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A simple kit for rapid field diagnosis of potato virus Y by latex serological test

AARNE KURPPA and MATTI VUENTO

Department of Plant Pathology, Agricultural Research Centre
SF-31600 Jokioinen, Finland

Department of Biochemistry, University of Helsinki
Unioninkatu 35, SF-00170 Helsinki, Finland

Abstract. A simple kit for rapid detection of potato virus Y by latex serological test was developed. The test is carried out on a white cardboard sheet and the results can be read by naked eye in two minutes. A test card of 10 × 6 cm holds latex sensitized antibodies, buffers and other necessary ingredients as dry blue colored formulate on the ringed areas of the card. A test card includes space for six tests and positive and negative controls. The kit also includes disposable plastic sticks for mixing the samples with test reagents and a hand press with disposable plastic tips.

For testing, dried reagents are dissolved in drops of sample and mixed. After gentle rotation, samples containing virus appear clearly granulated while samples from healthy plants remain unagglutinated.

The testing of undiluted extracts of evenly developed tuber sprouts resulted in over 91 % identity with the results obtained with ELISA that was used as a control method. Testing of diluted leaf extracts reached the same reliability but undiluted leaf extracts from glasshouse grown potatoes were not well suitable as test samples because of their dark green color. No such problems occurred with field grown material and a complete identity with the ELISA readings was true when the samples included secondarily infected potato plants.

No reaction to other potato viruses than PVY was obtained by the test kit.

Index words: virus detection, serology, latex test, ELISA, potato virus Y, PVY

Introduction

Serological diagnosis of plant viruses has developed drastically during the past decade, particularly with the introduction of labelled antibody techniques, especially the ELISA (enzyme-linked immunosorbent assay, CLARK and ADAMS 1977). Also, the latest research interest in developing more advantageous serological techniques has mainly focused on labelled antibody techniques originating in medical immunology. However, interest in the

study of essentially simpler but sufficiently reliable tests has remained limited.

The ELISA has a number of advantageous properties over the methods previously used for routine detection of plant viruses, above all high sensitivity and specificity, and it is easy to be applied in any moderately equipped research laboratory. However, it cannot be used for field diagnosis, and it takes one to one and a half days to have the results.

At present, the ELISA is the only relatively rapid test method for the detection of potato virus Y (PVY). Other more traditional techniques such as the agglutination test, microprecipitin test (van SLOGTEREN 1957), and radial immunodiffusion test (SHEPARD 1972, RICHTER et al. 1979 a), are not sensitive enough for the routine detection of PVY in potato tubers or leaves.

The latex serological test (BERCKS 1967, ABU SALIH et al. 1968) offers potential sensitivity for the detection of barley yellow dwarf virus in plant extracts (AAPOLA and ROCHOW 1971). However, the test is too complicated with its preparatory steps, and thus has not aroused sufficient interest as a routine technique. The latest attempts of developing latex serological test kits for practical diagnosis of PVY (TALLEY et al. 1980, WIEDEMANN and FUCHS 1983, FRIBOURG and NAKASHIMA 1984) have been promising, but the tests are still to laborous or complicated for easy diagnosis in the field or on the farm.

The aim of this study was to develop a simple but highly sensitive and reliable test kit for the extremely rapid detection of potato virus Y by latex serological principle.

Materials and methods

Antibody production and characterization

For the production of PVY specific antibodies for the test kit, a Y^n strain isolate F43 was selected for an antigen. This isolate is serologically closely related to the isolate YSF15 (KURPPA 1983, KURPPA and KORHONEN 1984) and is thus suitable for raising up antibodies to recognize all isolates of potato virus Y found in Finland.

