

トマト・アルターナリア茎枯病菌が生産するAL毒素のトマト培養根に対する作用

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著者	Orolaza, N.P. 川口, 悦男 柘植, 尚志 道家, 紀志
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Effect of AL-toxin Produced by *Alternaria alternata* Tomato Pathotype on Cultured Roots of Tomato*

Noemi P. OROLAZA**, Itsuo KAWAGUCHI**, Takashi TSUGE**
and Noriyuki DOKE**

Abstract

The effect of AL-toxin produced by *Alternaria alternata* tomato pathotype on susceptible and resistant plants was investigated by employing a quantitative, simple and easy assay system using cultured roots of tomato. Root growth and root cell viability were examined by weighing and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] colorimetric assay, respectively. Cultured roots of susceptible cv. Mie First incubated in culture media containing different concentrations of AL-toxin I showed a detectable reduction and complete inhibition of the growth at toxin concentrations of 0.01 and 1 $\mu\text{g/ml}$, respectively. The growth of cultured roots of resistant tomato cv. Saturn was significantly and completely reduced at toxin concentrations of 0.1 and 10 $\mu\text{g/ml}$, respectively. When the root viability was determined by the colorimetric assay using MTT, the viability of susceptible cultivar was significantly reduced at a toxin concentration of 1 $\mu\text{g/ml}$, two days after treatment, while reduction in the viability of resistant cultivar was not detected even at a toxin concentration of 10 $\mu\text{g/ml}$. These results indicate that the use of cultured roots of tomato may contribute to a quantitative and qualitative determination for evaluating the host specific mode of action of AL-toxin.

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Key words: *Alternaria alternata* tomato pathotype, AL-toxin, *Lycopersicon esculentum*, cultured root, MTT assay.

INTRODUCTION

Alternaria alternata tomato pathotype [*A. alternata* (Fries) f. sp. *lycopersici*] is the causal agent of *Alternaria* stem canker of tomato^{3,11,14}. This fungus produces AL-toxins (AAL-toxins) which are essential for host-specific pathogenesis^{8,9,14,15,22}. Tomato genotypes which are susceptible to the fungus are highly sensitive to the toxins, showing stem canker and veinal necrosis of leaf, while genotypes resistant to the fungus are insensitive to the toxin^{3,10}.

In contrast to some host-specific toxins from other pathotypes of *Alternaria alternata*, AL-toxins do not induce rapid losses of electrolytes from sensitive tissues^{14,18}. It was suggested that aspartate carbamoyltransferase (ACTase; EC2.1.3.2), a key enzyme in pyrimidine biosynthesis which had been reported for several plants to be located in chloroplast^{4,20,21}, was a target enzyme of AL-toxins⁸. However, in our recent results¹²) and those of Fuson and Pratt⁶), the involvement of AL-toxins as an inhibitor of the ACTase was not demonstrated. Also, electron microscopic studies showed that AL-toxin caused aberrations on mitochondria and endoplasmic reticula of leaves of toxin-sensitive tomato cultivar 24 hr after AL-toxin treatment¹⁹), further suggesting that ACTase may not be the key site of action of AL-toxin. We also showed abnormal accumulations of ethanolamine and phosphoryleth-

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** School of Agricultural Sciences, Nagoya University, Nagoya 464, Japan 名古屋大学農学部

anolamine in AL-toxin treated tissues of susceptible tomato, suggesting that metabolism associated with them may be affected by AL-toxins¹²⁾.

To facilitate in assessing the mode of action of toxin produced by pathogens in disease development, convenient and quantitative assay systems are generally important. Most of the previous works on the effects of AL-toxins have been done using intact plants such as leaves, leaf discs and shoots^{3,8-11,14,15)}. Studies on the effect of AL-toxin on calli, suspension-cultured cells, protoplasts and pollen have also been done^{1,6,13,21,23)}. To gain further insight into cellular effects of AL-toxin, we developed a simple, easy and quantitative assay employing tomato cultured roots for evaluating the biological effect of AL-toxin.

MATERIALS AND METHODS

Plant materials. Plants were grown in pots under a glasshouse condition. Tomato (*Lycopersicon esculentum* Mill.) cv. Mie First and cv. Saturn, which are susceptible and resistant to *A. alternata* tomato pathotype, respectively, were used in the study.

Root culture. Cultured tomato roots were prepared according to the method of Butcher²⁾, with slight modifications. Plant seeds were incubated on 0.8% water agar at 23°C in dark under aseptic condition. Then, the growing roots were excised from the seedlings, cultured in 100 ml Erlenmeyer flask in liquid medium (MS-B5) consisting of MS salts¹⁷⁾, B5 vitamins⁷⁾ and 3% sucrose at 25°C in light on a gyratory shaker at 100 rpm and maintained by transferring several pieces of roots to fresh medium every week.

