

**PRODUCTION OF VIRUS - FREE
PLANTS USING TISSUE CULTURE
TECHNIQUE**

BY

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A thesis submitted in partial fulfilment
of
the requirement for the degree of

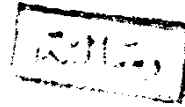
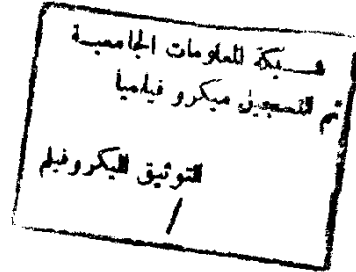
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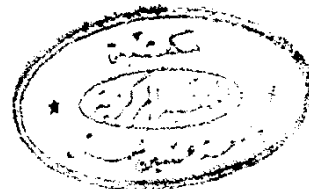
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**Production of virus - Free plants using
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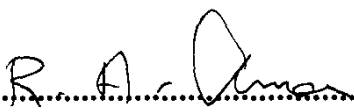
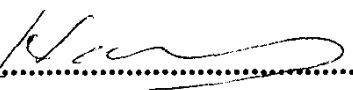

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ABSTRACT

Viruses infect many plants, the effects produced by these viruses are of many types and are found in many plant species including most that are economically important. for instance potato plants.

PVX, PVY, PLRV, the most important viruses that attack potato plants in Egypt .

The aim of this study was to establish an effective protocol to produce virus - free basic potato seed, this study invloved :

- I. Preparation of some potato viruses antisera.
- II. Eradication of some potato viruses using tissue culture technique
- III. Propagation of virus - free plantlets

The procedure could be summarized as follows :

1. Isolate and identification of potato virus X (PVX) and potato virus Y (PVY) according to host range, stability, transmission, morphology of viruse particles and serology.
2. The two viruses were purified by Allam's method (1967), for PVX and modified method similar described by Huttinga (1973); Makkouk and Gumpf (1974 b) and De-Laurdes et al. (1981) for PVY.
3. Purity of purified virus suspension was determined biologically, serologically and by spectrophotometr and electron microscop.

4. Antisera preparation was carried on by injecting purified virus suspension into rabbits. Sensitivity of antisera was determined by precipitin test tube
5. Potato viruses (PVX, PVY and PLRV) were eradicated by apical meristem culture technique, and chemotherapy using virazol.
6. Comparison between three methods of virus detection in plantlets regenerated from tissue culture.
7. The plantlets free of virus can be multiplied by, single nodal cutting on solid medium and /or overlaying on liquid medium.
8. Evaluation of five tuberization induce media, in Lab. was carried on.
9. Adaptation of microtubers and plantlets in field and greenhouse.

Key words : Potato, PVX, PVY, PLRV, isolation, identification, purification, serology, tissue culture, meristem culture chemotherapy, virazol, virus-free plantlet, microtubers, tuberization *in vitro*, *ex-vitro*, microtubers.

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LIST OF FIGURES

	Page
Fig. (1) : Potato plant cv. King Edward showing severe mosaic	54
Fig. (2) : Typical symptoms of PVY on potato cv. Alpha	
a. Right (healthy leaves) - left (infected).....	55
b. Necrosis on the vein of back of leaf	55
Fig. (3) : Initial plantlets regenerated from meristem tip	75
Fig. (4) : Single nodal cuttings	76
Fig. (5) : Severe mosaic of PVX on <i>Datura stramonium</i>	86
Fig. (6) : Mosaic of PVX on <i>Nicotina rustica</i>	86
Fig. (7) : Mosaic and mild mosaic of PVX on <i>N. tabacum</i> cv.	
a) White burely	87
b) Samsun	87
Fig. (8) : Local lesion of PVX on <i>Gomphrena globosa</i>	88
Fig. (9) : Local lesion op PVX on <i>Chenopodium amaranticolor</i>	88
Fig.(10): Vein clearing symptoms caused by PVY on <i>N. glutinosa</i>	91
Fig.(11): Symptoms of PVY on <i>D. metal</i>	91
Fig.(12): Systemic necrotic lesion of PVY on <i>Nicandra physalodis</i>	92
Fig.(13): Absorption spectra of PVX purified suspension	98
Fig.(14): Absorption spectra of PVY purified suspension	99
Fig.(15): An electron micrograph of PVX particles showing flexuous rods particles negatively stained by phosphotungstic acid	102
Fig.(16): An electron micrograph of PVY particles showing filamentous virus particles negatively stained by uranylacetate ..	103
Fig.(17): Effect of size of excised part of potato meristem tip on some potato viruses elimination.....	107
Fig.(18): Effect of Virazol concentration on some potato viruses elimination from meristem tip of potato	111
Fig.(19): Effect of Virazol treatment on some potato viruses elimination from meristem tip	112
Fig.(20): Effect of Virazol treatment on virus elimination from meristem tip of potato	113
Fig.(21): Average relative growth over time of meristem tip cultures from potato plants exposed to each of four Virazol levels	118
Fig.(22): Growth data of meristem tip grown on Virazol medium for 28 weeks.....	119
Fig.(23): Comparison between three methods of detection of PVX in tissue culture	122
Fig.(24): Comparison between three methods of detection of PVY in tissue culture	123
Fig.(25): Comparison between two methods of detection of PLRV in tissue culture	125

