Construction of a Taiwan Mild Strain of Papaya Ringspot Virus by Site-directed Mutagenesis in HC-Pro Gene

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ABSTRACT

Papaya ring spot virus (PRSV) was first found in southern Taiwan in 1975, and within 4 years, the virus had destroyed most of the papaya production areas in Taiwan. Development of virus-resistant cultivars by classical breeding or by genetically engineering of the plant is difficult and slow. The alternative method is to use cross protection to against virus diseases. In cross protection, plants infected with a mild strain of virus are protected by a second related severe strain of the same virus. Previous studies have shown that the mild strains of a plant virus used for cross protection were derived either from field collection or from artificial mutagenesis. In PRSV, the attenuated strain of PRSV HA5-1 that infects papaya without conspicuous symptoms has been applied for cross protection against its parental severe HA strain. The strain-specific protection limits its application in Taiwan. Therefore, a Taiwan mild strain must be developed for effectively protecting papaya plants in Taiwan. Previous study had shown that the P1 and HC-Pro proteins of PRSV are involved in symptom expression and viral accumulation in infected plants. Comparison of the P1 and HC-Pro sequences from a Taiwan severe strain PRSV YK with a Hawaii severe strain PRSV HA, its mild mutant HA5-1 and other potyviruses revealed that two amino acid changes (I309→S and K481→Q) in P1 region and two amino acid changes (F753→L and D944→N) in HC-Pro region may be responsible for the attenuation of PRSV from severe to mild symptoms. Site-directed mutagenesis was carried out through polymerase chain reaction to change the corresponding sites of PRSVYK in vivo transcripts. Two single-mutated viruses derived from pPYK753L and pPYK944N and one double-mutated virus derived from pPYK753L944N were constructed and were tested by mechanical inoculation on papaya seedlings for the virulence. The pPYK753L944N clone induced mild symptoms on papaya. The pPYK753L and pPYK944N clone induced no symptoms on the papaya plants one-month post-inoculation. However, the accumulation of the double-mutated pPYK753L944N virus was as 50% lower as that of the wild type PRSV YK inoculated plants according to the reading from Enzyme-Linked Immunosorbent Assay (ELISA) using PRSV polyclonal antibody. The ability of pPYK753L944N to protect papaya plants again the severe PRSV YK was further evaluated under green house conditions. One month after the challenge of the severe YK strain, the pPYK753L944N protected papaya plants showed very mild symptom, and the accumulation was higher than pPYK753L944N inoculated papaya for 60%. The results suggest that the two amino acid changes in the HC-Pro gene are the major virulent determinants of PRSV YK. The double-mutated virus pPYK753L944N is a potentially useful Taiwan attenuated PRSV and can be use to reduce the damage of severe strain by cross protection.

