Histology of Primary Infection of *Brassica* Species by *Albugo candida* Race 7

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INRODUCTION

Albugo candida (Pers. ex Lev.) Kuntze is an obligate parasite of cruciferous plants. Little is known of the mechanisms responsible for establishing and maintaining such a relationship, or how an incompatible relationship is determined when the fungus confronts host cultivars carrying resistance genes. Histological studies on the sequence of fungal pathogenesis on compatible and incompatible cultivars may reveal critical events in the early infection process. This information is essential for interpreting results of biochemical and physiological research on mechanisms of resistance.

Verma et al. (1975) studied the infection of four *Brassica* species (*B. campestris*, susceptible; *B. napus*, resistant; *B. hirta*, moderately susceptible and *B. juncea*, (susceptible) by *A. candida*. Pidskalny (1984) has described some aspects of the infection process in a compatible interaction between *B. campestris* cultivars and *A. candida* race 7. In these studies observations on fungal pathogenesis were initiated 24 h after inoculation. In fact, the initial 24 h is probably the critical period for the establishment of this obligate parasite. It is logical to begin observations at the time of inoculation and observe the earliest host-parasite interactions until a functional relationship between host and parasite either is or is not established. This study describes the sequence and timing of the pathogenesis of zoospores of *A. candida* race 7 on cotyledons of susceptible and resistant rapeseed lines.

MATERIALS AND METHODS

A. candida race 7 obtained from naturally occurring infections on *B. campestris* cv. Torch was used to inoculate *B. napus* cv. Regent, (resistant) and two lines, 2282-9 and GCL (susceptible) and *B. campestris* cv. Torch, which is highly susceptible to *A. candida* race 7. Plants were grown in a controlled environment chamber at day/night temperature of 22/17C under 16 h photoperiod. Inoculation was performed approximately 6 days after seeding. A 10 ul droplet of zoospore suspension containing 1 x 10^5 zoospores/ml was placed on the centre of the abaxial surface of each half cotyledon. The inoculated seedlings were incubated at 20C and 100% RH, for 24 h with initial 12 h in dark.

Whole cotyledons were sampled at 1, 2, 3, 4, 8, 12, 24, 36, 48, 60, 72, 84 and 96 h after inoculation. They were cleared and stained according to the methods of Bruzzese and Hasan (1983). The cleared specimens were mounted in lactophenol and examined using differential interference contrast (DIC) microscopy. The following data were obtained at given sampling times: germ tube length, primary hyphal length, the number of haustoria per infection site and colony area. Only isolated infection sites were examined.

RESULTS AND DISCUSSION

The sequential process of infection of A. candida on compatible rapeseed lines was encystment of zoospores on cotyledon surfaces, cyst germination and germ tube elongation, stomatal penetration, primary hyphal formation and elongation, haustorial development, secondary hyphal formation, mycelial ramification, sporangiophore formation, and sporangial production.

One hour after inoculation, the majority of zoospore cysts accumulated in the stomata on the upper epidermal surface. Zoospore cysts germinated within 2-3 h by producing single germ tubes. Stomatal penetration was soon initiated by the germ tubes (Figure 1). On entering the substomatal chamber, the germ tubes swelled to form a substomatal vesicle and then elongated to form primary hypha which extended to the palisade mesophyll. Primary haustoria usually formed at the tip of the young hyphae and were first detected in the palisade mesophyll cells adjacent to the substomatal chamber 8 h after inoculation (Figure 2). The haustoria were spherical ranging from 2.0 to 4.0 um in diameter and connected to the hyphae by narrow necks approximately 1.8 x 0.4 um. Within 24 h after inoculation, secondary hyphae formed as side branches from the primary hyphae at the sites which previously gave rise to the initial haustoria.

On susceptible lines, primary haustoria formation followed by successful development of the secondary hyphae seems to represent the establishment of a functional relationship between rape and A. candida. The fungal growth accelerated dramatically resulting in ramification of hyphae as downward spirals into the spongy mesophyll. By 48 h, hyphal lengths in three susceptible lines investigated averaged 67 um with production of 1-8 haustoria per infection site (Table 1). Intercellular hyphal growth continued with more haustorial formation in mesophyll cells. By 84 h following inoculation, some of the fungal colonies became so extensive that they merged to form large compact mycelial masses. By 96 h, almost all the intercellular spaces were occupied by mycelia. Even at this stage, the host cells did not seem to be disrupted to any degree. Numerous club-shaped sporangiophores arose from the dense mycelial mat beneath the lower epidermis. White pustules were macroscopically visible within 5-6 days after inoculation.

The average germ tube lengths of 2282-9, GCL, Regent and Torch at 4 h after inoculation were 18.3, 17.4, 18.9 and 18.7 um, respectively. This implies that the incompatible line possesses no morphological or physiological features which could prevent cyst germination or subsequent penetration through stomata. Differences occurred between compatible and incompatible reactions 12 h after inoculation. The greatest increase in mean primary hyphal lengths occurred between 12-36 h for the compatible lines. On the resistant line fungal growth

did not increase significantly after this time. Hyphal growth ceased between 12 and 48 h after inoculation.

Haustoria were first formed in all lines at 8 h after inoculation. The average and maximum numbers of haustoria formed on each line at each time is shown in Table 2. Usually only one haustorium was formed per primary hypha on the resistant line. A resistant response, involving cell necrosis of the host, first appeared 12 h after inoculation (Figure 3). The number of infection sites showing cell necrosis then increased rapidly and amounted to 95% within 48 h. At this stage, it was no longer possible to make an accurate measurement of incompatible reaction since the fungal structure was obscured by collapsed host cells. Only the cells penetrated by haustoria became necrotic; adjacent uninfected cells and mesophyll cells below the dead cells remained healthy.

