TECHNICAL SHEET No. 27

Virus Detection: *Prune dwarf virus*

Method: ELISA

General

Virus detected: *Prune Dwarf Virus* (PDV)
General method: ELISA.

Developed by

Name of researcher: Hana Sobh and Yusuf Abou-Jawdah, American University of Beirut, Lebanon
Address (email): hs05@aub.edu.lb and abujawyf@aub.edu.lb
Date: November 26, 2002

Goals

Identification of PDV

Introduction

Prune Dwarf virus belongs to the Genus Ilarvirus family Bromoviridae. Virus particles of this genus are isometric to short bacilliform and labile. It is a single-stranded RNA virus composed of three RNAs (but four RNAs are needed for infection to occur). The two largest RNAs code for the RNA polymerase enzyme (2 proteins, 120 and 100 Kd), the third RNA codes for a 34 Kd protein, the movement protein. RNA 4 codes for the coat protein but the genetic code for this gene is also present in RNA 3. The capsid is composed of one type of protein subunits, with a MW between 24 to 30 Kd, depending on virus isolate.

PDV is transmitted by seed, pollen, and grafting. PDV causes a yield reduction of 50% and the remaining fruits are large and firm. In chronically infected trees, the reduced numbers of fruit spurs give a bare wood appearance. PDV-infected plums and prunes develop narrow, strap-like leaves that are thicker than normal and the internodes tend to be rosette. In most peach cultivars, PDV produces mild stunting and no leaf symptoms while in the Muir peach cultivar, PDV infections produce a dense canopy due to the shortening of the internodes. PDV is considered by Martelli and Savino (1997) to be the most damaging and widespread Ilarvirus.

Materials and Methods

A. ELISA: The standard double-antibody sandwich enzyme-linked immuno-sorbent assay (DAS-ELISA) was used for the detection of PNRSV. IgGs and conjugated IgGs were purchased from Sanofi, France or Agdia, USA. Extraction and detection of the infected leaves by ELISA are as follows:

Phosphate Buffer Saline (PBS)	**Washing Buffer pH 7.4**	**Coating Buffer pH 9.6**
Distilled water 1.0 l	PBS 1.0 l	Distilled water 1.0 l
NaCl 8.0 g	TWEEN 20 0.5 ml	Na_2CO_3 1.6 g
$Na_2HPO_4.12H_2O$ 2.9 g		$NaHCO_3$ 3.0 g
KH_2PO_4 0.2 g		
KCl 0.2 g		

Conjugate Buffer pH 7.1-7.3	Substrate Buffer pH 9.6 adjusted by concentrated HCl
PBS TWEEN 100 ml	Distilled water 80.0 ml
PVP 2 g	Diethanolamine 9.7 ml
Ovalbumine 0.2 g	

Coating of ELISA plates

1. Dilute antibodies in coating buffer as recommended by the supplier
2. Add 100 µl to each well, cover the plate
3. Incubate for 3-4 h at 37ºC
4. Wash three times with PBS-TWEEN

Antigen extraction and binding

1. Samples were either stored at 4ºC until processed, normally 3-4 days, or stored at -80ºC for few weeks.
2. Leaf tissues (1:10 w/v) were extracted in 0.1 M phosphate buffer Saline-Tween-Polyvinylpyrolidone (PBST-PVP), pH 7.4 containing 2% PVP 0.05% Tween-20, 0.15 M NaCl, and 4 mM KCl.
3. Add 100 µl extracted sample/well; and two wells were used per sample
4. Incubate overnight at 4ºC
5. Wash three times with PBS-TWEEN

Conjugate

1. Dilute conjugated antibodies in conjugate buffer as recommended by the supplier and add 100 µl to each well
2. Incubate for 3-4 hr at 37ºC
3. Wash three times with PBS-TWEEN

Color reaction

1. Dissolve 10 mg pNPP in 10 ml substrate buffer just before use, transfer 100 µl to each well, incubate for 1 h
2. After incubation, the reaction may be detected visually or at A_{405} nm using an ELISA reader.
3. The test was considered positive when the mean absorbance value of a sample was over twice that of healthy controls

References

Martelli, G.P. and Savino V. 1997. Infectious diseases of almond with special reference to the Mediterranean area. EPPO Bulletin. 27 (4) 525-534.