

ナシ黒斑病菌の宿主特異的作用機構VIII

AK-毒素のK⁺透過機能障害に対する各種カチオンの効果

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Nature of Specific Susceptibility to *Alternaria kikuchiana* in Nijisseiki Cultivar among Japanese Pears VIII.* Effects of Other Cations on Losses of K^+ after Exposure to AK-Toxin

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尾谷 浩**・森川通明**・西村正暘**・甲元啓介** : ナシ黒斑病菌の宿主特異的作用機構(VIII)* AK-毒素の K^+ 透過機能障害に対する各種カチオンの効果

Abstract

The host-specific toxin (AK-toxin) of *Alternaria kikuchiana* caused rapid increases in efflux of K^+ from susceptible leaves of Japanese pear (*Pyrus serotina*). On the contrary, toxin caused a decrease in effluxes of Na^+ , Mg^{2+} and Ca^{2+} from susceptible tissues, as compared to efflux from control tissue. Toxin-induced loss of K^+ was much greater in the presence of Na^+ , Mg^{2+} or Ca^{2+} in the ambient solution. Stimulation of toxin-induced leakage was evident immediately after adding these cations to toxin-treated tissues; when these cations were removed, loss of K^+ reverted to the lower level. The stimulative effect of Na^+ and Mg^{2+} increased as the concentrations were increased from 0.01 to 10 mM; the effects of Ca^{2+} reached a plateau at 0.1 to 1.0 mM and then rapidly decreased. Susceptible tissues, in the presence of Mg^{2+} at 10 mM, lost most of their K^+ by 3 hr after exposure to toxin. When toxin-induced K^+ efflux was stimulated by other cations, the tissues took up such cations. Na^+ and Mg^{2+} uptakes by toxin-treated tissues continued to increase at a constant rate, but Ca^{2+} uptake leveled off gradually with time. These data show that AK-toxin affects permeability of susceptible plasma membrane to K^+ ; the loss of K^+ may be associated with the presence of certain other inorganic cations.

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Introduction

Alternaria kikuchiana Tanaka produces a host-specific toxin (AK-toxin), which selectively affects the Japanese pear (*Pyrus serotina* Rehd.) cultivars that are susceptible to the fungus^{5,14,18}. AK-toxin is an essential determinant of pathogenicity which causes the same visible and biochemical symptoms as those caused by the fungus^{10,11,16}. In addition, susceptibility in pears to the fungus and to its toxin

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is controlled by a single dominant gene⁶⁾. Thus, an understanding of specificity in this plant-parasite interaction should be possible from studies of the action of AK-toxin.

AK-toxin has a rapid effect on the plasma membrane^{5,10,11)}. There is an almost instantaneous increase in losses of electrolytes from toxin-treated susceptible tissues^{5,14,15)}. Phosphate and K^+ appear to be the major electrolytes lost from tissues following exposure to toxin; increased effluxes of these substances were detected within 5 min after exposure to toxin⁹⁾. Recently, we happened to find out an interesting fact that the effect of AK-toxin on efflux of K^+ can be enhanced by some inorganic cations. Such an enhancement by cations may be helpful in evaluating the mechanism of AK-toxin action. In present paper, therefore, we have investigated the status of cation effects on K^+ efflux from susceptible pear leaves treated with AK-toxin. Abstracts of some of this work were published^{8,12,13)}.

Materials and Methods

Japanese pear cultivars susceptible (Nijisseiki) and resistant (Chojuro) to *A. kikuchiana* and to AK-toxin were used in all experiments. AK-toxin was prepared from culture filtrates of a virulent isolate (No. 17) of *A. kikuchiana*, by previously described procedures^{14,15)}. This preparation caused black veinal necrosis on susceptible Nijisseiki pear leaves at 0.01 $\mu\text{g/ml}$.

Five leaf disks (total fresh wt., ca. 50 mg), 1.0 cm in diameter, were cut from freshly-harvested young leaves. The disks were vacuum-infiltrated for 20 min in AK-toxin solution (5 $\mu\text{g/ml}$) or in deionized water as controls. After rinsing with deionized water, the leaf tissues were placed in flasks containing 10 ml deionized water or in solutions of the inorganic cation to be tested, and incubated on a shaker at 27 C. At the end of each hour, the leaching solutions were removed and 10 ml fresh solution was placed in each flask. The fresh solutions contained the same species of electrolytes; in some cases, different ions were added with fresh solutions. Content of cations in each leaching solution was measured by an atomic absorption spectrophotometer (Hitachi 170-30).