Fungal growth in the compatible lines was rapid following production of functional haustoria. Hyphae ramified intercellularly forming numerous secondary haustoria in the host mesophyll cells. As many as 44 haustoria per infection site were observed in Torch at 84 h after inoculation. More haustoria were present at 96 h or later, but the coalescence of fungal thalli made it impossible to obtain the accurate numbers of haustoria per infection site.

This study reveals that differences in reaction to A. candida race 7 between resistant and susceptible rapeseed lines only become pronounced after primary haustorial formation in the host palisade mesophyll cells. This suggests that successful formation of the primary haustoria is essential for the establishment and maintenance of a compatible relationship between A. candida and its host. The fact that the sequence and timing of pre- and post-penetration events were similar on resistant and susceptible lines implies that specific recognition is prerequisite to an incompatible reaction.

REFERENCES

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Table 1. Effect of rapeseed lines and hours after inoculation on the length of primary hyphae.

Hours after Inoculation	Mean length of primary hyphae (um)@			
	Regent	GCL	2282-9	Torch
8	22.7 a*	21.6 a	24.2 a	24.4 a
12	23.6 a	25.1 a	26.6 a	28.5 a
24	24.3 a	39.4 b	42.5 b	45.1 b
36	24.8 a	60.0 c	61.6 c	63.5 c
48	25.0 a	66.4 c	66.9 c	68.2 c

@ Average of four replications and five sampling units except that values of Regent at 36 and 48 hours after inoculation were average of 18 and 1 observations, respectively due to necrosis of host cells.

* Values in each column and each row followed by different letters differ significantly according to Duncan's Multiple Range Test, P=0.05.

Table 2. Mean and maximum numbers of haustoria per infection site in cotyledons of susceptible and resistant rapeseed lines.@

	Line								
	Torch		2282-9		GCL		Regent		
Hours	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Nec.*
8	0.7	1	0.7	1	0.5	1	0.6	1	0 %
12	1.0	1	0.8	1	0.7	1	0.7	1	26 %
24	1.1	2	0.9	2	0.9	1	0.3	1	53 %
36	1.6	3	1.5	3	1.3	3	0.2	1	80 %
48	3.1	8	1.7	3	1.5	3	0.2	2	95 %
60	6.3	16	4.8	9	4.9	10	--	--	--
72	6.0	18	5.8	12	5.6	12	--	--	--
84	22.8	44	19.9	38	13.5	31	--	--	--

@ Observations made on 5 infection sites on each of 4 cotyledons for all 4 rapeseed lines.

* Percentage of infected host cells which were necrotic.

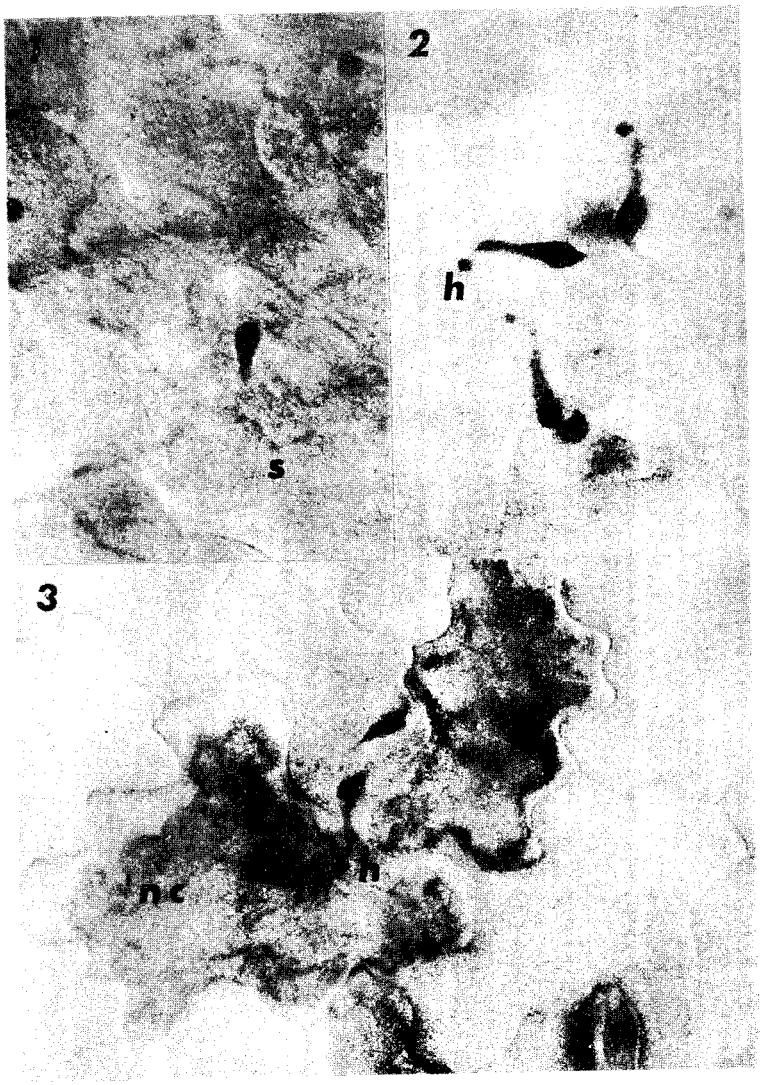

Figure 1. Encysted zoospore with germ tube and penetration of germ tube into host stomate (3h after inoculation).
Figure 2. Primary haustoria formed in mesophyll cells from tips of germ tubes (8h after inoculation).
Figure 3. Host cell necrosis on cv. Regent following primary haustorial formation (12h after inoculation).