Effect of Triton X-100 Inclusion in Extraction Buffers in Reducing Non-specific Backgrounds in Dot -Blot Immuno-Binding Assay (DBIA) of Plant Viruses

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ABSTRACT

Several buffers were compared in dot blot immunoassay (DBIA) technique for their efficacy in removing the non-specific background color (NBC) formed in healthy control plants, upon using polyclonal antibodies (PAB) against tomato yellow leaf curl virus (TYLCV). Only those buffers containing the non-ionic detergent Triton X-100 (TX-100) were able to prevent the NBC formation in healthy controls. The present technique enabled the use of PABs with DBIA tests without any fear of NBC formation in controls.

Key words: *Dot blot immunobinding assay, non-specific binding, tomato yellow leaf curl virus, geminiviruses, Triton X-100.*

INTRODUCTION

The technique of DBIA on nitrocellulose membranes (NCM) has recently been used for detection of many plant viruses. It is very sensitive, economic, and time saving (Makkouk *et al.*, 1993; Abdel-Salam and El-Sharkawy, 1996;, Abdel-Salam, *et al.*, 1997a, and b,; Figuieira, 1997; Mahmood and Hein, 1997 and Abdel – Salam *et al.* , 1998).

Due to the high degree of sensitivity of DBIA, the presence of NBC in healthy controls (not detectable by ELISA) represents a problem in reading results. This NBC has been attributed to the presence of non-specific binding of antibodies from plant origin, whichinterfere with the color reactions elicited by the antigen under-study. Monoclonal antbodies (MAB) have been suggested as a replacement for PAB to overcome this problem. However, MAB are not feasible to all laboratories due to complexity, non - availability, and cost. Another alternative is to cross absorb PAB with healthy plant antigens. However, this technique, although successful with tissue blotting assay, is not successful enough with DBIA since traces of NBC formation are often seen in healthy controls.

Stott (1989) suggested that the physicochemical basis of binding of protein to NCM was believed to be largely due to hydrophobic interactions and hydrogen bonding. Ionic interactions were unlikely to