STUDIES ON INCLUSION BODIES INDUCED BY SOME PLANT VIRUSES

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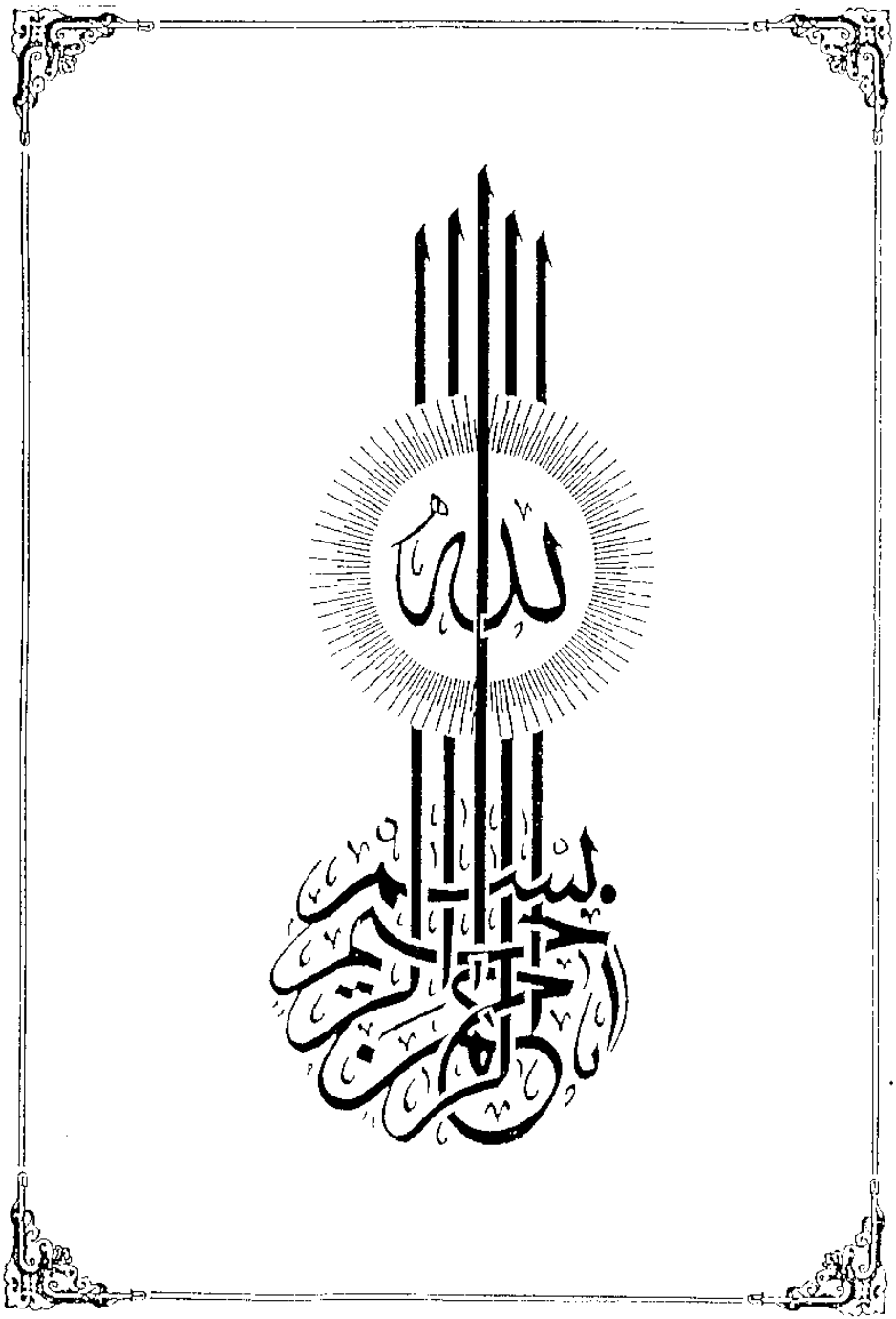

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INTRODUCTION

Tobacco (tomato) mosaic virus (Tobamo-virus group), soybean mosaic virus and potato virus Y (Potato virus Y group) are found to be common in tomato, soybean and potato crops respectively (Smith, 1957), causing a considerable reduction in plant yield (Smith, 1957).