The virus was propagated in *Nicotiana tabacum* cv. Samsun and purified with a slightly modified method of RICHTER et al. (1979 b). The final purification step was density gradient centrifugation in nuclease-free sucrose gradients (5—35 %) prepared in 0.1 M borate buffer at pH 8.2. The purified material showed a single polypeptide band having a mol. wt. of 33 000 when analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate (SDS-PAGE, LAEMMLI 1970). This value, although higher than that calculated from the amino acid sequence (SHUKLA et al. 1986), agrees well with the values obtained by other investigators using SDS-PAGE (deBOKX 1981, ARUTA 1983). The preparations had A_{260}/A_{280}-ratio of 1.13—1.23, which is well in line with the value previously described for the purified virus (deBOKX 1981). Electron microscopy of the purified virus was carried out after negative staining with phosphomolyblic acid. It revealed long, thin flexible structures characteristic of potato virus Y (Fig. 1).

Rabbits were immunized by subcutaneous injections of purified virus (100 μg) emulsified in Freund's incomplete adjuvant. The injections were given on days 0, 21 and 35, and blood was collected on day 44. Sera showing good titres (below) were pooled and immunoglobulin fraction was precipitated with ammonium sulphate at a 50 % saturation. The precipitated immunoglobulins were dialyzed against 0.9 % NaCl and stored at —20°C. For the characterization of antibodies in the ELISA, immunoglobulins were separated from the antisera using columns of protein A-Sepharose CL 4B and Sephadex G-25 (Pharmasia). Immunoglobulin fractions were used at concentrations of 0.25, 1 and 4 μg/ml for coating the plates. Comparative dilutions from enzyme conjugates of Y^n and Y^o strain antibodies were made for the test. Test samples included known isolates of PVY in potato leaf extracts, and the double sandwich procedure of CLARK and ADAMS (1977) was used.

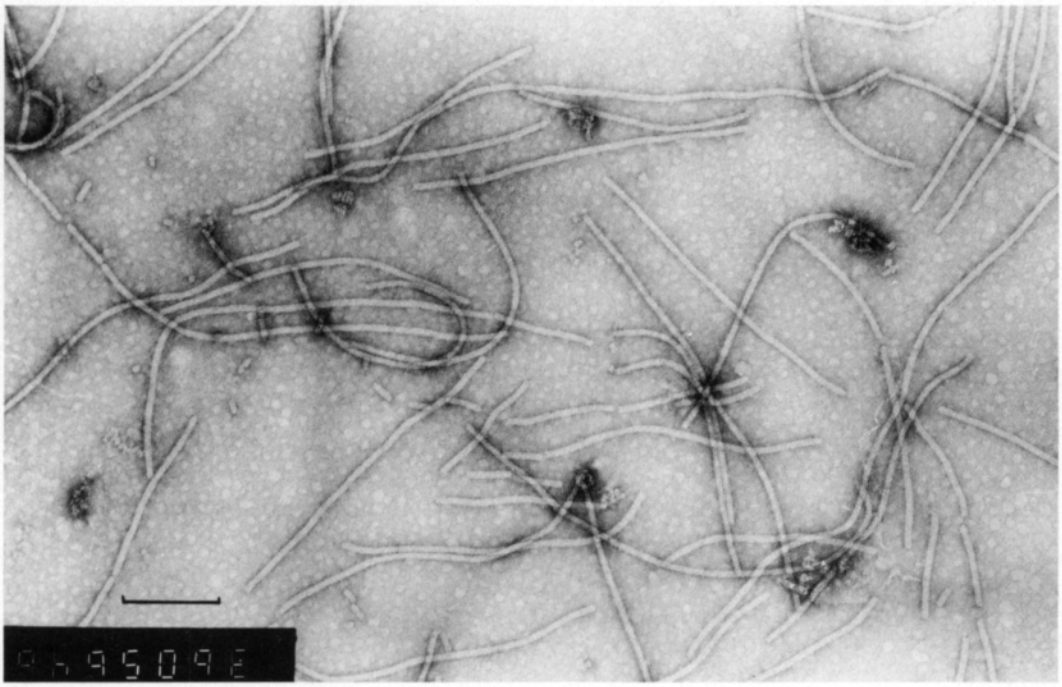

Fig. 1. Electron micrograph of purified particles of PVY^n strain F43 negatively stained with 1 % uranyl acetate. Bar represents 200 nm.

