STUDIES ON A RING SPOT VIRUS OF DOUBLE TROPAEOLUM (*TROPAEOLUM MAJUS* L.)

By

MAHENDRA DEO MISHRA

A thesis submitted to the Faculty of Science, Aligarh Muslim University, Aligarh (U.P.) for the degree of

DOCTOR OF PHILOSOPHY

IN

BOTANY

1977

In my frail canoe I struggle to cross the sea
of desire, and forget that I too am playing a game.

From 'The Crescent Moon'
by Rabindranath Tagore

Abstract

STUDIES ON A RING SPOT VIRUS OF DOUBLE TROPAEOLUM (TROPAEOLUM MAJUS L.)

Double tropaeolum plants (Tropaeolum majus L.), exhibiting abnormal flora morphology leading to complete antholysis and suppression of sexual morphogenesis, are found to be invariably infected by a virus showing chlorotic and necrotic rings, mottling, curling and puckering of their leaves and colour breaking of flowers.

The virus is sap-inoculable when mixed with celite and Na_2SO_3 and is transmitted by aphids (Aphis gossypii Glov., A. craccivora Koch. and Myzus persicae Sulz.) from tropaeolum. From tobacco, the virus is not transmitted by aphids but readily through soil. There is strong evidence of its being transmitted by nematode (Xiphinema americanum Cobb.). The thermal-inactivation-point of the virus lies between 60 and 62°C, dilution end-point 10^{-4} and 15 x 10^{-3}, longevity in vitro between 48 and 75 hrs at 19-27°C and between 168 and 240 hrs at 8-10°C. The infectivity is maximum at pH 6.0. Freeze-dried leaves when kept 4-7°C do not loose their infectivity upto 11 months. The wide host range of the virus includes plant species belonging to families: Cucurbitaceae, Moraceae, Umbelliferae, Oxalidaceae, Labiatae, Violaceae, Balsaminaceae, Chenopodiaceae, Amaranthaceae, Compositae, Leguminosae, Polemoniaceae, Ranunculaceae, Scrophulariaceae, Solanaceae, Tropaeolaceae and Verbenaceae, indicating different degrees of susceptibility. Statistical analysis shows random distribution of susceptibility in these families. The 'mean host reaction' of the virus does not differ significantly from

both TRSV (tobacco ring spot virus) or NRSV (tropaeolum ring spot virus), thereby indicating a close relationship with them.

Inclusions have been observed in virus infected tropaeolum leaves when stained with Acradine Orange indicating their RNA nature. The electron microscopic studies of purified virus preparation reveal the existence of hexagonal particles having an average diameter of 29 nm. Sucrose gradient centrifugation resolves the purified virus preparation into three components. Ultraviolet-absorption spectrum studies shows high proportion of RNA content in the bottom component which is highly infectious. On the basis of extinction values at 260 and 280 nm, the purified virus preparation indicates high proportion of RNA, approximately 36.91%. Positive precipitin-reaction and gel-diffusion test with TRSV antisera reveals a close relationship of the two viruses.

Considering similarities in symptom expression, host range, physical properties, structure and function of virus particles and serological relationship, the virus under study (DNRSV) appears to be quite close to TRSV. Efficiency of aphids as vectors of DNRSV and differences in the symptom expression on some of the common hosts, suggest DNRSV to be a strain of TRSV affecting tropaeolum plants.

A comparison of infectivity of different parts of tobacco plants infected with DNRSV indicated that roots and younger leaves are highly infectious, while the meristems are not. The infectivity of the inoculated plants is maximum around 9th day of inoculation. The petals of double tropaeolum flowers are highly infectious, whereas the infectivity is altogether absent

in the petals of single tropaeolum and are reduced considerably in the leaves of both. The inhibitory effect of tropaeolum leaves is neutralised by soaking them in solutions of Na_2SO_3, caffeic acid or EDTA. Coumarin and sodium salicylate inhibit DNRSV infectivity in vitro.

Callus cultures of virus affected tropaeolum, turkish tobacco and N. glutinosa explants shows decrease of infectivity in subsequent transfers. With respect to turkish tobacco, the leaf callus is more infectious than the callus developed from stem. Dedifferentiated cultures give rise to both infectious as well as non-infectious shoots, but infectivity of these cultures as a whole is more than that of the undifferentiated callus cultures. [redacted]. The histogenetic dedifferentiation and production of chlorophyll in the callus has no effect on the infectivity.

