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Testing seeds for viruses by Dot Immuno Binding (DIB) directly on plain paper

Påvisning af virus i frø ved Dot Immuno Binding (DIB) direkte på almindeligt papir

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Summary

The dot immuno binding (DIB) technique has been simplified so that it can be performed on plain paper. The usefulness of the technique is demonstrated by detecting the seed-borne viruses Pea seed-borne mosaic (PSbMV), Pea early browning (PEBV), Squash mosaic (SqMV), Bean common mosaic (BCMV) and Barley stripe mosaic (BSMV) directly from seed. This DIB technique does not require specialized equipment and is suitable for use in seed testing stations and quarantine services in industrialized as well as in developing countries.

Key words: Seed-borne viruses, serological testing.

Resumé

Dot Immuno Binding (DIB) metoden er blevet forenklet, så den kan udføres på selv almindeligt papir. Metodens anvendelighed demonstreres ved, at den benyttes til at påvise de frøbårne virus: ærte brunsotvirus, frøbåren ærmemosaikvirus, almindelig bønnemosaikvirus, græskarmosaikvirus og bygstribemosaikvirus direkte fra frø.

DIB-metoden forudsætter ikke rådighed over specialiseret teknisk udstyr. Metoden er direkte anvendelig i forbindelse med frøkontrol og plantetilsyn i såvel industrialiserede som udviklingslande.

Nøgleord: Frøbårne virus, serologisk test.

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Introduction

Recently, improved methods for detection of viruses in seed and other plant materials have been published (reviewed in 3, 4, 5). The least demanding of the techniques is the DIB test as it can be done with crude, specific antiserum and a single general enzyme conjugate for all viruses tested. Moreover, the test can be scored with the unaided eye (5).

The present paper reports on further improvements and simplifications of the DIB technique for detection of virus infections directly from seed.

Materials and methods

The DIB procedure was carried out with five different seed-borne viruses on various types of paper and on nitrocellulose membranes (Fig. 1). The following seed samples were used: garden pea (*Pisum arvense* L.) infected with pea seed-borne mosaic virus (PSbMV) or with pea early browning virus (PEBV), French bean (*Phaseolus vulgaris* L. cv. Pinto 111) infected with bean common mosaic virus (BCMV strain N.Y. 15), barley (*Hordeum vulgare* L. cv. Prentice) infected with barley stripe mosaic virus (BSMV) and squash (*Cucurbita melo* L.) infected with squash mosaic virus (SqMV). Healthy seed samples of each cultivar were used as controls.

The titers of the antisera used were between 1:100 and 1:300 as determined by the microprecipitin test and were used as whole sera diluted 1:500 in TST buffer (Tris saline Tween buffer; 0.05 M Tris HCL, 0.5M NaCl, 0.5%. Tween 20, pH 10.3). The alkaline phosphatase conjugated swine anti-rabbit gamma globulin was used in a dilution of 1:1000 in TST.

The seed samples were ground to a powder in an electric (or manual) coffee mill and homogenized in 1:10 (w/v) PBS (phosphate buffered saline; 0.05M Na_2HPO_4, 0.4M NaCl, pH 7.0) in a mortar. After settling, 5 μl aliquots of the supernatant were loaded onto various types of paper or nitrocellulose membranes (types and sources listed in Fig. 1). After drying at room temperature, the loaded membranes or papers were incubated in a blocking solution (5% (v:v) of horse serum in TST buffer) for 30 min, rinsed in TST buffer, and then incubated in the crude, specific antiserum (diluted 1:500 in TST buffer in which 1% horse serum was added) either for 2 hrs at 38°C or overnight at 4°C. The papers or membranes were then rinsed and submerged in the enzyme conjugate (diluted 1:1000 in TST), incubated as the primary antiserum, rinsed again under running tap water or in TST and rinsed in ethanolamine buffer (0.1M, pH 9.6).

Staining was done with nitroblue-tetrazolium in ethanolamine buffer according to the description of *Blake et al.* (2), and was stopped after 10–15 min by rinsing in distilled water.