Results of all experiments were calculated on the basis of fresh weight of leaf tissue. Results shown in tables and figures are cumulative values to each time interval used.

Results

Effect of AK-toxin on efflux of inorganic cations from tissues

Our preliminary data suggested that AK-toxin caused a large increase in rate of K^+ loss from susceptible tissues, but did not change the rates of Na^+ , Mg^{2+} and Ca^{2+} losses⁹⁾. We now have more careful experiments to determine whether or not AK-toxin specifically affects K^+ efflux. Results indicate that susceptible tissues treated with AK-toxin lost more K^+ than did control tissues; toxin-induced efflux of K^+ increased at a constant rate after exposure (Fig. 1, A). Efflux of Mg^{2+} from toxin-treated susceptible tissues increased slightly during first hour, and then gradually decreased (Fig. 1, B). The efflux patterns of Na^+ and Ca^{2+} were similar to that of Mg^{2+} . After 4 hr, the amount of Mg^{2+} , Na^+ and Ca^{2+} leaked from toxin-treated susceptible tissues was less than that leaked from the respective control tissues (Table 1). Cation efflux from resistant tissues was not affected by AK-toxin (Fig. 1 and Table 1).

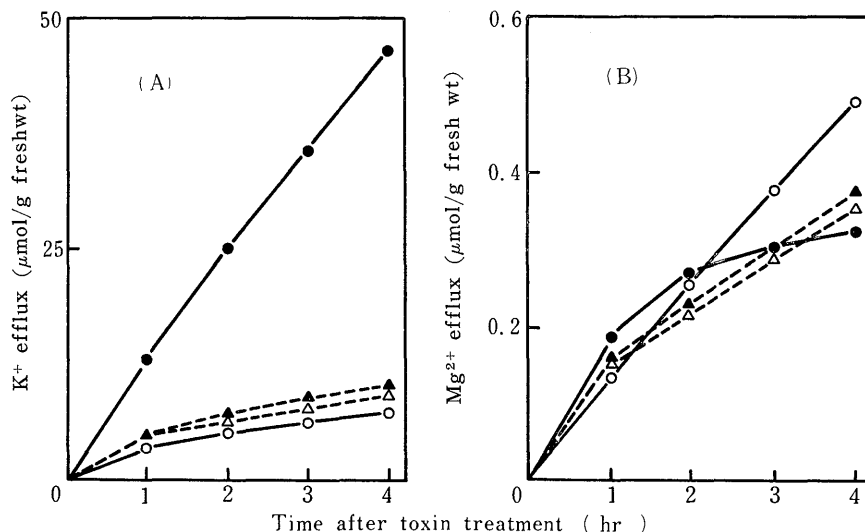


Fig. 1. Efflux of K^+ (A) and Mg^{2+} (B) from pear leaf tissues treated with AK-toxin. Susceptible (—●—) and resistant (---▲---) leaf tissues were vacuum-infiltrated with AK-toxin solution and leached in deionized water. The leaching solutions were analyzed at intervals for K^+ and Mg^{2+} . Efflux from susceptible (—○—) and resistant (---△---) controls without toxin is shown.

Table 1. Effect of AK-toxin on efflux of inorganic cations from pear tissues

Treatment ^{a)}	Cation ($\mu\text{mol/g fresh wt}$) ^{b)}			
	K^+	Na^+	Mg^{2+}	Ca^{2+}
Susceptible				
Toxin	46.54 ± 0.13	0.31 ± 0.04	0.34 ± 0.02	0.36 ± 0.06
Water	7.44 ± 0.83	0.45 ± 0.02	0.54 ± 0.02	0.48 ± 0.04
Resistant				
Toxin	9.36 ± 0.90	0.36 ± 0.05	0.38 ± 0.01	0.24 ± 0.03
Water	8.72 ± 0.51	0.34 ± 0.07	0.36 ± 0.02	0.24 ± 0.04

a) Pear leaf tissues were treated with AK-toxin or deionized water and leached as indicated in Figure 1.

b) The value of each cation in the leaching solutions was taken 4 hr after first exposure to AK-toxin.

Effect of inorganic cations on AK-toxin-induced efflux of K^+

After AK-toxin treatment, pear tissues were leached in 1 mM solutions of $NaCl$, $MgCl_2$ or $CaCl_2$, and monitored for K^+ efflux. These compounds stimulated further toxin-induced efflux of K^+ from susceptible leaves (Fig. 2). $MgCl_2$ plus toxin gave the greatest increase in loss of K^+ . The stimulative effects of $CaCl_2$ decreased gradually with time. K^+ efflux from resistant tissues was not affected by toxin, or by toxin plus $NaCl$, $MgCl_2$ or $CaCl_2$.