Preparation of latex reagent and test card

For preparation of test latex reagent, polystyrene latex particles (diameter 0.8 μm, 1 % final concentration) were incubated with anti-potato virus Y antibodies at varying concentrations in 0.1 M-glycine buffer pH 8.2 for 2 h at 37°C. After the incubation, bovine serum albumin was added to a concentration of 0.1 %. The latex reagent was sedimented by centrifugation and resuspended in the above buffer containing 0.1 % albumin. This washing procedure was repeated twice. Control latex reagent was prepared as above but with immunoglobulins isolated from nonimmune rabbit serum.

The prepared latex reagents were tested for agglutination by purified potato virus Y and by plant sap from potato sprouts and potato leaves infected with the virus as well as by plant sap from healthy plants. Latex reagent (30 μl 1 % latex) was mixed with 30 μl of sample on a white test card at room temperature. The test card was tilted back and forth for exactly 2 min, after which time the result was read. Test latex reagent showing high sensitivity and a minimum of nonspecific reactivity was selected for the preparation of test cards.

Preparation of test cards that contained the test and control latex reagents in dry form was carried out by Ani Biotech Ltd, Helsinki, Finland. The test cards also contained a dried positive control reagent that, when dissolved together with the dried test latex reagent, caused an agglutination of the latex reagent. Each card contained reagents for six tests. In addition, one positive control (dried latex reagent together with dried positive control reagent) and one negative control (dried control latex) were included in each test card. The cards were delivered in sets of ten cards sealed in air-tight bags together with a silica desiccator.

Comparative virus testing

For the latex test kit a simple hand press was

designed. With disposable plastic tips a standard volume of c 30 μl of liquid sample could be laid on the test card. Dried latex-antibody dots on the card were dissolved in a sample drop and were mixed carefully but quickly with a disposable plastic stick. The test was ready to be read with the naked eye after two minutes of gentle rotation of the test card. A PVY positive sample resulted in granulated accumulation of sensitized latex particles, similar to the positive test control. Negative samples as well as negative test controls stayed unagglutinated (Fig. 2).

To characterize the practical properties and reliability of the latex test a comparison with the standard double sandwich ELISA (CLARK and ADAMS 1977) was done. The same extracts from potato sprouts and leaves were tested with both methods. In the ELISA antibodies and their enzyme conjugates to Y^n and Y^o strain isolates were used.

Test groups of 18 sprouted tubers were formed. Each group included 6 to 12 tubers naturally infected with PVY, and the remaining tubers were healthy or infected with other potato viruses (Tables 1 and 2). The number of potato cultivars tested totalled eleven. The tubers within the groups were mixed to avoid expected results in the latex test. After testing of sprouts, the tubers were planted in peat substrate in a greenhouse. A few sample tubers from the preceding test had to be replaced because no emerging sprouts were left after sample taking.

The comparative tests from potato leaf samples were done three to four weeks later. At first undiluted leaf extracts were used but because of the harmful effect of dark green color on the visibility of latex agglutination reaction, the test samples were later diluted 1:5 in unbuffered saline (0.15 M NaCl).

For preliminary experience in testing of field grown potato plants, a comparative test with 120 leaf samples was done in late July.

The test results obtained with the latex test and with the ELISA were compared in each test group by counting identical and different recordings and trying to find out the reason for disagreement in each case.

