Biolistic transfection of plants by infectious cDNA clones of *Plum pox virus*

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Abstract

Plant biolistic transfection by two *Plum pox virus* (PPV) infectious cDNA clones (strains PPV-M and PPV-D) using the gene gun apparatus PDS 1000-He was optimized. *Nicotiana benthamiana* plants were germinated on Petri dishes with MS growth medium. At the age of four weeks the plants were subjected to biolistic transfection and three days later were transplanted into common soil substrate. The plant survival after transplantation was about 70 %, the transfection efficiency was over 80 % (compared to 6 – 10 % efficiency reached by mechanical plant inoculation). The plants showed typical PPV symptoms two weeks post transfection. The virus presence was confirmed by immunoblotting, RT-PCR, as well as by successful transmission by sap to healthy plants. The co-transfection of *N. benthamiana* plants by PPV-M and PPV-D led to mixed infections with PPV-D strongly prevalent. We assumed the properties of cDNA constructs responsible for this behaviour.

Keywords: gene gun, PPV strains, immunoblotting

Introduction

Infectious clones of plant RNA viruses are excellent tools for research of the intracellular infection process (virus-host interactions), as well as the base for preparation of viral vectors for transient expression of exogenous genes in plants. Agroinfection, biolistic transfection, electroporation and mechanic inoculation are the most common methods for introducing foreign DNA into plant cells (Nagyová and Šubr, 2007).

PPV causes detrimental Sharka disease of stone fruit trees in many countries, especially in European and Mediterranean regions. Three of six recognized PPV strains have an economic impact, namely PPV-M, PPV-D and PPV-Rec (Candresse and Cambra, 2006). The relatively recently recognized strain PPV-Rec (evolved by homologous recombination of PPV-D and PPV-M) is commonly spread in central and southern Europe (Glasa et al., 2004). Detailed genomic functional analysis could help elucidate the high fitness of this strain under natural conditions. Therefore in our laboratory attempts are done to prepare an infectious cDNA clone of PPV-Rec. An efficient method of plant transfection is essential for this project. In this work we used infectious clones of PPV-D and PPV-M to optimize a simple biolistic transfection protocol and compare it to mechanical inoculation.

Material and methods

PPV cDNA clones designated pIC PPV-D (isolate Rankovic) and pIC PPV-M (isolate SK68) were kindly provided by Prof. García (CSIC Madrid, Spain) and Prof. Palkovics (CU Budapest, Hungary), respectively (López-Moya and García, 2000; Raughpathy et al., 2006). The plasmids were maintained in *Escherichia coli* DH5α and isolated by alkaline lysis. The *N. benthamiana* plants were transfected mechanically or biolistically using the PDS-1000 He apparatus (Biorad) after DNA binding to the tungsten microparticles M-10 according the manufacturer's recommendation. Four weeks old seedlings of *N. benthamiana* (5 – 6 leaf stage, total leaf surface about 1.5 cm^2 per plant) grown aseptically by two – five on Petri dishes with MS medium were used as target for biolistic transfection. Three days after bombardment the plants were transfered to common soil substrate. The transfection efficiency was evaluated visually (symptom recording) and by immunoblots of crude plant extracts using monoclonal (MAb) or polyclonal antibodies against PPV (Boscia et al., 1997; Šubr and Matisová, 1999). RT-PCR amplifying the NIb-CP viral genome region was performed too. Reverse transcription as described by Glasa et al. (2002), was followed by PCR using strain-unspecific primers

NCuniFor (5'-GAGGCAATTTGTGCTTCAATGG-3') and
NCuniRev (5'-CGCTTAACTCCTTCATACCAAG-3')

under following conditions: 95ºC/5 min, 40 x (95ºC/15 s, 60ºC/30 s, 72ºC/1 min), 72ºC/10 min.

Results and discussion

PDS-1000 He is the only type of biolistic instrument available in Slovakia today. Because its construction with a vacuum box limits target size small plant seedlings arranged on Petri dishes were used. About 70 % of plants survived the subsequent transplanting procedure. Two weeks later typical symptoms of PPV infection were observed (leaf distortion and mosaic). The presence of the virus in systemically infected (not bombarded) leaves was verified by RT-PCR with PPV-specific primers (Fig. 1), and its infectivity was proved by succesful mechanical sap transmission to healthy plants. Results of the immunoblot analyses generally agreed with symptom manifestation.

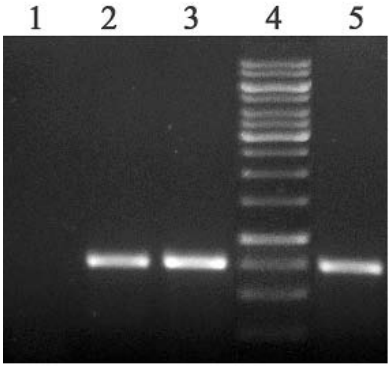

Fig. 1 RT-PCR detection of PPV from biolistically transfected *N. benthamiana*. Lane 1 – negative control (healthy plant), lane 2 – pIC PPV-D-transfected plant, lane 3 – pIC PPV-M-transfected plant, lane 4 – 1 kbp DNA ladder, lane 5 – positive control (PPV-D-infected plant).

