Phytopathol. Mediterr. (2002) 41, 275–278

Low-cost paper can be used in tissue-blot immunoassay for detection of cereal and legume viruses

KHALED M. MAKKOUK and SAFAA G. KUMARI

Virology Laboratory, Germplasm Program, International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria

Summary. In an effort to reduce the cost of virus assays, different types of regular paper were evaluated as possible replacements for the commonly used nitrocellulose membrane (NCM) as the solid phase in the tissue-blot immunoassay (TBIA) used to detect *Alfalfa mosaic virus*, *Bean yellow mosaic virus* and *Broad bean stain virus* (BBSV) in faba bean tissue, and *Barley yellow striate mosaic virus* in barley tissue. Among the many types of paper evaluated, Hewlett-Packard (HP) non-glossy plotter paper proved to be adequate for detection of all the above viruses. After printing (blotting) of the samples to be tested and blocking with either 2% gelatin (for one hour at 37°C) or using 0.1% Roche blocking reagent (for one hour at room temperature), satisfactory results were produced. This paper could also be used to detect BBSV in groups consisting of 15 young lentil seedlings. HP non-glossy plotter paper was not as effective when testing for phloem-limited legume viruses such as *Bean leaf roll virus* (BLRV), *Faba bean necrotic yellows virus* (FBNYV) and *Barley yellow dwarf virus* (BYDV). White paper (manufactured by Soporcel, Portugal) was slightly more sensitive when used for BLRV and FBNYV detection in faba bean tissues and for BYDV in barley tissues. Since NCM represents 40–50% of the cost of test reagents, using ordinary paper reduced costs considerably.

Key words: TBIA, regular paper, viruses, serology.

Introduction

To control virus diseases it is necessary to know the identity of the viruses involved, their ecology and epidemiology, and to have practical methods to detect these viruses in different plant tissues, including seed. Tests for detecting viruses should be simple, sensitive, reliable, and inexpensive. It is not always possible, however, for a single test to have all the above features.

The development of enzyme-linked immunosorbent assay (ELISA) for plant viruses in 1977 (Clark and Adams, 1977) was a significant improvement in this field, making virus testing easier and also improving its sensitivity. Since that time, significant efforts have been made to improve the procedure still further. Tissue-blot immunoassay (TBIA), has, over the last decade, proved a reliable, fast and sensitive test for the detection of many viruses (Lin *et al*., 1990; Hsu and Lawson, 1991; Makkouk and Comeau, 1994; Makkouk and Kumari, 1996). The simplicity of this test, not needing sophisticated and expensive equipment (e.g. an ELISA reader) makes it the test of choice for many laboratories, especially in developing countries. The most important feature of TBIA is that it does not require sample extraction, a very time-consuming step needed in other procedures.

Corresponding author: K. Makkouk
Fax: +963 21 2213490
E-mail: K.Makkouk@cgiar.org

To reduce the cost of TBIA further, the use of regular paper as a replacement for expensive nitrocellulose membrane (NCM) as the solid phase in TBIA was evaluated for the detection of a number of general and phloem-limited legume and cereal viruses.

Materials and methods

Virus cultures

This evaluation used faba bean plants experimentally infected with *Alfalfa mosaic virus* (AMV, genus *Alfamovirus*, family *Bromoviridae*), *Bean yellow mosaic virus* (BYMV, genus *Potyvirus*, family *Potyviridae*), *Broad bean stain virus* (BBSV, genus *Comovirus*, family *Comoviridae*), *Bean leaf roll virus* (BLRV, family *Luteoviridae*) and *Faba bean necrotic yellows virus* (FBNYV, genus *Nanovirus*, family *Nanoviridae*). Lentil seedlings experimentally infected with BBSV, and barley plants experimentally infected with *Barley yellow striate mosaic virus* (BYSMV, genus *Cytorhabdovirus*, family *Rhabdoviridae*) and *Barley yellow dwarf virus-PAV* (BYDV-PAV, genus *Luteovirus*, family *Luteoviridae*) were also used. Healthy faba bean, lentil and barley plants were used as controls.

Antisera used

Rabbit polyclonal antisera for AMV, BYMV, BBSV, BYSMV and BYDV-PAV were used; these were obtained from the Virology Laboratory of International Center for Agricultural Research in the Dry Areas, Aleppo, Syria. In addition, two monoclonal antibodies for FBNYV and BLRV (4B10) were provided by A. Franz and L. Katul, Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA), Braunschweig, Germany.