Strong caulogenic response is observed in meristem-tip-cultures grown on medium incorporated with 7.0 mg/l of adenine and 100 mg/l of myoinositol.

...

To

my wife Usha and daughter Rajita
who have shared many anxious moments in my pursuit to earn the degree

ACKNOWLEDGEMENT

I am indebted to Prof. Abrar M. Khan, Head, Department of Botany, Aligarh Muslim University, for his keen interest, critical guidance and constant encouragement. I would also like to express my sincere gratitude to Dr. S.P. Raychaudhuri, Ex-Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, for his inspiring interest, helpful suggestions, valuable criticism and guidance during the course of these investigations.

I am grateful to Prof. Reyat Khan, Ex-Head of the Department of Botany, Aligarh Muslim University, for extending the facilities for my work in his department and to Dr. M.S. Swaminathan, then Director, Indian Agricultural Research Institute, for permitting me to work for the Ph.D. degree.

Thanks are also due to Prof. Dr. J.P.H. van der Want, Dr. (Mrs.) Jean Dijkstra (Singh), Dr. Dick Peters of Laboratorium voor Virologie, Dr. J.G. ten Houten and Drs.Fredrica Quak of the Instituut voor Plantenziektenkundig Onderzoek, Wageningen, The Netherlands for providing me the facilities to work in their laboratories and many helpful suggestions. I also gratefully acknowledge the help given to me by the Food and Agricultural Organization of the United Nations for selecting me as their fellow. It enabled me to do a part of my work in the above laboratories.

I also wish to express my sincere gratitude to Dr. S.K. Saxena, Reader, Department of Botany, Aligarh Muslim University and Prof. V.V. Chenulu, Division of Mycology and Plant Pathology,

I.A.R.I., and Dr. B.B. Nagaich, Central Potato Research Institute, Simla, for various critical suggestions and help during these investigations.

It is my privilege to thank my friends and colleagues at the Department of Botany, Aligarh Muslim University and Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi and at other places for their ungrudging help on various occasions. Dr. G.C. Rath, Department of Plant Pathology, Agricultural University, Bhubneswar helped me in statistical analysis and interpretations. Mr. Ram Nath extended his valuable criticism in the preparation of the manuscript. Dr. V.S. Verma, Dr. Hemant C. Phatak, Dr. A.K. Lambat, Dr. Nam Prakash, Dr. A.S.Summanwar, Mr. A. Ghosh, Mr. K. Jagadish Chandra helped me in various ways. Thanks are also due to Mr. F.R. Niazi, Mr. Vinod Kumar, Mr. H.K. Gorkha, Mr. Y.P. Sehgal and Mr. Vijay Sharma, for their help from time to time.

Dated: October, 1977.

(Mahendra Deo Mishra)

CONTENTS

contd..

CONTENTS CONTD.

contd..

CONTENTS CONTD.

LIST OF TABLES

contd..

LIST OF TABLES CONTD.

Contd..

LIST OF TABLES CONTD.

contd..

LIST OF TABLES CONTD.

LIST OF FIGURES

contd..

LIST OF FIGURES CONTD.

Number

Fig.1.1. A. Symptoms on double tropaeolum plants. Leaves showing curling, puckering, green vein-banding, chlorotic rings and mottling.

B. Symptoms on turkish tobacco plants with severe distortion and mottling of young leaves, with concentric rings, line-pattern and occasional necrosis.

C. Symptoms on Datura stramonium showing systemic mottling chlorotic patches and occasional concentric rings on the inoculated leaves.

A

B

C

FIG. 1.1

I INTRODUCTION

Tropaeolum majus L., the Indian cress or garden nasturtium is widely grown ornamental and is native of South America. The plant belongs to family Tropaeolaceae and derives its name from classical Greek world, 'Tropae' or 'Tropaion' equivalent to 'Trophy' of English, since shape of the flower resembles the halmet and leaves, a miniature shield. It is a tall trailing species with yellow, orange, red or scarlet coloured flowers. As a result of incessant hybridization, many strains of garden nasturtium have evolved in nature. Varieties with semi-double flowers are known as 'Glen-hybrids', having orange, salmon, scarlet and mahogany colour.

