Alteration of Membrane Permeability of Potato-tuber Tissue Infected by Incompatible and Compatible Races of Phytophthora infestans During the Initial Phase of Infection

N. MATSUMOTO*, K. TOMIYAMA** and N. DOKE**

Abstract

Electric potential difference (EPD) between the surfaces of potato-tuber disks infected by compatible and incompatible races of Phytophthora infestans and between each of them and noninfected ones were determined during the initial period of infection. EPD began to increase almost simultaneously with host wall penetration by both the incompatible and compatible races and the increase was already larger in the former than in the latter. Electric conductivity (EC) measurements of the exudates from the infected tuber disks showed that an increase in EC was also found in both the compatible and incompatible infections. The increase in EC was larger in the incompatible combination before hypersensitive death of infected cells occurred. In both incompatible and compatible combinations the increasing rate in EC was larger when the disks were inoculated 20 hr after cutting rather than just after cutting. Leakage of preabsorbed $^{32}$P from potato-tuber disks was increased by infection with both the incompatible and compatible races, and the increase was higher in the former than in the latter. All these results suggested that the permeability of cell membranes of potato tuber disks increased very soon after the infection (or at the same time as penetration occurred) when they were inoculated with either incompatible or compatible races of P. infestans. However, the rate of increase seemed to be larger in the incompatible than in the compatible combination before hypersensitive cell death of the former occurred.

(Received September 22, 1975)

Introduction

It has been reported that potato cells infected by an incompatible race of Phytophthora infestans may die hypersensitively within about 30 min after penetration, in the quickest case$^{8,17}$. Those infected by a compatible race, however, lived in coexistence with the parasite for more than 3 days. Microscopic$^{16}$ and cinephotomicrographic observations$^{8}$ indicated that the cytoplasmic response of potato to infection by an incompatible race of P. infestans differed from the response to infection by a compatible race at about the time of penetration. These

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This research was supported in part by research grants No. 948101 and No. 936004 from the Ministry of Education, Japan.
observations presented a clue that infection by an incompatible race of *P. infestans* may affect the cell membrane of potato very soon after contact between the fungus and the host cell membrane.

So far, in many diseases, an increase in leakage of electrolyte from plant tissues caused by infection has been reported. This phenomenon has been supposed to be due to change of permeability of cell membrane. In the experiments reported here, we tried to examine whether or not physiological activity of cell membrane was altered just after the penetration by means of determinations of electric potential difference, electrolytic conductivity and leakage of preabsorbed $^{32}$P.

**Materials and Methods**

**Materials.** Potato cultivars Rishiri bearing the $R_1$-gene, Pentland Ace ($R_3$) and Irish Cobbler ($r$) were used. Tubers were stored at 4°C for 3-12 months until use. Three races of *Phytophthora infestans* race 0, race 1 and race 3 were used. Zoosporangia were collected on Toyo filter paper No. 2, washed three times with water, and then suspended in either Knop’s solution (for determination of electric potential difference) or in distilled water for other experiments. The sporangia were allowed to germinate at low temperature and the resulting zoospore suspension was adjusted to $2 \times 10^6$ zoospores/ml for inoculation. In each experiment, the pathogenicity was checked by inoculation experiments. Responses of potato cultivars to the races are shown in Table 1.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Race 0</th>
<th>Race 1</th>
<th>Race 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irish Cobbler ($r$)</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Rishiri ($R_1$)</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Pentland Ace ($R_3$)</td>
<td>R*</td>
<td>R*</td>
<td>S</td>
</tr>
</tbody>
</table>

* Degree of resistance (Field resistance) was weak and sparse sporulation was observed.

**Microscopic observations.** Tissue cylinders were cut out with a cork borer, 10 mm in diameter, from the central parenchymatous tissues of tubers of Rishiri, and then sliced into disks 1.5 mm in thickness. They were washed thoroughly with water and incubated at 18°C in a moist condition for 20 hr. Then one side of each disk was inoculated with 0.1 ml of the zoospore suspensions of race 0 or race 1. Microscopic sections were made and were stained with 0.05% neutral red solution containing 0.8 M sucrose. Observations were made at intervals of 1 hr. Plasmolyzed cells were regarded as living.

