14 or 15. The present results also show that *P. megasperma* var. *sojae*, races 1 and 3 are type 9 or 11, while *P. cactorum* and *P. katsurae* can be type 10 or 12. Currently, available data do not permit the precise designation of sexuality type among members of group II. Those fungi belonging to group III (neuter) cannot stimulate others, nor can they be stimulated by others to produce oospores. There is only one type, 16, belonging to this group. Pratt *et al.* (1972) reported that 1 of 372 isolates of *P. cinnamomi* from Australia failed to form oospores when mated with either A¹ or A² isolates. Chee (1969) also found that 8 of 194 isolates of *P. botryosa* and 3 of 24 isolates of *P. palmivora* from Thailand and West Malaysia were 'non-complementary' because they were not able to form oospores with either A¹ or A² strains. These isolates can all be considered type 16. Type 16 was also found in single-oospore cultures of *P. drechsleri* (Khaki & Shaw, 1974), *P. palmivora* (Chee, 1973), *P. botryosa* (Chee, 1973), *P. capsici* (Timmer *et al.*, 1970) and *P. cinnamomi* (Brasier & Sansome, 1975).

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