PRODUCTION OF VIRUS - FREE PLANTS USING TISSUE CULTURE TECHNIQUE

BY

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Production of virus - Free plants using tissue culture technique

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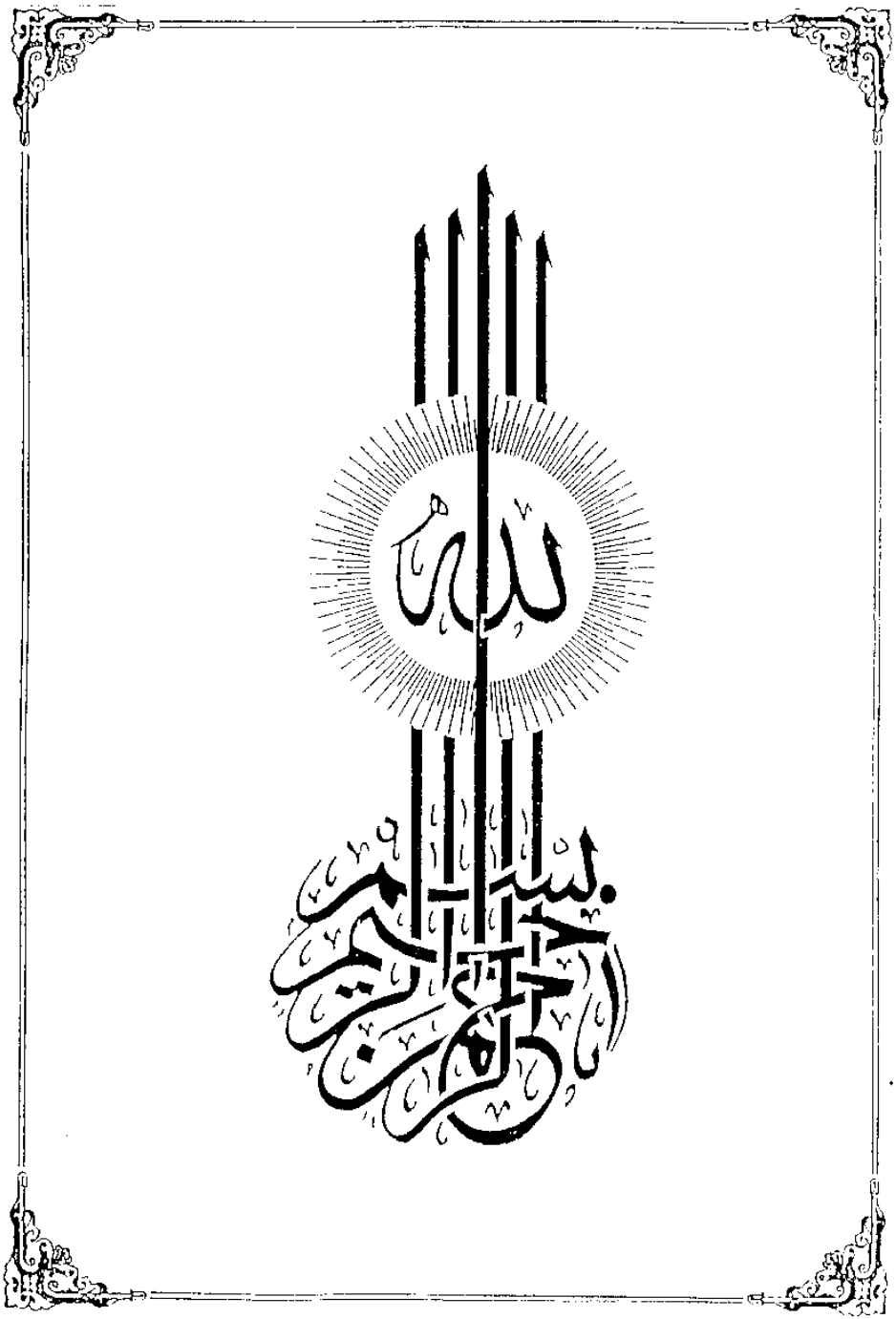

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ABSTRACT

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 effecive 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 cultrure 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 nodel cutting on solid medium and / or overlayring on liquid medium.
8. Evaluation of five tuberization induce media, in Lab. was carred 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,* minitubers.

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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), *Chenpodium amaranticolor* (Horvath, 1969; Allam *et al.*, 1980 and Erkan & Yorganci, 1982), *Chenpodium 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 longivity 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} (Labedurg *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 mμ respectively and mean length was 500-600 mμ. 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 mμ. However, a smaller value, about 10 mμ, 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 mμ long and 12 mμ wide and also had a helical struction. Bercks (1970) reported that the members of potato virus X group with normal lengths of 480-580 mμ. 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-preciptin 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