Journal of Virological Methods 220 (2015) 13–17

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Development of an immunochromatographic strip test for rapid detection of lily symptomless virus

Yubao Zhang[a], Yajun Wang[a], Jing Meng[b], Zhongkui Xie[a,*], Ruoyu Wang[a], Hadley Randal Kutcher[c], Zhihong Guo[a]

[a] *Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Sciences, Lanzhou 730000, China*
[b] *Ningxia Agricultural Comprehensive Development Office, Yinchuan 750002, China*
[c] *College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK S7N 5A8, Canada*

Article history:
Received 14 January 2015
Received in revised form 26 March 2015
Accepted 26 March 2015
Available online 4 April 2015

Keywords:
Immunonochromatographic strip
LSV
PCR
Rapid detection

ABSTRACT

A rapid immunochromatographic strip (ICS) test for specific detection of lily symptomless virus (LSV) was developed. The test is based on a double-antibody sandwich format and employs two distinct anti-LSV polyclonal antibodies (IgG_1 and IgG_2). The first antibody, IgG_1 was used as the detection antibody conjugated to colloidal gold and the second antibody, IgG_2 was used to 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 LSV were tested, the LSV detection limit of the ICS test was 6.0×10^{-8} mg/mL, which was the same as the quadruplex RT-PCR assay. Relative to quadruplex RT-PCR, the specificity and sensitivity of the ICS were 98.6% and 100%, respectively for field leaf samples. There was significant agreement between the results of the ICS and quadruplex RT-PCR tests ($\kappa = 0.983$). Compared with conventional lily virus detection methods, our ICS test has many advantages: simple, fast, low cost, high sensitivity and specificity, and has applications in the laboratory and in the field to detect and control LSV.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Lanzhou lily (*Lilium davidii* var. *unicolor*) is an important edible bulb crop as well as a traditional medicinal plant with a 150-year cultivation history in the Lanzhou area of northwestern China (Wang et al., 2010). It is famous for its large size, thick white flesh, and sweet taste. It is also is an important ornamental plant because of its flaming and flamboyant coloration. The economic importance of the Lanzhou lily has increased greatly during the past decade because of the rapid increase in demand for this plant. However, cultivars of the Lanzhou lily are seriously affected by viral infections, which have decreased lily production by 50% in recent years.

Lily symptomless virus (LSV; Genus *Carlavirus*, family Flexiviridae) is the most common virus that infects Lanzhou lily (Wang et al., 2007); it has been reported in the United States, Europe, Australia, and Asia (Singh, 2005). LSV is a filamentous particle, 640 nm in length and 17–18 nm in diameter. The genomic RNA of LSV comprises 8394 nucleotides and contains six open reading frames (ORFs). The ORF5 (7140–8015 nucleotides) encodes a coat protein (CP) of 291 amino acids, and the genomic RNA of LSV is encapsulated in a single type of CP with a molecular weight of 32 kDa (Choi and Ryu, 2003). The host range of LSV is restricted to Liliaceae (Singh, 2005). It is one of the most prevalent viruses of lilies that causes quantitative and qualitative aspects of yield reduction of bulbs and flowers (Asjes, 2000).

Viral diseases represent some of the most dangerous threats to Lanzhou lily, so it is important to develop fast and effective diagnostic techniques for early detection. However, the most commonly used methods to detect viruses, including LSV in lily samples are electron microscopy (Wang et al., 2007), enzyme-linked immunosorbent assay (ELISA; Sharma et al., 2005), the polymerase chain reaction (PCR; Niimi et al., 2003; Zhang et al., 2010), and the real-time PCR (Nesi et al., 2013). However, these methods are limited by their lengthy test time, the technical expertise required, and the necessity for specialized laboratory equipment. Therefore, these methods are unsuitable for widespread use to support the production of Lanzhou lily, and it is necessary to

* Corresponding author at: Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Sciences, No. 320 Dong Gang West Road, Lanzhou 730000, China. Tel.: +86 931 496 7206; fax: +86 931 827 3894.
E-mail addresses: zyubao@yahoo.com (Y. Zhang), w.yajun@yahoo.com (Y. Wang), 13909573359@163.com (J. Meng), wxhcas@lzb.ac.cn, zyubao@yahoo.com (Z. Xie), wangruoyu@lzb.ac.cn (R. Wang), randy.kutcher@usask.ca (H.R. Kutcher), guozhhong@hotmail.com (Z. Guo).

http://dx.doi.org/10.1016/j.jviromet.2015.03.021
0166-0934/© 2015 Elsevier B.V. All rights reserved.

develop a rapid, specific, and easily performed LSV assay. The immunochromatographic assay offers several advantages over traditional immunoassays, such as its low cost, procedural simplicity, limited requirements for special skills or expensive equipment, and rapid results (Alvarez et al., 2010). This assay has thus been widely applied to detect hormones, bioactive molecules, contagious human diseases, animal and plant viruses, bacteria and parasite antigens, and their antibodies (Zhang et al., 2008; Sun et al., 2013).