Preparation of toxin. AL-toxins were isolated according to the method¹²⁾ based from those reported elsewhere^{3,9,11,14)}. In the present experiment, AL-toxin I was used for the assay.

Bioassay. The phytotoxicity of AL-toxin was evaluated using root-growth assay and MTT-colorimetric assay systems. Five-day-cultured tomato roots (10 mg) of cvs. Mie First and Saturn were incubated in MS-B5 medium containing various concentrations of AL-toxin I at 25°C in light on a gyratory shaker at 100 rpm. In the latter assay, root viability was determined by a colorimetric assay using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Chemical International Inc.) according to the standard procedure^{5,16)} with some modifications adapted for cultured roots. Roots (60 mg) were transferred to test tubes containing 500 μ l distilled water and 50 μ l of solution A (5 mg MTT dissolved in 1 ml phosphate buffered saline, pH 7.4) and then incubated at 28°C. After incubation for 4 hr, 500 μ l of solution B (isopropanol containing 0.04 N HCl) was added to the root suspension, and then roots were homogenized by using a teflon homogenizer for 2 min. The resulting root homogenate was centrifuged at 1,500 \times g for 5 min, and the absorbance of the supernatant was measured at 570 and 630 nm using a Ubest-30 spectrophotometer (Japan Spectrophotometric Co. Ltd.).

RESULTS AND DISCUSSION

Root growth

The inhibitory effect of the toxin on the growth of roots of susceptible cv. Mie First, which was determined by measuring the fresh weight, was detectable at a toxin concentration of 0.01 μ g/ml (Fig. 1). A partial and complete inhibition of the root growth was observed at ca. 0.1 and 1 μ g/ml, respectively. Slight browning was only observed in the susceptible ones, and necroses sometimes appeared at the root tips. In the case of the cultured roots of resistant tomato cv. Saturn, the growth inhibition was significantly detected at the toxin concentration of 0.1 μ g/ml (ca. 30% inhibition). Complete inhibition was observed at 10 μ g/ml similar to that of the susceptible one. The difference in toxin sensitivity between susceptible and resistant cultivars was apparent at the toxin concentration ranging from 0.01 to 0.1 μ g/ml.

Quantification of root cell viability using MTT

When MTT assay was employed, a linear relationship between the absorbance resulted from the production of formazan and quantities of root tissues was demonstrated at a level of 10 to 40 mg tissues

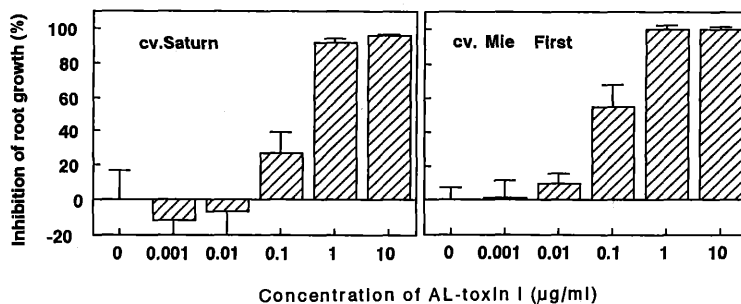


Fig. 1. Effect of AL-toxin I on the growth of tomato cultured roots of susceptible and resistant cvs. Mie First and Saturn, respectively. Cultured roots were incubated in MS-B5 media containing different concentrations of AL-toxin I. The cultured roots were weighed 6 days after incubation at 25°C. Each value represents the average of 5 experiments with standard deviation.

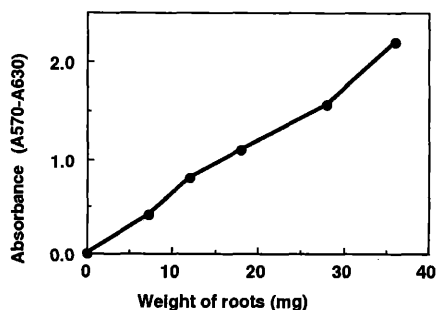


Fig. 2. Relationship between MTT-formazan production by cultured roots of tomato cv. Mie First and fresh weight of the roots. Different quantities (mg in 500 μ l of MS-B5 medium) of cultured roots were incubated with MTT for 4 hr at 28°C, and then subjected to spectrophotometric measurement of the content of MTT-formazan.