Keywords : Papaya ringspot virus(PRSV) ; Point mutantino ; Cross protection

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REFERENCES

1.李皇照. (2006). 全球木瓜生產與貿易. 行政院統計出版品農政與農情會刊 172. 2.Adams, M. J., Antoniw, J. F. & Beaudoin, F. (2005). Overview and analysis of the polyprotein cleavage sites in the family Potyviridae Molecular Plant Pathology 6, 471-487. Anandalakshmi, R., Pruss, G. J., Ge, X., Marathe, R., Mallory, A. C., Smith, T. H. & Vance, V. B. (1998). A viral suppressor of gene silencing in plants. Proc Natl Acad Sci U S A 95, 13079-13084. Blanc, S., Ammar, E. D., Garcia-Lampasona, S., Dolja, V. V., Llave, C., Baker, J. & Pirone, T. P. (1998). Mutations in the potyvirus helper component protein: effects on interactions with virions and aphid stylets. J Gen Virol 79 (Pt 12), 3119-3122. Branch, A. D., Benenfeld, B. J., Franck, E. R., Shaw, J. F., Varban, M. L., Willis, K. K., Rosen, D. L. & Robertson, H. D. (1988). Interference between coinoculated viroids. Virology 163, 538-546. Brigneti, G., Voinnet, O., Li, W. X., Ji, L. H., Ding, S. W. & Baulcombe, D. C. (1998). Viral pathogenicity determinants are suppressors of transgene silencing in Nicotiana benthamiana. Embo J 17, 6739-6746. Broadbent, L. (1976). Epidemiology and control of tomato mosaic virus. Annu Rev Phytopathol 14, 75-96. Chiang, C.-H., Lee, C.-Y., Wang, C.-H., Jan, F.-J., Lin, S.-S., Chen, T.-C., Raja, J. A. J. & Yeh, S.-D. (2007). Genetic analysis of an attenuated Papaya ringspot virus strain applied for cross-protection. European Journal of Plant Pathology 118, 333-348. Choi, I. R., Horken, K. M., Stenger, D. C. & French, R. (2005). An internal RNA element in the P3 cistron of Wheat streak mosaic virus revealed by synonymous mutations that affect both movement and replication. J Gen Virol 86, 2605-2614. Chu, M., Lopez-Moya, J. J., Llave-Correas, C. & Pirone, T. P. (1997). Two separate regions in the genome of the tobacco etch virus contain determinants of the wilting response of Tabasco pepper. Mol Plant Microbe Interact 10, 472-480. Cost, A. S. & Muller, G. W. (1980). Tristeza control by cross protection:. A U.S.-Brazil cooperative success. Plant Dis 64, 538-541. Cronin, S., Verchot, J., Haldeman-Cahill, R., Schaad, M. C. & Carrington, J. C. (1995). Long-distance movement factor: a transport function of the potyvirus helper component proteinase. Plant Cell 7, 549-559. De Zoeten, G. A. & Fulton, R. W. (1975). Understanding generates possibilities. Phytopathology 65, 221-222. Dolja, V. V., Haldeman, R., Robertson, N. L., Dougherty, W. G. & Carrington, J. C. (1994). Distinct functions of capsid protein in assembly and movement of tobacco etch potyvirus in plants. EMBO 13, 1482-1491. Fan, Z., Chen, H., Liang, X. & Li, H. (2003). Complete sequence of the genomic RNA of the prevalent strain of a potyvirus infecting maize in China. Arch Virol 148, 773-782. Fletcher, J. T. (1978). The use of avirulent strains to protect plants. against the effects of virulent strains. . Ann Appl Biol 89, 110-114 Gal-On, A. (2000). A Point Mutation in the FRNK Motif of the Potyvirus Helper Component-Protease Gene Alters Symptom Expression in Cucurbits and Elicits Protection Against the Severe Homologous Virus. Phytopathology 90, 467-473. Ghabrial, S. A., Smith, H. A., Parks, T. D. & Dougherty, W. G. (1990). Molecular genetic analyses of the soybean mosaic virus NIa proteinase. J Gen Virol 71 (Pt 9), 1921-1927. Gibbs, A. (1969). Plant virus classification. Adv Virus Res 14, 263-328. Hamilton, A. J. & Baulcombe, D. C. (1999). A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 286, 950-952. Harrison, B. D. (1958). Ability of single aphids to transmit both avirulent and virulent strains of potato leaf roll virus. Virology 6, 278-286. Hsu, Y. C., T.J, Y. & Y.C, C. (2005). A new combination of RT-PCR and reverse dot blot hybridization for rapid detection and identification of potyviruses. Journal of virological methods 128, 54-60. Kasschau, K. D. & Carrington, J. C. (1998). A counterdefensive strategy of plant viruses: suppression of posttranscriptional gene silencing. Cell 95, 461-470. Kim, K.