

Citrinin (モデルAK-toxin)処理した日本ナシ葉細胞における微細構造変化

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Ultrastructural Changes of Cells in Leaf Tissues of Japanese Pear Treated with Citrinin, a Model Compound for AK-toxin

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朴 杓允 : Citrinin (モデルAK-toxin) 処理した日本ナシ葉細胞における微細構造変化

Abstract

Young leaves of cultivar Nijisseiki and Chojuro of Japanese pear were treated with 100ppm citrinin which exerts a pathological effect similar to that of AK-toxin and the ultrastructural changes in the treated leaves were observed. Within 3 h of treatment, citrinin caused a rapid electrolyte-loss in Nijisseiki leaves without any visible symptoms. The invagination of plasma membrane at the both ends of plasmodesmata in the vascular bundle sheath cells was the first obvious change in the ultrastructure. By 8 h after treatment, the veinal necrosis appeared and invaginations of plasma membrane became more extensive in both vascular bundle sheath and mesophyll cells. The spaces between cell wall and invaginated plasma membrane contained many lomasome-like vesicles, the central cores extended from plasmodesmata and electron-lucent materials. Cellular membranes were disrupted in necrotic cells of Nijisseiki leaves treated with citrinin for 12 h. Citrinin did not affect the cells of resistant Chojuro leaves even after 12 h of treatment. These results suggest that citrinin acts upon plasma membrane of Nijisseiki cells and that permeability changes are correlated with the plasma membrane modifications.

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Introduction

AK-toxin, a host-specific toxin produced by *Alternaria kikuchiana* Tanaka which is the causal fungus of the black spot disease of Japanese pear, causes permeability changes in susceptible but not in resistant pear leaves as does the virulent fungus^{5,6,7,11}). The permeability changes appeared to be associated with the invagination, fragmentation and vesiculation of plasma membrane in susceptible pear leaves caused by treatment of AK-toxin^{8,10,11}) and inoculation of spores of *A. kikuchiana*⁹).

Nishimura and Kohmoto³) found a fungal metabolite in culture filtrate of *Penicillium citrinum*, which exerted a pathological effect similar to that of AK-toxin against pear leaves, and identified it as citrinin which had been known as one of mycotoxins¹⁴). Although selective toxicity of citrinin to pear plants was not always compatible with that of *A. kikuchiana* and AK-toxin, it exhibited host-specificity against cultivar Nijisseiki and Chojuro unless a massive concentration was applied⁴); citrinin caused necrosis and permeability changes in Nijisseiki pear but did not in Chojuro pear⁴).

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The purpose of this study is to elucidate ultrastructural changes of cellular membranes in pear leaves treated with citrinin to induce permeability changes.

Materials and Methods

Plants and citrinin treatment. Two Japanese pear cultivars, Nijisseiki and Chojuro, susceptible and resistant respectively to infecting by *A. kikuchiana* were used in this study. Citrinin provided by Professor Syoyo Nishimura of Tottori University, was dissolved to give a concentration of 100 ppm. The veins of the young leaves of cultivars Nijisseiki and Chojuro were injured by pricking with a hypodermic needle. Several drops of citrinin solution were placed on the wounded portions of veins. Leaves placed with distilled water served as controls. The citrinin-treated and water-treated leaves were incubated for 3 hr, 8 hr and 12 hr respectively at 28 C in a moist chamber.

Fixation, dehydration and embedding. Four mm² leaf tissues 1 cm distant from the treated veins were trimmed with a razor blade. These sections of leaves were prefixed at 4 C for 4 hr in a mixture of 3% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.25 and postfixed at 4 C for 2 hr in 1.5% osmic acid in 0.1 M cacodylate buffer at pH 7.25²⁾. The tissues were dehydrated with ethanol and embedded in an Epon mixture. Ultrathin sections were stained with 2% uranyl acetate for 30 min followed by counterstaining with lead citrate for 10 min. The sections were observed with an electron microscope of JEM-7 type (JEOL Ltd).

Results

Ultrastructure of citrinin-treated pear leaves

Nishimura & Kohmoto³⁾ reported that citrinin caused permeability changes in Nijisseiki leaves, but did not in Chojuro. The effect of citrinin on permeability was re-examined by measuring the leakage of electrolytes in order to determine the response time of tissues with respect to permeability changes. Citrinin caused a rapid loss of electrolytes from Nijisseiki leaves within 1 hr of treatment and a slow loss of electrolytes from Chojuro leaves at 3 hr after treatment. Therefore, pear leaves treated with citrinin for 3 hr, 8 hr and 12 hr were subjected to examination of ultrastructural changes.

