

ジャガイモ植物における *Phytophthora infestans* に対する全身誘導抵抗性の誘導と関連したO₂-²生成反応, スーパーオキシドジスムターゼ, およびペルオキシターゼの全身的活性化

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Systemic Activation of O_2^- Generating Reaction, Superoxide Dismutase, and Peroxidase in Potato Plants in Relation to Induction of Systemic Resistance to *Phytophthora infestans*

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Abstract

Treatment of the lower three leaves of potato plants with hyphal wall components of *Phytophthora infestans* systemically activated in upper leaves an O_2^- generating reaction from 1 to at least 7 days after treatment. In the same leaves activation of superoxide dismutase immediately followed the enhanced O_2^- generating reaction. Peroxidase activity increased linearly from 3 days after treatment. In the leaves activated for O_2^- generation, superoxide dismutase and peroxidase, challenge inoculation with a zoospore suspension of *P. infestans* caused further enhancement of the O_2^- generating reaction from 1 to 5 hr after inoculation. The percentage of successful penetrations by the fungi and the appearance of susceptible lesions were greatly reduced in such tissues. The systemic activation of O_2^- generating reaction is discussed in relation to induction of systemic resistance.

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Key words : systemic resistance, O_2^- generating reaction, potato plant, superoxide dismutase, peroxidase.

Introduction

Systemic resistance to *Phytophthora infestans* has been shown to be induced in potato plants by treating lower leaves with hyphal wall components of the fungus¹⁻³⁾. The induced systemic resistance was characterized by reduction of cystospore germination, appressorium formation and fungal penetration^{2,3)}, although the physiological mechanisms of the induced resistance have not been elucidated.

Recently, a steroidalkaloid saponin, digitonin, was shown to induce resistance in potato leaves to a compatible race of *P. infestans*. Digitonin also activated an O_2^- generating reaction⁴⁾. The physiology of the resistance response induced by digitonin treatment was quite similar to that of systemic resistance⁴⁾. Therefore, we assumed that an O_2^- generating reaction may be associated with systemic resistance in potato.

Some studies have correlated O_2^- generation with enhanced activities of superoxide dismutase (SOD) and peroxidase (PO)⁵⁻⁷⁾. Other studies on systemic resistance have demonstrated an activation of PO⁸⁻¹¹⁾, and have proposed lignification to be a key

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mechanism^{12,13}). These observations were consistent with the notion that superoxide play a role in the mechanism of systemic resistance.

This paper reports that activation of an O_2^- generating reaction occurs in association with systemic resistance to *P. infestans*. The response was triggered by treatment of potato with hyphal wall components of the fungus. Activation of superoxide dismutase and peroxidase followed the O_2^- generating reaction. Preliminary reports of this work have been published^{1,2}).

Materials and Methods

Plant. Potato cv. "Irish Cobbler", which has no major resistance genes to *P. infestans*, was used. Seed potato tubers were longitudinally cut into two: one half was used for induction of systemic resistance and the other for the control. The plants were grown in pots (22.5 cm in diameter, 18 cm depth) under greenhouse conditions through April and May. Plants were used when about seven leaves had developed.

Fungus and inoculation. Race 0 of *P. infestans* was used for inoculum and for the preparation of hyphal wall components (HWC) as the inducer of systemic resistance. A zoospore suspension was prepared to give a concentration of 3×10^5 spores/ml as described previously¹⁴) and 100 μ l of the suspension was applied to about 10 cm² of leaf surface.

Induction of systemic resistance. HWC were prepared as described previously¹⁵) and stored at -20°C as a lyophilized powder until used as an inducer of systemic resistance. HWC were suspended in 0.05 M Tris-HCl buffer (pH 7.4) at a concentration of 5 mg/ml, mixed with carborundum (600 mesh, 0.1 mg/ml) and applied to the surface of the three lowest potato leaves by abrading with a paint brush. Control plants were treated identically by abrading with a suspension of carborundum in the same buffer. Fungal penetration and lesions were observed 3, 7 and 20 days after HWC or control treatments.

