Hypersensitive Reactivity in Potato: Transition from Inactive to Active State Induced by Infection with an Incompatible Race of *Phytophthora infestans*

N. Furuichi, K. Tomiyama, and N. Doke

Plant Pathology Laboratory, Faculty of Agriculture, Nagoya, Japan.
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ABSTRACT

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We reported previously that surface cells of fresh potato tuber disks had low ability to react hypersensitively to infection with an incompatible race of *Phytophthora infestans*. These cells acquired hypersensitive reactivity gradually and attained almost full ability to react about 16–20 hr after cutting. We now report that when the surface tissue zone (0.2–0.3 mm thick) from aged disks was removed with a razor blade, cells of the freshly cut surface also had high ability to react. This indicates that the low ability of freshly cut disks to react is not caused by wounding but is due to the inherent low ability of intact cells. Treating the cut ends of petioles of unfolding young leaflets with 5 ppm blasticidin S and inoculating the intact epidermis with the incompatible race greatly reduced hypersensitive cell death. These results indicate that an intact potato cell, from tubers as well as from leaves, has very low initial ability to react hypersensitively to infection. The ability is induced by infection with an incompatible race of *P. infestans* or by wounding. We propose to name these two states "inactive" (state I) and "active" (state II). Transition from state I to state II requires protein synthesis.

Additional key words: potato late blight, resistance.

It has been reported (2,4–7) that surface cells of freshly cut disks of potato tubers do not respond with rapid hypersensitive death when infected with an incompatible race of *Phytophthora infestans*. The cells acquire this ability within 16–20 hr after cutting (2), and de novo protein synthesis is necessary. Once the hypersensitive reactivity has developed to a high level, however, de novo protein synthesis is not necessary for hypersensitive cell death (1,2,4). A similar phenomenon has been observed in freshly cut leaf petioles (5).

The question raised is whether the ability for the hypersensitive reaction is present initially in tissue and is removed by cutting or wounding or whether it is inherently low in intact tissue and increases only after wounding. If the latter is true, (i) why does the intact cell subsequently react hypersensitively to infection and (ii) does infection per se induce the ability of an intact cell to react hypersensitively? We carried out experiments to answer these questions.

MATERIALS AND METHODS

Plant materials and inoculation. The potato cultivar Rishiri, which carries the R_1 gene for resistance to *Phytophthora infestans*, was used throughout. The tuber disks (10 mm in diameter, 10 mm thick) were prepared as reported previously (4). Potato plants were grown from tubers planted in soil and placed in a greenhouse at 18 C. After the plants were about 40 cm in height, the youngest unfolding leaflets were excised and used for the experiments.

Zoospore suspensions were prepared and tuber disks were inoculated according to methods reported previously (1,4). The intact epidermal cells of petioles were inoculated by flooding them with the zoospore suspension ($7–9 \times 10^5$ spores/ml).

Treatment with blasticidin S. Unfolding leaflets were cut and the cut ends of the petioles were pressed against absorbent cotton soaked in 5 ppm blasticidin S in a petri dish.

Microscopic observations. Penetration by the parasite and death of the infected cells were observed microscopically according to methods described previously (3,4).

RESULTS

Hypersensitive reactivity of cells neighboring the aged cut surface cells. After the surface cells of cut potato tubers had acquired a high ability to react hypersensitively to infection by an incompatible race of *P. infestans*, the surface tissues (0.2–0.3, 1, or 5 mm thick) were removed. The freshly cut surfaces then were inoculated with the incompatible race 0. When 0.2–0.3 mm was removed, cells of the freshly cut surface responded to infection with

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race 0 at almost the same rate as those of the aged cut surface (Fig. 1). Hypersensitive death occurred much later, however, on freshly cut surfaces after 1 or 5 mm of the aged surface tissue was removed

Induction of hypersensitive reactivity of intact cells by infection. Hypersensitive death of intact epidermal cells of midrib of unfolding leaflet petioles was greatly inhibited by preinfection treatment of the cut ends of the petioles with 5 ppm blasticidin S for 1 hr immediately after excision (Fig. 2). Almost all infected cells of water-treated petioles died within 8 hr after inoculation, but only a small number of those treated with blasticidin S died. In a second experiment, the cut ends of petioles were treated with 5 ppm blasticidin S for 1 hr beginning 4 hr after excision; the intact epidermis of both water- and blasticidin S-treated petioles then was inoculated with race 0. Results were similar to those in petioles treated with blasticidin S immediately after excision. Treatment with 5 ppm blasticidin S for 1 hr before inoculation had little effect on penetration by *P. infestans* or on intracellular hyphal growth, as reported previously (1,4). We also confirmed, by using ^{3}H-leucine, that protein synthesis in leaf petioles was inhibited more than 90% by blasticidin S under this experimental condition.

DISCUSSION

Results show that once the ability of the aged cut surface tissue to react hypersensitively has been raised to a high level, the tissue appearing after removal of 0.2–0.3 mm of the aged cut surface also has a high ability (Fig. 1). Induction is limited to tissue near the wound site and does not extend very deeply (Fig. 1). This indicates clearly that ability of the aged cut surface tissue to respond to infection is not removed by wounding and also that freshly cut surface tissue has an inherent low ability. A previous paper (8) reported that in potato tuber tissue neighboring a wound or an infection, the level of accelerated metabolic activity is highest in the cut surface tissue and decreases toward the inner tissue. The decreasing activity is related to distance from the lesion in a negative exponential manner.

