Occurrence of Resistant Reaction by Exogenous DNA Fraction on Potato Leaves Infected with *Phytophthora infestans*

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疫病感染ジャガイモの外生 DNA フラクションによる抵抗性反応の誘導

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Introduction

There are already many reports on the experimental induction of resistant reaction to the susceptible cultivar. For example, ethylene and phenylalanine treatments induce the resistant reaction on the susceptible cultivars[1,2], and the decrease of diseased spots was reported on the plants treated with high molecular substance[3].

The writers[4,5,6] already reported the coexistence of hypersensitive flecks and susceptible lesions on potato leaves inoculated with *Phytophthora infestans* after treating with the DNA fraction from resistant hybrid. However, the rate of coexistence of hypersensitive flecks and susceptible lesions suddenly decreased by heat treatment over 90°C. The writers[7] also recognized the coexistence of hypersensitive flecks and susceptible lesions on susceptible potato leaves painted with the DNA fraction from *Datura alba* and *Nicotiana tabacum* which showed hypersensitive flecks to the invasion of *P. infestans*. From these results, it is assumed that the induction of resistant reaction on susceptible cultivar is derived from the treatment of the DNA fraction of the resistant hybrid.

This paper deals with the results of experiments on the changes of enzymatic activities on susceptible cultivar infected with *P. infestans* after treating with the DNA fraction from the resistant hybrid.

Materials and methods

Plants and pathogen

Susceptible cultivar Benimaru (gene r) to *Phytophthora infestans* and resistant inter-specific hybrid 96-56 (gene R_1) were cultivated on experimental farm of Shimane University, and the 3rd and 4th leaves from the top were cut and placed in the moist Petri dishes in an artificial atmospheric apparatus for 12 hr (20°C, 2,800−3,200 lx). Zoospore suspension of *P. infestans* (race 0) (6.0−8.0 x 10^5 zoospores/ml) was

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inoculated (0.2 ml/drop). Inoculated leaves were again placed in the moist Petri dishes in an artificial atmospheric apparatus for a definite time and were used for the material of enzymatic measurements.

Preparation and measurement of peroxidase

Two g of potato leaves were homogenized with mortar and pestle in 0.1 M Tris-HCl buffer (pH 8.4) containing 6 mM ascorbic acid, 6 mM cysteine-HCl and sucrose in a cold room (3-4°C), and the homogenate was centrifuged at 23,000 x g for 20 min at 0-4°C. The supernatant was dialyzed against 0.1 M phosphate buffer (pH 7.4), and the dialyzate was used as crude enzyme solution. The measurement of the activity was conducted by following the method of Wood and Barbara.[8)]

Preparation and measurement of PAL

Two g of potato leaves were homogenized with mortar and pestle in 20 ml of cold 25 mM borate buffer (pH 8.8) containing 5 mM 2-mercaptoethanol and filtrated through four layers of gauze, and the filtrate was centrifuged (23,000 x g, for 20 min at 0-4°C). The supernatant was passed through the column of Sephadex G-25 (medium, bed volume 180 ml) in order to remove phenylalanine and phenolic compounds. Protein fractions were collected and used as crude phenylalanine ammonia-lyase (PAL) enzyme solution.

The measurement of PAL activity was conducted as follows ; 1 ml of 180 mM L-phenylalanine solution was added to 5 ml of crude enzyme solution and incubated for 1 hr at 37°C with shaking in a water bath. The control was incubated with distilled water without substrate.

The reaction was stopped by acidifying with 0.6 ml of 6 N HCl, and the acidified solution was extracted twice with 10 ml of ethyl ether. The combined ethyl ether was evaporated to dryness and the residue was dissolved in 5 ml of 0.1 N NaOH solution. The amount of t-cinnamic acid was determined by reading the absorption at 268 nm with a spectrophotometer (Hitachi spectrophotometer model 124).

Measurement of protein

Protein contents were measured by the method of Lowry et al.[9)] using Bovin Serum Albumin as a control.