The three mentioned viruses induce intracellular cytoplasmic inclusion bodies, so-called amorphous inclusions; in the infected cells; Christie (1967) for TMV, El-Afifi (1978) and Hunst and Tolin (1982) for SMV and Edwardson (1966) and Christie (1967) for PVY. In addition, TMV induce crystalline cytoplasmic inclusions (Warmke, 1968; Foglein *et al*, 1976 and Milici, 1977). Rapid diagnosis of virus infection could be maintained by the light microscopic examination to reveal the crystalline inclusions in the infected leaf strips mounted in water (Goldin and Rustambekov, 1971 and Bos, 1972) and with the help of special stains to illustrate the amorphous inclusions (Mcwhorter, 1957; Christie, 1967; Quiniones and Dunleavy, 1970; Harrison *et al*, 1970; Huertos and Bos, 1973 and Martelli and Russo, 1976).

Examination of ultrathin sections by the electron microscope is a good mean for the detection of viral crystalline inclusions (Milne, 1966) and amorphous inclusions (Edwardson, 1966; Dunleavy *et al*, 1970; Tu, 1974; Nicolaescu and Titu, 1976; Edwardson *et al*, 1984 and El-Afifi and Meherashan, 1984).

In the present investigation, the cytoplasmic inclusions induced by the TMV, SMV and PVY were studied according to the following items :-

1. Detection of the amorphous inclusion bodies by the cytochemical techniques.

2. Period required for the detection of inclusion bodies induced by TMV and PVY in different hosts and chosen the suitable host for the detection of their inclusions.

3. Chosen the best part of the plant for the detection of the inclusions induced by TMV, SMV and PVY.

4. Relationship between virus concentration in different leaves and the intensity of the inclusion bodies.

5. Detection of viral inclusions using the electron microscope.

In addition, purification of the cytoplasmic clyindrical inclusions induce by SMV and production of the specific antiserum was also carried out.

REVIEW OF LITERATURE

Many plant viruses induce inclusion bodies as a response to local and systemic infection (Milne, 1966; Warmke, 1968 and Vela, 1972), however inclusions formed in local infection are fewer than that found in systemic infection (Milne, 1966).

Some plant viruses induce two types of inclusions in the cytoplasm of the infected cells i.e. crystalline and amorphous inclusions; tobacco mosaic virus (Christie ,1967 and Vela,1972), petunia ring-spot virus (Huertos, 1962) and Atropa mild mosaic virus (Harrison and Roberts, 1971).

Other viruses induce amorphous (or cytoplasmic cylindrical) inclusions; soybean mosaic virus (Tu, 1974; Nicolaescu and Titu, 1976; El-Afifi, 1978; Sabek *et al* ,1979; Hunst and Tolin, 1982 and El-Afifi and Meherashan 1984); potato virus Y (Shepard, 1968; Hirbert *et al* ,1971; El-Hammady *et al* ,1983; Eskarous *et al*,1983 and Edwardson *et al* ,1984); tobacco etch virus (Edwardson *et al* ,1968; (Hiebert *et al*, 1971; Andrews and Shalla, 1974 and Dougherty, 1983); pepper mottle, bidens mottle and turnip mosaic viruses Hiebert *et al*1971 and Hiebert and Mcdonald, 1973); Tobacco vein mottling virus (Hellmann *et al*, 1983); Papaya ringspot virus and water-melon mosaic virus I (Yeh and Gonsalves, 1984) and Parsnip mosaic virus (Murant *et al*, 1971).