Assay of O_2^- generating reaction. O_2^- generating reaction of leaf tissues was assayed using 5 leaf discs (10 mm in diameter) punched out from leaflets. Superoxide-dismutase-inhibitory reduction of cytochrome c was determined using the spectrophotometric method described previously¹⁶).

Assay of superoxide dismutase (SOD) and peroxidase (PO) activities. Leaf tissues were homogenized with a cold 0.05 M potassium-phosphate buffer (pH 8.0) containing 0.01% 2-mercaptoethanol (1 ml/g fresh tissue) with a chilled motor and pestle. The homogenate was centrifuged at $20,000 \times g$ for 30 min, and the resultant supernatant was dialyzed against 0.05 M potassium-phosphate buffer (pH 8.0) for 24 h at 4°C . The dialyzed solution was used as crude SOD and PO. Protein content was determined according to the method of Lowry *et al.* using albumin as a standard¹⁸).

SOD activity was assayed by modifications of the method of Macord and Fridovich¹⁹). The reaction mixture consisted of 0.1 mM xanthine, 0.1 mM EDTA, 20 μ M cytochrome c (Horse heart, Type IV, Sigma), 50 mM potassium phosphate (pH 7.8), test solution (0.1 ml) and xanthine oxidase solution (0.04 ml) in a total volume of 2 ml. Concentration of

xanthine oxidase in the reaction mixture was adjusted to provide an increase of optical density of 0.020/min at 550 nm. Under these conditions, a 50% inhibition of cytochrome c reduction was referred to as one unit of SOD activity.

PO activity was spectrophotometrically assayed using guaiacol as substrate according to the method of Hammerschmidt *et al.*¹¹⁾.

Assay of fungal development. Fungi on leaf tissues were observed under a fluorescent microscope after staining leaf tissues with Calcofluor White according to the method of Rohringer *et al.*²¹⁾ as was previously described¹⁶⁾.

Results

Systemic induction of O_2^- generating reaction, and SOD and PO activities

O_2^- generating activity, determined as cytochrome c reducing activity, increased from 1 day after HWC treatment and was maintained for at least 7 days (Fig. 1 B). Such enhanced O_2^- generating reaction was not observed in the control plants (Fig. 1 A).

Increased SOD-activity was detected from 1 day after HWC treatment and achieved maximum level by the second day (Fig. 1 B). The elevated level was sustained for at least 4 further days. Little SOD activity was detectable in control plants.

PO activity was detected in control leaves at a constant level. However, after 3 days the HWC-treated plants displayed increasing activity of PO (Fig. 1).

