

Production of Transgenic Squash Plants Resistant to Zucchini Yellow Mosaic *Potyvirus*

(Received : 28.06.1999)

Said M. Khalil*, Atef S. Sadik**, Hamdy El-Doweny*** and Magdy A. Madkour*

* Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt.

** Dept. Agric. Microbiol. (Virology Lab.), Fac. Agric., Ain Shams Univ., Cairo, Egypt.

*** Horticultural Research Institute (HRI), ARC, Giza, Egypt.

ABSTRACT

Zucchini yellow mosaic potyvirus (ZYMV) is one of the most serious viruses affecting cucurbits, particularly squash, in Egypt. For the production of transgenic squash plants (*Cucurbita pepo* L. cv. *Eskandarani*) resistant to ZYMV, a regeneration system was developed through organogenesis using shoot tips as explants. Transformation was performed using *Agrobacterium tumefaciens* LBA4404 strain containing the pGA643 plasmid carrying the coat protein gene (CP) of ZYMV Connecticut (ZYMV-CT) strain and NPT II gene as a selectable marker. Twenty-two R_0 Kanamycin resistant lines were evaluated for the presence of ZYMV-CT-CP gene by the PCR and Southern blot hybridization with a DNA probe labelled with ^{32}P . In addition, the expression of the same gene was detected by DAS-ELISA technique. Fifteen out of the 22 R_0 lines were found to contain the ZYMV-CT-CP gene. These lines proved to be resistant to the virus when tested with the DAS-ELISA technique 2, 4 and 6 weeks post twice mechanical inoculation with an Egyptian isolate of ZYMV (ZYMV-Eg), under biocontainment conditions. Six out of the 15 lines were found to be tolerant to ZYMV-Eg infection 6 weeks post virus inoculation, while the control plants were completely destroyed after a maximum of 3 weeks. Three lines, namely L5, L6 and L17 appeared to be highly tolerant as mild symptoms were shown after 10 weeks from virus inoculation. Plants in R_1 (lines 5, 6, 9, 11, 17 and 22), R_2 (4 families from each of lines 5 and 17) and R_3 (4 sub-families from each of lines 5 and 17) were also evaluated for virus resistance under greenhouse as mentioned above. The H-family of line 17 was found to be the most promising since a delay in developing ZYMV-Eg characteristic symptoms until the tenth week from virus inoculation was observed.

Key Words: ZYMV, Squash, Regeneration, *Agrobacterium*-mediated gene transformation, Kanamycin resistance, ZYMV-CT-coat protein gene, Transgenic plants, Virus resistance, PCR, ELISA, Virus evaluation.