Table 1. Comparative frequency of cells with invaginated plasma membrane, in Nijisseiki and Chojuro pear leaves treated with 100ppm of citrinin for 3 h and 12 h

Cultivars	Treat- ment	Treat- ment time(h)	Percentage of cells with invaginated plasma membrane								
			Vascular bundle sheath cells			Mesophyll cells adja- cent to bundle sheath			Mesophyll cells remote from bundle sheath		
			No.of observed cells	No.of cells with invag- ination	%	No. of observed cells	No.of cells with invag- ination	%	No. of observed cells	No.of with invag- ination	%
Nijisseiki	Citrinin	3	162	76	47	90	3	<4	72	1	<2
Nijisseiki	Water	3	167	0	0	68	0	0	100	0	0
Chojuro	Citrinin	3	75	2	<3	52	1	<2	111	0	0
Chojuro	Water	3	86	0	0	50	0	0	95	0	0
Nijisseiki	Citrinin	12	61	39	64	55	21	38	104	1	<1
Nijisseiki	Water	12	122	0	0	106	1	<1	121	1	<1
Chojuro	Citrinin	12	105	1	<1	104	1	<1	131	2	<2
Chojuro	Water	12	91	0	0	79	0	0	104	0	0

Table 2. Comparative frequency of plasmodesmata with invaginated plasma membrane, in Nijisseiki and Chojuro pear leaves treated with 100ppm of citrinin for 3 hr

Cultivars	Treat- ment	Percentage of plasmodesmata with invaginated plasma membrane								
		Vascular bundle sheath cells			Mesophyll cells adjacent to bundle sheath			Mesophyll cells remote from bundle sheath		
		No. of observed PD*	No. of PD* with invagination	%	No. of observed PD*	No. of PD* with invagination	%	No. of observed PD*	No. of PD* with invagination	%
Nijisseiki	Citrinin	120	82	68	126	51	40	88	14	16
Nijisseiki	Water	118	0	0	99	0	0	98	0	0
Chojuro	Citrinin	136	0	0	107	0	0	97	0	0
Chojuro	Water	122	0	0	96	0	0	103	0	0

* The abbreviation of PD indicates plasmodesmata.

Citrinin-induced necrotic symptoms on Nijisseiki leaves were very similar to those produced by AK-toxin. In Nijisseiki leaves, primary symptoms appeared as veinal necrosis as early as 8 hr after treatment. The necrosis extended from veins to the mesophyll area by 12 hr of treatment. The necrosis, however, did not occur in Chojuro leaves even after 12 hr of treatment.

Plasma membrane of cells in untreated leaves of both cultivars was tightly appressed to cell wall; the invagination of plasma membrane was rarely observed (Figs. 1 and 2) (Table 1). The first change in ultrastructure was the invagination of plasma membrane which was found at both ends of plasmodesmata in vascular bundle sheath cells of Nijisseiki leaves treated with citrinin for 3 hr (Fig. 3) (Table 2). The invaginations became much extensive and more frequent between vascular bundle sheath and mesophyll cells as time of treatment was prolonged (Figs. 4 to 6) (Table 1). Eight hours after treatment, the spaces between cell wall and invaginated plasma membrane contained lomasome-like vesicles, electron-lucent materials and central cores extended from plasmodesmata (Fig. 6). The central cores appeared to be embedded by the electron-lucent materials (Fig. 6). Twelve hours after treatment, mesophyll cells in the vicinity of necrotic tissues showed the elongation of outer layer of nuclear envelope and the swelling of chloroplasts in addition to plasma membrane-invaginations (Fig. 5). The adjacent vascular bundle sheath cells were completely disorganized (Fig. 5). Necrotic cells showed disruptions of cellular membranes (Figs. 8 to 10). A disrupted nucleus was represented by the occurrence of lysigenous spaces in nuclear matrix and indistinguishable nuclear envelope (Fig. 9). The disorganization of the fretwork systems of grana lamellae was evident in chloroplasts (Fig. 10).

Ultrastructural changes were not observed in Chojuro cells treated with citrinin for 3 hr, 8 hr and 12 hr (Fig. 7).

Comparative frequency of occurrence of cells with the invagination of plasma membrane

The invaginations of plasma membrane were used as a marker for modifications of plasma membrane. The frequency of occurrence in cells with the invaginated plasma membrane was examined of leaf tissues treated with citrinin for 3 hr and 12 hr as described previously¹¹⁾. The frequency was high in vascular bundle sheath cells, but was very low in mesophyll cells of citrinin-treated Nijisseiki leaves (Table 1). The percentage increased in vascular bundle sheath cells and the adjacent mesophyll cells in the lapse of time of treatment. The invaginations were

not almost found in citrinin-treated Chojuro leaves and untreated leaves (Table 1).