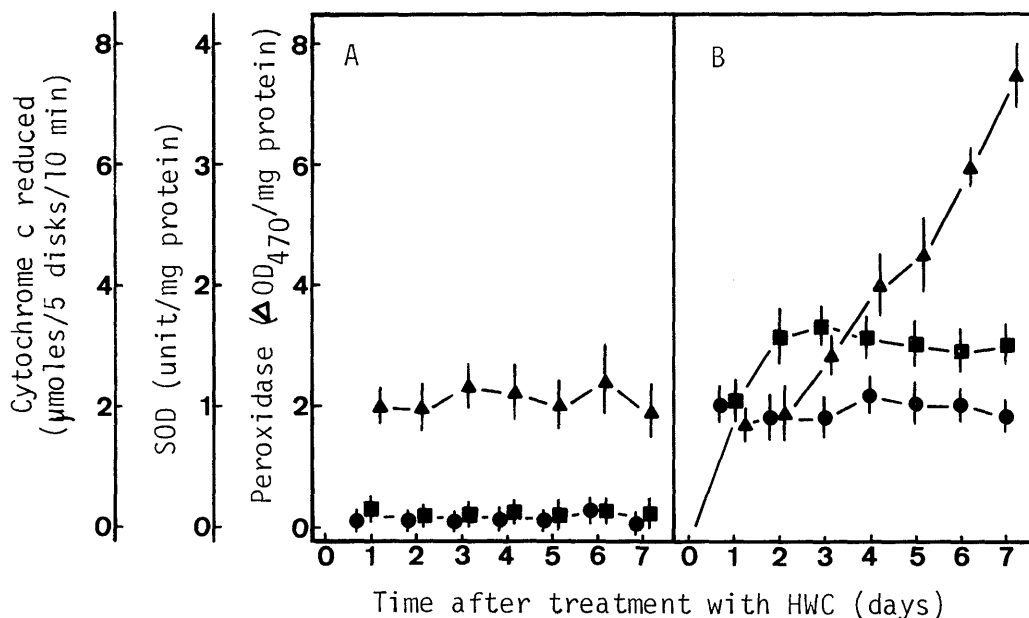


Fig. 1. Systemic activation of O_2^- generating reaction, superoxide dismutase (SOD) and peroxidase (PO) in upper leaf (leaf 5) of potato plants (cv. Irish Cobbler) by treatment of 3 lower leaves with hyphal wall components (HWC) of *Phytophthora infestans* (race 0). A: treated with 0.01 M Tris-HCl buffer (pH 7.4), B: treated with HWC dissolved in the buffer. ● : O_2^- generating reaction, ■ : SOD, ▲ : PO. Each value represents the mean of three experiments with S. D.

Systemic induction of resistance

Inoculation of HWC-treated plants with the fungal zoospores further enhanced the O_2^- generating reaction (Fig. 2). The effect was already observed 1 hr after inoculation, continued until 5 hr, and then declined to the level of noninoculated HWC-treated plants until 10 hr after inoculation. With control plants, inoculation with zoospores increased the O_2^- generation level to that of noninoculated HWC-treated plants (Fig. 2).

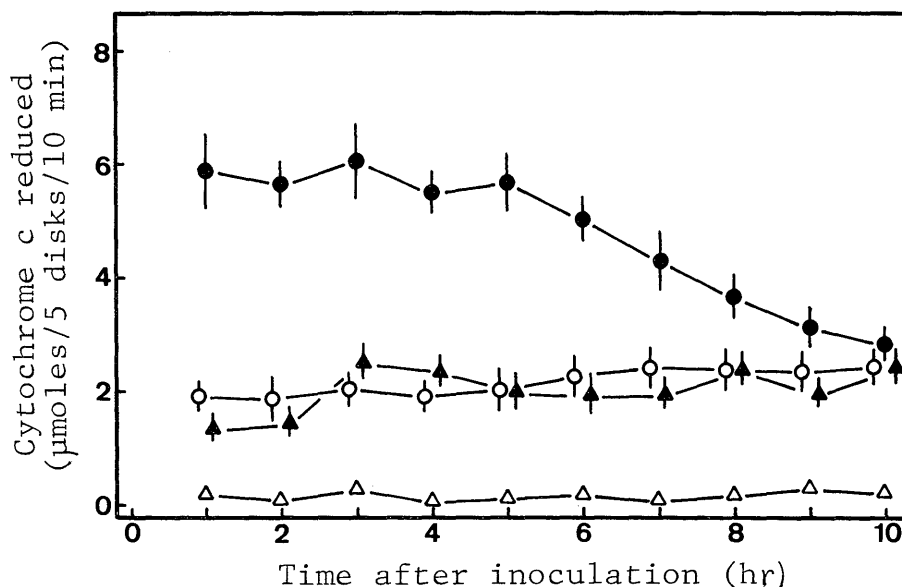


Fig. 2. Effect of challenge inoculation with zoospore suspension of *Phytophthora infestans* (race 0) on O_2^- generating reaction in systemic resistance-induced leaves of potato plants. The assayed leaves were used 5 days after treatment of lower leaves with hyphal wall components of the fungus. ● : challenged on induced leaves, ○ : not challenged on induced leaves, ▲ : challenged on noninduced leaves (sham induction-treatment), △ : not challenged on noninduced leaves. Each value represents the mean of three experiments with S. D.