Types of paper

Various types of regular paper were evaluated as replacements for NCM (0.45 µm) as the solid phase in TBIA: (i) 'HP non-glossy plotter' paper (made by Hewlett-Packard, Palo Alto, CA, USA, Cat. No. 17803P), (ii) white paper, 80 g m^{-2} (made by Soporcel, Lavos, Portugal), (iii) photocopy paper, 80 g m^{-2} (manufactured by Excell Laser Copy, Indonesia), and (iv) filter paper (Whatman No. 1; Maidstone, UK, Cat. No. 1001090). Three different brands of standard photocopy paper available in Aleppo, Syria, were also evaluated.

Blocking reagents

Five blocking reagents were evaluated: (i) 2% gelatin solution (Sigma, St. Louis, MO, USA, Cat. No. G-9382) for 1 h at 37°C, (ii) 1% I-Block (Tropix System, Bedford, MA, USA), (iii) 0.1% blocking reagent (Roche, Manneheim, Germany, Cat. No. 1096176), (iv) 3% bovine serum albumin (BSA) (Sigma, Cat. No. A-4503) for 1 h at room temperature, and (v) 1 µg ml^{-1} polvinyl alcohol (Sigma, Cat. No. P-8136) for 1 min at room temperature. PBS-Tween was the buffer used with all the above blocking agents.

TBIA

The TBIA procedure followed was that of Makkouk and Comeau (1994) with the following steps and washes in between: (1) cutting the plant samples (leaves, petioles, stems etc.) with a razor blade and immediately blotting the cut surface on NCM or paper, (2) soaking the NCM/paper placed in a plastic container with a blocking agent, depending on the blocking reagents used (see above), (3) soaking the NCM/paper with the virus-antibody solution for 1 h, (4) adding the antibody-alkaline phosphatase conjugate and leaving it to stand for 1 h and (5) adding the enzyme substrate mixture of nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate. The enzymatic reaction was terminated after 15–30 min by rinsing the NCM/paper in distilled water, and air-drying it before reading.

Results and discussion

Filter and photocopy papers were found to be very delicate, and had to be handled with great care during processing. Among the many types of paper evaluated, HP non-glossy plotter paper and Soporcel white paper proved to be adequate for the detection of systemic and phloem-limited viruses. Highly sensitive detection of BBSV, AMV and BYMV in faba bean stem and BYSMV in barley tissue was possible with both HP non-glossy plotter paper and Soporcel white paper (Fig. 1). However, detection of BLRV and FBNYV in faba bean stems and BYDV in barley stems was weak with HP non-glossy plotter paper and was slight-

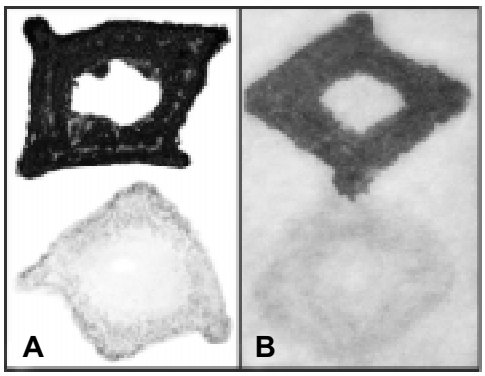

Fig. 1. Detection of *Alfalfa mosaic virus* (AMV) by TBIA in infected faba bean tissue blotted on nitrocellulose membrane (A) and on HP non-glossy plotter paper (B). In all duplicate stem blots, the healthy tissue is at the bottom of the picture and the AMV-infected tissue is at the top.

