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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THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences
(Pomology)

Department of Pomology
Faculty of Agriculture
Cairo University
EGYPT

2014

APPROVAL SHEET

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Approval: / / 2014

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.

ACKNOWLEDGMENT

I wish to express my sincere thanks to ***Dr. Amina Hamed Gomaa,*** *professor of pomology, Faculty of Agriculture, Cairo University for her supervision and guidance through the course of my study and for her revision of the manuscript of this thesis. Grateful thanks are due to* ***Dr.Mohamed Helmy Abd El- Zaher****, professor of pomology, Faculty of Agriculture, Cairo University for his supervision and help and valuable advice throughout the investigation. Sincere thanks is due to* ***Dr. Adel El-Sawy Mohamed****, researcher professor of Plant Biotechnology, National Research Center for planning this work, active supervision and for his help in preparation of this manuscript. Sincere thanks are extending to* ***Dr. Abd El-Salam Abd El-Hameed Reda,*** *researcher professor of Plant Biotechnology, National Research Center for his guidance and supervision. Sincere thanks are extending to* ***Dr. Narman Tawfeek,*** *researcher professor in Plant Biotechnology Department, National Research Center for her supervision.*

Grateful appreciation is also extended to all staff members and colleagues and assistants of the Plant Biotechnology Department, National Research Center for being always ready to help when needed.

Special deep appreciation is expressed to all my family for their patience and continuous support during this work.

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