DETECTION OF POTATO SPINDLE TUBER VIRUS (PSTV)

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Viroids are a group of important plant pathogens consisting of low molecular weight RNA capable of replicating autonomously (Diener, 1971). PSTV is known to cause a severe disease in infected plants. Yield reduction can be as high as 64% (Singh et al., 1971). PSTV is readily transmitted by contact and sometimes up to 100% through the botanical seed of infected plants. Unlike most viruses PSTV cannot be eradicated by heat treatment but work in CIP suggest that 'cold treatment' of infected materials follows by meristem culture offers a possibility for its eradication.

Symptomatology

Symptoms of the disease vary considerably depending on the potato cultivar and environmental conditions. The foliage of infected plants sometimes turns slate gray with dull leaf surface (Diener, 1979). The tubers are elongated with prominent 'eyebrows' having in many instances severe growth cracks.

Vines have sometimes upright habit of growth or are stunted. These vines usually die earlier than healthy ones and leaves are narrower and pointed in some cultivars. Marginal Leafrolling has been observed in some cultivars resembling initial PLRV symptoms. Purple tips is other symptom which has been observed in some affected cultivars always associated to leafrolling. Severe rugosity of leaves and stunting have been observed in some Solanum acaule accessions at CIP (unpublished)

Methods of diagnosis

1. Diagnosis of PSTV infections in tomato.

Following the discovery of tomato as a source of and indicator plant for PSTV (Raymer, O'Brien & Merriam, 1964) most advances in the study and recognition of infections have been done in this host. No special care is needed to grow tomato seedlings but it is important that they be inoculated in the cotyledonary leaf stage-preferably using phenol extracts (Singh, 1977). After inoculation tomato plants are kept at high temperature (27-35 °C) to promote development of severe symptoms. The presence of manganese in the growth medium enhances symptom expression and should be included if possible (Lee & Singh, 1972). PSTV symptoms appear in 2-4 weeks as a characteristic bunching of the tops and extensive vein necrosis in the developed leaves. Mild strains however, show very light or no symptoms in infected tomatoes.

a) Challenge Test. Farnow (1967) developed a system to detect mild strains based on the well known cross-protection phenomenon. Tomato seedlings in the cotyledonary stage are rubbed (inoculated) with the test sample. Fourteen days later the test plants are inoculated with a severe strain of PSTV. If this doubly inoculated plants show typical symptoms of the severe strain indicate that the test sample (first inoculated) was free of PSTV. Conversely, those which do not develop the symptoms were first inoculated with an infected test sample.
b) Yang and Hooker procedure. Recently Yang et al. (1977) found
that tomato seedlings inoculated either with a mild or a severe strain of
PSTV developed a distinct albinism when they were maintained after in-
oculation under a continuous light regime of 1000 - 2000 ft-c at a tempe-
rature of 27-31 C and a relative humidity over 60%. This procedure
has shown advantages over the other tests on tomatoes under CIP condi-
tions. However, careful observations should be made on the quality of
light before use. Fluorescent lamps and not incandescent ones must be
used. Reaction on tomatoes usually appear in two weeks with severe
strains and up to 4 weeks with mild strains.
The variety of tomato used is not very critical but best results are obtained
in cv. Rutgers. Seeds should be obtained from mother plants tested for
freedom of PSTV.

2. The use of Scopolia sinensis

Singh (1971) showed that Scopolia sinensis, a solanaceous species,
reacted with local lesion production after inoculation with mild or severe
strains of PSTV. Seedlings should be grown in relatively low light intensity
(300-400 ft-c) at a temperature of 18-23 C with a relative humidity of over
60%.

It is important that the plants be regularly fertilized to ensure vigorous
growth and only fully-developed succulent leaves should be used for the
inoculation.

After inoculation plants should be maintained under the same environmental
conditions as described above. With severe strains local lesions usually
develop in 6-10 days whereas with mild strains they appear between 14-18
days. Local lesions are invariably followed by systemic symptoms of extensive
veinal necrosis and finally leaf drop.

3. Electrophoresis

PSTV occurs as free ribonucleic acid of low molecular weight and migrates
discretely in the gel electrophoresis compared to other cellular RNA.

