Hypersensitive Reactivity of Various Host and Nonhost Plant Leaves to Cell Wall Components and Soluble Glucan Isoalted from *Phytophthora infestans*

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道家紀志**・酒井 進**・富山宏平** : *Phytophthora infestans* の細胞壁成分と可溶性グルカンに対する各種宿主及び非宿主植物葉の過敏感反応能

Abstract

Cell wall components (CW) and water soluble glucan (WSG) from *Phytophthora infestans*, that have been known to elicit and suppress the hypersensitive response of potato tissue, respectively, were applied to leaves of various host and nonhost plants by rubbing with carborundum to study how they respond hypersensitively to the fungal components. Leaves of *Solanaceae* plants : potato, tomato, sweet pepper, egg plant, red pepper, datura, and Japanese bladder cherry ; *Leguminosae* plants : soybean, kidney-bean, garden pea, broad bean, and cowpea ; and *Lileaceae* plants : onion, welsh onion, and white trumpet lily showed necrotic lesions 24 hr after treatment with CW. On the contrary, leaves of *Solanaceae* plants : tobacco ; *Chenopodiaceae* plants : goose-foot, white-goose-foot, spinach, and beet ; *Compositae* plants : chrysanthemum, garland chrysanthemum, and lettuce ; *Cruciferae* plants : cabbage, chinese cabbage, turnip, and radish ; *Gramineae* plants : rice, barley, wheat, and corn ; and *Rosaceae* plants : strawberry, Japanese pear, and rose did not show any visible necrotic cells within 48 hr after the treatment with CW. Red pepper and Japanese bladder cherry (*Solanaceae*), soybean and cowpea (*Leguminosae*) and white goose-foot (*Chenopodiaceae*) showed almost the same necrotic lesion by treatment with WSG as those by CW. In the cases of plant leaves in which necrotic lesions were elicited by these fungal components, an abnormal leakage of electrolytes occurred within 5 hr after treatment with them. These resulsits suggested that specific families of plant have a nature to respond hypersensitively to the cell wall components and water soluble glucan of *P. infestans* regardless of host or nonhost relationship.

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Introduction

It has reported that some fungal components elicited the hypersensitive reaction of host plants including tissue browning and accumulation of phytoalexins in several host-parasite system^{1, 2, 3, 4, 5, 11, 13, 14, 18}. In potato-*Phytophthora infestans* system, sonicate of mycelia^{6, 7, 12, 19, 20}, homogenate of zoospores¹⁰ and cell wall components isolated from *P. infestans*^{5, 9, 15, 16} elicited the hypersensitive reaction in potato tissues regardless of their resistance genes. It was also reported that autoclaved sonicate of *Oomycetes* (*P. infestans*, *P. parasitica*, *Phythium aphanidermatum*, *Achlga flagelata*

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and *Aphanomyces euteiches*) that contained glucan cellulose as major cell wall components elicited the accumulation of phytoalexin in potato tuber tissues, but those of other fungi containing glucan-chitin, glucan-chitosan, chitin-mannan and glucan-mannan did not¹⁷⁾. Water soluble glucan fraction isolated from *P. infestans* did not elicit the hypersensitive reaction in potato tuber tissues while the glucan had an activity to suppress the hypersensitive reaction elicited by infection with incompatible races or treatment with the cell wall fraction of *P. infestans*^{6,7,8,9,12)}.

On the other hand, glucan isolated from *P. megasperma* var *sojae* elicited the accumulation of phytoalexin in soybean tissue^{2,3)}. These experimental evidences suggested that some polysaccharide components of fungi may elicit the hypersensitive reaction in some particular plant tissues.

In the present paper, what sort of families, genera and species of host or nonhost plants have hypersensitive reactivity to polysaccharide fractions of *P. infestans* was investigated to know whether there are a specificity in recognition of fungal components by plant tissues or not.

