Crop Protection 61 (2014) 51–57

Contents lists available at ScienceDirect

Crop Protection

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

Modification of serological techniques and their evaluation for detection of potato viruses in seed certification related activities

Jamil Samsatly, Maan Jawhari, Carole Najjar, Hana Sobh, Yusuf Abou-Jawdah*

Department of Agricultural Sciences, American University of Beirut, Riad El-Solh, Beirut 1107 2020, Lebanon

ARTICLE INFO

Article history:
Received 30 August 2013
Received in revised form
6 March 2014
Accepted 8 March 2014

Keywords:
Virus detection
Multiplexing
Tissue print immunoassay
Dot blot immunoassay
ELISA

ABSTRACT

Development of alternative serological techniques to ELISA for detection of potato viruses offers advantages for monitoring virus incidence and for seed potato certification systems. Several trials showed that multiplex tissue print immunoassay (TPIA) and dot blot immunoassay (DBIA) might represent fast, practical, and sensitive alternatives for the detection of: *Potato leaf roll virus* (PLRV), *Potato virus S* (PVS), *Potato virus X* (PVX) and *Potato virus Y* (PVY), from green and/or tuber tissues. In TPIA, the specific precipitation patterns in infected tissues of leaf petioles or stem cross sections, observed with each virus, allowed identification of the specific virus or mixed infections in a single multiplex assay. For detection of PVY in green tissues, DBIA was shown to be over 50 times more sensitive than ELISA. TPIA and ELISA from the tuber stem end or from eyes might be used for rapid detection of PVY and PVS in seed potato tubers without prior germination. PVS was evenly distributed in potato tuber tissue, while PVY was localized in the vascular tissue beneath the epidermis, with irregular distribution along the periphery of the potato tuber. For laboratories in developing countries lacking time and facilities for tests based on tuber germination, monitoring for PVS and PVY using TPIA in tuber tissue may be a suitable alternative to ELISA.

1. Introduction

Potato is the fourth major world food crop after corn, rice and wheat. Total world potato production is about 368 million tons cultivated on over 19.3 million ha (FAOSTAT, 2012). The transmission of viral diseases through seed potato has led to decline in potato yield year after year (Khurana, 1992; Bhat et al., 2010). Since potato is a vegetatively propagated crop, the quality of seed potato plays a major role for the sustainable production of good quality and high yield crop. Most countries have enacted legislations and certification schemes that set tolerances for accepted incidences of viruses and other damaging diseases (Callison et al., 1982; Lund and Sun, 1985; Ioannou and Vakis, 1988; Gudmestad, 1991). The continued increase in average yield per unit area may be partially correlated with the development of efficient seed certification systems in major production countries. Depending on the country, the tolerance to major potato viruses in certified seed potato varies between 1 and 5% (British and Wisconsin seed potato certification schemes, 2006 and 2007, respectively). The major viruses to be monitored are *Potato leaf roll virus* (PLRV), *Potato virus A* (PVA), *Potato virus M* (PVM), *Potato virus S* (PVS), *Potato virus X* (PVX) and *Potato virus Y* (PVY) (Salazar, 1996).

Most potato viruses can be detected by ELISA (Petrunak et al., 1991), which is the most popular technique that has been used in seed potato certification systems and in quarantine labs (Casper, 1979; Vetten et al., 1983; Singh and Somerville, 1983, 1986; Maat and de Bokx, 1978; Petrunak et al., 1991). However, new advanced techniques were developed using nucleic acid hybridization (Singh et al., 1999; Hsu et al., 2005; Agindotan and Perry, 2007, 2008), multiplex RT-PCR (Singh et al., 2000; Nie and Singh, 2000, 2001; Du et al., 2006; Khan et al., 2009; Bostan and Elibuyuk, 2010), real-time PCR (Agindotan and Perry, 2007, 2008), macroarrays (Agindotan et al., 2007) and microarrays (Zhang et al., 2010; Nicolaisen, 2011). While most of these techniques are highly sensitive, they are relatively expensive and are preferably used to screen relatively small sample sizes. Several studies have proved that tissue print immunoassay (TPIA) is a suitable technique for use in field surveys for detection of viruses in several crops (Lin et al., 1990; Abou-Jawdah et al., 2008). Although Samson et al. (1993) reported that TPIA is superior to ELISA in several criteria, only a limited number of papers were recently published on the detection of potato viruses using TPIA (Whitworth et al., 1993; Krzymowska and Hennig, 1997). In 1993, TPIA was shown to be as accurate as ELISA for potato virus detection whether from stems, petioles, leaves, or tubers. Figures of the precipitation patterns obtained by