Fig.(26): Comparison between three methods of detection of some potato viruses in tissue culture	125
Fig.(27): Different sizes (mm) of excised meristem of potato on MS medium	128
Fig.(28): Effect of meristem size on the % of survived meristem of four potato varieties in - vitro.....	130
Fig.(29): Effect of meristem size on the % of regenerated plantlets of four potato varieties in - vitro.....	132
Fig.(30): Effect of meristem size on the regeneration time of four potato varieties in vitro.....	134
Fig.(31): Effect of meristem size on the growth scale rate of four potato varieties in - vitro.....	137
Fig.(32): Mass production of potato Crop in vitro by nodel cutting on MS solid Medium.....	140
Fig.(33): Initial plantlets from nodel cuttings on solid medium of MS.....	141
Fig.(34): Plantlets initiated on liquid medium of MS.....	142
Fig.(35): Tuberization curve of microtubers of four potato varieties inducing in vitro on different media.....	154
Fig.(36): Microtubers inducing in-vitro	
a. Tuberized plantlet into medium.....	155
b. Different sizes of microtubers.....	155
Fig.(37): Plants derived from microtubers	
a. green haulms.....	159
b. tubers.....	159
Fig.(38): The growth rate of plantlets in green - house.....	162
Fig.(39): Transplantation procedures	
a. plantlet under plastic cap (one week old after ex-vitro).....	164
b. Plantlet after removal the plastic cap. (two week old after ex-vitro).....	164
c. Ex-vitro plantlet 2 weeks from the removal of plastic cap (4-weeks after ex-vitro).....	165
d. Ex-vitro plantlet in a big pot. (10 - weeks old after ex-vitro).....	165

Contents

	Page
- Introduction	1
- Review of Literature	2
- Materials and Methods	53
- Results	83
I. Preparation of some Potato viruses antisera	
A. Isolation and identification	83
1. Host range and symptomology	83
2. Stability of viruses	93
3. Mode of Transmission.....	93
4. Serodiagnosis of viruses.....	93
B. Purification of PVX & PVY.....	96
C. Antisera preparation.....	100
II. Eradication of some potato viruses using tissue culture technique	105
A. By Apical meristem culture	105
Effect of size of excised meristem on virus elimination	105
B. By Chemotherapy.....	109
C. Comparisen of ELISA, slide agglutination test and indicator plant for detection of PVX, PVY and PLRV in meristem plantlets.....	120
III- Propagation of virus-free plantlets	127
1. Establishment of an aseptic culture.....	127
2. Rapid multiplication of plantlets in vitro.....	135
3. Microtubers production in vitro.....	143
4. Field performance of micropropagated of potato ...	157
a. Performance of microtubers.....	157
b. Performance of plantlets.....	160
5. Production of super elite - I of tubers.....	167
- Discussion.....	149
- English Summary.....	195
- References.....	197
- Arabic Summary.....	