Preparation and application of DNA fraction

The preparation of DNA fraction from resistant interspecific hybrid was conducted by Thomas method.[10)] Twenty g of potato leaves were homogenized with mortar and pestle in 80 ml of SSC-buffer (0.5 M sucrose, 0.15 M NaCl and 0.05 M sodium citrate, pH 7.8) and 2% (w/w) sodium dodecyl sulfate (SDS), and filtrated through four layers of gauze. The filtrate was centrifuged (3,000 x g, for 30 min). Equal volume of SSC-buffer saturated phenol was added to the supernatant and stirred gently. After the centrifugation (3,000 x g, for 30 min), three volumes of cold ethanol were added to the water phase and chilled (−10°C) for more than 4 hr. The white precipitate was collected by centrifugation (1,500 x g, 20 min), and dissolved in 0.15 M NaCl solution, and dialyzed against 0.15 M NaCl solution for overnight. The ratio of OD 260 nm/OD 280 nm of this DNA fraction was 2.0-2.1.

Petato leaves (susceptible cultivar Benimaru) were applied on the DNA fraction solution (500 μg/ml) from resistent interspecific hybrid 96-56 and immersed in vacuo with an aspirator for 3-5 min. These leaves were kept in the moist chamber at 20°C for 2 hr, and washed with tap water in order to exclude the outer DNA. *P. infestans* (race 0) was inoculated on these leaves. 0.15 M NaCl solution was treated on other leaves and the fungus was inoculated as the control.

Results

Change of peroxidase activity on potato leaves inoculated with P. infestans after treating with the DNA fraction

In order to make clear the change of peroxidase (PO) activity by the treatment of the DNA fraction, the change of PO activity on the interspecific hybrid 96-56 leaves inoculated with *P. infestans* (race 0) was investigated first of all [Fig. 1].

By 3 hr after inoculation of *P. infestans*, the difference of PO activity on the inoculated leaves and the control was not recognized, but at 6 hr the activity on the inoculated leaves was twice as compared with that of non-inoculated leaves. Such striking difference of the activity between them was not observed after 9 hr and the difference of PO activity on the inoculated and non-inoculated control was not recognized.

The change of PO activity on susceptible cultivar Benimaru infected with *P. infestans* (race 0) was shown in Fig. 2.

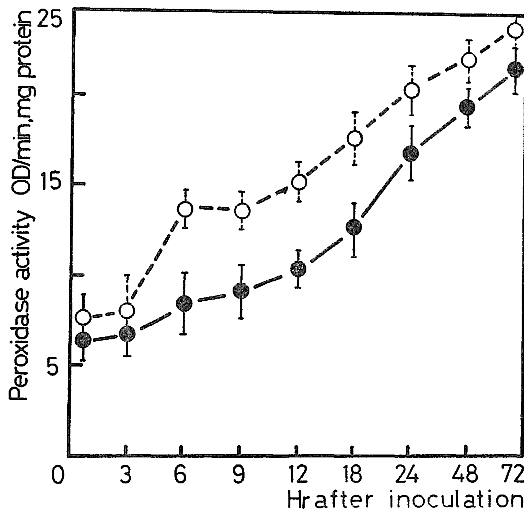

Fig. 1 Peroxidase activity on leaves of resistant interspecific hybrid 96−56 (R_1) infected with *Phytophthora infestans* (race 0). PO activity on inoculated leaves (○…○) ; non-inoculated (●—●). Vertical line drawn through a mean represents the range from the highest to the lowest activity of the enzyme. PO activity was measured by the method of Wood & Barbara.

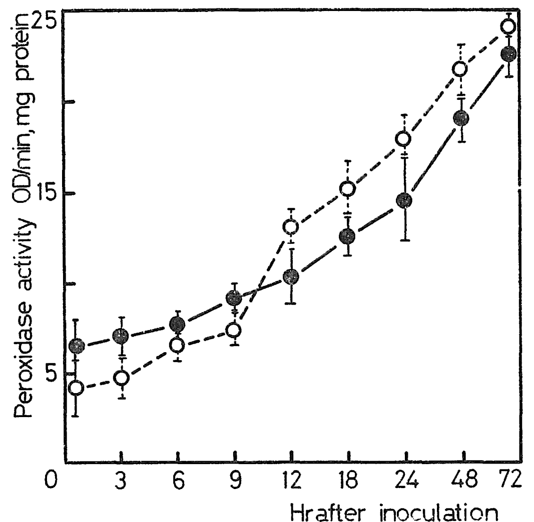

Fig. 2 Peroxidase activity on leaves of susceptible cultivar Benimaru (r) infected with *Phytophthora infestans* (race 0). PO activity on inoculated (○…○) ; non-inoculated (●—●). Vertical line drawn through a mean represents the range from the highest to the lowest activity of the enzyme. PO activity was measured by the method of Wood & Barbara.

