Developing Potatoes viral diseases rapid diagnostic tests

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Abstract
A protocol of potato virus Y- (PVY) accumulation in Nicotiana tabacum (N. tabacum) callus tissue has been developed to gain highly purified virus. Preparations of polyclonal and monoclonal antibodies specific to PVY have been obtained. The design has been defined, the main steps and manufacturing parameters of the test for PVY antigen detection in plant material has been worked out.

Keywords: potato virus Y, monoclonal antibody, lateral flow test

One of the effective diagnostic tests for potato virus determination is lateral flow immunochromatographic assay [1]. The test allows detecting viral infection in 10-15 minutes outside laboratory. The purpose of our research was to develop a lateral flow test for detection of PVY in plants.

During the research PVY accumulation protocol in tissue culture of N. tabacum has been worked out to obtain purified virus. N. tabacum variety Samsun inoculated with infectious sap of two potato's clones (varieties Cherie and Artemis) has been transferred in vitro. Callus has been induced from leaf-explants of N. tabacum in agar nutrient medium. After 4 passages 97 gr and 120 gr of each type of the infected callus has been obtained. Highly purified viral preparations have been obtained from the tissue culture of N. tabacum with concentration of 410 μg/ml and 120 μg/ml, which belong to the group of "necrotic" (PVYN-L) and the "normal" (PVYO-F) strains.

We have defined the optimum scheme of rabbits immunization with purified (PVYO-F) consisting of three subcutaneous and a single intravenous injections at a dose of 10 μg/ml with 14 days interval, which allows getting serum with high content of specific antibody. Purified Preparation of polyclonal antibodies has been obtained and their basic immunochemical properties have been studied.

As the result of BALB/c mice immunization with (PVYO-F) preparation in a dose of 5 μg/ml immune response with a titer higher than 1: 25600 of specific antibodies was recorded, which indicated active induction of B-cell clones producing antibodies of predetermined specificity. Hybridization of X63Ag8.653 myeloma cells and spleen cells were carried out by the method of Oi V., Herzenberg L [2]. 2 strains of hybridoma (2B4G9and4F6A3), stably producing monoclonal antibodies specific to PVY have been obtained. Basic immunobiological and immunochemical properties of hybridoma and monoclonal antibodies (Mab) have been studied. It has been determined that the Mab refers to immunoglobulin of G class, G1 subclass. The maximum titer in ELISA was 1:12800. The binding constant (affinity) of the antibodies against various strains of antigen which was used was from 2x10-8M to 2,5x10-8M. Mab (PVYO-F) conjugate with colloidal gold (20nm) have been prepared according to G. Frens [3] method with the use of sodium citrate (Sodium citrate dihydrate, Sigma) for reducing gold-hydrochloric acid (Gold (III) chloride hydrate, Sigma). The main manufacturing steps and parameters of immunoassay using specific reagents have been worked out. To construct the test a set of membrane for immunoassay Easypack Membranes Kit, Dipstick (Advanced Micro devices Pvt. Ltd) was used. In order to determine the specificity of the developed immunoassay test some laboratory tests have been conducted. As a result, technical indicator of the developed diagnostic kit meets the requirements of other similar tests and allows detection of PVY antigens in plant material.

References
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