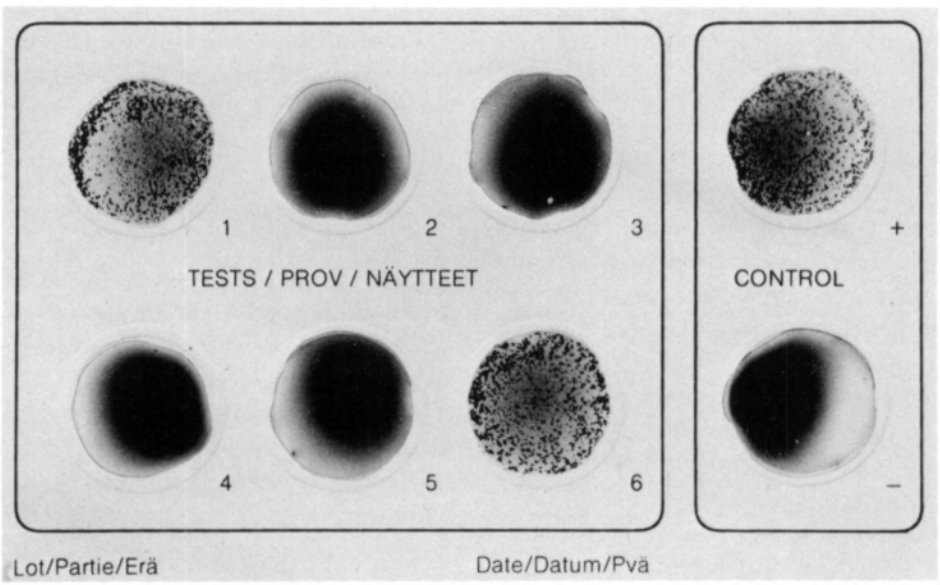

Fig. 2. A test card of 10 × 6 cm with clear granulated latex agglutination of virus positive samples (1 and 6) and the positive control. Samples were diluted 1:5 in unbuffered saline.

Table 1. Comparison of the latex test with the ELISA for the detection of PVY in eye sprouts of eleven potato cultivars.

Test group[1]	PVY positive samples		
	Latex	ELISA	Latex = ELISA
1	12/18	12	10
2	11	9	9
3	10	10	10
4[2]	4	10	4
5[2]	5	8	5
6	10	10	10
7	9	9	9
8	8	11	8
9	9	10	9
10	8	9	8
11	11	10	10
12[2]	6	10	6
13	8	10	8
14	8	8	8
15	10	10	10
16[2]	6	10	4
17	12	11	10
18	8	10	8
19	10	9	8
20	10	12	10
21	8	10	8
Totals	183/378	208	172
PVY-%	48.4	55.0	
Identical recordings (%)		82.7 (all test groups)	
		91.1 (groups 4, 5, 12 and 16 rejected	
False positive recordings in the latex test (%)		6.0 (all test groups)	

[1] Test groups 1—9 also included samples infected with PVM, PVS and PVX

[2] Poorly developed tiny sprouts, c 0.5 cm long

Results

Potential and optimising of the latex method

Antisera raised in the rabbits for the test kit were highly virus specific. Some strain specificity to PVY^n was present, as expected, and there were also minor differences between antisera from different animals. Specific IgG fractions from the antisera, when used for coating the ELISA plates, resulted in the following comparative absorbance readings from potato leaf samples (IgG, enzyme conjugate = 1 μg/ml, substrate incubation 20 mins at 20°C):

a) PVY^n conjugate, Y^n samples 1.553 (1.202—1.818), background 0.011
Y^o samples 1.227 (0.939—1.419)

b) PVY^O conjugate, Y^n samples 1.269 (0.880—1.373), background 0.016
Y^O samples 1.416 (0.927—1.506)

Immunoglobulins from our high-titre antisera were effective in coating latex particles at concentrations as low as 10—20 μg/ml. The resulting test latexes were agglutinated by sap from infected but not from healthy potato sprouts. However, using very low amounts of antibody for coating introduced a risk of getting false negative reactions by samples containing very high amounts of the virus (antigen excess). Therefore, we chose to coat the test latex with a slightly higher antibody concentration of 40 μg/ml. Test latexes pre-

Table 2. Comparison of the latex test with the ELISA for the detection of PVY in potato leaf samples diluted in unbuffered saline 1:5. The test included ten potato cultivars.