Comparative frequency of occurrence of plasmodesmata with the plasma membrane-invagination per the observed plasmodesmata.

The invaginations of plasma membrane were often found at the both ends of plasmodesmata. In Nijisseiki leaves treated with citrinin for 3 h, percentages of membrane-invaginated plasmodesmata in each tissues were as follows; vascular bundle sheath cells, 68%; mesophyll cells adjacent to the bundle sheath, 40%; mesophyll cells remote from the bundle sheath, 16% (Table 2). However, the invaginations at plasmodesmata were not found in citrinin-treated Chojuro leaves and untreated leaves (Table 2).

Discussion

This paper showed that citrinin was a model compound for AK-toxin with respect to its biological effect against cultivars Nijisseiki and Chojuro of Japanese pear as observed at cellular level. Citrinin did not cause any disruptions of cellular membranes in Chojuro cells, but induced some ultrastructural changes in Nijisseiki cells. The first effect of citrinin appeared on plasma membrane near plasmodesmata in vascular bundle sheath cells of the leaves, evident by 3 h of treatment. The changes were the invagination, fragmentation and vesiculation of plasma membrane which apparently represent modifications of the membranes^{8,10,11}. The modifications of plasma membrane in citrinin-treated Nijisseiki cells were very similar to those induced by AK-toxin^{8,10,11}. Cells of Nijisseiki leaves treated with citrinin for 8 hr and 12 hr showed the general disruptions of cellular membranes. However, it remains unknown whether the disruptions of the membranes are caused by the secondary effects of citrinin or it acts directly upon the membranes. In spite of the similar changes in ultrastructure, the mode of action of citrinin to pear plants is not always the same as that of AK-toxin because host specificity of citrinin to pear plants is not always compatible with that of AK-toxin⁴. Up to date, there is no evidence to interpret why Chojuro cells were not affected by citrinin.

This study further supported the evidence for a correlation between plasma membrane modification and permeability changes^{8,11,12,13}. Citrinin caused a rapid loss of electrolytes and the plasma membrane modification in Nijisseiki leaves whereas non-host-specific toxins such as tenuazonic acid and phenylacetic acid, both produced by *A. kikuchiana*, caused a slow loss of electrolytes from Nijisseiki and Chojuro leaves and did not induce the characteristic modifications of the membrane in cells of these cultivars⁸. The correlation was also recognized in other susceptible combinations of plants and host-specific toxins, i. e. AK-toxin-Nijisseiki pear^{8,11}, AM-toxin I-Indo apple¹² and AM-toxin I-Nijisseiki pear¹³. The specific leakage of electrolytes from the toxin-treated and citrinin-treated susceptible tissues was followed by the plasma membrane modifications which seem to be common changes in the permeability change-induced plant cells during pathogenesis.

The invagination of plasma membrane in citrinin-treated Nijisseiki cells occurred at most of plasmodesmata (Table 2). As the result of the invagination, central cores within plasmodesmata were extended from the structures. The results obtained in this study show that the deformed plasmodesma with the invagination is apparently one of characteristics in plasma membrane modifications caused by citrinin. Similar observations were obtained in the AK-toxin-treated susceptible

Japanese pear^{8,10,11,13)} and AM-toxin I-treated susceptible apple¹²⁾ and pear¹³⁾.

Citrinin has been known to cause a leakage of constituent pigments from a certain fungus¹⁾ and to induce dilation of lower nephrons of rat kidney^{15,16)}. These results, together with evidence presented in this paper, suggest that citrinin acts upon cellular membranes.