The efficiency of biolistic transfection was markedly higher (about 80 % for both clones) compared to mechanical inoculation (6 % for pIC PPV-M, 10 % for pIC PPV-D). We found no influence of helium pressure or target distance from the microparticle source on transfection efficacy (Tab. 1).

Tab. 1 Efficiency of biolistic transfection under various conditions. p – helium pressure, X – distance between the particle source and target tissue, η – transfection efficiency (% of infected plants).

Isolate	p [kPa]	X [cm]	η [%]
SK68	7580	9	73.3
		12	80
	9300	9	81.3
		12	75
Rankovic	7580	9	86.7
		12	75
	9300	9	80
		12	77.8

pIC PPV-D caused slightly more intensive symptoms and it gave a much stronger immunoblot signal than pIC PPV-M. Cotransfection by both infectious clones led to mixed infections with PPV-D being strongly prevalent (Fig. 2). Such difference in capsid protein concentration was not observed with mechanically passaged virus isolates Rankovic and SK68. We suggest different relative infectivity reflected rather properties of constructed cDNA clones than the PPV isolates. Stronger expression could be caused by the doubled 35S promoter in pIC PPV-D. Moreover, absence of the NOS terminator in the pIC PPV-M could lead to the production of longer primary transcripts and problems with their translocation to the cytoplasm where proteosynthesis takes place.

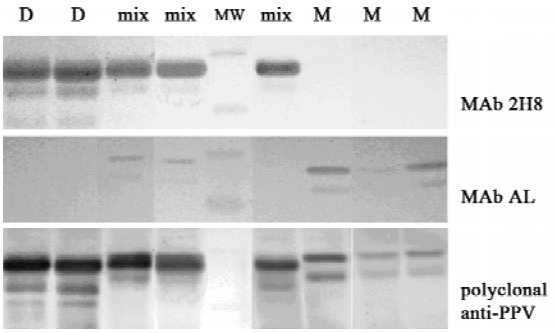

Fig. 2 Immunoblot analysis of *N. benthamiana* transfected with pIC PPV-M, pIC PPV-D and with a mixture of both. MW – molecular weight marker (bands correspond to 47 and 34 kDa); used antibodies are indicated on the right border.

Mechanic inoculation is simple and inexpensive method commonly used for the infection of plants by viruses in laboratory conditions. However, the transfer of isolated DNA into the nucleus without drastically disturbing the cell is statistically improbable using this method. Low transfection efficiency disables to detect changes of infectivity resulted e.g. from directed genome manipulation.

Biolistic techniques are more gentle and facilitate entry into the cell nuclei even in internal tissues. Of the two types of biolistic apparatuses the gun-shaped devices are more flexible and better suited for whole plant transfection. However, PDS-1000 He is the only type of biolistic device available presently in Slovakia. Our optimized protocol enabled a high efficiency of transfection of *N. benthamiana* plants by PPV cDNA clones.

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Literature

Boscia, D.; Zeramdini, H.; Cambra, M.; Potere, O.; Gorris, M.T.; Myrta, A.; DiTerlizzi, B.; Savino V.; 1997: Production a characterization of a monoclonal antibody specific to the M serotype of plum pox potyvirus. European Journal of Plant Pathology **103**, 477-480.

Candresse, T.; Cambra, M.; 2006: Causal agent of sharka disease: historical perspective and current status of Plum pox virus strains. EPPO Bulletin **36**, 239-246.

Glasa, M.; Marie-Jeanne, V.; Labonne, G. Šubr, Z. Kúdela, O.; Quiot, J.-B.; 2002: A natural population of recombinant Plum pox virus is viable and competitive under field conditions. European Journal of Plant Pathology **108**, 843-853.

Glasa, M.; Palkovics, L.; Kominek, P.; Labonne, G.; Pittnerova, S.; Kudela, O.;Candresse, T.; Subr Z.; 2004: Geographically and temporally distant natural recombinant isolates of Plum pox virus (PPV) are genetically very similar and form a unique PPV subgroup. Journal of General Virology **85**, 2671-2681.

López-Moya, J.J.; García, J.A.; 2000: Construction of a stable and highly infectious intron-containing cDNA clone of plum pox potyvirus and its use to infect plants by particle bombardment. Virus Research **68**, 99-107.

Nagyová, A.; Šubr Z.; 2007: Infectious full-length clones of plant viruses and their use for construction of viral vectors for heterologous expression in plants. Acta Virologica **51**, 223-237.

Raghupathy, M.B.; Griffiths, J.S.; Stobbs, L.W.; Brown, D.C.W.; Brandle, J.E.; Wang A.; 2006: Transfection of Arabidopsis protoplasts with a Plum pox virus (PPV) infectious clone for studying early molecular events associated with PPV infection. Journal of Virological Methods **136**, 147-153.

Šubr, Z.; Matisová, J.; 1999: Preparation of diagnostic monoclonal antibodies against two potyviruses. Acta Virologica **43**, 255-257.