Test group[1]	PVY positive samples		
	Latex	ELISA	Latex = ELISA
1	13/18	13	13
2	13	12	12
3	8	8	8
4	5	6	5
5	8	7	7
6	8	8	8
7	8	8	8
8	6	6	6
9	9	9	9
10	9	8	8
11	12	12	12
12	9	9	9
13	11	10	9
14	14	12	12
15	12	9	9
16	16	16	16
17	13	11	10
18	12	12	11
Totals	186/324	176	171
PVY-%	57.4	54.3	
Identical recordings (%)			91.9
False positive recordings in the latex test (%)			5.4
False negative	— » —		2.7

[1] The test groups were not exactly identical with those presented in Table 1.

pared by using this coating concentration were agglutinated by purified virus at concentrations of 1 μg/ml or higher; we tested the reagent for agglutination by high viral concentrations and found the reagent to be effectively agglutinated at the highest concentration tested (200 μg/ml). Control latexes prepared by using 40 μg/ml of nonimmune rabbit immunoglobulis for coating were not agglutinated with purified virus.

Polystyrene latexes prepared by several manufacturers in various colors were used in initial experiments. While the various latexes gave similar results with purified virus particles, the initial results varied when plant material was used as samples. The darkness of potato sap made it impossible to use white or any light colored latex in the test. After some experimentation, we selected dark blue latex to be used in the present test. Suitable material was supplied e.g. by Serva (Germany) or by Rhone-Poulanc (France).

The final dried reagent dots contain 2 moles of glycine buffered at pH 8.2. In addition, the dots contain reagents used in drying the latexes; however, they do not affect the ionic balance of dissolved reagents.

Comparative testing of sprout and leaf extracts

With the latex test the agglutination reaction was clear and easy to read if well developed sprouts grown in the darkness or under weak non-continuous light were used. The reaction was normally completely developed after two minutes' gentle rotation of the test card but in a few cases an extra minute of agitation increased the clearness of virus specific agglutination. Rotation of the card for five minutes or longer may result in non-specific reactions. In testing of samples from well developed pale and juicy sprouts, the comparability with the ELISA recordings was excellent, 91 per cent (Table 1).

Because of their violet color sprout samples from tubers kept under strong natural or artificial light caused some visibility problems of the virus specific reaction. The latex agglutination reaction on the white card was at least as intensive as with the pale sprout sap but it became partially covered with colored material. The reaction remained reliably readable, if the sample extract did not contain much crushed material with large plant cell particles. Coarce crushed opaque plant material may accumulate particularly at the edges of the ringed test area and completely cover the virus specific latex agglutination reaction. Similarly negative reactions may remain unconfirmed.

Potato tubers at the early stage of sprouting were not suitable for testing PVY in the sprouts with the latex test or with the ELISA. No more than c 50 % of the virus infected samples could be detected with the latex test (Table 1) and false negatives were also found with the ELISA from sprouts of c 5 millimeter long or shorter. Almost no false positive recordings were made, which indicates low virus concentration being responsible for the unsatisfactory results.

Exceptionally high ELISA absorbances did not necessarily indicate intensive agglutination in the latex test. In the range of absorbance values from c 0.3 to 2.0 (30 mins of substrate incubation at 20°C) all comparative latex agglutination reactions could have almost the same intensity.

Intense non-transparent green color in undiluted potato leaf sap disturbed the interpretation of the results in the latex test. Although virus positive reactions developed rapidly during card rotation, agglutinated latex particles were sometimes hardly visible through thick colored material. After ten test groups, testing was discontinued because only c 70 % of the positive recordings were true positives when compared with the ELISA. Insufficient reliability in the latex test was due to both false positive and negative recordings.