Susceptible, toxin-treated tissues were leached in $MgSO_4$ or $CaSO_4$ (1 mM), to determine whether the stimulation by inorganic compounds is caused by the cation

or by the anion. Results were comparable to those obtained with MgCl_2 and CaCl_2 . This indicates clearly that cations rather than anions have an effect on toxin-induced losses of K^+ .

Susceptible leaf tissues were exposed to toxin and leached in several concentrations of NaCl , MgCl_2 or CaCl_2 . Toxin-induced loss of K^+ increased with increasing concentrations of NaCl and MgCl_2 , from 0.01 to 10 mM (Fig. 3, A&B). Addition of MgCl_2 at 10 mM caused a four-fold increase in K^+ efflux, as compared with that induced by AK-toxin alone. In contrast, CaCl_2 at 0.1 to 1.0 mM caused increased losses of K^+ after exposure to toxin, but rapid decreases at higher levels (Fig. 3, C).

Total content of K^+ in pear tissues was determined in a supplementary experiment. Five leaf disks were ground in a mortar with 100 ml deionized water. The homogenates were centrifuged at $10,000 \times g$ for 30 min and K^+ content in the supernatant was determined. Susceptible and resistant pear leaves had 124 ± 18 and $102 \pm 35 \mu\text{mol K}^+$ per g fresh weight, respectively. Therefore, susceptible tissues exposed to toxin and leached

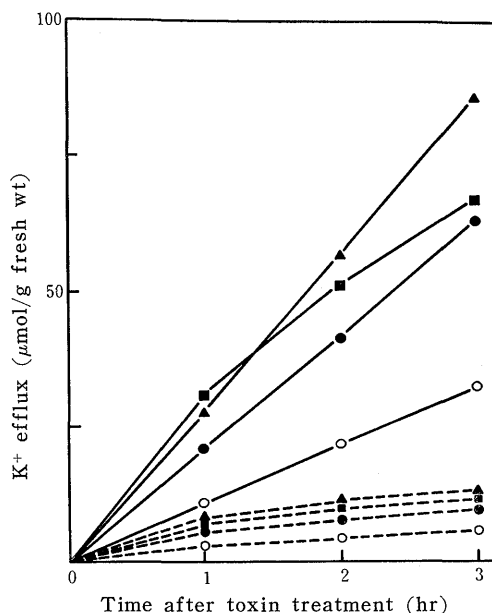


Fig. 2. Effect of several inorganic compounds on toxin-induced loss of K^+ from pear tissues. Susceptible leaf tissues were treated with AK-toxin, incubated in 1 mM solutions of NaCl (—●—), MgCl_2 (—▲—) or CaCl_2 (—■—), and monitored for K^+ efflux. The K^+ efflux from toxin-treated controls (—○—), and from water (---○---), NaCl (---□---), MgCl_2 (---△---) and CaCl_2 (---□---) controls is shown.

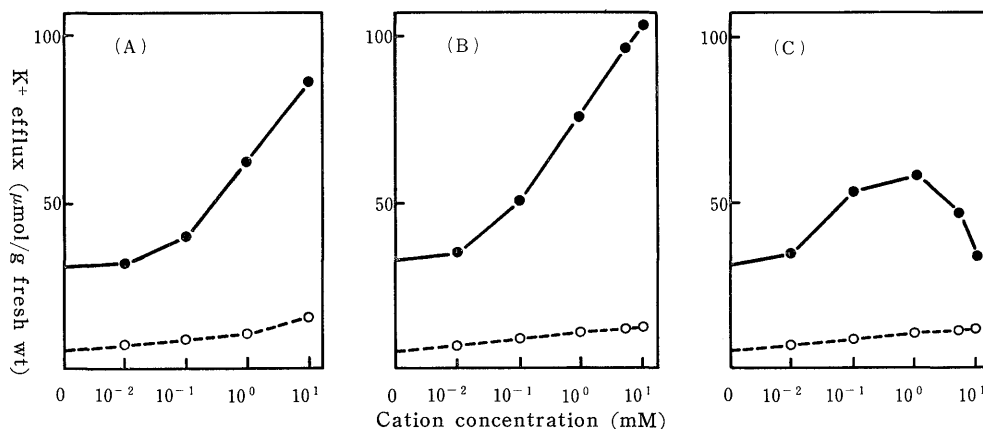


Fig. 3. Effect of several concentrations of NaCl (A), MgCl_2 (B) and CaCl_2 (C) on K^+ efflux induced by AK-toxin. Susceptible leaf tissues were vacuum-infiltrated in AK-toxin solution (—●—), or in deionized water (---○---), and placed in solutions of inorganic compounds at several concentrations. Each value of K^+ in the ambient solutions was taken after leaching for 3 hr.

in 10 mM MgCl_2 solution lost most of their total K^+ within 3 hr (Fig. 3, B).