Materials and Methods

Plant. The following plants were used. *Solanaceae*: potato (*Solanum tuberosum* L. vr. Irish Cobbler, Rishiri, Hokkai 53, and Pentland Ace), tomato (*Lycopersicon esculentum* Mill vr. Hard Top, Ogata-Fukuju, and Satan), egg plant (*Solanum melongena* L. vr. Kokuyo, Senryo, Manryo), sweet pepper (*Capicum frutescence* L. vr. California Wanda, Ace, and New Ace), red pepper (*Capicum annuum* L. vr. Shishito), tobacco (*Nicotiana tabacum* L. vr. Sumsun NN and Bright Yellow and *Nicotiana glutinosa* L.), Datura (*Datura stramonium* L.), and Japanese bladder cherry (*Physalis alkekengi* L. vr. francheti (Mast) Hurt.). *Leguminosae*: soybean (*Glycine max* Merr. vr. Tanba-black, Ogaki-brown, and Toei-white), kidney bean (*Phaseolus vulgaris* L. vr. Kairyo-otebo and Tebo), Pea (*Pisum sativum* L. vr. Sanukinagasaya and Wase), cowpea (*Dolichos sesquipedalis* Wight vr. Kurodanesanjaku, Juroku and Black-eye). *Cucurbitaceae*: water melon (*Citrullus vulgaris* Schrad vr. Shinfuji), melon (*Cucumis melo* L. vr. Mizuhoaoihi), cucumber (*Cucumis sativus* L. vr. Sagamihangetsu), pumpkin (*Cucubita moshata* Duch. vr. Hyuga No 14) and bottle gourd (*Legenavia sincerabia* Stan. vr. hispenda Hara cv. Daimaru). *Chenopodiaceae*: goose-foot (*Chenopodium album* L. vr. centrorubrum Makino), white goose-foot (*Chenopodium album* L.), spinach (*Spinacea oleracea* L. vr. Heian-hiyoshimaru), and beet (*Beta vulgaris* L. vr. Donyu No 2). *Liliaceae*: onion (*Allium cepa* L. vr. OK-kidama), welsh onion (*Allium fistulosum* L. vr. Kujofutonegi), and white trumpet lily (*Lilium auratum* L.). *Compositae*: chrysanthemum (*Chrisantemum molifolium* L.), garland chrysanthemum (*Chrisanthemum coronarium* L. vr. Taiyo), lettuce (*Lactuca sativa* L. vr. Alpen). *Cruciferae*: cabbage (*Brassica oleracea* var *captata* L. vr. Alpen), chinese cabbage (*Brassica pekinensis* Rupr. vr. Shinkashin), turnip (*Brassica rapa* L. vr. Tokyo-tokinashi), radish (*Raphanus sativus* L. vr. Natsuminowase No 1). *Gramineae*: rice (*Oryza sativa* vr. Asahi), barley (*Hordium vulgare* L), wheat (*Triticum vulgare* L.) and corn (*Zea mays* L. vr. Golden crossbantum). *Rosaceae*: strawberry (*Fragaria chiloensis* Duch. var. annansa Bail) and Japanese pear (*Pyrus serotina* Rehder vr. Chojuro). In the case of no description, unknown cultivated varieties were used. The 3rd to 4th leaves from the top ones of *Solanaceae*, *Chenopodiaceae*, *Compositae*, and *Graminea* plants, first true leaves of *Leguminosae* and *Cucurbitaceae* plants and appropriate developed leaves of the other plants were used.

Extraction of fungal components. The methods described in a previous paper

were used to isolate cell wall components^{5,9,15,16)} and water soluble glucan^{6,7)} of *P. infestans* mycelia (race 0 and race 1) that were grown on a rye seed-extract liquid medium for 13 days at 18 C. The former and latter compounds were known to be a hypersensitivity-eliciting and inhibiting factors of potato tuber tissues, respectively^{6,7,9,12)}.

Treatment with fungal component. Surface of detached leaves was washed by tap water and wiped with filter paper. Solution containing cell wall components or water soluble glucan (5 mg/ml) were applied on the upper surface of leaves by rubbing gently with a painting brush with carborundum (600 mesh). The treated leaves were incubated on a wet filter-paper in a petri dish at 25 C under a light condition (ca. 2,000 lux).

Observation of cell necrosis. Necrotic cells in all leaves were observed under a light microscope ($\times 150$) and the number of necrotic cell group per a definite area was counted 24 and 48 hr after treatment. Two or three leaves of each plant were used in each experiment and the experiment was repeated at least three times.

Measurement of loss of electrolyte. The loss of electrolytes from major leaves treated with the mycelial components was determined as follows: after measuring area of each leaf, the leaves were treated with the mycelial components as described above and kept in a moist chamber for 2 hr, and then the leaves were immersed in 50 ml of deionized distilled water. Electric conductivity of the immersion water (μ mhos/cm² leaf area) was measured by a electric conductivity meter (Conductivity bridge RC 216B2, Beckman Instrument Inc.) at intervals after treatment.