When the test comparisons were repeated using 1:5 diluted (in unbuffered saline) potato leaf extracts, the non-specificity problems almost disappeared and a comparability of 92 % with the ELISA was reached (Table 2,

Fig. 2). False positive recordings were more common than missing positive reactions, which indicated at least sufficient sensitivity of the latex test in the detection of PVY in diluted potato leaf extracts. False positive readings were mostly caused by accumulation of coarse plant cell material present in the sap samples.

Testing of leaf samples from field grown potatoes gave completely identical results compared with the ELISA. Agglutination reaction was always clear if a minimum sufficient volyme of undiluted sap (15—20 μl) to cover the test area on the card was used. An increased sample volume may result in antibody-antigen imbalance (antigen access), which appears in poor or prohibited agglutination. The petioles of the leaves were found as exellent material for testing.

Potato virus Y could be detected reliably with the latex test in potato leaf extracts diluted 1:50 in unbuffered saline. A dilution of 1:100 caused an increasing proportion of questionable reactions to occur. When dilutions of 1:3 or less were tested, false positive or masked reactions became increasingly common.

The latex test did not react to any other potato viruses than PVY.

Discussion

The results shown in Tables 1 and 2 indicate that our latex agglutination test was strictly specific for potato virus Y. This was no doubt very much due to the successful immunization procedure producing high-titered antisera with good specificity. We feel that a high antibody titer is essential in developing this type of a latex since it allows the antibody to be used at relatively low concentrations in the preparation of the latex reagent. Although low titre antisera have been successfully used with the antibody sensitized protein, A-coated latex (PAL) in some cases as reported by Torrance (1980), it always introduces a new source of increased non-specificity.

The low concentration of antibody used in the coating of the latex effectively dilutes out possible contaminating antibodies against plant tissue. The immunogen is never absolutely pure, and even minor impurities can give rise to significant production of contaminating antibodies. Some of our antisera did show traces of antibodies against potato sap components. Also, using high immunoglobulin concentrations to coat latex particles, we found that normal potato sap components (possibly lectins) agglutinated the latexes even in the absence of viral antigens.

As compared to other latex tests described for the detection of potato viruses X, S, and Y (Talley et al. 1980, Fribourg and Nakashima 1984, Franc and Banttari 1986), our test is significantly faster. Incubation times of 1—2 h have been used in previous tests, with our latex test, however, the result can be obtained in two minutes. Prolonged incubation time could possibly increase sensitivity, but this seems unnecessary in the light of our present results which show that our latex test has a predictive value close to that of the ELISA.

The buffer system used in our latex test did not give maximum sensitivity for the test. In initial experiments with purified virus particles we noted that lowering the pH of the assay system to pH 6 considerably increased its sensitivity. However, those buffers were poorly compatible with the drying system used. We also tried Tween 20 and mercaptoethanol (Fribourg and Nakashima 1984) in our test buffer, but this led to no improvement in the performance of the assay. However, the glycine buffer selected as the test buffer did give fair sensitivity, and it was compatible with the reagent drying procedure used by Ani Biotech.

The properties of the antibodies produced for the test kit were well suitable for the purpose with exellent recognition of heterologous isolates of Y^n and Y^o strains. Reliable detection of various PVY isolates with a single polyclonal antiserum agrees with the results of Torrance (1980) but disagrees with the data of Maat and deBokx (1978), who have reported distinct serological relations between

the two type strains of PVY. Perhaps the different assays used can partially explain this discrepancy. In our previous studies (KURPPA 1983) significant serological differences were present between Y^n and Y^o strain isolates, but with careful selection of an immunogen, much of this diversity could be avoided.

