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# High Level of Field Resistance of Transgenic Tobaccos Induced by Integrated Potato Virus Y Coat Protein Gene

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The coat protein gene (CP) from a highly virulent, necrotic and dominant strain of potato virus Y (PVY) originated from the Hungarian flora has been engineered via *Agrobacterium* infection into different Hungarian tobacco breeding lines and cultivars. The integration of the CP was confirmed by polymerase chain reaction (PCR) using genomic preparations. The transcription and the expression of the integrated CP gene were detected by Northern and Western analysis. The pathogen-derived resistance was demonstrated by inoculation of the R1 progeny of transformant plants with purified PVY. The efficiency of the protection varied between different transgenic tobaccos ranging from complete to no protection. The challenge infection of the plants was monitored by dot blot hybridisation at different intervals after mechanical inoculation.

Western blot analysis showed that there is no correlation between the level of expressed CP and the extent of resistance. From tobacco cultivars namely Virgin D, Stamm C2 and Hevesi 11, 38, 55 and 23 transformants were obtained, respectively. After several years of greenhouse experiments, only the extreme resistant tobaccos were planted field under the special licence, given by the competent authority. In field conditions, transgenic tobacco varieties showed extreme resistance against natural infection of PVY.

Keywords: field resistance, potato virus Y, transgenic tobacco.

Abbreviations: coat protein (CP), non-translated region (NTR), polymerase chain reaction (PCR), potato virus Y (PVY).

Since the first successful demonstration of genetically engineered resistance to a plant virus (Powell-Abel et al., 1986), the coat protein gene mediated resistance was demonstrated in a couple of dozen host virus systems (Beachy, 1997; Lawson et al., 1990; Ling et al., 1991; Lindbo and Dougherty, 1992; Namba et al., 1992; Stark and Beachy, 1989; Valkama-M. et al., 2000). Today, several transgenic plants bearing the viral coat protein gene are in an advanced stage of the R and D for commercialisation (James, 1998). Tobacco and potato productions are hampered in Central Europe due to the high incidence of potato virus Y. The aphid transmitted potyvirus is widely spread and is devastating these crops (de Bokx and Huttinga, 1981; Hollings and Brunt, 1981). In the last three decades the necrotic strains of this virus have became abundant and caused serious losses. Important and valuable breeding lines of tobaccos could not be brought into breeding program due to severe symptoms induced by the necrotic strain of this virus. Efficient pathogen derived resistance induced by the integrated PVY coat protein gene in tobacco was achieved and described earlier in our laboratory by Kollár et al. (1993).

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Transgenic plants of three tobacco cultivars, namely Virgin D, Stamm C2 and Hevesi 11 were produced via Agroinfections using the construct based on the cloned coat protein gene of the necrotic strain of PVY isolated from the Hungarian flora (Beczner et al., 1984; Kollár et al., 1993; Thole et al., 1993). Molecular characterisation of those R1 progeny was detailed earlier (Kollár et al., 1993). Transgenic tobaccos showing high levels of resistance in consecutive years in greenhouse conditions were further analysed and selected for field experiments to study the plants's field resistance and the sustainability of their resistance. Plant DNA from the same lot of plants was extracted essentially as described by Rubino et al. (1992), while the polymerase chain reaction was carried out in Perkin Elmer thermal cycler using the oligonucleotide primers 5'-CACTTGCTCGAGTATGCTCCACAGC-3' homologous to nucleotides 8776-8800 and 5'-CGTCCGGAGAGACACTACA-3' complementary to nucleotides 9376-9394 chosen from the CP sequences and the 3' NTR covering a 618 bp long DNA. PCR reactions were carried out as described by the manufacturer's instructions and the PCR products were analysed by gel electrophoresis and Southern hybridisation with $^{32}P$ labelled nick-translated CP DNA construct as a probe according to Sambrook et al. (1989) *Fig. 1* demonstrates the Southern hybridisation of a set of Virgin D transformants. Northern blot analysis was performed after the total nucleic acids isolation from the same plants using essentially of the method of White and Kaper (1989) and the separated nucleic acids were