Table 1. Fungal penetration and appearance of susceptible lesions in systemic resistance-induced leaves of potato plants following inoculation by zoospore suspension of a compatible race of *Phytophthora infestans*^{a)}

Time after HWC treatment (days)	Control leaves		HWC-treated leaves	
	Penetration ^{b)} (%)	No. of lesions ^{c)}	Penetration ^{b)}	No. of lesions ^{c)}
3	57 ± 6	105 ± 20	4 ± 6	2 ± 3
7	55 ± 7	93 ± 15	8 ± 5	1 ± 3
20	N. T. ^{d)}	85 ± 12	N. T. ^{d)}	3 ± 5

a) The systemic resistance was induced by treatment of 3 lower leaves of the plants with hyphal wall components (HWC) of the fungus.

b) Number of germinated cystospores penetrating into leaf cells per total number of spores observed in a definite area.

c) Number of susceptible lesions per 10 cm² leaf 3 days after challenge inoculation.

d) N. T. : not tested.

Fungal penetration and susceptible lesions per leaf were greatly reduced in HWC-treated plants in comparison to control plants (Table 1).

Discussion

A reaction generating O_2^- was systemically activated in potato by treatment of lower leaves with HWC from *P. infestans*. The response was apparent in upper leaves from 1 day and continued until at least 7 days after HWC treatment. The present and previous works have demonstrated that systemic resistance to *P. infestans* was also evident from 1 day^{2,3)} (Table 1). Therefore, the systemically activated O_2^- generating reaction seems to be closely associated with systemically induced resistance.

Sequential activation of SOD, which converts O_2^- to H_2O_2 , and PO, which degrades H_2O_2 , occurred subsequent to the activation of O_2^- generating reaction. Our preliminary experiments showed no changes in catalase activity in leaves with systemic resistance. These data are consistent with previous reports of PO activation in systemic resistance⁸⁻¹¹⁾. These activated enzymes may seem to participate in detoxifying the superoxide anion and its derivatives in non-challenged leaves with systemic resistance.

Although it is unclear from the present experiments how the O_2^- generation contributes to systemically induced resistance, SOD and/or catalase added into zoospore inoculum has been shown to enhance infection rate in leaves where O_2^- generation had been induced by digitonin⁴⁾. The generation of O_2^- appears to disturb fungal infection. Several possible mechanisms for induced resistance may exist: lignification of cell walls by enhanced H_2O_2 production and activation of PO; production of toxic lipid peroxides²⁰⁾ as a function of O_2^- ^{21,22)}; disturbance of compatible systems by generated superoxides.

HWC from *P. infestans* have been shown to activate the O_2^- generating reaction in tissues²³⁾ or protoplasts²⁴⁾. In the present study, the HWC was not brought to direct contact to the upper leaves. Therefore, some signals for activation of O_2^- generating reaction must be translocated from the lower treated leaves to the upper leaves although the mechanisms by which the O_2^- generating reaction is systemically activated remains to be elucidated.

In the upper leaves of HWC treated plants, inoculation with zoospores caused further enhancement of O_2^- generating reaction at infection stages before the inoculated fungus would begin penetration in noninduced leaves. In noninduced leaves, a little activation of O_2^- generating reaction occurred prior to fungal penetration, probably due to germination fluid prior to fungal penetration. Therefore, the ability of leaves to produce O_2^- seems to be much enhanced prior to penetration, thus resulting in disturbance of infection behavior of compatible races in leaves with systemic resistance.

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和 文 摘 要

蔡 熙乗・道家紀志：ジャガイモ植物における *Phytophthora infestans* に対する全身誘導抵抗性の誘導と関連した O_2^- 生成反応，スーパーオキシドジスムターゼ，およびペルオキシダーゼの全身的活性化

ジャガイモ植物の下葉3枚に *Phytophthora infestans* の菌体壁成分を処理すると，その上葉に，処理後1日目から少なくとも7日にわたって， O_2^- 生成反応が活性化された。また，その活性化に続きスーパーオキシドジスムターゼの活性化がおこり，さらに処理後3日目からペルオキシダーゼの活性が増加した。これらの活性化した葉に *P. infestans* の親和性レースを接種すると，接種後1～5時間にわたって O_2^- 生成反応が更に高まり，菌の侵入率並びに病斑形成率は著しく減少した。これらの結果から，全身抵抗性の誘導には活性酸素代謝系の活性化が伴い，これが抵抗性獲得機構に重要な意味を持つものと推察された。