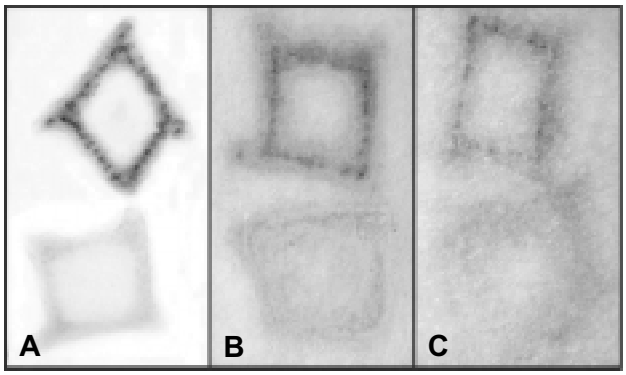

Fig. 2. Detection of *Faba bean necrotic yellows virus* (FBNYV) by TBIA in phloem vessels of infected faba bean tissue blotted on nitrocellulose membrane (A), Soporcel white paper (B) and HP non-glossy plotter paper (C). In all duplicate stem blots, the healthy tissue is at the bottom of the picture and the FBNYV-infected faba bean tissue at the top.

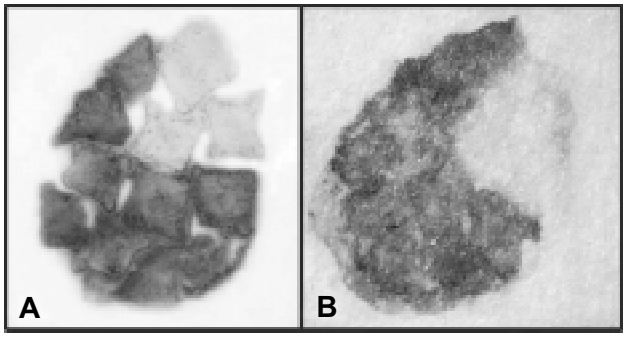

Fig. 3. Detection of *Broad bean stain virus* by TBIA in a group of 12 infected lentil seedlings, blotted together as one sample on nitrocellulose membrane (A) and on HP non-glossy plotter paper (B).

ly better with Soporcel white paper (Fig. 2). Moreover, it was possible to use HP non-glossy plotter paper for detecting BBSV in groups of 12–15 young lentil seedlings (Fig. 3).

Four blocking solutions, gelatin (used for 1 h at 37°C), I-Block (Tropix), Roche blocking reagent, and BSA (used for 1 h at room temperature), were all equally effective in producing a clean background on the paper. However, the fifth blocking solution, polyvinyl alcohol (which is an adequate blocking agent when used with NCM), did not work well with HP non-glossy plotter paper or Soporcel white paper. Sensitivity of virus detection was greatest when gelatin was used. This was also more economical, because gelatin is cheaper than the other blocking reagents tested.

NCM is relatively expensive and represents 40–50% of the TBIA reagents cost, which limits its use in large-scale testing programmes, especially in developing countries. Using HP non-glossy plotter paper or Soporcel white paper reduced test costs considerably. By our calculations, the cost per faba bean sample when using NCM was around 10 US cents, but only 6 US cents when ordinary paper was used. This saving should encourage a wider use of the TBIA technique, especially in large–scale testing programmes. The sensitivity of the test and the distinctness of the reaction were slightly reduced when HP plotter paper was used instead of NCM. However, such disadvantages do not much affect the results of large–scale testing programmes. At locations where either the cost or the availability of NCM is a limiting factor, TBIA can therefore still be conducted, using a cheap and widely available product. It is hoped that the results obtained in this study will encourage workers to evaluate other paper brands for their suitability in TBIA. Further evaluation for virus detection in naturally infected plants is needed, as virus concentration in such plants are often low and non-specific background reactions sometimes pose problems.

Literature cited

Clark M.F. and A.N. Adams, 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34, 475–483.

Hsu H.T. and R.H. Lawson, 1991. Direct tissue blotting for

detection of tomato spotted wilt virus in *Impatiens*. *Plant Disease* 75, 292–295.

Lin N.S., Y.H. Hsu and H.T. Hsu, 1990. Immunological detection of plant virus and a mycoplasmalike organism by direct tissue blotting on nitrocellulose membrane. *Phytopathology* 80, 824–828.

Makkouk K.M. and A. Comeau, 1994. Evaluation of various methods for the detection of barley yellow dwarf luteovirus by the tissue-blot immunoassay and its use for BYDV detection in cereals inoculated at different growth stages. *European Journal of Plant Pathology* 100, 71–80.

Makkouk K.M. and S.G. Kumari, 1996. Detection of ten viruses by the tissue-blot immunoassay (TBIA). *Arab Journal of Plant Protection* 14, 3–9.

Accepted for publication: October 11, 2002