* Corresponding author. Tel.: +961 1350000.
E-mail address: abujawyf@aub.edu.lb (Y. Abou-Jawdah).

http://dx.doi.org/10.1016/j.cropro.2014.03.007
0261-2194/

TPIA were only presented for PLRV (Whitworth et al., 1993; Samson et al., 1993) or PVY in a cross-section of potato stem (Krzymowska and Hennig, 1997), and the localization of potato viruses in potato tissues were mostly done using electron microscopy (Ramajäki and Valkonen, 2002, 2003; Kogovšek et al., 2011). The ability to reliably detect potato viruses by dot blot immunoassay (DBIA) with an equal sensitivity to that of DAS-ELISA was also reported (Banttari, 1985; Weidemann, 1988; Lizarraga and Fernandez-Northcote, 1989; Palma et al., 2013).

Testing for potato viruses can be conducted in the production fields, or on seed potato. For testing imported seed potato, the recommended method is to incubate potato tubers until they germinate and use the sprouts to test for virus incidence. Many laboratories in developing countries and in remote areas may lack the facilities to conduct such tests. This paper evaluates TPIA and DBIA for detection of the four potato viruses in seed potato fields and seed potato lots as a practical and simple alternative diagnostic technique to ELISA.

2. Materials and methods

2.1. Plant material and serological kits

Potato tissue samples were collected from healthy plants or plants singly infected with PVY, PVX, PLRV, or PVS or mixtures of these viruses; the plants were grown in pots or in soil in insect-proof greenhouses. For virus detection in seed potato, potato tubers were collected from three commercial potato fields showing a high incidence of virus infection by visual inspection. The fields are located in the Bekaa plain, the major potato production area in Lebanon. Serological kits for detection of the four potato viruses were purchased from Agdia (USA), Adgen Phytodiagnostics (UK), or BioReba (Switzerland).

2.2. Virus detection by TPIA

Several TPIA assays were conducted to evaluate the precipitation pattern of each of the four viruses. In the first assay, cross sections from both stem and leaf petiole of potato tissues infected singly with one of the four viruses were tested. In another assay, multiplex TPIA was conducted on potato tissues infected either with one of the four viruses or with mixed infections using a mixture of the four antibodies for PVY, PVS, PVX, and PLRV. An assay was also conducted to check the practicality of multiplex TPIA for screening a large number of samples and its sensitivity was compared to ELISA. For this purpose, thirty samples of infected or healthy potato leaves were collected and tested for the presence of the four viruses using ELISA and multiplex TPIA, concurrently.

