ラテックス凝集反応法および Protein A-coated latex-linked antisera 法によるズッキーニ黄斑モザイクウイルス (ZYMV) の検出

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Detection of Zucchini Yellow Mosaic Virus by Latex Flocculation and Protein A-coated Latex-linked Antisera Tests*

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Key words : zucchini yellow mosaic virus, latex flocculation, PALLAS.

The latex flocculation (LF) test was found to be a useful procedure for detecting plant viruses[1-6]. This relatively simple procedure gives very rapid results and can be applied readily to small or large testing program. However, it is not readily adaptable if the available virus antisera have low titer or contain some inhibitory components which prevent the flocculation reaction[2]. Recently, these disadvantages could be successfully overcome by coating the latex particles using protein A before sensitization with antibody, and the modified procedure was called protein A-coated latex-linked antisera (PALLAS) test[7]. This paper described a comparison of the sensitivity of LF and PALLAS tests for detecting zucchini yellow mosaic virus (ZYMV).

ZYMV (isolate 169) and it's rabbit antiserum were the same as in the previous studies[8,9]. The tests were done with purified preparations and crude extracts from infected pumpkin (*Cucurbita maxima* cv. Hōkōaokawa) leaves. The crude extracts used in LF test were prepared by grinding 1.0 g leaves with 1.0 ml 0.05 M Tris-HCl buffer, pH 7.2, containing 0.02% Tween 20 and 0.02% polyvinylpyrrolidone (PVP) 40,000 (Tris-HCl buffer-TPO) in a mortar with pestle. After squeezing through two layers of gause, the sap was then centrifuged at 6,000 rpm for 15 min and was used as antigen in the tests. The procedure was also used to prepare antigen in PALLAS test, except that the extraction medium was 0.1 M glycine buffer, pH 8.2, containing 1.0% saline, 0.02% Tween 20, and 0.02% PVP (glycine buffer-STPO). Gamma-globulin from rabbit antiserum against ZYMV was prepared by a method as described by Clark and Adams[10].

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The LF test of Bercks and Querfurth[2] was adopted in this experiment. The tests were carried out with a suspension of Bacto latex 0.81 μm (Difco Laboratories, Detroit, Michigan) which had been sensitized with gamma-globulin from rabbit antiserum. In the preliminary tests, 100 μg/ml resulted in the best detection out of several concentrations of sensitizing gamma-globulin. Latex was sensitized by firstly diluting 1 part of latex suspension with 14 parts of 0.15 M NaCl. The mixture of an equal volume of gamma-globulin in 0.05 M Tris-HCl buffer, pH 7.2, and the diluted latex suspension was incubated for 1 hr at 25 C with occasional stirring. It was then twice washed by subjecting to a centrifugal force at 6,000 rpm for 30 min, sucking the supernatant, and resuspending the pellet with Tris-HCl buffer-TPO. The final pellet was resuspended in the same buffer containing 0.02% sodium azide and was stored at 4 C before use. The tests were done in polystyrene microtiter plates (Wako Pure Chem. Ind. Ltd.). The sensitized latex was used by adding 25 μl suspension to 50 μl antigen preparation. The plates were shaken at about 100 oscillations per minute for 15 min at 30 C. Detection end point was determined as the highest dilution of extracts from infected leaves or the minimum concentration of purified virus which resulted in visible flocculation. Doubtful reactions were checked under a microscope.

The procedure of Querfurth and Paul[7] was employed for PALLAS test. In this procedure, the latex particles were previously coated with protein A before sensitization with antibody. Protein A (Zymer Laboratories) was dissolved in distilled water containing 0.05% sodium azide to the concentration of 1.0 mg/ml. Before use, it was further diluted to 200 times with 0.1 M glycine buffer, pH 8.2, containing 1% NaCl. Latex particles were coated with protein A by mixing an equal volume of latex suspension diluted at 1 : 14 with 0.15 M NaCl and the diluted protein A. The mixture was incubated for 4 hr at about 20 C with occasional stirring. It was then further incubated overnight at 4 C. The mixture was twice washed with glycine buffer-STPO in the same way as described in the latex preparation. The protein A-latex complex was coupled with gamma-globulin by mixing an equal volume of the solution and 100 μg/ml gamma-globulin from rabbit antiserum diluted in glycine buffer-STPO. The mixture was then incubated and washed in the same way as those for coating the latex with protein A. The procedure of PALLAS test was principally identical to that of LF test.