Review of Literature

REVIEW OF LITERATURE

I. Preparation of Some Potato Viruses Antisera:

A. Virus isolation and identification:

1. Potato virus X (PVX)

PVX occurs worldwide, potato affected by PVX may yield slightly fewer and slightly smaller tubers than those from healthy plants. Yield depression differs according to virus strain and potato cultivar. Some necrosis-evoking strains induce yield losses of over 50% in some potato cultivars. In Egypt the virus was isolated from potato plants by (Allam et al., 1973), PVX depressed the potato yield by 11-30% than healthy ones (Omar et al., 1967). Its effect is serious in double infection with TMV (Smith, 1972).

Host range:

Potato virus X was reported to have a wide range of hosts belong to different plant families (about 15 angiosperm families, Rich, 1983). Plants which can be systemically infected with PVX include: *Nicotiana tabacum* var. white burely (Salaman, 1938 and Gracia et al, 1983), *Nicotiana tabacum* var. Samson (Kohler, 1973; Allam et al, 1967, 1973, 1980), *Nicotiana glutinosa* (Bawden, 1948; and Jones, 1985), *Nicotiana rustica* (Ladeburg et al, and Allam et al, 1973, 1980), *Nicotiana chinensis* (Ladeburg et al, 1950 and Allam, 1973) *Nicotiana longiflora* (Asuyama et al, 1951), *Nicotiana debineyi* (Kohler, 1947; Bagnall, 1961 and Moore et al, 1965), *Capsicum annum* L. (Salaman, 1938; Krotechanova & Ivanova, 1979; Horvath &

Nienhaus, 1982 and Erkan & Yorganci, 1982) *Capsicum frutescens* (El-Hammady et al, 1977), *Petunia vialacea* (Bohme, 1933; and Allam et al, 1980), *Lycopersicon esculantum* (Suhov et al, 1956 and Allam et al 1973), *Physalis floridana* (Ross, 1948, and Tein et al, 1966), *Nicandra physaloides* (Ladeburg et al, 1950 and Allam et al, 1973), *Datura stramonium* (Ainsworth, 1934; Suhov et al, 1956; Reunov & Legs, 1983 and Allam et al, 1987), *D. meteloides* (Neuton et al, 1936, and Mallozzi & Drummond, 1984), *Datura metal* (Bode et al, 1965), *Datura tatula* (Matthews, 1949; Hooker et al., 1960 and Goth & Webb, 1985), *Gomphrena globosa* (Wilkinson et al, 1948; Thomson, 1956; Sharma, 1964; Allam, 1980; Klein Livingston, 1983; Goth & Webb, 1985 and Allam et al, 1987) *Amaranthus caudatus*, *A. hybridus*, *A. retroflexus* and *A. tricolor* (Ladeburg et al, 1950 and Allam et al., 1980), *Chenopodium amaranticolor* (Horvath, 1969; Allam et al., 1980 and Erkan & Yorganci, 1982), *Chenopodium quinoa* (Bode et al, 1965 Attathom et al, 1978 and Kiratiya-angul et al, 1988), *Chenopodium album* (Thomson, 1956 and Allam et al, 1980), *Spinacia oleraceae* (Allam et al, 1973), *Beta vulgaris* (Chester; 1935 and Allam et al, 1973), *Phaseolus vulgaris* (Kohler, 1958), *Physalis lanceifolia* (Horvath, 1985), *Physalis glabripes* (Horvath, 1984), *Vicia fabae* (Salaman, 1938), *Cassia occidentalis* (Allam et al, 1973), *Trifolium pratense* L. (Goth et al, 1960), *Chrysanthemum* (Kvicola et al, 1961), *Annulus dubins* (Frank, 1948), *Lycium holimifolium* (Ladeburg et al, 1950) and *Hyoscyamus muticus* (El-Hammady et al., 1977).

Stability:

Thermal inactivation point, dilution end point and longevity in vitro of potato virus X (PVX) are known to be extremally variable, this is due, mainly to the existance of this virus in several and numerous strains. Thermal inactivation point was recorded: 70°C (Salaman, 1938), 68°C (Clinch, 1944), 71-73°C (Ladeburg *et al*, 1950), 90-95°C (Gracia *et al*, 1983), 75°C (Allam *et al*, 1987) and 68-76°C (Beemster & de Bokx, 1987).

