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Application of Direct Tissue Blot Immunoassay in Comparison with DAS-ELISA for Detection of Turkish Isolates of Citrus Tristeza Closterovirus (CTV)

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Abstract: In this study, the direct tissue blot immunoassay (DTBIA) procedure was tested for the detection of 10 different Turkish isolates of the citrus tristeza virus (CTV) by employing monoclonal and polyclonal antibodies. The DAS-ELISA and DTBIA procedures were compared using two different monoclonal antibodies (3E10 and MCA-13) for 10 CTV isolates. The storage effects of blotted membranes on the blot assay were also investigated by using different temperatures (4 and 25 °C) and storage intervals (1, 2, 3 and 4 weeks). In addition, 258 Satsuma trees in one orchard were tested using ELISA and DTBIA. All polyclonal (1212-1, 981-1 and 908-7) and monoclonal (ECTV-175, ECTV-176, 11B1-3 and 3E10-6) antibodies reacted to CTV isolates, except MCA-13, which only reacted to Igdir and Cyprus CTV isolates. DAS-ELISA was also positive for all CTV isolates that reacted to DTBIA. Membranes kept at 25 °C gave better results than those kept at 4 °C, whereas there were no clear differences between storage periods for the same treatment combinations. Assays of 258 Satsuma trees by ELISA and DTBIA indicated similar rates of CTV infection. Seven trees were infected and 251 trees were virus-free in both tests. DTBIA is a rapid, sensible and reliable procedure for the detection of CTV. It requires little sample preparation and tissue blots can be stored at 25 °C for at least 4 weeks prior to the assay. Moreover, blotted membranes can be sent safely to another place for testing.

Key Words: Tissue blot, nitrocellulose membrane, citrus tristeza virus, ELISA

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