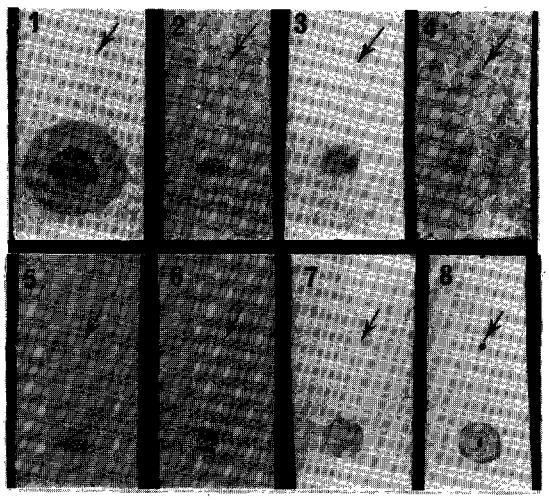

Fig. 1. Strips of eight different types of papers or nitrocellulose membranes used for detection of squash mosaic virus (SqMV) in squash seed suspension. Arrows indicate healthy controls. 1) Whatman No. 1 filter paper; 2) note pad paper (405 H, BICP, Glostrup, Denmark; 3) art paper (150 g/m^2); 4) air mail paper (45 g/m^2); 5) typing paper (bank post, 80 g/m^2); 6) xerographic paper (80 g/m^2) – Papers 3–6 available from Borch Papir, Brøndby, Denmark; 7) nitrocellulose membrane, Schleicher & Schüll, BA 85; 8) nitrocellulose, Agdia, No. 481.

Strimler af otte typer papir samt nitrocellulosemembraner brugt til påvisning af græskarmosaikvirus (SqMV) direkte fra en suspension af knuste græskarfrø. Pilene angiver sundt græskarfrø. 1) Whatman No. 1 filterpapir; 2) Linieret skrivepapir (405 H, BICP, Glostrup Danmark), 3) tegnepapir (150 g/m^2; 4) luftpostpapir (45 g/m^2); 5) skrivemaskinepapir (Bank post, 80 g/m^2); 6) Fotokopieringspapir (80 g/m^2) – papirtyperne 3–6 forhandles af Borch Papir, Brøndby, Danmark; 7) Nitrocellulosemembran, Schleicher & Schüll, BA 85; 8) Nitrocellulosemembran, Agdia, No. 481.

Results

The various papers used for the DIB procedure all gave significantly higher signal from diseased material as compared to healthy seed samples of peas, beans, squash and barley. Fig. 1 illustrates the results obtained with SqMV from squash seed. Similar results were obtained for pea seed infected with PEBV and PSbMV, French bean seed infected with BCMV and barley seed infected with BSMV. The most important information in this figure is not which paper is optimal but rather that various sorts of papers can be used with good results. The reaction observed on the nitrocellulose sheets is very distinct, but the reaction on the filter paper is the most prominent. The small dot size which develops on typing paper has the advantage that it will allow incorporation of many tests on small test strips.

The volume of specific antiserum, after dilution, needed for 100 tests made on typing paper is 10 ml; 100 tests carried out on nitrocellulose require 12 ml, and filter paper (Whatman No. 1) require 20 ml. Similar figures are also obtained for the volume of enzyme conjugate and substrate solution required.

The nitrocellulose sheets and the very light weight papers (e.g. air mail paper) were very delicate and fragile and had to be handled with great care during processing and some discoloration took place (Fig. 1).

In further experiments a simplified DIB procedure in which the specific antiserum was added directly to the blocking solution was used successfully.

Discussion

The present results show that the DIB method carried out on even plain paper is suitable for detection of PEBV, PSbMV, BCMV, BSMV and SqMV, representing flexuous, rod shaped and spherical virus particles in seed. Coating the typing paper with formvar or collodion was not found to improve the results (*Lange* and *Heide*, unpublished).

Of the various kinds of papers tested, typing paper (bank post, 80 g/m^2) (No. 5 in Fig. 1) was found to represent the best compromise between sensitivity, low reagent requirements and easy handling.

The sensitivity of the DIB technique has been characterized as being comparable to the sensitivity of ELISA (1, 6, 7). Experience with DIB detection of potato viruses in green leaves confirmed this (3). The results obtained with seed suspensions made directly from the dry seeds indicate a somewhat lower sensitivity (5).

In plant quarantine testing, infected reference material is often not available. The use of the DIB procedure in seed health testing along with the acquisition of infected samples, dried down and immobilized on paper, may represent a possible solution to this problem without violating quarantine regulations.

The promising aspect of the DIB test described here is that it is very rapid and can be carried out without the use of specialized equipment. By using the DIB technique on plain paper, testing for seed-borne viruses can be done in seed testing stations, quarantine services and in production units for monitoring micro-propagated plants in industrialized as well as in developing countries.

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