Dilution end point of PVX was found to be 10^{-4} - 10^{-5} (Koch, 1933), 10^{-6} (Ladeburg *et al*, 1950), 10^{-6} - 10^{-7} (Allam *et al*, 1967) 10^{-8} - 10^{-9} (Gracia *et al*, 1983), and 10^{-5} - 10^{-6} (Beemster & de Bokx *et al*, 1987).

Longevity in vitro of PVX has been reported and was found to be, 120 days (Salaman, 1938), 60-90 days (Kassanis, 1949), 360 days (Ladeburg, *et al*, 1950), 35-42 days (Allam *et al*, 1967), 32-64 days (Gracia, *et al*, 1983), 56-63 days (Allam *et al*, 1987), and from several weeks to one year (Beemster & de Bokx, 1987).

Transmision:

Most investigators reported that, PVX was transmitted easily by mechanical means (Koch, 1933; Bawden 1948; and Beemster & de Bokx, 1987), including infectious sap, the cutting knife, mechanical planter, cultivating and spraying equipments, animals and by contact of sprouts leaves and roots (Mai, 1947; Manzer & Merriam, 1961 and Beemster & de Bokx,

1987). Transmission of PVX through the infected tubers is a common phenomenon. Experimentally, the virus was transmitted by dodder (Bennett, 1940 and Ladeburg, *et al*, 1950). Most investigators reported that no insect had been found to transmitt PVX (Dykstra, 1939; Roberts, 1946; Roberts, 1953 and Beemster & de Bokx, 1987). But some of grasshoppers (*Melanoplus differentials*) transmitted PVX (Walter, 1951 and Roberts, 1952).

Concerning the grafting manner, Heath, (1956) reported that, tomato necrosis (a strain of potato virus X) was transmitted to *Nicotiana glutinosa* by grafting but not by aphids.

As for seed transmission, the PVX is not transmissible through potato true seeds (Ladeburg *et al*, 1950; Bercks, 1970 and Beemster & de Bokx, 1987).

Virus particles:

Potato virus X have been examined in the electron microscope. The certain conclusions that can be reached are that; potato virus X has rod like particles that vary greatly in length; these seem to be more flexible than tobacco mosaic virus particles and Takahashi and Rowlin (1946) gave the mean width as 15.7 and 16.1 m μ respectively and mean length was 500-600 m μ . Bawden and Crook (1947) indicated that only a little virus X can be obtained by grinding the fibrous residus from infected leaves, but this is obtained in particles of a shorter average length than those in sap, few exceeding 250 m μ . However, a smaller value, about 10 m μ , has been reported by

Kleczkowskii and Nixon (1950) who noted no differences in width between the various strains of virus X they examined. And Electron microscopy studies (Varma *et al*, 1968), showed that PVX particles is a flexible rod, about 515 μ long and 12 μ wide and also had a helical struction. Bercks (1970) reported that the members of potato virus X group with normal lengths of 480-580 μ . Kozar *et al* (1976) reported that, in ultrathin section of infected cells with PVX, it is baciliform and 240 X 80 nm. In Philippines, Talens (1979) reported that electron microscopy demonstration of flexuous rods of 15 x 550 nm in partially purified preparation. But Mondy *et al* (1980) indicated the presence of PVX as fibrous particles in cytoplasm of potato meristem tip cells. Ashoub *et al* (1993) found that electronmicroscopy examination of dip preparation of PVX infected sap, demonstrated filaments flexuous virus particles.

Serodiagnosis:

PVX is serologically active (Rich, 1983) several serological tests are widely used in detecting the presence of PVX in plants or in tubers such as: slide agglutination test (Dounin and Popova, 1937; Bercks, 1949; 1956; Omar, 1967; Allam *et al*, 1972 and Apaclaza & Baeriswyl, 1986), Agar gel double diffusion test (Shepard & Secor, 1969; McCrum, *et al*, 1971), micro-precipitin test (Van slogteren, 1959 and Scherbakova & Ganzhina, 1969), tube-precipitation (Allam *et al*, 1972), Latex agglutination test (Polak *et al*, 1983 and Gallenberg & Jone, 1985) and ELISA test (Gallenberg & Jone, 1985). However, ELISA