STUDIES ON THE CONTROL OF SOME PLANT VIRUSES USING BIOTECHNOLOGY

BY

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B.Sc. Agric. Sc. (Agric. Microbiology), Ain Shams University, 1995
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ABSTRACT

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The main target of the present study is to control tomato mosaic *tobamovirus* (ToMV) and potato Y *potyvirus* (common strain) PVY^{O} depending on biotechnological methods. Confirmation of virus isolates was carried out by testing differential hosts susceptibility, symptomatology, detecting inclusion bodies by light microscopic examination and serologically by indirect ELISA. Viruses were purified using differential ultra centrifugation and purified preparations were evaluated by infectivity assay, indirect ELISA, UV absorption and electron microscopy. Purified preparations were used for antisera production by rabbit immunization, and immunoglobulins G (IgGs) were purified from the crude antisera. Viral coat proteins molecular weights were determined using the SDS-PAGE. Purified IgGs preparations were evaluated by detecting the viruses under study in infected tobacco sap using double diffusion test in agar, tissue and dot blot immunoassays. Sequencing of ToMV was carried out using Sanger's method and the nucleotide sequence was compared with five overseas isolates. PVY^{O}-free potato plantlets were produced by meristem tip culture, also a combination between meristem tip culture and ribavirin chemotherapy was performed and finally virus-free plantlets were used to produce microtubers. PVY^{O} under study was used to protect tobacco plants cv. White Burley from the necrotic symptoms caused by PVY necrotic strain (PVY^{N}). Coat protein genes (*cp*) for both viruses were isolated and amplified using immunocapture-reverse transcriptase-polymerase chain reaction (IC-RT-PCR) using primers with add-on restriction sites for *SmaI* and *SacI* enzymes.

The genes were cloned in pBI121 vector plasmid between the CaMV 35S promoter and the *nos* terminator after removing the *gus* gene by restriction enzymes (*Sma*I & *Sac*I) digestion. The new constructs were used for *Agrobacterium tumefaciens* (strain EHA101) transformation which was then used for tobacco transformation. Plants transformation success was confirmed using indirect ELISA, PCR, and RT-PCR. Plants resistance was tested by challenging with the viruses under study, remarkable success was obtained as 20% and 36% of transformed tobacco plants blocked viral infection for ToMV and PVY^{O}, respectively.

Key Words: ToMV, PVY^{O}, Sequencing, Genome, Coat protein gene (*cp*), Meristem tip culture, Cross protection, Transformation, pBI121, *Agrobacterium tumefaciens*, IC-RT-PCR.

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1. INTRODUCTION

Tomato (*Lycopersicon esculantum* L.) is one of the most economical vegetable crops worldwide which is subjected to different pathogens **(Broadbent, 1964 and Brunt *et al*, 1996)**. In Egypt, the cultivated area of such crop at the year 2004 was about 450000 feddans and gave a yield of about 6400000 tons **(Egyptian National Agricultural Library site: http://nile. enal.sci.eg/)**. The virus diseases were found to be the most serious diseases as they reduce the yield as well as the quality of such crop **(El-Hammady *et al*, 1983).**

Tobamoviruses were reported to be an economically impo-rtant virus group infecting tomato **(Van Regenmortel *et al*, 2000). Brunt *et al* (1996)** reported that tobacco or tomato mosaic *tobmovirus* (TMV or ToMV) causes mosaic symptoms with financial loss ranged from 12 to 33 % of tomato yield. Such viruses have wide host range, extremely stable and present in relatively high concentration in infected plant cells **(Brunt *et al*, 1996)**.

Potato (*Solanum tuberosum* L.) is considered as an important vegetable crop in Egypt, 80% of the potatoes in Africa are produced by Egypt, South Africa, Algeria and Morocco. Growth rates have been strong, led by Egypt at 5% annually **(FAO reports, 2003)**. The cultivated area at the year 2004 reached about 230000 feddans, yielded about 2200000 tons (**http://nile. enal. sci.eg/)**.

Virus diseases are the most responsible factors for the loss caused in potato production. Potato was reported to be a suitable host for more than 25 plant virus **(Salazar, 1996)**. Potato Y *potyvirus* (PVY) is considered the most damaging virus infecting potato crop causing great yield quantity and quality reduction **(Smith, 1972)**. PVY has a relatively wide host range, with low concentration inside the virus-infected cells and less stable compared with ToMV **(Hollings *et al*, 1976)**.

Virus disease can not be cured and so searchers were focusing on controlling the infection by different ways, i.e., removal of weed hosts, starting with virus-free planting materials and breeding of plants resistant to virus infection **(Fraser, 1990).** Recently advances in biotechnology are creating opportunities to increase food production in developing countries and reduce environmental problems associated with conventional farming practices **(Sawyer, 1992).**

Techniques of modern molecular biology and tissue culture were used and grow for more than decade, mostly for elimination of virus disease in planting material **(Trigiano and Gray, 1996 and Foster and Taylor, 1998).** For vegetatively propagated crops such as potato, production of virus-free starting materials (tubers) is very important to reduce yield loss due to over-viral infection in field (**Trigiano and Gray, 1996).**

Cross protection is a traditional way to protect plants from aggressive virus strains in field which is still used effectively. This is carried out by pre-inoculation of seedlings with a mild virus protective strain related to the aggressive one **(Pennazio *et al,* 2001).**

Some strategies of molecular biology were used for virus infection control, i.e., coat protein-mediated resistance **(Powell-Abell *et al,* 1989; Guo *et al,* 2003 and Higgins *et al,* 2004)**, movement protein (**Lapidot *et al,* 1993)**, replicase-mediated resistance **(Carr *et al,* 1990 and Quemada *et al,* 1991)**.

Therefore, the present investigation aimed to control ToMV and PVY^{O} (common strain) depending on biotechnological methods (meristem tip culture, chemotherapy, cross protection and coat protein-mediated transformation) by carrying out the following scheme of work:

1- Confirmation of virus isolates, i.e., ToMV and PVY^{O}.
2- Virus purification.
3- Electron microscopy.
4- SDS-PAGE analyses of purified virus preparations.

5- Raising polyclonal antisera and their evaluation.
6- Determination of the nucleotide sequences of the full genome of ToMV.
7- Sequence analysis.
8- Production of virus-free potato minitubers.
9- Establishment of regeneration and transformation systems for tobacco cv. White Burley.
10- IC-RT-PCR isolation of the *cp* genes of the viruses under investigation.
11- Preparation of two constructs containing the interest genes for *Agrobacterium* transformation.
12- *Agrobacterium* transformation of tobacco plant cv. White Burley.
13- Acclimatization of the putative transgenic plants.
14- Evaluation of the virus resistance of the tobacco transgenic lines.