Higher PO activity on the non-inoculated leaves was shown by 9 hr than the inoculated leaves, but after 12 hr the inoculated leaves showed higher activity than the control. However, there was no striking difference of PO activity on the inoculated leaves between the inoculated and non-inoculated control. PO activity of the inoculated leaves increased after 24 hr parallel to that of the non-inoculated control. From these results, that increase of PO activity on the resistant hybrid 96-56 infected with *P. infestans* (race O) was observed 6 hr after the infection and the increase was recognized on susceptible cultivar Benimaru after 12 hr.

Fig. 3 shows the change of PO activity on leaves of susceptible cultivar Benimaru infected with *P. infestans* (race 0) after treating with the DNA fraction from the resistant hybrid 96-56 [Fig. 3].

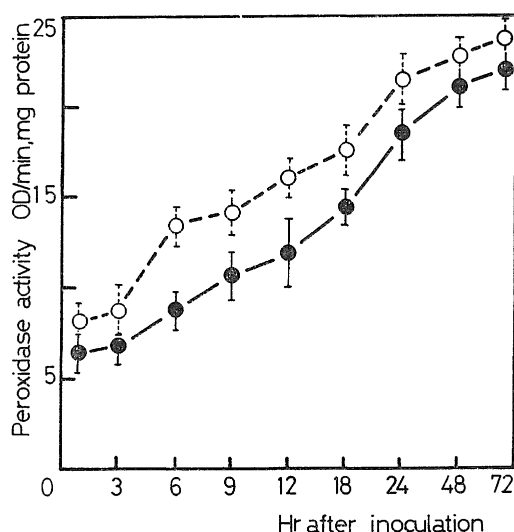

Fig. 3 Peroxidase activity on leaves of susceptible cultivar Benimaru (r) infected with *Phytophthora infestans* after treating with the DNA fraction from resistant interspecific hybrid 96−56 (R_1). PO activity on DNA treated-inoculated leaves (○…○) ; non-treated-inoculated leaves (●—●). Vertical line drawn through a mean represents the range from the highest to the lowest activity of the enzyme. PO activity was measured by the method of Wood & Barbara.

A slight increase of PO activity was recognized on potato leaves treated with the DNA fraction during 6-12 hr. The activity of the treated leaves showed 1.5 times that of the control 6 hr after inoculation. The difference of the activity between the DNA fraction treated leaves and non-treated control became smaller by 24 hr. The in crease of PO activity in the leaves infected *P. infestans* after treating with the DNA fraction was similar to that shown in the resistant interspecific hybrid 96-56 infected with *P. infestans*.

Change of PAL activity on potato leaves infected with P. infestans and treated with the DNA fraction

The change of PAL activity was investigated on the leaves of susceptible cultivar Benimaru and resistant interspecific hybrid 96-56 infected with *P. infestans* (race 0) [Fig. 4].

PAL activity on leaves of susceptible cultivar Benimaru infected with *P. infestans* (race 0) did not increase by 24 hr after inoculation and maintained almost constant activity, and the activity increased gradually after 24 hr. Meanwhile the activity on leaves of resistant hybrid 96-56 infected with *P. infestans* (race 0) increased 3 hr after

inoculation, and the increase continued till 72 hr after inoculation.

PAL activity on susceptible cultivar Benimaru infected with *P. infestans* (race 0) after treating with the DNA fraction from resistant interspecific hybrid 96-56 was investigated [Fig. 5].

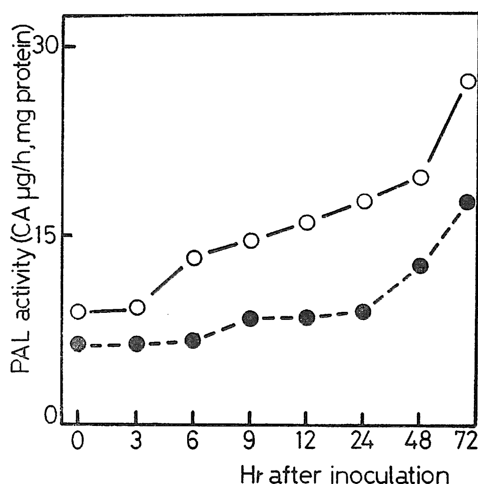

Fig. 4 PAL activity on leaves of susceptible cultivar Benimaru (r) and resistant interspecific hybrid 96−56 (R_1) infected with *Phytophthora infestans* (race 0). PAL activity on susceptible cultivar Benimaru infected (●…●); PAL activity on leaves of resistant interspecific hybrid 96−56 infected (○—○). PAL activity was showed by the amount of t-cinnamic acid.