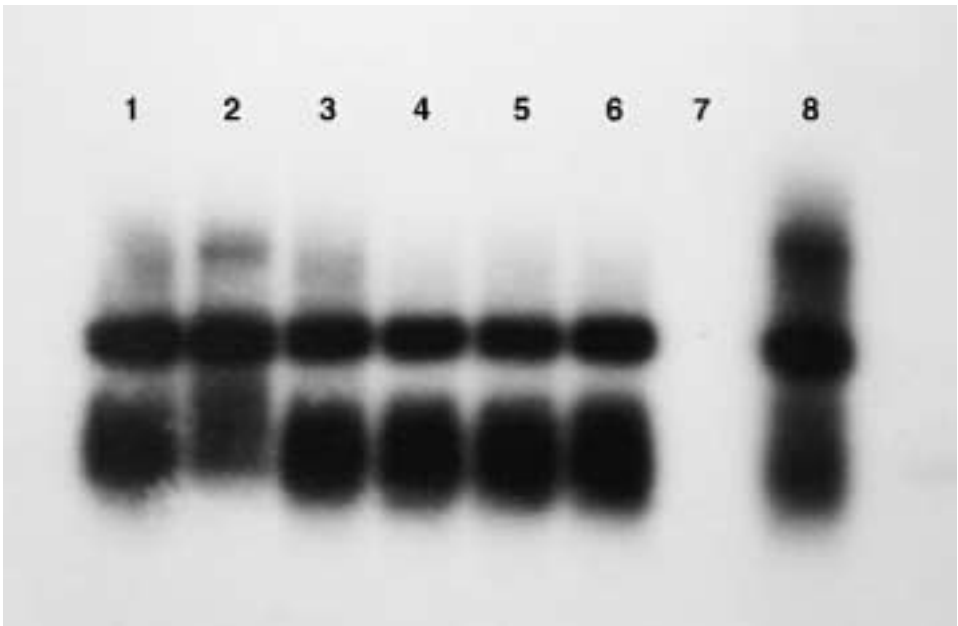


Fig. 1. Southern blot analysis. DNA extracts prepared from Virgin D transformants and were amplified with PCR. Lanes: 1. VD1, 2. VD2, 3. VD3, 4. VD4, 5. VD5, 6. VD6, 7. non-transformed tobacco as negative control, 8. PVY infected, non-transgenic tobacco as positive control

transferred from the formaldehyde gel to Hybond membranes. Hybridisation reactions revealed that all transgenic plants producing the same amount of transcripts (data not shown). The expression of the coat protein in these transgenic plants was demonstrated by Western blot analysis according to the protocol described in Sambrook et al. (1989). The 12% polyacrylamide gel separated protein extracts of the plants were transferred into Hybond C membrane. The membrane bound proteins were visualised by using alkaline phosphatase conjugated anti-PVY CP antibody and 5-bromo-4-chloro-3-indolyl-phosphate-para-toluidine and para-nitro-blue-tetrazolium-cholride chromogenic substrate.

*Fig. 2* shows the Western blot analysis from the Virgin D transformant lines demonstrating few differences among the transformant lines. Plants transferred to field conditions were monitored visually and as shown with molecular techniques regarding their characteristics. In the breeding station, where the transgenics were in experimental plots, plants were monitored regularly and samples were taken for testing their resistance. Virus replication was monitored with dot blot showing no sign for virus replication of the

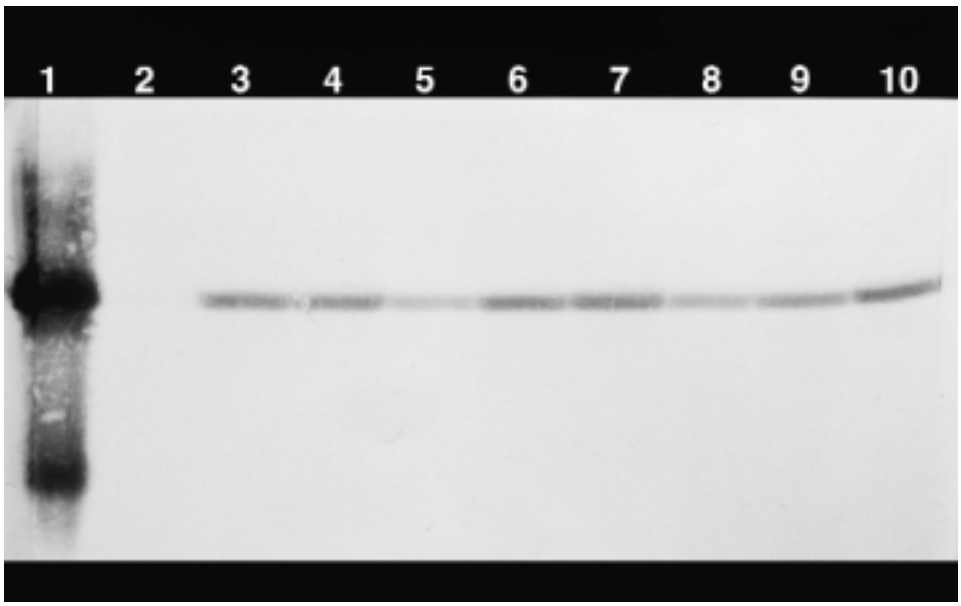


Fig. 2. Western blot analysis. Protein samples were prepared from transgenic Virgin D plants. Lanes: 1. PVY infected, non-transgenic tobacco as positive control, 2. non-transformed tobacco as negative control, 3. VD2, 4. VD3, 5. VD4, 6. VD5, 7. VD6, 8. VD7, 9. VD8, 10. VD9

transgenics reported here. All plants which proved earlier with extreme resistance against mechanical inoculation of PVY exhibited high resistance in natural conditions (see *Fig. 3*). Here above we summarise our preliminary reports that those transgenic tobaccos, which exhibited high level of resistance against PVY in laboratory and greenhouse conditions