-S., Oh, H.-Y., Suranto, S., Nurhayati, E., Gough, K. H., Shukla, D. D. & Pallaghy, C. K. (2003). nfectivity of in vitro transcripts of Johnsongrass mosaic potyvirus full-length cDNA clones in maize and sorghum. Archives of virology 148, 563-574. Klein, P. G., Klein, R. R., Rodriguez-Cerezo, E., Hunt, A. G. & Shaw, J. G. (1994). Mutational analysis of the tobacco vein mottling virus genome. Virology 204, 759-769. Lain, S., Martin, M. T., Riechmann, J. L. & Garcia, J. A. (1991). Novel catalytic activity associated with positive-strand RNA virus infection: nucleic acid-stimulated ATPase activity of the plum pox potyvirus helicaselike protein. J Virol 65, 1-6. Lain, S., Riechmann, J. & Garcia, J. (1990). RNA helicase: a novel activity associated with a protein encoded by a positive strand RNA virus. Nucleic Acid Research 18, 7003-7006. Lecoq, H., Lemaire, J. M., Bourdin, D. & Wipf-Scheibel, C. (1991a). Control of zucchini yellow mosaic virus in squash by cross protection. Plant Dis 75, 208-211. Lecoq, H., Lemaire, J. M. & Wipf-Scheibel, C. (1991b). Control of zucchini yellow mosaic virus in squash by cross protection. plant disease 75, 208-211. Li, X., Valdez, P., Olvera, R. & Carrington, J. (1997). Functions of the tobacco etch virus RNA polymerase (NIb): subcellular transport and protein-protein interaction with VPg/proteinase (NIa). J Virol, 71, 1598-1607. Lin, S.-S., Wu, H.-W., Jan, F.-J., Hou, R. F. & Yeh, S.-D. (2007). Modifications of the helper component-protease of Zucchini yellow mosaic virus for generation of attenuated mutants for cross protection against severe infection Phytopathology 97, 287-296 Lin, S. S., Hou, R. F. & Yeh, S. D. (2001). Complete Genome Sequence and Genetic Organization of a Taiwan Isolate of Zucchini yellow mosaic virus. Botanical Bulletin of Academia Sinica 42, 243-250. Maia, I. G., Haenni, A. & Bernardi, F. (1996). Potyviral HC-Pro: a multifunctional protein. J Gen Virol 77 (Pt 7), 1335-1341. McKinney, H. H. (1929). Mosaic diseases in the Canary Islands, West Africa and Gibraltar. J Agric Res 39, 557-578. Osbourn, J. K., Plaskitt, K. A., Watts, J. W. & Wilson, T. M. A. (1989). Tobacco mosaic virus coat protein and reporter gene transcripts containing tobacco plants; implications for coat protein-mediated protection. Molec Plant Microbe Interact 2, 340-345. Palukaitis, P. & Zaitlin, M. (1984). A model to explain the cross protection phenomenon shown by plant viruses and viroids, in Plant-Microbe Interactions: Molecular and Genetic Perspectives (Kosuge, T. and Nester, E. W., eds.), MacMillan, New York, p. 420. Peng, Y. H., Kadoury, D., Gal-On, A., Huet, H., Wang, Y. & Raccah, B. (1998). Mutations in the HC-Pro gene of zucchini yellow mosaic potyvirus: effects on aphid transmission and binding to purified virions. Journal of General Virology 79, 897-904. Plisson, C., Drucker, M., Blanc, S., German-Retana, S., Le Gall, O., Thomas, D. & Bron, P. (2003). Structural characterization of HC-Pro, a plant virus multifunctional protein. J Biol Chem 278, 23753-23761. Ponz, F., . & Bruening, G. (1986). Mechanisms of resistance to plant viruses. Annu Rev Phytopathol 24, 336-381. Posnette, A. F. & Todd, J. M. (1955). Virus disease of cacao in West Africa. IX. Strain variant and