The effects of addition or removal of cations after toxin treatment were tested. Susceptible tissues were vacuum-infiltrated with AK-toxin and then leached in MgCl_2 solution (5 mM). After leaching for 2 hr, tissues were removed from MgCl_2 solution and placed in deionized water. Two hours later, the leaves were again placed in MgCl_2 solutions. The stimulative effect of MgCl_2 on toxin-induced K^+ efflux was lost when tissues were removed from MgCl_2 solutions (Fig. 4). When these tissues were replaced in MgCl_2 solutions, the stimulative effect of the cation was again evident. Similar results were obtained in experiments with NaCl and CaCl_2 . When tissues were held continuously in MgCl_2 solutions, the K^+ efflux rate decreased rapidly after 4 hr of toxin treatment, because K^+ in the tissues was depleted.

Uptake of inorganic cations by pear tissues treated with AK-toxin

We considered the possibility that Na^+ , Mg^{2+} and Ca^{2+} are taken up by toxin-treated tissues, at the time when K^+ is lost. Thus, concentrations of these three cations in the leaching solutions were measured. The disappearance of cations from the leaching solutions was considered to reflect the amount of cations taken up by the leaf tissues. Results showed that within 1 hr, toxin-treated susceptible tissues took up more Na^+ , Mg^{2+} and Ca^{2+}

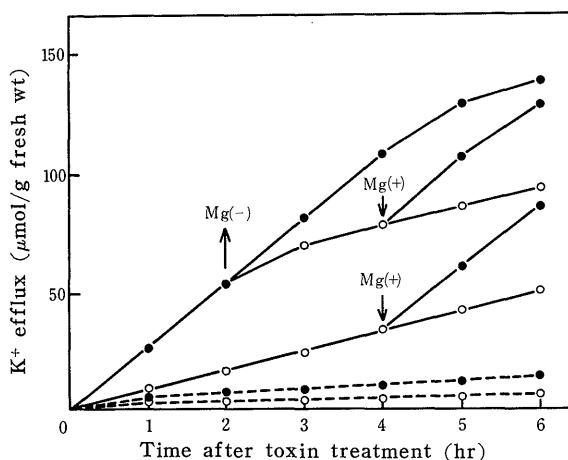


Fig. 4. Reversal and recovery of the effects of Mg^{2+} on toxin-induced losses of K^+ from pear leaves. Susceptible tissues were treated with AK-toxin and leached in MgCl_2 solution (—●—) or in deionized water (—○—). At times indicated, the tissues were removed from MgCl_2 (↑) or placed in MgCl_2 (↓). The K^+ efflux from MgCl_2 (---●---) and water (---○---) controls without toxin is shown.

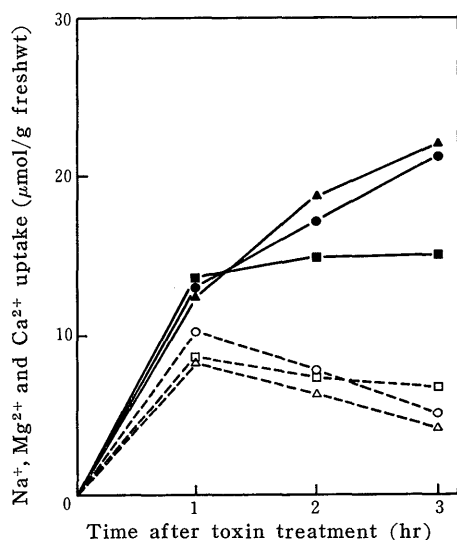


Fig. 5. Uptake of Na^+ (—●—), Mg^{2+} (—▲—) and Ca^{2+} (—■—) by susceptible pear tissues treated with AK-toxin. Cation uptake was measured by determining concentrations of cations in the ambient solutions shown in Figure 2. Uptake of Na^+ (---○---), Mg^{2+} (---△---) and Ca^{2+} (---□---) controls without toxin is shown.

than did control tissues (Fig. 5). The rate of Na^+ and Mg^{2+} uptake by toxin-treated tissues continued to increase, but that of Ca^{2+} uptake leveled off gradually with time. Control tissues did not continue to take up the cations after 2 hr.

