P15: The use of Immunocaptured Polymerase Chain Reaction (IC-PCR) to Study the Translocation of Tomato Yellow Leaf Curl Virus (TYLCV) in Tomato Plants

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Abstract

Translocation of TYLCV was studied in tomato plants after whitefly-mediated inoculation at 3-4 true leaf stage. Therefore, twenty healthy tomato plants were grown under laboratory conditions at 25-30 degree Celsius. Whitefly-mediated inoculated was employed using adult insects raised for two generation on TYLCV–immune plants including eggplant and pumpkin. The virus was acquired by the whiteflies after an access period of 48-hr on TYLCV-infected jimsonweeds. Inoculation of tomato test plants was done by caging the third top leaf of each plant with ten whiteflies using leaf cages or perforated plastic bottles. After 24 hr feeding access, the whiteflies were killed (Pico *et. al.* 1996, Sawalha, 2009b).

Tissue samples were collected at different intervals from the inoculated leaves, leaf petioles, stems, roots and top leaves. The samples were kept frozen at -20 degree Celsius and then tested by IC-PCR. Healthy tissues were obtained from control plants (eight tomato plants) grown and treated similarly (Sawalha, 2009c).

The IC-PCR was employed as described by Sawalha (2000) using TYLCV-specific polyclonal IgG. The reaction was employed as described by Navot *et. al.* (1992), Campbell and Reece (2005) and Tortora *et. al.* (2002) using TYLCV-specific oligonucleotide primers. Sub-genomic fragments of the virus genome were amplified. The primers were purchased from the Alltech Company, Paisley, UK. The primer sequences were from 5' to 3', P1V, ATACTTGGACACCTAATGGC, nucleotides (nt) 61-80, and P4C, TGGACATCTAGACCTAAG, nt. 2054-2071. The sequence of the P1V corresponds to the viron positive strand, whereas the P4C is complementary to the viron strand. Results were recorded as described by Sawalha (2000) and Sawalha (2009a).

Based on the PCR results, the virus needed 12 hours to pass through the petioles of the inoculated leaves then two days to reach the tap root of the inoculated plants. In addition, the virus translocated upward and needed three days after inoculation to invade the upper most top leaves then one and two days later to arrive the second and the third most upper leaves. Furthermore, the virus needed twelve days to make an invasion for the most plant parts. Determining the translocation rate of the virus particles revealed that they move in average rates of 78 mm/day from inoculated leaves downward toward the root and 182 mm/day upward from root to the top part of shoot

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