interference in virus 1A. Ann Appl Biol 43, 433-453. Pruss, G., Ge, X., Shi, X., Carrington, J. & Bowman, V. V. (1997). Plant Vira1 Synergism: The Potyviral Genome Encodes a Broad-Range Pathogenicity Enhancer That Transactivates Replication of Heterologous Viruses. Plant Cell 9, 859-868. Restrepo-Hartwig, M. A. & Carrington, J. C. (1992). Regulation of nuclear transport of a plant potyvirus protein by autoproteolysis. J Virol 66, 5662-5666. Restrepo-Hartwig, M. A. & Carrington, J. C. (1994). The tobacco etch potyvirus 6-kilodalton protein is membrane associated and involved in viral replication. J Virol 68, 2388-2397. Riechmann, J. L., Cervera, M. T. & Garcia, J. A. (1995). Processing of the plum pox virus polyprotein at the P3-6K1 junction is not required for virus viability. J Gen Virol 76 (Pt 4), 951-956. Rodriguez-Cerezo, E., Findlay, K., Shaw, J. G., Lomonossoff, G. P., Qiu, S. G., Linstead, P., Shanks, M. & Risco, C. (1997). The coat and cylindrical inclusion proteins of a potyvirus are associated with connections between plant cells. Virology 236, 296-306. Rojas, M. R., Zerbini, F. M., Allison, R. F., Gilbertson, R. L. & Lucas, W. J. (1997). Capsid protein and helper component-proteinase function as potyvirus cell-to-cell movement proteins. Virology 237, 283-295. Schaad, M. C., Jensen, P. E. & Carrington, J. C. (1997a). Formation of plant RNA virus replication complexes on membranes: role of an endoplasmic reticulum-targeted viral protein. Embo J 16, 4049-4059. Sheen, T.-F., Wang, H.-L. & Wang, D.-N. (1998). Control of Papaya Ringspot Virus by Cross Protection and Cultivation Techniques. Japanese Society for Horticultural Science 67, 1232-1235. Shukla, D. D. & Ward, C. W. (1989). Identification and classification of potyviruses on the basis of coat protein sequence data and serology. Brief review. Arch Virol 106, 171-200. Tribodet, M., Glais, L., Kerlan, C. & Jacquot, E. (2005). Characterization of Potato virus Y (PVY) molecular determinants involved in the vein necrosis symptom induced by PVYN isolates in infected Nicotiana tabacum cv. Xanthi. J Gen Virol 86, 2101-2105. Urcuqui-Inchima, S., Haenni, A. L. & Bernardi, F. (2001). Potyvirus proteins: a wealth of functions. Virus Res 74, 157-175. Varrelmann, M. & Maiss, E. (2000). Mutations in the coat protein gene of Plum pox virus suppress particle assembly, heterologous encapsidation and complementation in transgenic plants of Nicotiana benthamiana Journal of General Virology 81, 567-576. Wang, C.-H. & Yeh, S.-D. (1997). Divergence and conservation of the genomic RNAs of Taiwan and Hawaii strains of papaya ringspot potyvirus. Archives of virology 142, 271-285. Wilson, T. M. A. & Watkins, A. C. (1986). Influence of exogenous viral coat protein in the cotranslational disassembly of tobacco mosaic virus (TMV) particles in vitro. Virology 149, 132-135. Yeh, S.-D. & Gonsalves, D. (1984b). Purification and immunological analyses of cylindrical-inclusion protein induced by papaya ringspot virus and watermelon mosaic virus. Phytopathology 74, 1273-1278. Yeh, S. D. & Gonsalves, D. (1984a). Evaluation of induced mutants of Papaya ringspot virus for control by cross protection. Phytopathology. Phytopathology 74, 1086-1091. Yeh, S. D., Jan, F. J., Chiang, C. H., Doong, T. J., Chen, M. C., Chung, P. H. & Bau, H. J. (1992). Complete nucleotide sequence and genetic organization of papaya ringspot virus RNA. J Gen Virol 73 (Pt 10), 2531-2541.