TECHNICAL SHEET No. 35

Virus Detection: *Tomato Yellow Leaf Curl Virus* (TYLCV)

Method: Non-radioactive hybridization using the Enhanced Chemi Luminescence labeling system (ECL).

General

Virus detected: TYLCV from tomato leave and whiteflies.
Method: Non-radioactive hybridization using the Enhanced Chemi Luminescence labeling system (ECL).

Developed by

Name of researchers: Omar Dar-Issa, and Naim Iraki, UNESCO Biotechnology, Center at Bethlehem University.
Address (Email): omari@bethlehem.edu; niraki@behtlehem.edu
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Goals

To develop a sensitive method for TYLCV detection.

Introduction

Tomato (*Lycopersicon esculentum)* is one of the most important vegetable crops of the family Solanaceae, which includes about 1,500 tropical and subtropical species. The genus *Lycopersicon* consists of only eight species and it is subdivided into two subgenera: *Eulycopersicon* and *Eriopersicon*. Fruits of plants of *Eulycopersicon* are usually red or yellow in color when ripe. This genus includes the cultivated tomato (1). Fruits of the *Eriopersicon* on the other hand, remain green or purple green throughout development. Tomato fruits normally contain 5-10% dry matter, of which 1% is skin and seeds. Reducing sugars such as glucose and fructose constitute nearly 50% of the dry matter. In addition to these sugars, tomato fruit also contains potentially toxic glyco-alkaloids, such as tomatine and solanine. These alkaloids protect the tomato plant against microorganisms and pests. Tomato is native to South America and Mexico (1). Large-scale cultivation of tomato did not begin until about a century ago then it became generally cultivated only after World War I. Now it is consumed all over the world and is the second largest vegetable crop in terms of dollar value (1). A report from Food and Agriculture Organization (FAO) 1993, shows that the highest production of tomato is in the U.S.A. The percentages of total world production were 15%, and 0.62% for U.S.A. and the Middle East, respectively.

The most serious viral disease infecting tomatoes in the Mediterranean region is caused by the *Tomato Yellow Leaf Curl Virus* (TYLCV), which is the major factor limiting tomato production, during Summer, Fall and Winter cultivations. When necessary precautions are not taken (using nets or insecticides) infection may reach 100% depending on the age of plants and the time of infection. TYLCV is a monoparatite, circular, single-stranded DNA genome of approximately 2.8 kb (2). It belongs to the Geminiviridae family. Affected tomato plants are stunted, the shoots have short internodes (2). The leaves are small, curled, leathery and chlorotic (2). The most significant effect of TYLCV infection is flower abscission. Usually, less than one in ten flowers set fruit, thus severely reducing the yield.

This tomato virus attacks a great variety of hosts including lentil and tobacco. TYLCV is transmitted by cotton or tobacco whitefly (*Bemisia tabaci)* or silverleaf whitefly (*Bemisia argentifolii).* A single whitefly is able to transmit the virus and the rate of transmission increases as the population density of the vector increases (3). No other means of transmission, such as mechanical were observed for this virus. The widespread occurrence of epidemics associated with TYLCV and its potential threat to tomato production, make it essential to develop procedures for TYLCV detection in both *B. tabaci* and plants for disease management. Serological methods have met limited success with the whitefly-transmitted Geminiviruses. But recently, nucleic acid hybridization techniques and polymerase chain reaction (PCR) provide sensitive methods for the detection and identification of TYLCV in infected plants, or the whitefly vector.

Materials and Methods

Tomato yellow leaf curl virus was detected by enhanced chemiluminescence system (ECL direct nucleic acid labeling and detection system-Amersham. RPN 3000). Infected tomato leaves and frozen whiteflies, squashed onto a dry nylon membrane. Cloned viral template labeled with enzyme horseradish peroxidase. Positively charged peroxidase attached loosely to completely denatured negatively charged template. Addition of glutaraldehyde strengthens the attachment through formation of chemical cross-links, so that the probe becomes covalently attached to the enzyme. In hybridization, the probe hybridized with target DNA immobilized on the membrane. Viral infection is detected by oxidation reduction reactions, using detection reagents, which produced blue light. The light output is increased and prolonged by the presence of an enhancer, so that it can be detected on a blue-light sensitive film (see kit leaflet for more details).

Preparation of TYLCV specific non-radioactive probe

Denature 100 ng of cloned TYLCV CP gene (available from the Hebrew University/Rehovot) at 100ºC for 10 min. Cool in ice for 5 min. Add 10 µl of DNA labeling reagent and 10 µl of glutaraldehyde solution to the cold DNA. Incubate the probe at 37ºC for 1 h. Hybridize the squash blots with the labeled probe for 13 h. Wash the blot twice with (1X SSC and 0.1% SDS) for 15 min.

Detection procedure

Cover the blots with equal volumes of both detection reagents (supplied with the kit). Place the blots in the film cassette. Expose top of the blots to autoradiography film for 1 min. Finally, remove and develop the film.

Results

Autoradiographic detection of TYLCV-DNA with TYLCV-specific non-radioactive probe, in squashes of tomato leaf tissues and single insect (Bemisia tabaci). Healthy: squashes of healthy tomato leaf. Infected: squashes of TYLCV infected tomato leaf. V.W.: squash of viruliferous whitefly.

References

(1) Nelson, P. 1996. Quality attributes of processed tomato products. Food Rev. Int. 12:375-401.
(2) http://hammock.ifas.ufl.edu/new/pg08400.htm
(3) Mansour, A., and Al-Musa, A. 1992. Tomato yellow leaf curl virus: host range and virus-vector relationship. Plant Pathol. 41:122 -125.