

**REGENERATION AND TRANSFORMATION
USING GLUTAREDOXIN-2 GENE ON
GRAPEVINE ROOTSTOCKS**

By

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THESIS

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ABSTRACT

This study was carried out to establish reliable techniques to be used for introducing *GRX-2* gene into the two grapevine rootstocks (SO4 and Freedom). This approach was performed using *Agrobacterium tumefaciens* during the period from 2015 to 2019 at the Biotechnology Research Lab. and Plant Tissue Culture Lab. Horticulture Research Institute, Agricultural Research Center, Egypt. The aim of this work was to optimize an efficient transformation system for of the two rootstocks involving the *GRX-2* gene by using *Agrobacterium tumefaciens* mediated technique. This gene donates recipient plant salinity tolerance and subjecting two rootstocks transgenic plants to salinity stress to evaluate their stress tolerance. Genetic transformation of an elite white poplar genotype SO4 and Freedom rootstocks was performed with the vector *pRI 101-ON* DNA in *Agrobacterium tumefaciens* strain LBA4404 containing the *Glutaredoxin-2* gene in order to enhance the resistance to abiotic stress. The time period of axillary buds infection with *Agrobacterium tumefaciens* suspension culture (0, 10, 20 and 25 min with different concentration of kanamycin in $\frac{3}{4}$ MS media) was tested for the transformation frequency. The highest frequency of transformation was obtained with axillary bud explants infected with *Agrobacterium* culture for 10 minutes at the concentration of $OD_{600} = 0.8$ with Kanamycin 100mg/l. Molecular analysis using PCR proved the presence and integration of the transgenes in the genome of the transgenic plants. Field experiments were conducted to investigate the effect of the introduced *GRX-2* gene on two rootstocks at salt concentrations (0, 2000, 2500 and 3000 ppm). The data showed that the transgenic lines expressed different levels of salt tolerance as expressed by the performance of vegetative characters (plants plant height, number of leaves, root length and stem diameter), pigments contents (chlorophyll A, B and carotenoids), total Phenol, proline content and enzymes activity. These results show that the *GRX-2* gene enhance salt tolerance character.

Key words: *Agrobacterium*, rootstocks, SO4, Freedom, Glutaredoxin-2, isozymes, salinity, pigment content, proline.

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