Results

Elicitation of necrotic cells

Leaves of 43 plants (9 families, 13 genera, 43 species) were treated with cell wall components and water soluble glucan isolated from mycelia of *P. infestans* (race 0 and 1). Leaves of *Solanaceae* plants (potato, tomato, egg plant, sweet pepper, datura and Japanese bladder cherry) (Table 1), *Leguminosae* plants (soybean, kidney bean, pea, broad bean and cowpea) (Table 2), *Lilaceae* plants (onion, welsh onion and white trumpet lily) and *Chenopodiaceae* plants (white goose-foot) (Table 3) showed necrotic lesions 24 hr after treatment with the cell wall components. In most of the cases the necrotic lesion was made up of 3 to 10 browned cells. Treatment with water soluble glucan elicited necrotic lesions in the leaves of red pepper (*Solanaceae*), soybean (*Leguminosae*), cowpea (*Leguminosae*) and white goose-foot (*Chenopodiaceae*) (Table 1-3). On the other hand, leaves of *Solanaceae* plants (tobacco), *Cucurbitaceae* plants (water melon, cucumber, pumpkin, and bottle gourd), *Chenopodiaceae* plants (goose-foot, spinach, and beet), *Compositae* plants (chrysanthemum, lettuce and garland chrysanthemum), *Cruciferae* plants (cabbage, chinese cabbage, Japanese radish and turnip), *Gramineae* plants (rice, barley, wheat and corn) and *Rosaceae* plants (strawberry, Japanese pear and rose) showed no visible necrotic lesions 72 hr after the treatments with both the cell wall components and water soluble glucan (Table 1-3).

In all the cases that necrotic lesions were elicited by the fungal components, visible lesions were already observed 24 hr after the treatment. However, thereafter the necrotic lesions did not develop larger during incubation at least until 72 hr after the treatment. There was no significant difference in the response of plant leaves to the fungal components isolated from race 0 and 1 of *P. infestans*. It was also found that there was no significant difference in the mode of response of leaves

Table 1. Response of *Solanaceae* plant leaves to cell wall components and water soluble glucan isolated from *Phytophthora infestans* mycelia

<i>Solanaceae</i> plant	Variety	Necrotic lesion ^a			
		Cell wall comp.		Soluble glucan	
		Race 0	Race 1	Race 0	Race 1
Potato (<i>Solanum tuberosum</i> L.)	Irish Cobbler	+	+	—	—
	Rishiri	+	+	—	—
	Hokkai 53	+	+	—	—
	Pentland Ace	+	+	—	—
Tomato (<i>Lycopersicon esculentum</i> Mill.)	Hard Top	+	+	—	—
	Ogata Fukuju	+	+	—	—
	Satan	+	+	—	—
Egg plant (<i>Solanum elongata</i> L.)	Kokuyo	+	+	—	—
	Senryo	+	+	—	—
	Manryo	+	+	—	—
Sweet pepper (<i>Capicum frutescence</i> L.)	California Wander	+	+	—	—
	Ace	+	+	—	—
	New Ace	+	+	—	—
Red pepper (<i>Capicum annuum</i> L.)	Shishito	+	+	+	+
Tobacco (<i>Nicotiana tabacum</i> L.)	Sumsun NN	—	—	—	—
	Bright Yellow	—	—	—	—
	(<i>Nicotiana glutinosa</i> L.)	—	—	—	—
Datura (<i>Datura stramonium</i> L.)		+	+	—	—
Japanese bladder cherry (<i>Rhynchos alpestris</i> L.)	Francheti	+	+	+	+

^a Observed 24 and 48 hr after treatment. + : Necrotic lesions were observed.Table 2. Response of *Leguminosae* plant leaves to cell wall component and soluble glucan isolated from *Phytophthora infestans* mycelia

Leguminosae plant		Necrotic lesion ^a			
		Cell wall comp.		Soluble glucan	
		Race 0	Race 1	Race 0	Race 1
Soybean (<i>Glycine max</i> Merr.)	Tanba-black	+	+	+	+
	Ogaki-brown	+	+	+	+
	Toei-white	+	+	+	+
Kidney bean (<i>Phaseolus vulgaris</i> L.)	Kairyotebo	+	+	—	—
	Tebo	+	+	—	—
Gardenpea (<i>Pisum sativum</i> L.)	Waiseiakabana	+	+	—	—
	Kinusaya	+	+	—	—
	Frenchosaya	+	+	—	—
Broad bean (<i>Vicia faba</i> L.)	Sankingasaya	+	+	—	—
	Wase	+	+	—	—
Cowpea (<i>Dolichos sesquipedalis</i> Wight)	Kurodanesanjaku	+	+	+	+
	Juroku	+	+	+	+
	Blackeye	+	+	+	+

