



Optimization of factors affecting *Agrobacterium*-mediated transformation of Micro-Tom tomatoes

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ABSTRACT. Micro-Tom is the smallest known variety of tomatoes. An orthogonal experimental design $L_{16}(4^5)$ was used to optimize *Agrobacterium*-mediated transformation of cotyledon explants of *Lycopersicon esculentum* cv. Micro-Tom. Four parameters were investigated to determine their effect on transformation frequency: the concentration of bacterial suspension, time of dip in bacterial suspension, co-cultivation time, and concentration of carbenicillin. We also examined the effect of these parameters on contamination rate, necrosis rate, mortality, cut-surface browning rate, and undamaged explant rate. Both the bacterial and carbenicillin concentrations had a significant influence on the rate of infected explants. The time of co-cultivation also had a significant influence on the transformation parameters. The optimal transformation protocol consisted of an *Agrobacterium* suspension of 0.5×10^8 cells/mL ($OD_{600} = 0.5$) and an infection time of 5 min, one day of co-cultivation and 500 mg/L carbenicillin. Under these conditions, the transformation efficiency of the shoots reached 5.1%; the mean transfor-

mation frequency was 3.9% (N = 838).

Key words: Orthogonal design; Micro-Tom; Tissue culture; Hygromycin phosphotransferase gene