**Determination of electric potential difference.** Tuber disks of the cultivar Rishiri, 2 mm thick and 18 mm in diameter, were prepared and were kept at 18°C in a moist condition for 20 hr. Two disks were placed on a gauze wetted with Knop’s solution. The upper surface of one disk was inoculated with 0.1 ml of zoospore suspension in Knop’s solution, and that of the other disk was wetted with 0.1 ml of Knop’s solution as a control. In other experiments, one disk was inoculated with race 0 and the other with race 1. Vaseline was used to coat the edges of the disks. A small piece of agar containing Knop’s solution was placed on the
disk and attached to the calomel electrode. The apparatus used to determine the electric potential difference is shown in Fig. 1. Electrodes were connected to an amplifier (Nihon Koden, AD 3-2) equipped with an oscillograph (Nihon Koden WI-130).

**Measurement of electrical conductance.** Tubers of three cultivars were cut into disks, 18 mm in diameter and 1.5 mm thick as before. The disks were inoculated with race 0, race 1 and race 3, respectively, just after/or 20 hr after cutting. Distilled water served as a control. Inoculated disks were incubated at 18 C in a moist condition and then 5 disks were immersed in 10 ml of distilled water at intervals of 1 or 2 hr. After shaking for 10 minutes (5 rev./minute), the disks were discarded and conductivity of the water was determined by using an electrical conductivity meter (Towa Denpa Co. Model CM-3M).

The electrical conductivity shown in each figure is the increment obtained by subtracting the value measured about 30 min after inoculation from each value measured later.

**Determination of 32P-leakage from infected disks.** Tuber disks 10 mm in diameter and 1.5 mm thick were immersed in a solution containing H₃¹³PO₄ (1.7 µCi/ml) for 30 min just after preparation of the disks (cutting out) or after exposing them to air at 18 C for 20 hr. After immersion, they were washed three times in distilled water and then inoculated with race 0, race 1. Water served as a control. The inoculated disks were immersed in 10 ml of distilled water at intervals of 1 hr. After 10 min of shaking, 1 ml of the water was poured into a vial containing 10 ml of distilled water. Radioactivity was counted in a liquid scintillation spectrometer (Packard model 3320).

**Results**

**Time course of penetration and cell death**

Fig. 4 indicates that the cut surface cells of tuber disks of Rishiri infected by the incompatible race, race O, began to die hypersensitively within 2 hr after inoculation, when inoculated 20 hr after inoculation. They are about to die 6 hr after inoculation, on the contrary, when inoculated just after cutting. It has been reported that the penetration of cell walls of tuber tissues of Rishiri began about 50 min after inoculation. When inoculated with the compatible race 1 no cell death occurred in response to infection. There is no difference in the time intervals from inoculation to penetration between the compatible and incompatible races.

**Electrical potential difference**

When disks were inoculated with *P. infestans* 20 hr after cutting, differences
in electrical potential between the surfaces of the disks inoculated with both the incompatible and compatible races and the noninfected one began to increase about 50-60 min after inoculation (Fig. 2). The time of initiation of the increase almost coincided with the time when some of the appressoria began to penetrate the cell wall. It seemed that the increasing rate was more rapid in the disks infected by the incompatible race than in those infected by the compatible race. Furthermore the difference between the disks inoculated with the incompatible race and those inoculated with compatible race seemed to begin almost simultaneously with the initiation of penetration (Fig. 3).

Electrical conductivity measurements

Tuber disks of Rishiri inoculated with the races 20 hr after cutting were immersed in water and the electrical conductivity (EC) of the water was measured. The EC in the case of the incompatible combination was already higher than the compatible one 2 hr after inoculation (Fig. 4). With the disks inoculated just after cutting and washing, the conductivity of the immersion water was higher in the incompatible combination than the compatible one before the hypersensitive death of cells in the former combination began (Fig. 5). Although the disks of Pentland Ace had the R3 gene, little difference in conductivities of the immersion water was observed between the compatible and incompatible races (Fig. 6).