^a Observed 24 and 48 hr after treatment. + : Necrotic lesions were observed

Table 3. Response of *Cucubitaceae*, *Chenopodiaceae*, *Liliaceae*, *Compositae*, *Cruciferae*, *Graminea* and *Rosaceae* plant leaves to cell wall component and soluble glucan isolated from *Phytophthora infestans* mycelia

Plant	Necrotic lesion ^a			
	Cell wall comp.		Soluble glucan	
	Race 0	Race 1	Race 0	Race 1
<i>Cucubitaceae</i>				
Watermelon (<i>Citrullus vulgaris</i> Schrad)	—	—	—	—
Melon (<i>Cucumis melo</i> L.)	—	—	—	—
Cucumber (<i>Cucumis sativas</i> L.)	—	—	—	—
Pumpkin (<i>Cucubita moshata</i> Duch.)	—	—	—	—
Bottle gourd (<i>Lagenavia sicerabia</i> Stan. var <i>Hispeda</i> Hara)	—	—	—	—
<i>Chenopodiaceae</i>				
Goose-foot (<i>Chenopodium album</i> L. var. <i>centrorubrum</i> Makino)	—	—	—	—
White goose-foot (<i>Chenopodium album</i> L.)	+	+	+	+
Spinach (<i>Spinacea oleracea</i> L.)	—	—	—	—
Beet (<i>Beta vulgaris</i> L.)	—	—	—	—
<i>Liliaceae</i>				
Onion (<i>Allium cepa</i> L.)	+	+	—	—
Welsh onion (<i>Allium fistulosum</i> L.)	+	+	—	—
White trumpet lily (<i>Lilium auratum</i> L.)	+	+	—	—
<i>Compositae</i>				
Chrisanthemum (<i>Chrisanthemum molifolium</i> Ram.)	—	—	—	—
Garland chrysanthemum (<i>Chrisanthemum coronarium</i> L.)	—	—	—	—
Lettus (<i>Lactuca sativa</i> L.)	—	—	—	—
<i>Gruciferae</i>				
Cabbage (<i>Brassica oleraceae</i> var. <i>capita</i> L.)	—	—	—	—
Chinese cabbage (<i>Brassica pekinensis</i> Rupr.)	—	—	—	—
Turnip (<i>Brassica rapa</i> L.)	—	—	—	—
Radish (<i>Raphnus sativas</i> L.)	—	—	—	—
<i>Graminea</i>				
Rice (<i>Oryzae sativa</i> L.)	—	—	—	—
Barley (<i>Hordium vulgare</i> L.)	—	—	—	—
Wheat (<i>Tritichum vulgare</i> L.)	—	—	—	—
Corn (<i>Zea mays</i> L.)	—	—	—	—
<i>Rosaceae</i>				
Strawberry (<i>Fragaria chiloensis</i> Duch. var <i>ananasa</i> Bail)	—	—	—	—
Japanese pear (<i>Pyrus serotina</i> Rehder)	—	—	—	—

^a Observed 24 and 48 hr after treatment. + : Necrotic lesions were observed

to the fungal components between cultivated varieties in the same species. When these leaves were treated with the fungal components without rubbing with carborundum using painting brush, a small number of necrotic lesions was found at the site of stomata. The rubbing with the brush with distilled water containing carborundum did not cause any visible necrotic cells.

Loss of electrolyte

To measure a response of leaves to the fungal components before necrotic lesions appeared, loss of electrolytes from the leaves of potato, sweet pepper, tomato, tobacco,

Fig. 1. Leakage of electrolytes from plant leaf tissues treated with cell wall components (a) or water soluble glucans (b) isolated from *Phytophthora infestans* (race 1). The following plant leaves were used: potato (cv. Rishiri), tomato (cv. Hard Top), Sweet pepper (cv. New Ace), Tobacco (cv. Sumsun NN), Soybean (cv. Tanba-black), Cowpea (cv. Kurodanesanjaku), melon (cv. Mizuhaoai), cucumber (cv. Sagami-hangetsu). Electric conductivity of the immersion water is expressed as μ mhos/cm² leaf area in 50 ml of deionized distilled water. Open circles: treated, filled circles: control.

soybean, cowpea, melon and cucumber treated with the fungal components were determined (Fig. 1). All plant leaves that responded to the fungal components with showing necrotic lesions later released electrolytes from leaves more than nontreated ones almost 4 to 5 hr after the treatment when the necrotic cells has not yet appeared. The leakages from the leaves that any necrotic lesion was induced by the treatment with the fungal components were almost the same as that of non-treated leaves (Fig. 1).