The amorphous inclusion bodies induced by some plant viruses were detected by the light microscopic examination using the cytochemical techniques (Christie, 1967; Bos, 1969; Martelli and

Russo, 1976; Kishtah *et al* ,1978; Sabek *et al*.1979; El-Hammady *et al* ,1983; Eskarous *et al* ,1983 and El-Kady, 1984); by examination the ultrathin section prepared from virus-infected leaves, in the electron microscope (Edwardson, 1966; Warmke, 1968; *et al* Harrison and Roberts, 1971; Murant *et al*,1971; Moldovan *et al*, 1974; Nicolaescu and Titu, 1976; Kishtah *et al*,1978; Carr and Kim, 1983; Dougherty, 1983; Eskarous *et al*,1983; Edwardson *et al* 1984; Yeh and Gonslaves, 1984 and Ammar, *et al* ,1985) and by serological techniques (Hiebert *et al*1971; Hiebert and Mcdonald, 1973; El-Afifi, 1978; El-Afifi and Mehreshan, 1984 and Yih and Ross, 1984).

1- Detection of viral cytoplasmic inclusions:

1.a. Detection of cytoplasmic inclusion by the cytochemical techniques:

The cytochemical staining was developed for differentiating plant virus inclusions in epidermal leaf strips through the light microscope (Christie, 1967; Bos, 1969; Kishtah *et al* , 1978 ; El-Hammady *et al* , 1983 and Eskarous *et al*,1983).

Mcwhorter (1957) found that crystalline nucleolar inclusions induce by bean yellow mosaic virus and the nucleoli stained black with 0.5% solution of trypan blue while nucleoplasm stained blue. The crystalline or amorphous inclusions induced by petunia ringspot virus (Huertos ,1962); sever etch virus (Huertos and

Hidalgo, 1964) and radish mosaic virus (Stefanac and Ljubesic, 1971) were easily demonstrated by immersing epidermal strips in a 1% phloxine solution without previous fixation. It was found that nucleoli and inclusions stained very bright red while the nucleoplasm stained pink.

A mixture of phloxine and methylene blue at concentration 1% was used in ratio of (1:20) (V/V) for the detection of both crystalline and amorphous inclusions induce by bean yellow mosaic, tobacco etch and tobacco mosaic viruses and the amorphous inclusions induced by potato virus X, potato virus Y and watermelon mosaic virus (Christie, 1967). Christie (1967) found that the crystalline inclusions induced by TMV remain unstained by the previous mixture. He also found that the crystalline and/or amorphous inclusions induced by the previous six mentioned viruses stained red; while nucleoplasm stained blue and nucleoli stained violet; indicating that they are consisted of protein. Bos (1969) obtained the same result for the crystalline and amorphous inclusions induce by bean yellow and clover yellow vein viruses using the same previous mixture but at ratio of (5:1) (V/V).

The PVY-amorphous inclusions stained blue with bromophenol blue indicating the presence of protein (El-Hammady *et al*, 1983 and Eskarous *et al*, 1983). Martelli and Maria (1971) and Martelli and Russo (1976) used the mercuric bromophenol blue for detection the amorphous inclusions induced by cauliflower mosaic and watermelon mosaic viruses while El-Kady (1984) used it for the

detection of amorphous inclusions induced by bean yellow mosaic virus. It found that the inclusions show deep blue colouration (indicating the presence of protein) while the nucleoplasm and nucleoli stained with pale blue colour.

The presence of fatty acids and/or phospholipids in viral cytoplasmic amorphous inclusions was checked using sudan black B. The amorphous inclusions induced by tobacco rattle virus (Harrison *et al*, 1970), and watermelon mosaic virus (Martelli and Russo, 1976) were stained dark blue or black with the previous mentioned stain after treating the unfixed strips of virus-infected leaves with 5-10% Triton X-100. In addition, El-Kady (1984) found that the amorphous inclusions induced by bean yellow mosaic virus were stained black or remain colourless using sudan black B.