These and previous results (2,4) indicate that in intact cells from leaves and tubers of potato, de novo protein synthesis is required before the ability to respond hypersensitively to infection is established. This ability is induced by either infection or wounding.

We propose to name the inability of an intact potato cell to react hypersensitively "inactive" (state I) and the state of the wounded or infected cell "active" (state II). De novo protein synthesis apparently is necessary for transition from state I to state II. The work reported here and elsewhere (2,4) shows that blasticidin S inhibition of the hypersensitive response can be used to differentiate the two states.

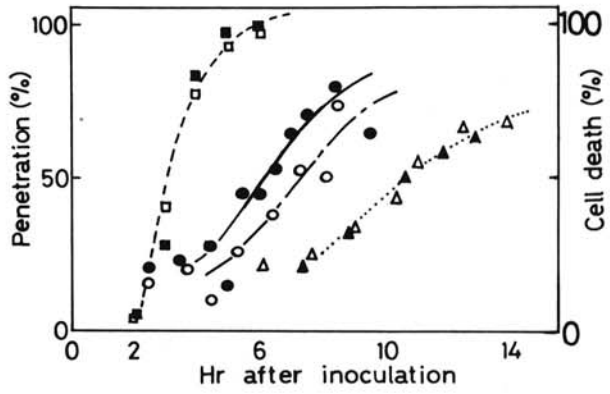

Fig. 1. Comparison of time for penetration and hypersensitive death of infected cells of aged cut surface tissues and those of neighboring tissues. Surface tissues 0, 0.2–0.3, 1, or 5 mm thick were removed from tuber disks, and the freshly cut surfaces were inoculated with an incompatible race of *Phytophthora infestans*. Each value represents the average of three experiments. Penetration: ■ = control, □ = 5 mm removed. Cell death: ● = control, o = 0.2–0.3 mm removed, ▲ = 1 mm removed, Δ = 5 mm removed.

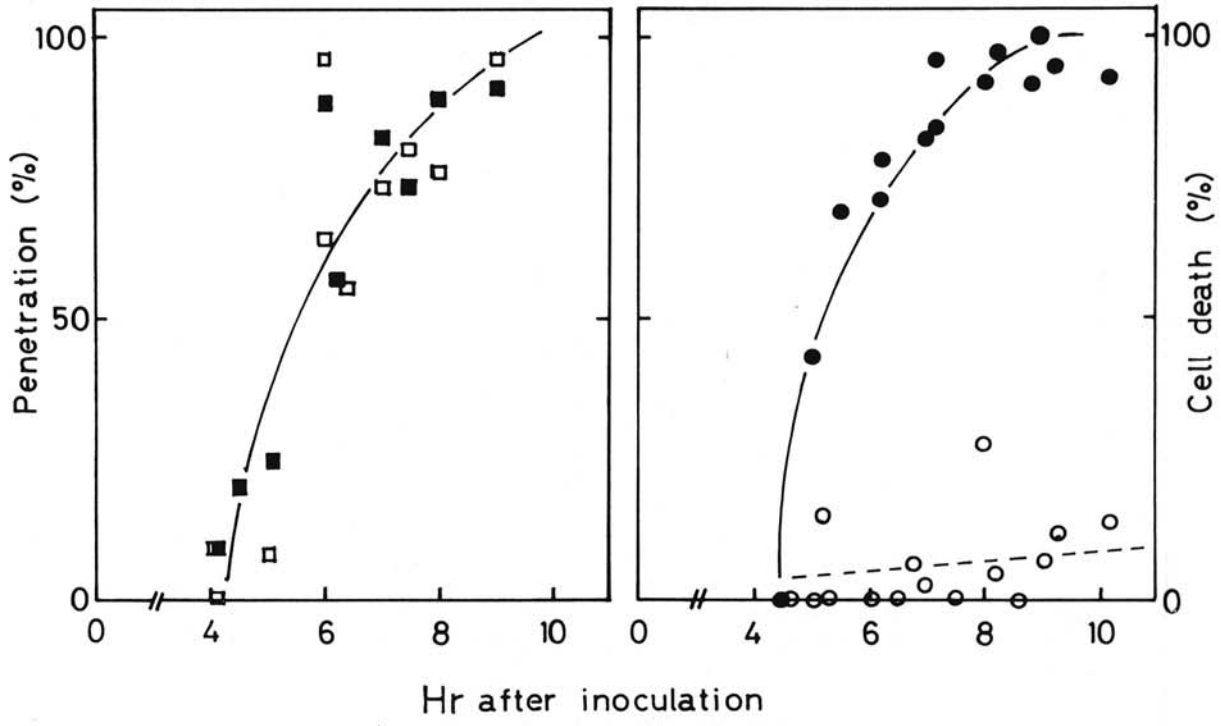

Fig. 2. Effect of blasticidin S on penetration of potato petiole cells by *Phytophthora infestans* and on time from penetration to hypersensitive death of the cells. Cut ends of petioles were treated with 5 ppm blasticidin S for 1 hr immediately after excision, and intact surfaces of the petioles were inoculated with an incompatible race of *P. infestans*. Penetration: ■ = control, □ = blasticidin S. Cell death: ● = control, o = blasticidin S.

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