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Improving apple stocks and cultivars through tissue culture techniques and elimination of chlorotic leaf spot virus.

By

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M.Sc. Agric., Zagazig University 1996

Dissertation

Submitted in Partial Fulfillment of The Requirements For
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Approval sheet

Improving apple stocks and cultivars through tissue culture techniques and elimination of chlorotic leaf spot virus.

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has been approved

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ABSTRACT

Apple production, in Egypt, faces many problems among which are viral diseases which affects production, quality of fruits and cause crop deterioration. Lately, apple trees (cv. Anna) and M.M.106 rootstock showed chlorotic spots symptoms. An isolate of apple chlorotic leaf spot virus (ACLSV) was isolated from apple trees growing in commercial orchards in Menofia governorate. The isolated virus was identified on the basis of differential hosts, graft transmisson, serological detections (ELISA and TBIA), electron microscopy and immunosorbent electron microscopy (ISEM).

Egyptian isolate of ACLSV was transmitted to limited hosts including *Chenopodium quinoa* willd. *Ch. Amerancicolor* Coste &Reyn and *Phaseolus Vulgaris* L. All the tested plants reacted by local lesion symptoms. ACLSV was successfully transmitted from infected apple trees to M.M.106 apple rootstock. ACLSV–Pab antiserum detected the presence of ACLSV - antigen in the infected leaves petioles and stem of apple using different immunosorbent techniques. Electron microscopy of partially purified virus preparations of ACLSV showed the presence of flexuous particles 740 x 12 nm. in diameter. Also, ACLSV could be detected and decorated upon using immunosorbent electron microscopy.

In vitro studies showed that shoot tips taken from April to June are the most promising explants. The most efficient sterilization was carried by dipping in 0.5% NaOcl + ascorbic & citric 150/0.06 mg/l + 0.1% Hgcl$_2$. Activated charcoal at 3g/l or reducing agents such as ascorbic and citric reduced browning. Best establishment media was ¾ MS with 0.5mg/l BA + 0.2mg/l IBA. However, 1.0mg/l BA, 1.0mg/l kinetin gave the highest number of proliferated shoots after four subcultures. Sucrose was at 60mM was found to be the best sugar added to the media. Half strength MS with 1.0 mg/l IBA and 162mg/l PG gave the highest percentage of rooting.

Virus elimination procedure was performed to determine the efficacy and preferable concentrations of the antiviral chemicals i.e. ribavirin (virazole), acetyl salicylic acid (ASA) and jasmonic acid (JA) for their antiviral effects on production of ACLSV - free apples (cv. Anna and M.M.106 rootstock). All the concentrations used of tested substances proved to be beneficial for both explants regeneration and percentages of virus – free plants. Incorporation of virazole into culture medium resulted in a high percentages of ACLSV – Free plants. These percentages were 73.3, 82.2, 86.7, and 76.3, 80.0 and 86.7, where the corresponding concentrations were 10, 20 and 30 mg/l respectively for apple cv. Anna and M.M.106 rootstock. Further, supplementing of ASA at concentrations of 10, 20, and 30 mg/l resulted in 74.0, 84.3, 89.1 % and 77.7, 85.7 and 91.4 respectively in case of using cv. Anna and M.M.106 rootstock apple explants. Jasmonic acid (JA) at very low concentrations was much the same as ASA.

Dedication

I would like to dedicate this thesis
to the soul of my beloved Father and Mother.

ACKNOWLEDGMENT

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CONTENTS

CONTENTS

CONTENTS