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A rapid immunochromatographic test to detect the lily mottle virus

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A B S T R A C T

We developed a rapid immunochromatographic strip (ICS) test for lily mottle virus (LMoV). The test is based on a double-antibody sandwich format and employs two distinct anti-LMoV polyclonal antibodies (IgG_3 and IgG_4). The first antibody, IgG_3 was conjugated with colloidal gold, and the second antibody, IgG_4 was used as the capture antibody at the test line. The performance of the ICS test was evaluated and the results obtained were compared with a quadruplex RT-PCR assay. When serial dilutions of purified LMoV were tested, the LMoV detection limit of the ICS test was 8.0×10^{-9} mg/mL, which was in complete agreement with the results of quadruplex RT-PCR. Compared with quadruplex RT-PCR, the specificity and sensitivity of ICS were 98.7 and 100%, respectively. There was therefore significant agreement between the results obtained from the two tests ($\kappa = 0.982$). The ICS test therefore appears to be broadly applicable, and will be especially useful in the field, as well as in areas without laboratory facilities, to support efforts to detect and control LMoV.

1. Introduction

Lily (*Lilium* spp.) is an important economic crop in the floricultural industry, and additional value is obtained from the bulbs, which are edible and have medicinal properties. Almost all lilies are propagated vegetatively. Therefore, viruses may accumulate in bulbs from one generation to the next. Viruses cause quantitative and qualitative yield reductions of lilies around the world (Wang et al., 2010). More than 10 different viruses have been reported to infect lilies worldwide, and the lily mottle virus (LMoV) is one of the most common (Ryu et al., 2002; Zhang et al., 2014). LMoV is closely related to the tulip breaking virus (TBV; Alper et al., 1982), and is a member of the *Potyvirus* genus within the Potyviridae family. LMoV is flexuous, non-enveloped, and rod-shaped; it is 680–900 nm long and 11–15 nm wide (King et al., 2011). LMoV is composed of a single-stranded 9.7 kb RNA with a positive polarity, and is surrounded by about 2000 copies of the coat protein (CP). The virus encodes a polyprotein of 3095 aa with a molecular weight of 351.0 kDa (King et al., 2011). The polyprotein self-cleaves and form proteins of different sizes and functions, including CP and a cytoplasmic inclusion protein (CI). The CP and CI contain 274 and 635 amino acids of 30 and 70 kDa in size, respectively (Urcuqui-Inchima et al., 2001). Symptoms of LMoV may vary from vein clearing, leaf mottle, leaf mosaic, chlorotic and yellow streaking, leaf curling, and narrowing and reddish-brownish-necrotic spots, to milder forms of leaf symptoms, or plants may even be symptomless at some stages of growth in the field (Fig. 1). Diseased plants are often shorter than healthy plants and the symptoms may be more severe if plants are also infected by Lily symptomless virus (LSV) (Asjes, 2000).

The most commonly used LMoV detection methods for lily samples are electron microscopy (Wang et al., 2007), enzyme-linked immunosorbent assay (ELISA; Sharma et al., 2005), reverse-transcriptase polymerase chain reaction (RT-PCR; Zhang et al., 2010), and real-time PCR (Zhang et al., 2014). However, these methods are time consuming, require technical expertise and specialized laboratory equipment. Therefore, these methods are not suitable for rapid detection during the cultivation of lily, it is necessary to develop a rapid, specific, and easily performed assay to detect LMoV. The immunochromatographic assay is one possibility. This technique offers several advantages over traditional immunoassays, such as its low cost, procedural simplicity, limited requirement for special skills or expensive equipment, and rapid results (Alvarez et al., 2010). The immunochromatographic assay has been widely applied to detect hormones, bioactive molecules,

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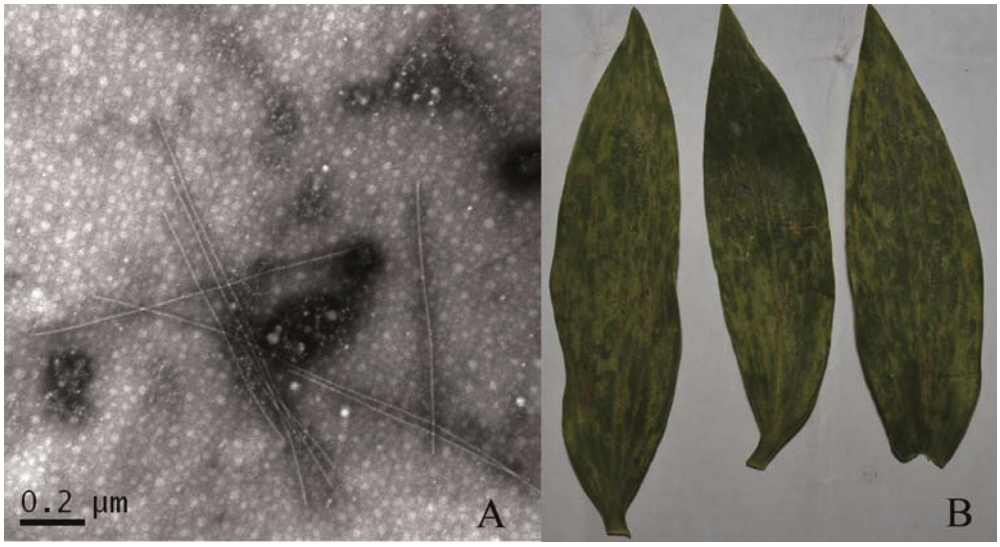

Fig. 1. (A) Electron microscopy image of LMoV particles and (B) leaves showing mottle symptoms.

contagious human diseases, animal and plant viruses, bacteria and parasite antigens as well as specific antibodies (Weiss, 1999; Alvarez et al., 2010; Sun et al., 2013).