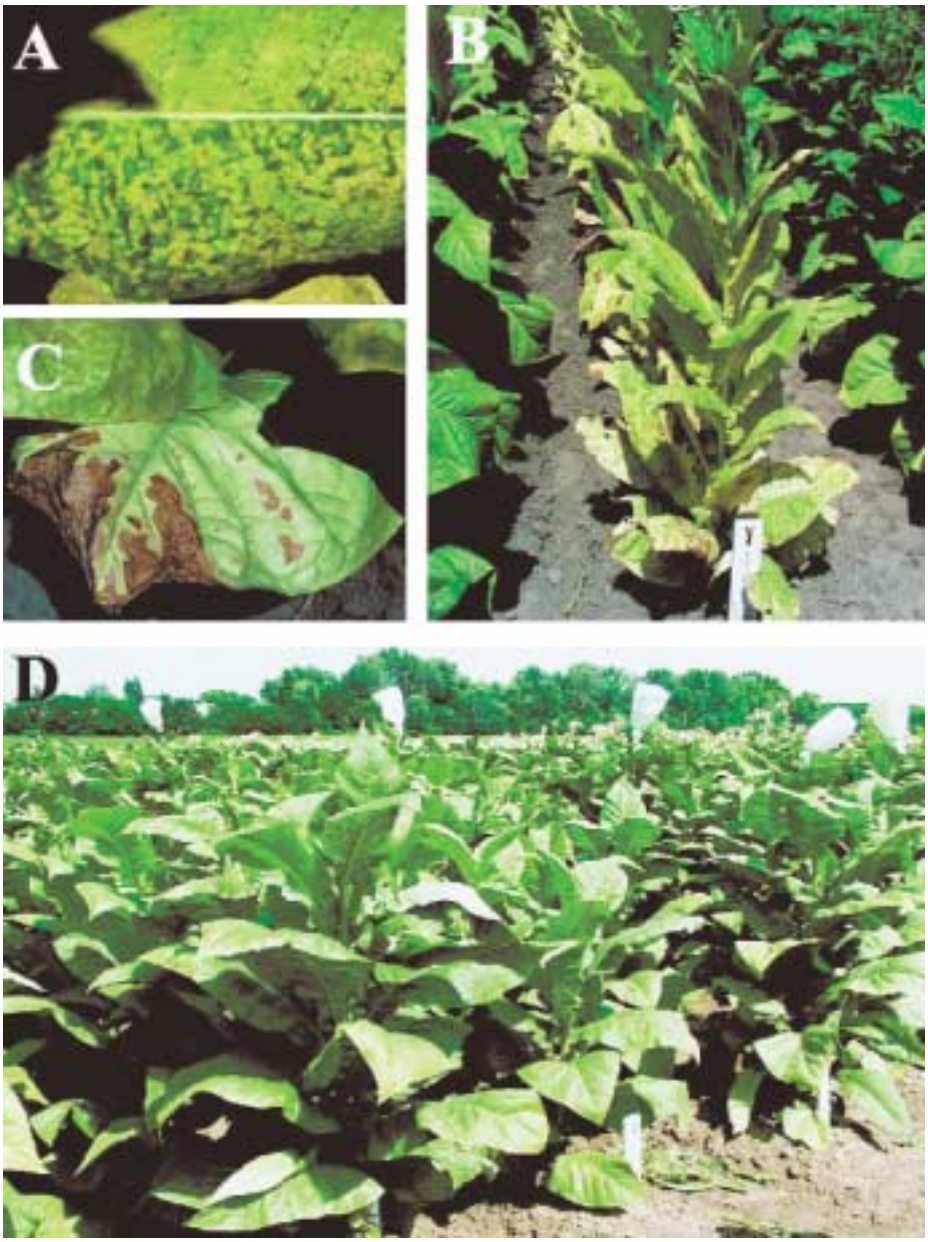


Fig. 3. PVY induced symptoms on tobaccoes grown in field.
A: systemic symptoms on non-transgenic tobbacco leaf.
B: PVY symptoms on a non-transgenic row of plants planted between transgenic plants.
C: Nectrotization of infected old leaf after infection.
D: View on transgenic plants showing extreme resistance against PVY

are expressing high levels of resistance against the same virus in natural conditions. As we have an enormous number of different transformant lines with different levels of resistance against this virus, detailed analysis of those plants are in progress regarding the mechanisms of the resistance and also the role of the copy number of the integrated gene in the development of the resistance.

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