Using the mixture of methyl green and pyronine, Khan *et al* (1977) found that the inclusions induced by red clover vein mosaic virus were stained red with faint blue shades (suggesting the presence of RNA and possibly some DNA), while the nucleoplasm and the nucleoli were stained blue and bright red respectively.

Sabek *et al* (1979) found that the inclusions of SMV stained red with methyl green-pyronine indicating the presence of RNA.

El-Kady (1984) found that the amorphous inclusions induce by bean yellow mosaic virus were stained red without faint blue shades (suggesting the presence of RNA only).

1.b. Detection of viral cytoplasmic inclusions induced by TMV.SMV and PVY in different hosts :

a) Inclusion bodies induced by TMV :

Tobacco mosaic virus (TMV) induce three types of inclusions i.e. crystalline, paracrystalline (Warmke, 1968) and amorphous inclusions (Christie, 1967 and Kassanis and Milne, 1971). The crystalline inclusions were found in the central areas of local lesions formed on *Nicotiana glutinosa*, *Chenopodium amaranticolor* (Milne, 1966 and Vela, 1972) and protoplast of *N.tabacum* cv. Xanthi, two weeks after the virus mechanical inoculation(Folgein, *et al* 1976). TMV induce crystalline inclusions in systemic infected leaves of *Petunia hybrida* (Vela, 1972), *N. tabacum* cv. White Burley (Vela, 1972 and El-Hammady *et al* ,1983) and *Lycopersicon esculantum* (Larina, 1974). It was found that strain Ni 118 of TMV induce amorphous and crystalline inclusions in tobacco cultivar Samsun (Kassanis and Milne, 1971). It induces also the two types of inclusions in *N. tabacum* cv. Turkish (Christie, 1967) and in *Digitalis thapsi* (Vela, 1972). The crystalline inclusions induce by TMV in systemically infected leaves show different shapes. Multilayer crystals, crystalline needles and hexagonal crystals were found in leaves of *Capsicum annum* obtained 10 days after the virus inoculation and remain till 60 days (Herold and Munz, 1967). Crystalline and paracrystalline needles, complexely formed virus spike or spindles and tubular crystals were found in *Nicotiana tabacum* cv. Turkish inoculated with the virus (Warmke, 1968 and Milicic, 1977).

b) Inclusion bodies induced by SMV :

Soybean mosaic virus (SMV) induces cytoplasmic cylindrical inclusions in infected leaves of *Glycine max* Merr plants(El-Afifi,1978 & Sabek *et al*,1979).The cylindrical inclusions appear;when sectioned under different angles;as: pinwheels (Dunleavy *et al*,1970; Yih and Ross, 1974; Nicolaescu and Titu, 1976; El-Afifi, 1978; Hunst and Tolin, 1982 and El-Afifi and Mehreshan 1984) and Scrolls or circular in cross sections (Dunleavy *et al*,1970 and Yih and Ross, 1972). While they appear as laminated aggregates in longitudinal sections (Tu, 1974, Nicolaescu and Titu, 1976 and El-Afifi and Mehreshan, 1984) and bundle inclusions (Yih and Ross, 1972). Amorphous membrane-bound bodies were induced by Glycine mosaic virus in pea plants and not in soybean ones (Bowyer *et al*,1970). However, Dunleavy *et al* (1970) found that SMV induced membrane-bound in soybean plants.

c) Inclusion bodies induced by PVY :

Potato virus Y and its strains induce cytoplasmic inclusions which appear in cross sections as pinwheels in the infected leaves of *N. glutinosa* (Skofenko and Kushuirenko, 1975 and Eskarous *et al*, 1983); tobacco (Edwardson, 1966; Hiebert *et al*,1971, Eskarous *et al*,1983 and Edwardson *et al* 1984); *N. rustica*,, *N.aebneyi*, *Solanium nigrum* cv. judaicum, *S. nigrum* cv. nigrum, *D. metel*, *N. physaloides* and *Capsicum annum* cv. Yolo Wonder A (Eskarous *et al*,1983) and scrolls in tobacco leaves (Hiebert and

Mcdonald, 1973 and Edwardson *et al*, 1984). In longitudinal sections, the inclusions induced by PVY appear as bundle (Edwardson, 1966 and Eskarous *et al*, 1983), short curved laminated aggregates (Edwardson *et al*, 1984).