Increases in conductivity were also found in compatible combinations during an initial period of infection, and the conductivity continued to increase (Fig. 4, 5, 6, 7). The increasing rates of conductivity in immersion water of Irish cobbler disks inoculated with the compatible races, race O, race 1 or race 3, were different but parallel with each other (Fig. 7). This phenomenon suggested that the aggressiveness of each race on this r-cultivar was almost the same. In spite of the same rate in the increase, the rise in the permeability of the disks inoculated with race 1 always occurred earlier than that of those inoculated with race O or race 3 (Fig. 7).
Fig. 4. Changes in electric conductivity of water in which tuber disks of Rishiri inoculated with incompatible races 0 and 3 and compatible race 1 were immersed. The broken line indicates the time required for hypersensitive death of cells in potato-tuber disks inoculated with race 0. Inoculation was made 20 hr after cutting out the tuber disks. r-0=race 0, r-1=race 1, r-3=race 3.

Fig. 5. Electric conductivity of water in which tuber disks of Rishiri inoculated with compatible and incompatible races of Phytophthora infestans were immersed. Inoculation was made 1 hr after cutting. All other experimental conditions were the same as Fig. 4.

Fig. 6. Changes in electric conductivity of water in which tuber disks of Pentland ace inoculated with incompatible races 0 and 1 and compatible race 3 (2×10^6 zoospores/ml) were immersed. Inoculation was made 20 hr after cutting.

Fig. 7. Changes in electric conductivity of water in which tuber disks of Irish cobbler inoculated with compatible races 0, 1 and 3 (2×10^6 zoospores/ml) were immersed. Inoculation was made 20 hr after cutting.
This may be due to the condition of the zoospores used, although the zoospores were adjusted to the same concentration. The increasing rates in conductivity were higher in the disks inoculated with the compatible races 20 hr after cutting than those inoculated just after cutting (Table 2).

Table 2. Increases in electrical conductivity of water in which potato-tuber disks inoculated with the compatible races just after cutting or 20 hr after cutting were immersed

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Races of P. infestans</th>
<th>Inoculated about 1 hr after cutting</th>
<th>Inoculated 20 hr after cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Increase in conductivity</td>
<td>(A) (A)-(control)</td>
</tr>
<tr>
<td>Irish Cobbler</td>
<td>r-0</td>
<td>5.6*</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>r-1</td>
<td>3.8</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>r-3</td>
<td>7.9</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>2.1</td>
<td>—</td>
</tr>
<tr>
<td>Rishiri</td>
<td>r-1</td>
<td>0.3</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>-3.7</td>
<td>—</td>
</tr>
<tr>
<td>Pentland</td>
<td>r-3</td>
<td>7.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Ace</td>
<td>Cont.</td>
<td>3.0</td>
<td>—</td>
</tr>
</tbody>
</table>

* Increment in electrical conductivity from 0.5 hr to 10 hr after inoculation (μmho).

\[ ^{32}P \text{-leakage from inoculated disks} \]

Leakage of preabsorbed \(^{32}P\) from the disks inoculated with compatible and incompatible races showed a similar pattern as the electrical conductivity (Fig. 8).

**Discussion**

The difference in electrical potential between surfaces of intact and wounded plant tissue is known to be caused by alteration in the permeability of cell membranes of the wounded tissue. The electrical potential difference observed (Fig. 3) suggested that permeability of the cell membranes to ionic substances increased more in incompatible than in compatible combinations. This occurred almost simultaneously with the beginning of penetration of the host cell walls. At this time, death of infected cells had not yet occurred (Fig. 4). A similar phenomenon was observed in the electrical conductivity measurements. The data from disks inoculated just after cutting (Fig. 6) indicated clearly that permea-
bility of the cell membrane increased more in the incompatible combinations than in compatible ones during the initial period of infection. In a previous paper\textsuperscript{13}, it was reported that the hypersensitive cell death did not occur during this period of infection when inoculated with the incompatible race just after cutting. These results showed that permeability of the cell membrane increased more in the incompatible combinations than in compatible ones before hypersensitive cell death in the former combination occurred. Little differences in electrical conductivity in disks of Pentland Ace inoculated with compatible and incompatible races (Fig. 6) may be due to weakness of the resistance, since the browning occurred very late, and even sparse sporulation of the incompatible races was observed.

