Transgenic Watermelon Lines Expressing Chimeric Construct Containing Antifungal Protein and Chitinase Confer Resistance

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ABSTRACT

Watermelon (Citrullus lanatus) is an economically important crop worldwide. Fungal diseases often cause serious economic loss of watermelon and people usually spray tons of agricultural chemicals or bacteria inhibitor to protect against watermelon fungal diseases. In consideration of the harmful and dangerous effects to the environment ecosystem, we are trying to introduce antifungal protein and chitinase genes into watermelons to control watermelon diseases. This investigation tried to set up an approach of Agrobacterium-mediated transformation of watermelon carrying with chimeric gene, cp-AFP3-CHI. The anti-fungal protein genes, cp-AFP3 and PR-protein genes cp-CHI chimeric gene from Caria papaya L., were kindly provided by Dr. Xiao, Chiehfu of Academia Sinica. Seeds were treated for 3 days, and cotyledons were cut into six parts as explants. The explants co-cultured with Agrobacteria for 3 days and then transferred to the selection medium. There were 7 putative cp-AFP3-CHI transgenic lines obtained. PCR analysis confirmed that the foreign gene was present in the regenerates. RT-PCR analysis indicated using primers specific for the mRNA sequence of cp-CHI-AFP to monitor expression of the gene at the mRNA level and showed 0.85 kb and 0.25 kb. Three transgenic lines (F27, F47, F49) conferred better degrees against Rhizoctonia solani, and we confirmed protein expression increase after Rhizoctonia solani by inoculation in vitro in western blot. One transgenic watermelon (F47 line) from cp-AFP3-CHI lines exhibited resistance to infection fungi under greenhouse conditions.

Keywords: antifungal protein, chitinase, transgenic watermelon
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 REFERENCES
李豐在。1985。花蓮區農業專訊 54:14-15。
杜金池、張義璋。1992。作物抗病品種之培育。病蟲害非農藥防治技術研討會專刊。台中。霧峰。 11 (28-29)。
余聰安。2001。木瓜微體繁殖與營養器官基因轉殖。國立中興大學植物學系博士論文。
陳任芳。1996。花蓮區農業專訊 55:15-16。
陳玉婷。1993。木瓜熟變相關基因之研究。國防醫學院生命科學研究所博士論文。
黃怡萍。2004。木瓜幾丁質之選殖與分析。私立東海大學食品科學研究所食品科技組碩士論文。
曾國欽。2004。植物重要防疫檢疫病害診斷鑑定技術研習會專刊 (三) 23-34。
蔡竹固。1999。甜瓜病害的診斷及其防治。國立嘉義技術學院農業推廣委員會。
蔡竹固、陳瑞祥。2000。本省瓜類作物之重要病害及其管理。農業世界雜誌。200:12-19。
楊秀珠。1991。植物病原真菌抗藥性問題探討。中華民國雜草學會會刊 12:135-154。
蘇宗振。1999。植物基因轉殖之研究。科學農業 47 (3, 4): 112-119。