The cylindrical inclusions induced by PVY were found in the cytoplasm of infected leaf cells of *N. tabacum* cv. Samsum NN 1-4 months after the virus inoculation (Hiebert *et al*, 1971 and Hiebert and Mcdonald, 1973). In addition, Skofenko *et al* (1977) found that the anomalous electron-dense formations; which are accumulation of straight or curved bands in different lengths and thickness near the cylindrical inclusions in *N. glutinosa*. leaves infected with PVY. Eskarous *et al* (1983) followed up the appearance of PVY inclusions in *S. nigrum* cv. judacium and they found that these inclusions remained in the infected cells till 45 days after inoculation.

1.c) Detection of inclusion bodies of TMV, SMV and PVY in different parts of virus-inoculated plants :

TMV induce crystalline and amorphous inclusions in the infected leaves of *N. tabacum* cv. Turkish (Christie, 1967), Samsun tobacco (Kassanis and Milne, 1971) and *Digitalis thaspi* (Vela, 1972). The crystalline inclusions induced by TMV were found in the epidermal strips of fruits and leaves of *Capsicum annum* L. infected with the virus (Herold and Munz, 1967) and *N. tabacum* cv. White Burley (El-Hammady *et al*, 1983).

Amorphous inclusions were found in the cytoplasm of the epidermal strips obtained from leaves of *Glycine max* Merr infected with SMV (Yih and Ross, 1972 and Sabek *et al*, 1979) and leaves of *N. glutinosa* infected with PVY (Skofenko and Kushuirenko, 1975 and Eskarous *et al*, 1983). Lamellar inclusions (pinwheels) were observed in *Glycine max* root nodule cells infected with SMV (Tu, 1974 and El-Afifi and Mehershan, 1984).

Inclusion bodies (cytoplasmic granular and crystalline nucleolar inclusions) induced by bean yellow mosaic virus were found in the epidermal strips obtained from stem, petioles and the undersides of the virus-infected leaves (Bos, 1969).

Tubular structures were seen close contact with pinwheels and dense bands in the cytoplasm of stem and leaf cells of *Dianthus barbatus* L. and *Silene armeria* L. infected with carnation vein mottle virus (Begtrup, 1976).

2- Detection of the cytoplasmic inclusions by electron microscopic examination :

The ultrathin sections obtained from *N.tabacum* L cv Turkish infected with TMV(Warmke, 1968), *N.tabacum* L.cv."Havana 425" infected with TEV and *Cucurbita pepo* cv small sugar infected with watermelon mosaic virus(Edwardson and Christie, 1978) were fixed with 6.5% glutaraldehyde, followed by post fixation in 1% osmium tetroxide (OsO4).

On the other hand, samples obtained from the leaves of *Chenepodium amaranticolor* inoculated with TMV (Milne, 1966), *Nicotiana glutinosa* (obtained 8-12 weeks after inoculation with PVX) (Stols and Toen, 1969) and *Vicia faba* infected with bean yellow mosaic virus (Weintraub and Ragetli, 1966) were fixed with 5% glutaraldehyde, followed by post-fixation with 1% OSO_4 ,dehydrated in graded series of alcohol or cold acetone and embedded in Epon 812, then stained with uranyl acetate and lead citrate perior to the examination in the electron microscope.

Harrison *et al*, (1970) fixed leaf tissue obtained from *Nicotiana clevelandii* infected with tobacco rattle virus with 5% glutaraldehyde followed by post-fixation with 2% OSO_4.

The ultrathin sections obtained from *Solanum nigrum* cv. judaicum leaves infected with PVY were fixed in 4% glutaraldehyde followed by post-fixation in 1% osmic acid (Eskarous *et al* ,1983)