In both tests, shaking and incubation times before observation were critical[7]. Preliminary tests showed that shaking time of 15 min and incubation time of 30 min at 25 C were the best of several conditions tested. As shown in Table 1, minimum detectable concentration of purified ZYMV by LF test was 25 μg/ml compared with 0.1 μg/ml for PALLAS test. When the antigen in crude extracts was examined, PALLAS test proved to be about 8 times more sensitive than LF test, resulting in the dilution end point of 4,000-5,000 times compared with 500-1,000 times for LF test (Table 2). Additionally, non-specific reaction was observed with LF test when the extracts were tested in the dilution of less than 50 times.

In agreement with the previous evidence[11], it was found that the sensitivity of PALLAS test for detecting ZYMV was higher than that of LF test. It is likely that in

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Table 1. Detection of purified ZYMV by latex flocculation (LF) and protein A-coated latex-linked antisera (PALLAS) tests[a)]

Test	Visible flocculation at virus concentration, μg/ml[b)]						
	50	25	10	1.0	0.1	0.01	0/0
LF	9/9	9/9	0/9	0/9	0/9	0/9	0/9
PALLAS	9/9	9/9	9/9	9/9	9/9	0/9	0/9

a) Data are total numbers of wells from three time experiments.
b) Numerator=Number of wells showed flocculation.
Denominator=Number of wells used.

Table 2. Detection of ZYMV in crude extracts of infected pumpkin leaves by latex flocculation (LF) and protein A-coated latex-linked antisera (PALLAS) tests[a)]

Test	Sample	Visible flocculation at dilution of[b)]						
		50	100	500	1,000	2,000	4,000	5,000
LF	Infected	9/9	9/9	9/9	0/9	0/9	0/9	0/9
	Healthy	3/9	0/9	0/9	0/9	0/9	0/9	0/9
PALLAS	Infected	9/9	9/9	9/9	9/9	9/9	9/9	0/9
	Healthy	0/9	0/9	0/9	0/9	0/9	0/9	0/9

a) Data are total numbers of wells from three time experiments.
b) Numerator=Number of wells showed flocculation.
Denominator=Number of wells used.

PALLAS test, protein A enable gamma-globulin to attach perfectly onto latex paticles. It is also possible that if protein A becomes attached to the Fc portion of gamma-globulin, the antigenic sites of all the globulin molecules should be free to react with antigen[11)].

Since another serological procedures were also found to be useful for detecting ZYMV[9,12,13)], the present results will extend the possibilities to choose the best detection method for ZYMV that achieves the most accuracy and efficiency under a certain experimental condition.

和 文 摘 要

Susamto Somowiyarjo・佐古宣道・野中福次：ラテックス凝集反応法および Protein A-coated latex-linked antisera 法によるズッキーニ黄斑モザイクウイルス（ZYMV）の検出

ラテックス凝集反応法（LF）と Protein A-coated latex-linked antisera 法（PALLAS）により ZYMV（分離株169）の検出試験を行った。マイクロプレートのウェルを反応槽として用い，25C下で15分間の振とう，30分間の静置を行い，反応の有無を判定した。純化ウイルスは LF 法により 25μg/ml，PALLAS 法により 0.1μg/ml まで検出された。罹病カボチャ葉の搾汁液では，前者の検出限界は500～1,000倍希釈，後者のそれは4,000～5,000倍希釈までであった。PALLAS 法は LF 法より感度が優れており，本ウイルスの検出法として有用であると考える。

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