

**PRODUCTION AND PRESERVATION OF VIRUS-
FREE CITRUS PLANTS BY USING
TISSUE CULTURE**

By

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B.Sc. Agric. Sci. (Horticulture), Fac. Agric., Menoufia Univ., 1998

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APPROVAL SHEET

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ABSTRACT

This study was carried out in Plant Biotechnology Department, National Research Center during the period from 2008 to 2013. Somatic embryogenesis from stigma and style explants of infected and uninfected trees with CPsV from different citrus genotypes showed significant effects on embryogenesis potentials on the same medium (MS + 3 mg/l BAP + 500 mg/l ME + 50 g/l sucrose) . Results indicated that stigma explants was better for callus and SE induction whereas, style explants produced callus only. Uninfected Washington navel orange produced the highest callus and SE formation percentages compared with infected ones or Valencia orange and local mandarin. Stigma-derived plantlets were CPsV-free as tested by RT-PCR and true to type as tested by RAPD-PCR. All virus-free microshoots preserved on media with 0.1 M sorbitol, sugar-free, GR-free and sugar & GR-free medium for 4 months able to proliferate new shoots. While, all explants preserved on media with 0.2 M, 0.4 M sorbitol or medium with 0.5 mg/l BA + 0.5 mg/l NAA proliferated new shoots when preserved for 5 months. Explants preserved on GR-free medium proliferated the longest shoots with the highest number of leaves/shoot when preserved for 9 and 12 months and all shoots formed roots after 9 months storage. 90%, 62.5% and 40% of encapsulated somatic embryos preserved for 1, 2 and 3 months, respectively regrowth after recovery period for 9, 4 and 10 months, respectively. 80%, 62.5% and 40% of encapsulated SEs preserved for 1, 2 and 3 months, respectively germinated completely after 10, 6 and 10 months, respectively. Encapsulated SEs preserved for 1 month developed the highest number of plantlets while those preserved for 2 months developed the longest plantlets and highest number of leaves/shoot after recovery period of 10 months. 40% of SEs pretreated with dehydration for 2 h before immersion in LN were able to produce secondary embryos when recovery on germination medium for 3 months. While, 40% of those pretreated with freezing for 2 days produce secondary embryos after 5 months of recovery. 60% of SE pretreated with encapsulation able to develop shoots and roots after 5 and 4 months on germination medium, respectively. 40% of SEs pretreated with dehydration able to form shoots and roots when recovery on germination medium for 5 months. Also, 40% of SEs pretreated with freezing were able to develop shoots and roots after 7 and 6 months on germination medium, respectively.

Key words: Citrus, stigma, style, embryogenesis, RAPD, CPsV, RT-PCR.

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