The aim of the present study was to develop an immunochromatographic test strip (ICS) for rapid and accurate detection of LSV. The sensitivity and specificity of the ICS were evaluated using a quadruplex reverse transcriptase (RT)-PCR assay as a reference test. In detection trials, 120 Lanzhou lily samples from the field were analyzed using both the quadruplex RT-PCR assay and the ICS test. The results showed excellent agreement between the tests ($\kappa = 0.983$).

2. Materials and methods

2.1. Reagents and materials

Naturally infected Lily plants showing typical dwarfing, or leaves displaying chlorotic, yellow spots or stripes, or mosaic symptoms were collected in fields of the Gaolan Research Station (36°05′ N, 103°31′ E) in Lanzhou, Gansu province, China. Leaves near the flower bud were tested by RT-PCR according to the method of Zhang et al. (2010). As a result, leaves from single plants that tested positive for LSV, CMV or LMoV served as sources of LSV, CMV and LMoV viruses and were stored at −70 °C. The first antibody, IgG_1-rabbit IgG anti-native LSV and the second antibody IgG_2-rabbit IgG anti-recombinant LSV CP were produced and purified following the methods of Wang et al. (2007, 2010). Both of the antibodies were kept in aliquots of 1 mg at −20 °C for 1 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 colloidal gold-IgG_1 conjugates

Colloidal gold particles with a mean diameter of 30 nm were prepared by the method of Hermanson (2008). Under constant stirring, 1.4 mL of 1% trisodium citrate (w/v) was 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, we maintained the pH at 7.5 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 the colloidal gold-IgG_1 conjugates, IgG_1 (160 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% (w/v) bovine serum albumin (BSA) was added to block excess reactivity of the gold colloid. The mixture was then stirred for an additional 30 min. After centrifuging the mixture at 10,000 × g and 4 °C for 30 min, the gold pellets were resuspended in 1 mL of dilution buffer (20 mM Tris/HCl buffer at pH 8.2 containing 1% (w/v) BSA, 3% (w/v) sucrose, and 0.02% (w/v) sodium azide). The colloidal gold conjugate was then 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 (PBS) containing 1% (w/v) BSA, 0.5% (v/v) Tween-20, and 0.05%(w/v) sodium azide at pH 7.4, and was then dried for 2 h at 37 °C. First, a control line was formed by carefully dragging a pipette tip containing 100 μL of the goat anti-rabbit IgG (at 2.0 mg/mL) along the long axis of the nitrocellulose membrane. The tip was guided against a ruler placed 6 mm from one end of the membrane. Immediately following this procedure, the same process was repeated to generate a test line using the IgG_2 (at 1.5 mg/mL). A 5-mm gap was left between two lines. Subsequently, the membrane was allowed to dry completely for at least 2 h at room temperature and was then attached to a backing plate. An 18 mm × 5 mm absorbent pad was applied at the downstream end of the nitrocellulose membrane. This material was used as the "sink pad". Next, a 5 mm × 5 mm cellulose filter pad was impregnated with 15 μL colloidal gold-IgG_1 solution and left to dry overnight at room temperature. The resulting "conjugate release pad" together with a 20 mm × 5 mm "sample pad" was placed at the opposite end of the sink pad to complete the test strip. These strips were housed in plastic cases and then stored at 4 °C under desiccated conditions until use.

2.4. The immunochromatographic assay

If the sample tested contained LSV, the LSV reacted with IgG_1 conjugated to colloidal gold. The complex IgG_1-LSV-gold then migrated into the nitrocellulose membrane by capillary action and subsequently reacted with the immobilized IgG_2 in the test line. Unbound IgG_1-colloidal gold particles ran over the test line and reacted with the goat anti-rabbit IgG at the control line to form a second visible purple–red band.

About 100 μL of the tested sample was applied to the plastic cassette sample window. The result was read between 5 and 10 min after the addition of the sample. The sample was considered positive if two distinct purple-red lines appeared, one in the test region and the other in the control region; negative when no line appeared in the test region, and invalid if the control line failed to appear.

2.5. Specificity and sensitivity of the ICS test

To evaluate the cross-reactivity of the ICS test strip, we tested LSV and two other common lily viruses (cucumber mosaic virus, CMV, and lily mosaic virus, LMoV) from field samples collected from the Gaolan Research Station, using the ICS. LSV positive leaves were used as a positive control and virus extraction buffer (0.2 M Tris/HCl buffer (pH 7.5) containing 10 mM EDTA-Na_2, 0.1% (w/v) polyvinyl polypyrrolidone and 0.1% (v/v) 2-mercaptoethanol) was used as a negative control. The sensitivity of the ICS test was evaluated by testing a series of 10-fold dilutions of the purified LSV solution (at 6.0×10^{-1} mg/mL), which was analyzed as virus antigen by means of western blotting using the IgG_2 protein at a concentration of 2.0×10^{-2} mg/mL. Each dilution was then added to an ICS test, and the sensitivity was determined from the end-point dilution.

In addition, we determined the specificity and the sensitivity of the ICS test compared with the quadruplex RT-PCR assay (Zhang et al., 2010). Briefly, we used four pairs of primers (Table 1) simultaneously to detect LMoV, LSV, CMV, and the lily 18S

Download English Version:

https://daneshyari.com/en/article/6133252

Download Persian Version:

https://daneshyari.com/article/6133252

Daneshyari.com