

Assessment of virus resistance in transgenic *Nicotiana tabacum* cv. Samsun lines against three Iranian isolates of potato virus Y

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ABSTRACT

Tobacco plants (*Nicotiana tabacum* cv. Samsun) were transformed by coat protein gene of potato virus Y necrotic strain (PVY^N-CP) using pBIN19 binary vector and agroinoculation method. The primers designed so that the inserted PVY-CP transgene had not any start codon and so, it was unable to be translated into coat protein product. Resistance of 31 transgenic *Nicotiana tabacum* cv. Samsun lines containing PVY^N coat protein transgene (PVY^N-CP), were evaluated against the mechanical challenge inoculation of 3 prevalent Iranian PVY strains. On the basis of biological and serological properties, 3 Iranian PVY isolates include PVYn-H, PVYn-Mz (necrotic strain of PVY) and PVYo-Ar (an Ordinary strain). Symptom evaluation and ELISA tests showed that after challenge inoculation of 31 transgenic lines with PVYn-H, PVYn-Mz and PVYo-Ar isolates, 5, 4 and 0 lines showed resistant phenotype, 9, 10 and 2 lines showed moderate symptoms of the disease and 17, 17 and 29 lines showed susceptible phenotype, respectively. Using TAS-ELISA and Western-blot analysis, the protein product of PVY^N-CP transgene could not be detected in any of 31 transgenic lines. It seems that an RNA mediated resistance is responsible for resistance in the PVY-CP transgenic tobacco plants. In *Nicotiana* species, natural resistance sources to PVY infection are poor and development of engineered resistance sources against PVY infections would be useful.

Key Words: Potato virus Y, Coat Protein, Cloning, Transformation, Agroinoculation, Transgenic Plant Resistance, Iranian PVY strains, Engineered resistance.

References

- ASSAD, F. F., TUCKER, K. L. and SIGNER, E. R., 1993. Epigenetic repeat-induced gene silencing (RIGS) in Arabidopsis. *Plant Mol. Biol.* 22, 1067-1085.

- BESTOR, T. H. and COXON, A., 1993. Cytosine methylation: The pros and cons of DNA methylation. *Curr. Biol.* 3: 384-386.
- BEVAN, M., 1984. Binary *Agrobacterium* vectors for plant transformation. *Nucl. Acids Res.* 12: 8711-8721.
- DeBOKX, J. A. and HUTTINGA, H., 1981. Potato virus Y.CMI/AAB Descriptions of plant viruses, No. 242, 6p.
- De AVILA, A. C., HUGUENOT, C., RESENDE, R. O., KITAJIMA, E. W., GOLDBACH, R. and PETERS, D., 1990, Serological differentiation of 20 isolates of tomato spotted wilt virus. *J. Gen. Virol.* 71: 2801-2807.
- DOUGHERTY, W. G. and PARKS, T. D., 1995. Transgenes and gene expression: telling us something new? *Curr. Opin. Plant Biol.* 7:399-405.
- FARINELLI, L., MALONE, P., and COLLET, G. F., 1992. Heterologous encapsidation of potato virus Y strain O (PVY^O) with the transgenic coat protein of PVY strain N (PVY^N) in *Solanum tuberosum* cv. Bintje. *Technology* 10: 1020-1025.
- FARINELLI, L., and Malnoe, P., 1993. Coat protein mediated resistance to potato virus Y in tobacco-Is the transgenic coat protein required for protection? *Mol. Plant Mic. Interact.* 6: 284-292.
- FITCH, M. M., MANSHARDT, R. M., GONSALVES, D., SLIGHTOM, J. L. and SANFORD, J. C., 1992. Virus resistant papaya plants derived from tissues bombarded with the coat protein of papaya ring spot virus. *Biotechnology* 10: 1466-1472.
- FRISCH, D. A., HARRIS-HALLER, L. W., YOKUBAITIS, N. T., THOMAS, T. L., HARDIN, S. H. and HALL, T. C., 1995. Complete sequence of the binary vector Bin19. *Plant Molecular Biology* 27:405-409.
- GAMBORG, O. L. and SHYLUK, J. P., 1981. Nutrient, media and characteristics of plant cell and tissue culture. pp. 21-44. *Plant Tissue Culture: Methodes and Applications in Agriculture.* By: Thorpe T. A. (ed.) Academic Press, New York.
- GONSALVES, D. and SLIGHTOM, J. L., 1993. Coat protein mediated protection: analysis of transgenic plants for resistance in a variety of crops. *Seminars in Virology* 4:397-405.
- HASHEMI, H., SHAHRAEEN, N. and DOMANSKY, N., 1998. Expression of potato virus X coat protein in transgenic potato. *Applied Entomology and Phytopathology* 66:1-3, (in Farsi with English summary).
- HOOKE, W. J., (Ed.), 1990. *Compendium of potato diseases.* APS Press, Minnesota, USA.