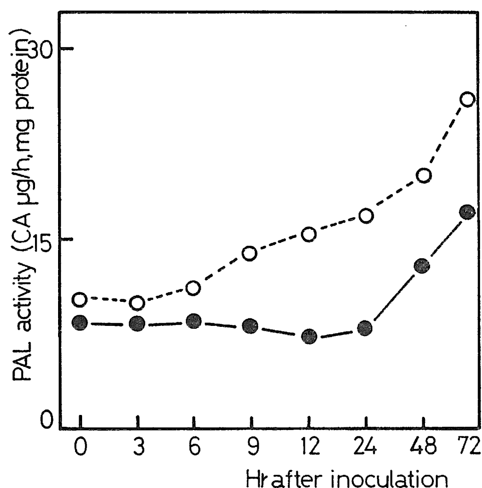

Fig. 5 PAL activity on leaves of susceptible cultivar Benimaru (r) infected with *Phytophthora infestans* (race 0) after treating with the DNA fraction from interspecific hybrid 96−56 (R_1). PAL activity on DNA treated-inoculated leaves (●—●); PAL activity on non-treated-inoculated leaves (○…○). PAL activity was showed by the amount of t-cinnamic acid.

The increase of PAL activity was recognized on leaves treated with the DNA fraction 3 hr after inoculation. The difference of PAL activity between the control and the DNA treated plot was recognized with the progress of time after inoculation. This pattern was similar to that of PAL activity on resistant hybrid inoculated with the incompatible race of *P. infestans*.

Discussion

There are few reports on the induction of resistance with exogenous DNA fraction. The writers[4,5,6] already reported that the coexistance of hypersensitive flecks and susceptible lesions on the leaves of susceptible cultivar infected with *P. infestans* after treatment with the DNA fraction from resistant hybrid was recognized. In this paper, the writers deal with the PO and PAL activities as an indicater of physiological change of potato leaves infected with *P. infestans* after treating with the DNA fraction. The pattern of enzyme activities on potato leaves infected with *P. infestans* after treating with the DNA fraction was similar to that of the resistant hybrid infected with an incompatible race of *P. infestans* at initial stage of infection. Hadwiger et al.[11] reported an accumula-

tion of pisatin, one of phytoalexins, and an increase of PAL activity on plant treated with substances intercalating into DNA. Ohyama et al.[12] reported that exogenous DNA combined with endogenous DNA in plant protoplast and showed the nature of exogenous DNA. From these considerations, it is assumed that the DNA in resistant hybrid which was treated the susceptible cultivar may have an action to the cells of susceptible cultivar. However, further investigation should be conducted on behaviour of DNA on potato leaves after treatment.

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Summary

The change of peroxidase and phenylalanine ammonia lyase activities was investigated on susceptible potato leaves inoculated with *Phytophthora infestans* after treating with exogenous DNA fraction from resistant interspecific hybrid. The increase of peroxidase activity of the leaves inoculated with *Phytophthora infestans* after treating with the DNA faction from the resistant hybrid was similar to that of the resistant interspecific hybrid inoculated with the incompatible race of *Phytophthora infestans*. Pattern of phenylalanine ammonia lyase activity in susceptible cultivar inoculated with *Phytophthora infestans* after treating with exogenous DNA fraction from resistant hybrid was similar to that of resistant hybrid inoculated with an incompatible race of *Phytophthora infestans*.

摘　要

ジャガイモ抵抗性種間雑種 96−56 葉から調製した DNA フラクションを罹病性品種紅丸葉に処理後，ジャガイモ疫病菌レース 0 を接種し，パーオキシデース (PO) とフェニールアラニンアンモニアリアーゼ (PAL) 活性を調べた．DNA 処理後疫病菌を接種した紅丸葉での PO 活性は，疫病菌を接種した抵抗性種間雑種 96−56 葉での活性変動パターンに類似していた．また PAL 活性についても PO 活性の場合と同じような傾向が認められた．