Discussion

Leaves of the most of *Solanaceae* and *Leguminosae* plants tested in the present experiments showed necrotic lesions when treated with cell wall components isolated from *P. infestans* that is known as an elicitor of the hypersensitive reaction in potato tuber tissues^{5,9,15,16}, and the reactions were accompanied by an increased loss of electrolytes before appearance of the necrotic lesions (Fig. 1). Plant leaves belonging to other families did not respond to the compounds with appearance of necrotic lesions and loss of electrolytes with a few exceptions (Table 3). These results indicated that such reactivity of plant leaves to the cell wall components of *P. infestans* is specific to *Solanaceae* and *Leguminosae* plants.

Very restricted plants (soybean, cowpea, red pepper, Japanese

bladder cherry and goose-foot) among those tested reacted hypersensitively to water soluble glucan fraction from *P. infestans*, followed by appearance of necrosis of the treated tissue (Table 1, 2, and 3). The glucan has been characterized by glucan containing β -(1 $\rightarrow$ 3) and β -(1 $\rightarrow$ 6) linkage and 17 to 23 glucose units^{6,7}.

It has been reported that potato tissues have ability to respond to the sonicate of certain species of fungi having glucan cell wall, but not to those of fungi having other kinds of polysaccharides as major cell wall components¹⁷, and also that glucan

fraction with β -(1 $\rightarrow$ 3) linkage isolated from *P. megasperma* var *sojae* elicited accumulation of phytoalexin in soybean tissues^{2,3)}. In the case of water soluble glucan from *P. infestans* suppressed the hypersensitive reaction in potato tuber tissue elicited by infection or treatment with the cell wall components^{6,7,9)}, however, did not cause any sign of necrosis or accumulation of phytoalexin. These results strongly suggest that plants may have reactivity to a specific group of mycelial wall components, and the physiologically active site of the mycelial components may be specific sugar residues or structures of polysaccharides consisting the mycelial wall.

Wound caused by carborundum was necessary to make the treatment of the leaves with mycelial components sufficiently effective. When the treatment was done without carborundum, necrosis occurred only in a few cells surrounding stomata. These observation indicated that in the case of the adult leaf surface composed of wax, cuticle and so on, the information of the mycelial components could hardly pass through it into cells. This presumption was also supported by the fact that protoplasts prepared from potato tissues reacted hypersensitively very soon after the treatment with the mycelial wall components (Doke and Tomiyama, unpublished).

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和 文 摘 要

Phytophthora infestans の細胞壁成分と可溶性グルカンに対する
各種宿主及び非宿主植物葉の過敏感反応能

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ジャガイモ組織に過敏感反応を誘導する *Phytophthora infestans* の細胞壁成分 (CW) とその誘導を抑制する水溶性グルカン (WSG) を各種宿主及び非宿主植物葉に、カーボランダムによる摩擦塗布処理をし、葉組織の過敏感反応性を調べた。ナス科のジャガイモ、トマト、ピーマン、ナス、トウガラシ、ダチュラ、ホーズキ、マメ科のダイズ、インゲン、エンドウ、ソラマメ、ササゲ、ユリ科のタマネギ、ネギ、テッポウユリの葉は CW に反応し、処理後24時間後には褐変死細胞を生じた。ナス科のタバコ、アカザ科の4種、キク科の3種、十字花科の4種、イネ科の4種、バラ科の3種の各植物葉はいずれも、処理後72時間以内にも肉眼的観察可能な細胞の反応は示さなかった。トウガラシ、ホーズキ、ダイズ、ササゲ及びシロザの葉は CW と同様に、WSG とも反応し褐変死細胞を生じた。それらの処理により過敏感反応を起し得る葉は、処理後、5時間以内に異常な電解質の漏出を起した。これらの結果は、特定な科に属する植物が、*P. infestans* の CW 及び WSG に対して、宿主・非宿主とにかかわらず過敏感反応を起す性質を持つことを示唆した。