- HORSCH, R. B., FRY, J. E., HOFFMANN, N. L., EICHHLTZ, D., ROGERS, S. G. and FRALEY, R. T., 1985. A simple and general method for transferring genes into plants. *Science* 227:1229-1231.
- INGELBRECHT, I., VAN HOUTT, H., VAN MONTAGU, M. and DEPICKER, A., 1994. Post-transcriptional silencing of reporter transgenes in tobacco correlates with DNA methylation. *Proc. Natl. Acad. Sci. USA* 91: 10502-10506.
- KOLLAR, A., THOLE, V., DALMAY, T., SALAMON, P., and BALAZS, E., 1993. Efficient pathogen derived resistance induced by integrated potato virus Y coat protein gene in tobacco. *Biochemie* 75:623-629.
- LAEMMLI, U. K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage. *Nature* 227: 680-685.
- LAWSON, C., KANIEWSKI, W., HALEY, L., ROZMAN, R., NEWELL, C., SANDERS, P. and TUMER, N.E., 1990. Engineering resistance to mixed virus infection in a commercial potato cultivars: Resistance to potato virus X and potato virus Y in transgenic Russett Burbank. *Bio/Technology* 8: 127-134.
- LINDBO, J. A., SILVA-ROSALES, L., PROEBSTING, W. M. and DOUGHERTY, W. G., 1993. Induction of a highly specific antiviral state in transgenic plants: implications for regulation of gene expression and virus resistance. *Plant Cell* 5: 1749-1759.
- MATZKE, M. A., PRIMING, M., TRNOVSKY, J., AND MATZKE, A. J. M., 1989. Reversible methylation and inactivation of marker genes in sequentially transformed tobacco plants. *EMBO J.* 8:643-649.
- MATZKE, M. A. and MATZKE, A. J. M., 1995. How and why do plants inactivate homologous (trans)genes? *Plant Physiol.* 107: 679-685.
- METZLAFF, M., O'DELL, M., CLUSTER, P.D. and FLAVELL, R. B., 1997. RNA mediated RNA degradation and chalcone synthase A silencing in *Petunia*. *Cell* 88:845-854.
- MEYER, P., 1995. Understanding and controlling transgene expression. *Trends Biotechnology* 13: 332-337.
- ROBAGLIA, C., DURAND-TARDIF, M., TRONCHET, M., BOUDAZIN, G., ASTIER-MANIFACIER, S. and CASSE-DELBART, F., 1989. Nucleotide sequence of potato virus Y(N strain) genomic RNA. *J.Gen. Virol.* 70:935-947.
- ROGERS, S. G. , HORSCH, R. B. and FRALEY, R. T., 1986. Gene transfer in plants: Production of transformed plants using Ti plasmid vectors. *Methods Enzymol.* 118:627-640.

- SAMBROOK, J., FRITSCH, E. F. and MANIATIS, T., 1989. *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbour Laboratory, NewYork.
- SHEW, H. D. and LUCAS, G. B., 1991. *Compendium of tobacco diseases*. APS Press,Minnesota,USA.
- SMITH, H. A., POWERS, H., SAWNEY, S., BROWN, Ch. and DOUGHERTY, W. G., 1995. Transgenic potato virus Y resistance in potato: Evidence for an RNA-mediated response. *Phytopathology* 85: 864-870.
- STARK, D. M., BEACHY, R. N., 1989. Protection against potyvirus infection in transgenic plants: Evidences for board resistance. *Bio/Technology*:1257-1262.
- SUDARSONO, YOUNG, J. B., WOLOSHUK, S. L., PARRY, D. C., HELLMANN, G. M., WERNSMAN, E. A., LOMMEL, S. A. and WEISSINGER, A. K., 1995. Transgenic burley and flue-cured tobacco with resistance to four necrotic isolates of potato virus Y. *Phytopathology* 85:1493-1499.
- TORRES, K. C., 1998. *Tissue culture techniques for horticultural crops*. Translated into persian by Khosh-Khui, 436p, Shiraz University Press.
- WATERHOUSE, P. M., GRAHAM, M. W. and WANG, M., 1998. Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. *Proc. Natl. Acad. Sci. USA* 95:13959-13964.
- WEBER, K. and OSBORN, M., 1969. The reliablity of molecular weight determinations by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244:4406-4412.
- Wilde, C. De., Van Houdt, H., De Buck, S., Angenon, G., De Jaeger G., Depicker, A. (2000) Plants as bioreactors for protein production: avoiding the problem of transgene silencing. *Plant Molecular Biology* 43: 347-359.

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