Discussion

Previous physiological evidence has shown that AK-toxin causes rapid and drastic changes in the characteristics of plasma membranes of susceptible but not resistant pear cells^{5,10,11}). The most striking evidence is the rapid increase in loss of electrolytes from host tissues^{5,14,15}). Our previous experiments indicated that much of the toxin-induced electrolyte loss was K^+ ; there was little or no loss of other cations, such as Na^+ , Mg^{2+} and Ca^{2+} ⁹). Efflux of inorganic cations other than K^+ tended to decrease in rate gradually after exposure to AK-toxin. In addition, toxin-induced K^+ efflux was stimulated by addition of the other cations, which were taken up by toxin-treated tissues. These results suggest that AK-toxin affects K^+ transport across plasma membranes of susceptible cells; the transport is closely associated with movement of other inorganic cations in the cells.

Plasma membrane ATPase is found in root cells of various higher Plants^{1,7}). This enzyme is stimulated by inorganic cations and is involved in cation transport across the plasma membrane^{1,7}). In oat roots, an ATPase is known to be stimulated by K^+ and Mg^{2+} , and inhibited by Ca^{2+} ¹). Our present experiments have shown that K^+ efflux induced by AK-toxin is stimulated by Na^+ , Mg^{2+} and Ca^{2+} , although Ca^{2+} at high concentrations inhibited the cation stimulation. Helminthosporoside, a host-specific toxin produced by *Helminthosporium sacchari*, activates membrane K, Mg-ATPase of susceptible sugarcane¹⁷). One possible explanation for toxin-induced loss of K^+ , and its stimulation by certain cations, is that a plasma membrane ATPase in host cells may be activated by AK-toxin, which may upset the ion balance of the cell. However, pretreatments of pear tissues with 2,4-dinitrophenol (uncoupler), oligomycin (energy transfer inhibitor), ouabain (ATPase inhibitor) and quinidine (inhibitor of passive transport of K^+) caused no changes in losses of K^+ following exposure to AK-toxin⁸). Furthermore, ATPase activity in the macromolecular fractions of susceptible pear tissues was not affected by AK-toxin, or by AK-toxin plus Mg^{2+} (unpublished data).

Host-specific toxins produced by *H. victoriae* and *Periconia circinata* induce K^+ efflux from oats and sorghum, respectively, but have no effect on efflux of Na^+ ^{3,4}). MnCl_2 and MgCl_2 are known to enhance the effect of *H. victoriae* toxin on permeability²). Our recent experiments indicated that AM-toxin I, a host-specific toxin from *A. mali*, induces a marked increase in K^+ efflux from susceptible apple tissues, and also causes effluxes of Na^+ , Mg^{2+} and Ca^{2+} ¹²). Efflux rates for Na^+ , Mg^{2+} and Ca^{2+} are much less than the efflux rates for K^+ ¹²). Marked further differences between the actions of AK-toxin and AM-toxin are indicated, because the activity of AM-toxin was stimulated by Ca^{2+} rather than by Mg^{2+} at 5 mM¹²). We have no satisfactory explanation for these differences. The finding that the activity of AK-toxin can be stimulated by specific cations could with further work lead to an understanding of the exact mechanism of action.

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和 文 摘 要

ナシ黒斑病菌の宿主特異的作用機構 (VIII) AK-毒素の
K⁺透過機能障害に対する各種カチオンの効果

尾谷 浩・森川通明・西村正暘・甲元啓介

ナシ黒斑病菌の分泌する宿主特異的毒素 (AK-毒素) は、感受性ナシ (二十世紀品種) の K⁺ 透過機能に障害を引き起すが、この AK-毒素作用に対する各種カチオンの効果について検討した。感受性ナシ組織では、AK-毒素処理後、直ちに K⁺ の急激な漏出を生じたが、Na⁺, Mg²⁺, Ca²⁺ などの漏出は、無処理ナシ組織からの漏出よりもむしろ減少する傾向にあった。一方、AK-毒素による K⁺ 異常漏出は、ナシ組織に Na⁺, Mg²⁺, Ca²⁺ などのカチオンを添加することによって、さらに著しく促進された。この促進効果は、添加カチオンの除去によって容易に消失した。Na⁺ と Mg²⁺ による促進効果は、処理濃度の上昇 (0.01 - 10 mM) とともに増大したが、Ca²⁺ の効果は、5 mM 以上の濃度で急激に減少した。以上の結果から、AK-毒素による宿主細胞膜の K⁺ 透過機能障害には、宿主組織に存在する数種のカチオンが関与しており、それは AK-毒素効果に対して促進的に働いているものと考えられる。