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Literature cited

1. Betina, V. & Barathova, H. (1968). J. Antibiot. 21 : 628-629.
2. Karnovsky, M. J. (1965). J. Cell Biol. 27 : 137.
3. Nishimura, S. & Kohmoto, K. (1974). Ann. Phytopath. Soc. Japan. 40 : 174. (Abstr.).
4. Nishimura, S., Kohmoto, K. & Otani, H. (1974). Rev. Plant Protec. Res. 7 : 21-32.
5. Otani, H., Nishimura, S. & Kohmoto, K. (1972). J. Fac. Agr. Tottori Univ. 7 : 5-12.
6. Otani, H., Nishimura, S. & Kohmoto, K. (1973). *Ibid.* 8 : 14-20.
7. Otani, H., Nishimura, S., Kohmoto, K., Yano, K. & Seno, T. (1975). Ann. Phytopath. Soc. Japan. 41 : 467. 476.
8. Park, P. (1977). *Ibid.* 43 : 15-25.
9. Park, P. (1977). *Ibid.* 43 : 26-32.
10. Park, P. (1976). *Physiol. Plant Pathol.* in press.
11. Park, P., Fukutomi, M., Akai, S. & Nishimura, S. (1976). *Ibid.* 9 : 167-174.
12. Park, P., Tsuda, M., Hayashi, Y. & Ueno, T. (1976). Ann. Phytopath. Soc. Japan. 42 : 87 (Abstr.).
13. Park, P., Tsuda, M., Hayashi, Y. & Ueno, T. (1976). *Ibid.* 42 : 353-354 (Abstr.).
14. Saito, M., Enomoto, M. & Tatsuno, T. (1971). In *Microbial Toxins* (Ciegler, A., Kadis, S. & Aji, S. J. eds.). Vol 6, pp357-367. Academic press, London and New York.
15. Sakai, F. (1955). *Nippon Yakugaku Zasshi* 51 : 451.
16. Sakai, F., Higuchi, T. & Uruguchi, K. (1955). *Ibid.* 51 : 105.

和 文 摘 要

Citrinin (モデル AK-toxin) 処理した日本ナシ葉細胞における微細構造変化

朴 杓 允

ナシ黒斑病菌 *Alternaria kikuchiana* Tanaka の宿主特異的毒素 (AK-toxin) と類似した病理学的活性を有する citrinin を二十世紀ナシ葉と長十郎ナシ葉に処理し、電子顕微鏡で観察した。処理後3時間目には、二十世紀ナシ葉はすでに原形質膜の透過性の変化が認められたが、まだ死は現われなかった。その二十世紀ナシ葉の維管束鞘細胞は最初の微細構造変化として原形質膜の陥入を示した。処理8時間後、葉脈死が生じている葉組織では、原形質膜の陥入が維管束鞘細胞と葉肉細胞において広範囲に認められた。原形質膜と細胞壁の間の陥入部位は、ロマゾーム様小胞と plasmodesma から突出した central core と低電子密度物質を含む。12時間 citrinin を処理した二十世紀ナシ葉の死細胞では崩壊した細胞内膜系が観察された。Citrinin は、処理後12時間においてさへ長十郎ナシ葉細胞になんら影響をおよぼさなかった。これらの結果は、citrinin が、AK-毒素と同様、二十世紀ナシ葉細胞の原形質膜に作用して形態の変化をおこし、それはまた透過性の変化と相関性があることを示している。

Explanation of Plates

Abbreviation

c:chloroplast; d: central core of plasmodesma; gr:grana lamella; iv: invagination of plasma membrane; llv: lomasome-like vesicle; ls: lysigenous space; m: mitochondrion; n: nucleus; pd: plasmodesma; pm: plasma membrane; v: vacuole; w: cell wall.

Fig. 1. A mesophyll cell of control Nijisseiki leaves. $\times 23400$.

Fig. 2. A vascular bundle sheath cell of untreated Chojuro leaves. $\times 5200$.

Fig. 3-4. Effect of citrinin on plasma membrane as observed at the both ends of plasmodesmata of vascular bundle sheath cells in Nijisseiki leaves treated with 100ppm citrinin for 3 hr (Fig. 3) ($\times 26600$) and 12 hr (Fig. 4) ($\times 8400$). The invagination of plasma membrane occurred at plasmodesmata, from which central cores were extended.

Fig. 5. Mesophyll cells of Nijisseiki leaves treated with 100ppm citrinin for 12 hr. Note the invagination of plasma membrane, swelling of chloroplasts and elongated outer layer of nuclear envelope. The adjacent vascular bundle sheath cells were disorganized. $\times 6500$.

Fig. 6. Nijisseiki leaves treated with 100ppm Citrinin for 8 hr. The invaginations of plasma membrane occurred at plasmodesmata between a mesophyll cell (upper) and a vascular bundle sheath (lower). Invaginated sites contained lomasome-like vesicles, the central cores extended from plasmodesmata and electron-lucent materials. $\times 12600$.

Fig. 7. Mesophyll and vascular bundle sheath cells of Chojuro leaves treated with 100ppm citrinin for 12 hr. No ultrastructural changes were observed. $\times 3200$.

Fig. 8-10. Necrotic cells in Nijisseiki leaves treated with 100ppm citrinin for 12 hr.

8) General views of disrupted mesophyll cells. $\times 8300$.

9) The degradation of nucleus. $\times 26000$.

10) The disorganization of grana lamella in chloroplasts. $\times 20800$.