Practical comparative tests clearly confirmed the high potential of our simple latex serological test for the detection of potato virus Y. The results obtained from naturally developed sprouts with the latex test demonstrated exellent comparability with the ELISA, which in the detection of PVY in potato sprouts has been found highly reliable by several researchers including MAAT and deBOKX (1978), GUGERLI and GEHRINGER (1980), and KURPPA (1983). The low virus concentration together with the irregular distribution in primarily infected tubers, reported by BEEMSTER (1967) and WEIDEMANN and WIGGER (1984) may cause inaccuracy in any testing procedure but this can be effectively avoided by breaking of dormancy with Rindite treatment as shown by GUGERLI and GEHRINGER (1980) and VETTEN et al. (1983). Low virus concentration was also clearly demonstrated in our experiments in some groups of samples at the early stage of sprouting but a sufficiently high concentration was reached with prolonged sprouting. The drastic effect of Rindite treatment in raising up PVY concentration is unquestionable, but for practical reasons it is an unwanted step here. Our previous experiments have shown that PVY is relatively evenly distributed and it achieves a sufficiently high concentration during tuber sprouting at a room temperature when the sprouts have reached the length of two to three centimeters.

The sufficient sensitivity of the latex serological test for the detection of potato virus Y in potato leaves has been previously reported by KHAN and SLACK (1978), TALLEY et al. (1980), TORRANCE (1980) and FRIBOURG and NAKASHIMA (1984). Thus the problem in the detection is not the lack of sensitivity but non-specificity arising from chlorophyll, lectins and other cell materials.

Masking of specific latex agglutination reactions or non-specific accumulation of green cell materials could be almost completely avoided by dilution of the leaf extracts in unbuffered saline (1:5). However, this is an unwanted extra step for field diagnosis, and alternatives to sample dilution have to be studied. The use of leaf petioles instead of laminae or even stems as test samples may offer essentially less chlorophyll containing extracts. Our experience in testing field grown potato plants is limited, but it seems very likely that no dilution of leaf sap is necessary for accurate virus detection in secondarily infected leaf tissue. Potato stems may also be suitable material for testing and at a late stage of the growing season they may also serve as exellent indicators for primary tuber infection.

At present our latex test kit offers accurate detection of potato virus Y in sprouts, but it can also be applied for virus detection in green plant samples, if a few precautions are taken.

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SELOSTUS

Yksinkertainen nopea lateksiagglutinaatiotesti perunan Y-viruksen määrittämiseksi

Aarne Kurppa

Kasvitautiosasto, Maatalouden tutkimuskeskus, 31600 Jokioinen

Matti Vuento

Biokemian laitos, Helsingin yliopisto, 00170 Helsinki

Perunan Y-virus aiheuttaa jatkuvasti tuntuvia sadonmenetyksiä ja vähentää täten perunanviljelyn kannattavuutta. Virus rasittaa erityisesti Etelä-Suomen ruokaperunan siementuotantoa. Ainoa mahdollisuus estää virustautivahingot on käyttää tervettä siemenperunaa. Terveyden varmistamiseksi siemeneksi käytettävä peruna on testattava. Nykyinen testauslaboratoriokapasiteetti riittää vain valio- ja kauppasiemenen tutkimiseen. Ruokaperunaa tuotetaan yhä yleisesti tarkastamattomalla siemenellä, mikä on usein pahoin Y-viruksen tartuttamaa. Tilanteen korjaamiseksi tarvitaan lisää siemenperunan testauskapasiteettia, mieluiten luotettavaa yksinkertaista testiä, joka soveltuisi myös neuvonnan ja perunatilojen käyttöön.

Tämä tutkimus Y-viruksen pikatestin kehittämiseksi tehtiin Maatalouden tutkimuskeskuksen ja Ani Biotech Oy:n (Helsinki) yhteistyönä. Alustavissa tutkimuksissa selvitettiin lateksiagglutinaatioperiaatteen sopivuus tarkoitukseen, minkä jälkeen valmistettiin tarkoitukseen erät vasta-aineseerumeita Y-viruksen Y^n-rotua antigeeninä käyttäen.

