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COMMUNICATION II

The Use of Antibody-sensitized Latex to Detect *Cymbidium* Mosaic Virus in Orchids

ABSTRAK

*Ujian agglutinasi lateks (L) dan satu modifikasi ujian lateks, bersalut protein A (PAL) telah dibandingkan untuk sensitisasi dengan antibodi kepada '*Cymbidium* mosaic virus' (CyMV). Satu siri kepekatan globulin telah diuji terhadap CyMV tulin. Kepekatan virus minimum yang boleh dikesan adalah sama untuk kedua-dua reaksi L dan PAL iaitu 0.104 µg/ml. Titik akhir yang boleh dikesan di sap orkid *Oncidium* *Gower Ramsey adalah serupa dipencairan 1/2560. PAL didapati lebih sensitif dari L di pencairan globulin lebih tinggi iaitu 1/512. PAL juga menunjukkan kesensitifan lebih tinggi dari L berasaskan kepada lima hibrid orkid dan menunjukkan persamaan dengan ujian mikroskop elektron.*

ABSTRACT

Latex agglutination test (L) and a modification of the latex test, protein A-coated latex (PAL) were compared for sensitization with antibodies to Cymbidium *mosaic virus (CyMV). A series of globulin concentrations was tested against purified CyMV. The minimum detectable virus concentration was similar for both L and PAL reactions at 0.104 µg/ml. The detectable end points in infected* Oncidium *Gower Ramsey orchid sap were similar at a dilution of 1/2560. PAL was found to be more sensitive than L at a higher globulin dilution of 1/512. The PAL test showed higher sensitivity than L based on five orchid hybrids and compared favourably with electron microscopy test.*

INTRODUCTION

Commercially produced orchids in Malaysia are frequently infected with *Cymbidium* mosaic virus (CyMV) and to a lesser extent with *Odontoglossum* ringspot virus (ORSV) (Abdul-Samad 1985, 1986, 1989a, 1989b). Detection and identification of plants infected with CyMV or ORSV or both have been difficult because the infected plants may be symptomless or they may show some chlorotic or necrotic patterns pending on the species or hybrids. At present there is no available method of detection for use in orchid viruses locally. Imported orchids are rarely tested for the presence of viruses. Therefore a simple and quick method of testing is needed that combines high specificity and sensitivity with practical value, especially in quarantine programs, and for the development and testing of virus-free stocks. The most obvious choice would be a serological test.

The latex agglutination test (L) and the protein A-coated latex (PAL), a modification of the latex test (Querfurth and Paul 1979) have been used widely for the detection of plant viruses in their naturally occurring host as well as in herbaceous plant indicator species (Bercks 1967; Abu Salih *et al.* 1968; de Sequera and Lister 1969; Mumford 1977; Khan and Slack 1978; Koenig and Bode 1978; Thomas 1980; Torrance 1980; Demski *et al.* 1986). Due to their simplicity and the fact they do not require elaborate instruments, they can be used to test large numbers of samples in the laboratory or in field situations. Therefore this study was undertaken to determine the sensitivity of L and PAL and their potential use in detecting CyMV in orchids. A comparison between L, PAL and electron microscopy was also carried out.

MATERIALS AND METHODS

Virus and Antiserum

Cymbidium mosaic virus (CyMV) isolate was originally obtained from the orchid *Cattleya* sp. (Abdul-Samad 1985) and propagated on *Dendrobium* sp. The virus was purified according to the method used by Wisler *et al.* (1982). Systemically infected leaves were homogenised in 0.05M phosphate buffer, pH 7.5 containing

0.125M Na_2SO_3. The extract was clarified by adding chloroform (1:1, v/v) and the virus was concentrated with polyethylene glycol 6000 (PEG 6000). It was further purified through one cycle of differential centrifugation and a sucrose density gradient. The virus zone was fractionated, collected and concentrated by ultracentrifugation and the purified virus was resuspended in the same buffer.

Antiserum was produced by immunising rabbits using a series of two intravenous, one subcutaneous and one booster injection. Approximately 250 µg of viral antigen was administered intravenously to each animal at the first injection. The second intravenous injection was given one week later with 500 µg of antigen. Four weeks later, 1 mg of antigen emulsified with an equal volume of Freund's complete adjuvant was injected subcutaneously at several sites on the flank of the animal. Booster injection was given subcutaneously with 450 µg of antigen emulsified with Freund's complete adjuvant one month later. The rabbits were bled at weekly intervals starting one week after the last injection. Blood samples were collected from the ear veins and the serum recovered after clotting and removal of cellular material by centrifugation at 2000 g for 10 mins.