When tuber disks were inoculated with the compatible race, no cell death occurred during the period of the experiments. Nevertheless, the difference in electrical potential, and also the electrical conductivity, increased almost simultaneously with penetration of host cell walls. There seemed to be a possibility that the increases were due to splitting of cell wall materials caused by the penetration or by exudates from the fungus. Endo-polygalacturonase activity has been found in filtrate from cultures of \textit{P. infestans}\textsuperscript{3,7}, but the activity seemed to be very low or hardly detectable\textsuperscript{2}. Pectinmethyl esterase activity was also found in the filtrate\textsuperscript{2,7}. Pectic lyase and macerating activities were not detected\textsuperscript{3}. Presence of galactanase activity in the filtrate was reported\textsuperscript{3,9}. However, the increases in electrical potential difference and electrical conductivity were assumed to be induced by a permeability change in the cell membranes due to infection, since (1) leakage of preabsorbed \textsuperscript{32}P from the disks also increased and (2) the increase in electrical conductivity was accelerated when the prepared disks were exposed to air for 20 hr before inoculation, in spite of the same growth rate of the intracellular hyphae under both inoculation conditions\textsuperscript{15}. These evidences also indicated that the most of increase in ion concentration in the immersion water might not be due to exudate from the fungi, although exudate from the fungi might contribute, to some extent, to the increase. The increased permeability of the cell membranes of potato tuber infected by the compatible race (Fig. 7) suggested that infection by a compatible race also affected the permeability of the host cell membranes.

Authors are grateful to Professor Dr. T. Tamura and Professor Dr. H. Okamoto, Nagoya University for their kind advice in the determination of electric potential difference, and Mr. S. Sakaguchi and Mr. Y. Umemura in Hokkaido Agr. Exp. Sta. for generous supply of the experimental materials. The authors are also indebted to Mr. H. S. Lee and Mr. N. Nishimura for their valuable discussion. The authors are indebted to Dr. J. R. Aist, Cornell University, for his help in preparation of the manuscript.

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疫病菌感染初期におけるジャガイモ塊茎細胞膜の透過性の変化について

松本直幸・冨山宏平・渡家紀志

ジャガイモ塊茎スライスに疫病菌親和性ならびに非親和性レースを接種し、感染が始まると同時に（1）表面電位差の測定（2）浸出イオンの電気伝導度による測定（3）あらかじめ吸収させた$^{32}$Pの漏出の測定を行った。これらは細胞から浸出したイオン濃度の測定と考えることができる。強い圃場抵抗性を併せもったR₁遺伝子抵抗品種リシリでは殆んど貯入が始まると同時に非親和性レース感染スライスの方が親和性レース感染スライスより細胞外イオン濃度の増加が著しい。諸実験結果は明らかに非親和性レースによる宿主細胞の過敏性細胞脳死以前に既に非親和性レース感染細胞の方が、親和性レース感染細胞より流体外のイオン濃度が増加することを示している。これに反して圃場抵抗性の著しく弱い（胞子形成が見られる）R₂遺伝子抵抗性品種ベントランドレースでは測定時間内には非親和性、親和性レース感染スライス間に差を見なかった。またすべての実験を通じて親和性レースの感染によっても貯入が始まると殆ど同時に細胞外浸出液のイオン濃度が増加する。

以上のべた非親和性及び親和性両レースによる感染ごく初期の細胞外液のイオン濃度の増加は次の2つの理由「(1) 塀茎を切断してスライスを調整してから20時間たちから接種した場合に非親和性、親和性ともに細胞内進展速度に差がないにかかわらず、切断直後接種よりイオン濃度の増加が著しい。（2）$^{32}$P一漏出も両レース感染で速やかに増加する。」から感染による細胞壁物質の分解流によるものではなく、また菌からのイオンの分泌でもなく、主として宿主細胞の原形質膜の透過性の変化によるものであろうと推定した。