Virusmääritys tehdään valkealla pahvikortilla. Tähän muotoiltujen kohoumarenkaiden keskelle on kiinnitetty pieni sininen täplä, mikä sisältää lateksi-vasta-ainekompleksin lisäaineineen. Kortilla (10 × 6 cm) on kuusi testipaikkaa sekä lisäksi paikat positiiviselle ja negatiiviselle kontrollille. Testattaessa kuiva reagnessitäplä liuotetaan noin 30 μl:n näytepisaraan ja sekoitetaan koko näytealueelle levittäen, minkä jälkeen näyte pidetään hitaassa liikkeessä korttia käännellen. Tulos luetaan kahden minuutin kuluttua silmävaraisesti. Y-virusta sisältävät näytteet sekä positiivinen kontrolli muuttuvat rakeisiksi kun taas viruksettomat näytteet ja negatiivinen kontrolli säilyvät hienojakoisen sileinä. Testi ei reagoi perunan muihin viruksiin.

Lateksitestillä päästiin yli 91 %:n yhdenmukaisuuteen ELISA-testin tulosten kanssa määritettäessä Y-virus perunan itujen puristemehusta. Virhe syntyi lähinnä joidenkin heikkojen positiivisten reaktioiden havaitsematta jäämisestä. Näytteiksi soveltuvat parhaiten pimeässä tai heikossa valossa kasvaneet mehukkaat vaaleat idut. Valossa kehittyneiden itujen violetti väri häiritsee tuloksen lukemista jonkin verran. Itämisen alussa olevia mukulanäytteitä ei pidä testata, koska viruskonsentraatio iduissa on tällöin vielä hyvin alhainen.

Kasvihuoneessa kasvatettujen perunantaimien laimennetuista lehtimehunäytteistä saatiin 92 %:sti oikeat viruspositiiviset tulokset. Virus voitiin määrittää luotettavasti vielä 1:50 laimennetusta näytteestä. Sopivaksi testilaimennokseksi katsottiin kuitenkin 1:5, vaikka tällöinkin syntyi joitakin voimakkaan vihreän värin aiheuttamia virhetulkintoja. Laimentamaton lehtimehu ei soveltunut ongelmitta näytteeksi, sillä väri saattoi peittää tai naamioida vahvankin agglutinaatioreaktion tai saostua vähäisenkin kuivumisen aikana. Lehtiruodeista tai varsista saatava puristemehu soveltuu lehtimehua paremmin näytteeksi.

Normaalien kasvustonäytteiden virusmäärityksistä tähän artikkeliin saatiin vasta alustavia tuloksia. Virusmääritys voitiin tehdä aina täysin luotettavasti myös laimentamattomasta lehtimehusta. Saostumareaktio oli erittäin selkeä, kun laimentamatonta mehua käytettiin minimimäärä (15—20 μl), mikä riitti peittämään tasaisesti testikortin näytetilan. Näytemäärän lisäännyttyä yli 30 μl:n reaktio heikkeni nopeasti, mikä aiheutui vasta-ainekonsentraation alenemisesta ja näin syntyvästä virus- ja vasta-ainekonsentraation epätasapainosta. Luotettavan määrityksen perusta on siten vakiotilavuuksinen näyte kortin kaikissa näytetiloissa.

Kehitetty testipaketti sisältää testikorttien lisäksi muovisia kertakäyttöisiä sekotustikkuja, pienen näytepuristimen sekä puristimen kertakäyttökärjet. Testikortit säilyvät käyttökelpoisina kuivassa viileässä paikassa vähintään vuoden. Testi soveltuu nykyisellään iduista tehtäviin määrityksiin ja myös muista kasvinäytteistä voidaan saavuttaa riittävä luotettavuus. Testiä tulisi vielä kuitenkin parantaa siten, että kasvustosta ennen nostoa otetusta versonäytteestä saataisiin luotettava tulos, jolloin viljelijä voisi hyvissä ajoin varautua mahdolliseen siemenkannan uusimiseen.