Morris and Wright (1975) developed a procedure which involved extraction of
cellular nucleic acids from infected tissue and their separation in polyacrylam-
ide gel in tubers. After staining with toluidine blue O, or Methylene ' 
blue, a 'PSTV band' was detected which was of diagnostic value because no
comparable bands were present in the healthy tissue. The technique was able
to detect both severe and mild strains. The technique was also able to detect
PSTV in dormant potato tubers. They also found that the presence of other
potato viruses did not affect position or appearance of PSTV band. Subsequently,
Morris and Smith (1977) published a modified procedure permitting rapid extraction of nucleic acid. The modification took only 2 days instead of 4 in the original procedure. PSTV can be detected from as little as 0.2 grs. of infected tissue.

Recently, however, Schumann et al. (1978) adopted previous reported procedure to the slab method and the use of a more sensitive staining with ethidium bromide. The advantages of using this procedure resides in the ease to prepare the gels and the possibility of a direct comparison of several samples. At the International Potato Center in Lima, Peru, electrophoresis has shown higher sensitivity to detect PSTV than the tomato test. However, it was found that plants to be tested should be grown at a temperature higher than 25°C in order to achieve its maximum sensitivity.

Extraction of RNA is performed by the well known phenol procedure and total cellular RNA fractionated with 2 M Litium chloride. Dialysis to remove Litium chloride has been found unnecessary and usually dangerous since risk of degradation of RNA by contaminating nuclease is increased. Instead, RNA is precipitated with ethanol. Other methods to extract RNA are being investigated.

Schedule for Electrophoresis of PSTV samples

A) Extraction of PSTV

grind 1 gr of infected tissue in clean mortar with 0.5 ml extraction buffer
(0.2 glycine + 0.1 M Na₂HPO₄ + 0.6 M NaCl + 1% SDS) and 2 ml water-
saturated phenol containing 10% m-cresol and 0.1% 8-hydroxyquinoline.
Pour in a clean test tube and add 2 ml butanol + chloroform (1:1, v/v),
agitate in mixer for 2-3 mins.
Centrifuge 10,000 rpm/20 min.
Recover supernatant and add 1/5 vol. of 10M LiCl.
Incubate in ice tray for 2 hrs.
Centrifuge 10,000 rpm/15 min.
Collect supernatant carefully with a clean Pasteur pipette and dialyze in the
cold overnight. (against destilled water).
Store under ethanol (2 1/2 vol.) with a drop 4M sodium acetate or add
directly to the gel.
B) Slab gel electrophoresis (for a 3 mm thick gel)

Prepare gel 5% Acrylamide by mixing in order:

- 50 ml Acrylamide solution (15% Acrylamide + 0.75% N,N methylene bisacrylamide)
- 30 ml stock buffer (5x) (0.18 M Tris (hydroxymethyl) methylenamine + 0.15 M NaH₂PO₄ · 2H₂O + 0.005 M EDTA)
- 67 ml distilled water
- 0.25 ml TEMED
- 2.5 ml 10% (NH₄)₂S₂O₈

Mix well and fast, pour in gel tank taking care to remove air bubbles and place slot former.

Leave at least 30 min, pre-run 1 hour in running buffer (stock diluted 1/5)

Loading samples:

if RNA is under ethanol, centrifuge 10,000 rpm/15 min, evaporate traces of ethanol and resuspend in required amount of buffer or distilled water. (50 ul is recommended).

- Add 2 drops of 10% RNASE-free sucrose containing enough bromophenol blue.

Apply samples with a Pasteur pipette or Hamilton-type syringe or any other clean, suitable device.

Running conditions:

Recirculate cold water (12C)

Set at 100 volts and recirculate buffer.

Run 3 hours or until tracer dye is c. 9 mm down the gel.

Unloading gel and staining:

Carefully remove gel, mark position of sample 1 and stain in 0.1% Toluidine blue O or methylene blue overnight.

D vastain with distilled or tap water (two or three changes will be enough).
CONCLUSIONS.

Unquestionably much more work is needed to find or develop new methods for detection of PSTV. Serology has been explored by some workers but results were negative excepting those of Dagnall (1969) who was able to prepare an antiserum which reacted differentially to an antigen present in infected plants. Unfortunately this antiserum was of a very low titer. Even though is very laborious, electrophoresis can yield results in 2 days compared to 2-4 weeks for the tomato or S. sinensis test. The occurrence of mild strains make bioassays somewhat unreliable, but electrophoresis is not either 100% reliable. In our hands electrophoresis showed slight advantages over the tomato test for testing single plants but for testing composite samples grown under screenhouses where no temperature control is possible we found the tomato test more sensitive.

REFERENCES