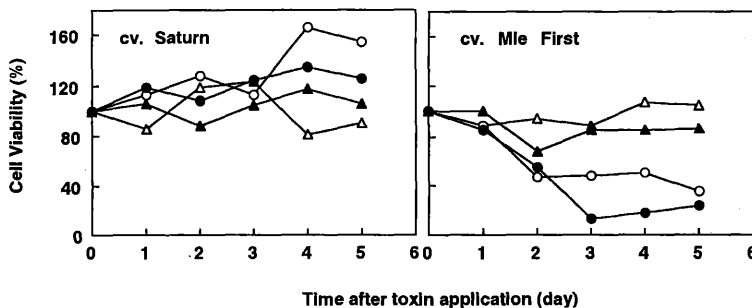


Fig. 3. Effect of AL-toxin I on the viability of tomato cultured roots of susceptible and resistant cvs. Mie First and Saturn, respectively. Cultured roots (mg) were incubated in MS-B5 media (500 μ l) containing different concentrations (●: 10, ○: 1, ▲: 0.1, △: 0.01 μ g/ml) of AL-toxin I for indicated periods at 28°C. After incubation, MTT-formazan production by the roots was measured by MTT assay, and cell viability was represented as the percentage of the production by the roots just before toxin treatment. Each value represents the average of 2 experiments.

(Fig. 2). As the tetrazolium salts is known to be converted to blue formazan by mitochondrial dehydrogenase in viable cells^{5,16}, dissociation of mitochondria or the resultant cell death could be efficiently detected by the MTT assay. The susceptible and resistant tomato cultivars showed a great difference in sensitivity to the toxin (Fig. 3). Cell viability of the susceptible cv. Mie First was significantly reduced

by the toxin treatment at a concentration of 1 $\mu\text{g/ml}$ from 2 days after incubation. Such reduction in the cell viability of resistant cv. Saturn was not detected even at the toxin concentration of 10 $\mu\text{g/ml}$. In our previous report, we have shown that leaves of susceptible tomato cv. Mie First was highly sensitive to AL-toxin as compared to the resistant one (Saturn). AL-toxin I induced veinal necroses on susceptible cv. Mie First at a concentration of 0.06 $\mu\text{g/ml}$, while on the leaves of intermediately susceptible cvs. First Power and Zuiko and resistant cv. Saturn, 100 and 2,000 times higher concentrations were needed, respectively, to cause veinal necroses¹²⁾. Essentially, cultured tomato roots are also applicable for evaluating the host-specific activity of AL-toxin and that MTT assay is effective in evaluating the toxicity in root tissues. Since root cells do not contain chloroplasts, their plastids may not be directly involved in the action site of AL-toxin.

There was no great difference in the influence of AL-toxin I on the fresh weight of cultured roots between susceptible and resistant cultivars: the toxin inhibited root growth not only in susceptible cultivar but also resistant one (Fig. 1). The sensitivity of root to AL-toxin seemed to be lower than leaf. However, the sensitivity of the root of susceptible cultivar to AL-toxin was much higher and selective when evaluated by MTT assay method. In the field, *A. alternata* tomato pathotype caused disease on stem and leaves but not on roots. One probable reason why the roots of tomato plants were not diseased could be due to the direction of movement of toxin in the tomato plant from stems of major infected sites.

The devised assay system using cultured roots offers some advantages: i) mutation of cultured roots is thought to be negligible, since the cultured roots were induced without dedifferentiation and thus, should retain the organization of an intact organ, ii) the MTT assay is simple, reproducible and efficient colorimetric method to evaluate the biological activity of AL-toxin at low concentration by determining the rate of living cells in toxin-treated roots.

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和 文 摘 要

Noemi P. OROLAZA・川口悦男・柘植尚志・道家紀志：トマト・アルターナリア茎枯病菌が生産するAL毒素のトマト培養根に対する作用

トマト・アルターナリア茎枯病菌が生産する宿主特異的毒素(AL毒素)のトマト培養根に対する作用について検討した。アルターナリア茎枯病感受性トマト品種三重ファーストおよび抵抗性品種サターンから培養根を調製した。両品種の培養液を種々の濃度のAL毒素Iを添加した培地中で6日間培養し、培養根の新鮮重量を測定した結果、感受性品種では、0.01 µg/ml以上の毒素によって根の生育異常が観察され、1 µg/ml以上では生育が完全に阻害された。また、抵抗性品種でも、0.1 µg/ml以上の毒素によって培養根生育が抑制された。次に、細胞の生存率検定法としてMTT比色定量法を用いて、培養根細胞の毒素反応性を調査した。その結果、感受性品種の培養根では、1 µg/mlの毒素によって2日目から細胞生存率が減少し、10 µg/mlの毒素添加培地中では、3日目以後生存率が約20%に低下した。また、細胞生存率の低下した培養根では、根全体の若干の褐変や根先端部のえ死が観察された。一方、抵抗性品種の培養根では、10 µg/mlの毒素添加培地中で5日間培養した場合にも細胞生存率の低下は認められなかった。以上の結果から、AL毒素は感受性品種だけでなく抵抗性品種の培養根に対しても顕著な生育阻害活性を示すが、培養根細胞に対するえ死毒性は宿主選択的であることが明らかとなった。