The aim of the present study was to develop an immunochromatographic test strip (ICS) that could be used to rapidly detect LMoV. The sensitivity and specificity of the ICS were evaluated using a quadruplex RT-PCR assay as a reference test with 118 lily samples from the field. The results showed strong agreement between the two assays ($\kappa = 0.982$).

2. Materials and methods

2.1. Reagents and materials

Leaves infected by LMoV, LSV or CMV served as sources of these viruses and were stored at −70 °C at the Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Science (Lanzhou, China). The first antibody, IgG_3-rabbit IgG anti-LMoV CP-CI200 (CPI200) and the second antibody IgG_4-rabbit IgG anti-LMoV CP were obtained following the procedure of Tong et al. (2010). Both of the antibodies were kept in aliquots of 1 mg at −20 °C for one year. We purchased chloroauric acid ($HAuCl_4$) and goat anti-rabbit IgG from the Sigma Company (St. Louis, MO, USA), nitrocellulose membranes (HiFlow-120) and cellulose filter from Millipore (Billerica, MA, USA), and special cellulose and absorbent papers from Jieyi Company (Shanghai, China).

2.2. Preparation of colloidal gold and a colloidal gold-IgG_3 conjugate

Colloidal gold particles with a mean diameter of 30 nm were prepared using the method of Hermanson (2008) in 1.4 mL of 1% trisodium citrate (w/v) which was rapidly added to 100 mL of 0.01% aqueous chloroauric acid solution (w/v) at 100 °C and boiled for 5 min. As the resulting colloidal gold cooled gradually to room temperature with continuous stirring, the pH was maintained at 7.4 by adding 0.1 M potassium carbonate. Sodium azide was added to a final concentration of 0.01% (w/v) before storage at 4 °C in a glass bottle covered with foil. The absorption maxima (λ_{max}) of the solutions were analyzed by means of ultraviolet/visible spectroscopy (UV/VIS) using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) to determine the approximate particle sizes, which were confirmed by transmission electron microscopy (TEM) measurements using a JEM-1230 transmission electron microscope (TEM; JEOL, Tokyo, Japan).

To prepare colloidal gold-IgG_3, the IgG_3 (180 μL at 1.0 mg/mL) was added drop-wise to 10 mL of the pH-adjusted colloidal gold solution. The mixture was stirred vigorously for 30 min, and then 2.5 mL of 10% bovine serum albumin (BSA) was added to stabilize the conjugate solution. The mixture was then stirred for an additional 30 min, then was centrifuged at 10,000 × *g* at 4 °C for 30 min, after which the gold pellets were suspended in 1 mL of dilution buffer: 20 mM Tris/HCl buffer (pH 8.2) containing 1% (w/v) BSA, 3% (w/v) sucrose, and 0.02% (w/v) sodium azide. The colloidal gold conjugate was stored at 4 °C until use.

2.3. Preparation of the immunochromatographic strip (ICS)

The ICS included four components: a sample pad (special cellulose paper), a conjugate release pad (cellulose filter), a nitrocellulose membrane, and an absorbent pad (absorbent paper). The sample pad was treated with 20 mM phosphate buffer containing 1% (w/v) BSA, 0.5% (v/v) Tween-20, and 0.05% (w/v) sodium azide (pH 7.4), and was then dried for 2 h at 37 °C. For the conjugated pad, we first impregnated the cellulose filter with 15 μL colloidal gold-IgG_3 and then dried the pad for 2 h at 37 °C. Subsequently, we formed a control line by carefully dragging a pipette tip containing 100 μL of the goat anti-rabbit IgG (1.5 mg/mL) along the long axis of the nitrocellulose membrane. This was done by leaning the tip against a ruler placed 6 mm from one end of the membrane. Immediately afterwards, we repeated this process to generate a test line using the IgG_4 (1.0 mg/mL). We used the rule to maintain a 5-mm gap between the two lines. The remaining active sites on the membrane were blocked by incubation with 2% (w/v) BSA in PBS for 30 min at room temperature. The membrane was washed once with PBS, a second time with distilled water, and was then dried for at least 2 h at room temperature. Next, the sample pad, the prepared conjugated pad, the nitrocellulose membrane, and the absorbent pad were adhered to a backing plate (300 mm × 70 mm) in the proper order, as described previously (Peng et al., 2008). The assembled plate was then cut into 4-mm-wide pieces with a pair of small scissors. The resulting strips were mounted in plastic cassettes with windows over the sample pad and the nitrocellulose membrane (Jieyi Company, Ltd., Shanghai China). The cassettes were then packaged in plastic bags containing desiccant, and stored at 4 °C until use.