For TPIA, leaf petioles or stems were cross-sectioned and pressed for few seconds on Hybond-N $^{+}$ nylon membranes or nitrocellulose membranes (GE Healthcare Life Sciences, UK). Membranes were blocked at 37 °C for one hour with Phosphate-Buffered Saline with Tween 20 (PBS-T) containing 5% skimmed milk (Regilait, Saint Martin Belle Roche, France) plus 1% bovine serum albumin (Fraction V, reagent grade, BIOREBA AG, Reinach, Switzerland). The membranes were incubated with single antibodies or a mixture of antibodies for PVY, PVX, PLRV and PVS. Membranes were then incubated at room temperature for an hour in alkaline phosphatase-conjugated goat anti-rabbit or anti-sheep IgGs, (Sigma, Germany) depending on the source of primary antibody (1:30,000). After each step, membranes were washed three times with PBS–Tween 20 (PBS–T). For colorimetric detection, membranes were incubated in a freshly prepared substrate of Nitroblue tetrazolium (NBT, 0.35 mg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP, 0.18 mg/ml) (Promega, USA), in 0.1 M Tris buffer, pH 8.5, containing 0.1 M NaCl and 0.1 M $MgCl_2$. The reaction was stopped by addition of distilled water. The dried membranes were examined for color development visually and under the stereoscope.

2.3. Sensitivity of detection by DBIA and comparison to ELISA (PVY as an example)

Samples collected from field surveys were screened for the presence of the four viruses using ELISA. Leaf samples were collected from PVY, PVX, PLRV or PVS infected potato leaves. Serial dilutions of leaf extracts from positive and negative samples were tested for the four viruses by DBIA and only for PVY by ELISA. About 1 g of leaf tissue was ground in 9 ml of 0.3 M Tris-HCl buffer pH 7.4 containing 137 mM NaCl, 3 mM KCl, 2% PVP, 0.5% sodium sulfite and 0.05% Tween 20. In DAS-ELISA tests, aliquots of 100 μl leaf extracts were applied in duplicate wells, while for DBIA, 2 μl were blotted on nylon membranes. Extracts from healthy samples were used as negative controls. DAS–ELISA was performed as described by Clark and Adams (1977). The antibodies and conjugated antibodies were used at the concentrations recommended by the manufacturer. The substrate (1 mg/ml p-nitrophenyl phosphate in diethanolamine buffer, pH 9.8) was added and incubated at room temperature for 1–3 h. The color reaction was monitored by measuring absorbance at 405 nm, using a microplate ELISA reader (Multiskan Ascent, Thermo Labsystems, Finland). A reaction was considered positive when the mean absorbance value for a sample was greater than three times the mean value of healthy controls. All serological tests were repeated at least three times. For DBIA, membranes were blocked and processed as mentioned above for TPIA. However, for DBIA, in addition to colorimetric detection, chemiluminescent detection was also attempted using the CDP-star substrate (Amersham Biosciences, UK) diluted (1:1) in water and applied to the membranes. The best exposure time of X-ray films (AGFA CP-BU, Medical X-ray film blue; Agfa-Gevaert N.V, Morstel, Belgium) was usually about 20 min.

2.4. Serological detection of potato viruses in seed potato lots (tubers)

A total of 530 tubers collected from potato fields, in 2008–2009 (230 tubers, Trial I) and 2009–2010 (300 tubers Trial II), were maintained at 4 °C for about two months then transferred to room temperature for a few days. All tubers were tested for PVY, PVX, PLRV, and PVS. Relatively large size tubers with more than four eyes were used. Each tuber was marked and used for comparison of the three virus detection methods from three different tissues: stem end parts, eyes and sprouts. The stem end part or three eyes were cut with the surrounding tissue and extracted as described above for ELISA; the extracts were tested by DAS-ELISA and by DBIA. Cross sections from these tissues were also tested by TPIA. For sprouts, the tubers were incubated in a growth chamber at 25 °C and 80% RH, when the sprouts reached over 2–3 cm in length they were tested by DAS-ELISA, TPIA and DBIA.

SPSS v.17 (SPSS Inc., Chicago IL) computing statistics was used to analyze and compare detection techniques and the types of tuber tissues. The comparison of frequencies was performed among the different treatments by CHI-square. Significant differences in the means and frequencies are reported at a $p = 0.05$.

3. Results

3.1. Serological detection of potato viruses from leaf or stem tissue

3.1.1. Virus detection by TPIA

Under experimental conditions, the TPIA technique delivered results within 4–5 h. Color development usually took about 30–