Preparation of Globulin and Sensitization of Latex

Globulin fractions were obtained from antiserum by precipitation with ammonium sulphate (equal volumes of saturated $(NH_4)_2SO_4$ with 0.5 ml antiserum diluted with 9.5 ml of distilled water). Solutions were incubated at room temperature for half an hour and centrifuged at low speed for 15 min. The precipitates were resuspended in saline and diluted to one-half of the original diluted antiserum and stored at 4°C after adding sodium azide to a concentration of 0.2% (Hill 1984). Antibody-sensitised latex (Sigma latex beads LB-8) and protein A-coated latex were prepared by the method described by Hill (1984).

Test Procedures

All tests were carried out in U-shaped microtiter plates (polystyrene) with 400µl capacity. Two-fold serial dilutions of the purified virus or infected plant sap to be tested were made with 0.05M Tris-HCl buffer, pH 7.2 containing 0.02% PVP 44,000. The latex was sensitised at a two-fold dilution of the globulin. For PAL the protein A solution was diluted 1:200 with glycine buffered saline and then mixed with the latex suspension which had been diluted with saline. Equal volumes (c. 50 µl) of L or PAL and the test samples were placed in wells. The plates were then shaken on a rotary shaker for 1 hr before observing agglutination reactions. The detection end point was recorded as the highest antigen dilution at which agglutination was visible (Ball 1974).

Electron Microscopy

A small piece of infected leaf tissue about 2-3 mm^2 was squashed on a glass slide in two drops of phosphotungstic acid (PTA), pH 6.8. A drop of sap mixture was placed on a 400 mesh carbon-strengthened Formvar coated grid, drained and dried and observed in a Philips HMG 400 electron microscope.

RESULTS AND DISCUSSION

Purified virus preparations and crude sap extracts reacted positively by forming visible loose aggregates with the latex-sensitised serum with or without protein A treatment. No agglutination of the sensitised latex preparations occurred against the control preparations where buffer and healthy orchid sap were used. The optimum antiserum dilution, which detected the highest dilution of antigen, for each serum globulin preparation for sensitising the latex particles for both L and PAL was determined by using purified virus. The dilution for both L and PAL was 1/512 in terms of the original globulin preparation. The minimum detectable virus concentration for the L and PAL reactions was similar at 0.104 µg/ml. Tests at higher globulin and antigen dilutions gave variable results. Determination of the detectable end-points by L and PAL was carried out by using crude sap extract from CyMV-infected *Oncidium* Gower Ramsey orchid. There was an increased detectable dilution end point from 1/128 to 1/512, a one-fold dilution between L and PAL. The PAL method showed higher sensitivity at a globulin dilution of 1/512. Therefore this would allow greater economy of use of antiserum globulins. No difference in sensitivity was detected when Tween 20 (Torrance 1980) was added to the extraction buffer for PAL. At a plant sap dilution of 1/2560 the virus could still be detected by L and PAL.

TABLE 1
Detection for CyMV in plants using L, PAL and electron microscopy

Orchid species/hybrid	L*	PAL*	E.M.#
Phalaenopsis Natasha	0	1+	+
Phalaenopsis sp.	1+	2+	+
Oncidium sp.	0	1+	+
Aerides Lorengga x *Vanda* Sanderiana	1+	2+	+
Dendrobium sp.	1+	3+	+
Vanda sp.	–	5+	+
Dendrobium utai	–	5+	+
Cattleya sp.	–	2+	+
Oncidium sp.	–	3+	+
Oncidium sp.	–	2+	+
Healthy sap (*Dendrobium* sp.)	0	0	0

* Tests were carried out using sample dilution at 1/320 and globulin dilution at 1/128. A minimum of three samples were taken from matured leaves

\# + Virus particles seen in at least three fields of view randomly selected at the illuminated viewing screen at an indicated 35,000 x magnification.

– Not tested

Agglutinations were ranked as follows:

0 = no reaction, 1+ = barely visible , 2+ = slight

3+ = moderate , 4+ = heavy , 5+ = very heavy

Both L and PAL were then used to test various orchid species and hybrids for presence of CyMV (Table 1) and sensitivity was compared with electron microscopy. Tests were carried out at sample dilution of 1/320 and globulin dilution at 1/128. The PAL test showed higher sensitivity than L, based on five orchid hybrids and compared favourably with electron microscopy.

The results from this study indicate that L and PAL constitute a sensitive, simple, rapid and reliable procedure for diagnosis of CyMV in orchids. Another advantage is that the sensitised latex has a long shelf-life when stored at 4°. Our test results showed that it could still be used after three months of storage without loss of sensitivity.

These techniques can be used in routine testing by growers advisory services for certification of healthy stocks. The technique does not require expensive equipment and biochemicals and has wider specificity where an antiserum to a single strain would be able to detect other strains of the same virus (Koenig *et al.* 1979). This technique could save cost, time and labour. The PAL method is very practical and particularly useful in the field even though it is not as sensitive as ELISA for detecting CyMV (N. Abdul-Samad, unpublished).

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