Transgenic watermelon lines with triple-virus resistance generated by agrobacterium-mediated transformation and ...

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ABSTRACT

Watermelon is an economically important crop of the tropic and subtropic regions. Virus disease often causes serious economic loss of watermelon and there is still no chemicals for prevention and therapy of virus damage. Watermelon silver mottle virus (WSMoV), Zuchini yellow mosaic virus (ZYMV) and Papaya ringspot virus type W (PRSV-W) are the most hazardous species among all kinds of viruses infected in cucurbit. There is no transgenic watermelon confers resistance against multiple virus. In this study, for the effective control of ZYMV, PRSV-W and WSMoV, an untranslatable chimera construct containing truncated ZYMV coat preton (CP), PRSV-W CP and WSMoV nucleocapsid protein (NP) genes were transferred into watermelon via Agrobacterium-mediated transformation. Three different WSMoV NP genes segments lines(203bp, 384bp, 650bp) were inserted to expression vector pBI121-ZYMV-PRSV-Cp. The different ZWP transgenic plant lines of Nicotiana benthamiana were obtained and separately challenged with WSMoV by mechanical inoculation under greenhouse conditions. We found that ZWP-650 transgenic lines had a better level of resistance than other lines (ZWP 348, 203). A total of 13 ZWP-650 transgenic watermelon lines was obtained and PCR analysis confirmed that the foreign gene was incorporated into the genomic DNA of the regenatants. When the transgenic lines were challenged with ZYMV and PRSV-W by mechanical inoculation, they showed different levels of resistance ranging from delay of symptom development to complete immunity. Three transgenic lines conferred complete immunity against ZYMV and PRSV-W. No virus was detected by indirect ELSIA , western blotting and RT-PCR in the three immunity transgenic lines 15 day postinoculation. The expression level of the transgene in the transgenic lines was detectable by RT-PCR before challenge inoculation, but the transcript of the transgene of immunity transgenic lines was disappeared or decreased apparenty after inoculation. For this reason we considered that the virus resistance is mediated by virus-induced gene silencing.

Keywords : Agrobacterium ; transgenic plant ; virus resistance ; greenhouse evaluation

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