The double garden nasturtium plant connected with the present studies is different in having predominantly petaloid flowers with complete suppression of sex organs. Indefinite number of bright scarlet coloured petals are produced with the suppression of androeceum and gynoeceum. The superior green sepal is devoid of its spur. These plants are propagated by cuttings as seeds are never produced. Besides, their leaves are universally affected with characteristic chlorotic and necrotic ring spot symptoms suggestive of viral infection. Bos (1957) reported Witches' broom like symptoms on certain garden nasturtium plants, along with breaking of the flower, known as antholysis. Occasional production of seeds from an otherwise phylloid

flower indicated an incomplete antholysis, wherein the sexual and vegetative developments are not altogether antagonistic but change only the morphogenesis of floral parts. These plants are now suspected to be affected by a mycoplasma (Personal communication from Dr. L. Bos).

Besides this, a number of plant viruses have been reported to infect T. majus. Six of them occur in nature and the remaining could be transmitted to garden nasturtium on artificial inoculation from their respective hosts. However, the symptoms they produce , vary (Table 1.1).

Tropaeolum mosaic virus with Zinnia elegans Jacq. as the only other known host was described by Jensen (1950) from California, USA and by Silberschmidt (1953) from Sao Paulo, Brazil. According to Smith (1957), tropaeolum mosaic virus described from USA and Brazil happen to be the same virus which is sap transmissible as well as transmitted by aphids, Myzus persicae Sulz., M. circumflexus Buckt., Aphis rumicis L., A. ferruginea-striata Essig and Rhopalosiphum prunifoliae (Fitsch). The virus is characterised by the production of vein-banding and chlorotic spots with necrotic blotches on the leaves. Breaking of flower colour is related to a stage of development of the disease syndrome. Moriondo (1958) also reported the occurrence of a mosaic virus on garden nasturtium and it is likely to be the same virus.

Smith (1949a,b) described another commonly occurring virus, tropaeolum ring spot virus from England, having a wide host range belonging to the families Solanaceae,

Table 1.1. Viruses infecting Tropaeolum majus L.

Viruses, synonyms and Cryptograms	Symptoms on Tropaeolum majus L.	Sap inoculation	Vectors	Shape of the virus particles	Reference
I Naturally infected					
A. Ring spot group of viruses					
1. Cabbage black ring spot virus Marmer brassicae Holmes Turnip mosaic virus Tropaeolum mosaic virus x/x x/x E/E S/Ap	Leaves: mosaic mottling flowers: colour breaking	Sap	Aphids	Flexuous rods	Smith (1950)
2. Tropaeolum ringspot virus Tropaeolum ring mosaic virus x/x x/x x/x S/Ap	Plants: stunted. Leaves: mosaic mottling with yellowish rings and line-pattern	Sap	Aphids	-	Smith (1949a & b) Schmelzer (1960) Bhargava and Joshi (1959) Bisht (1962)
B. Other viruses					
3. Beet curly top virus Chlorogenus entellicola Holmes x/x x/x x/x S/Au	Plants: stunted. Leaves: chlorotic and puckered. Petiole: withered. Flower buds: chlorotic and fail to open (Also on Tropaeolum peregrinum L.)	Sap	Leaf hoppers	-	Severin and Freitag (1933)

Contd..

Table 1.1 contd.

Viruses, synonyms and Cryptograms	Symptoms on *Tropaeolum majus* L.	Sap inoculation	Vectors	Shape of the virus particles	References
4. *Cucumber mosaic virus* *Marmer circumeris* Holmes. R/1 1/18 S/S S/Ap	Plants; Top necrosis, Leaves: Necrotic local lesions	Sap	Aphids	Spherical	Schmelzer (1960)
5. *Tomato spotted wilt virus* *Lethum anstraliense* Holmes R/x x/x S/S S/Th	Plants: Stunted Leaves: Mosaic mottling with yellowish to brown necrotic spots	Sap	Thrips	Spherical	Pittman (1934) Gardner and Whipple (1934)
6. *Tropaeolum mosaic virus* x/x x/x x/x S/Ap	Leaves: Mosaic mottling with chlorotic and necrotic spots. Flowers: Colour breaking	Sap	Aphids	-	Jensen (1950) Silberschmidt (1953) Smith (1957) Moriondo (1958)
7. *Tropaeolum witches' broom* x/x x/x x/x S/x	Plants: Stunted and bushy. Leaves: Chlorotic Flowers: phylloid	-	-	-	Bos (1957)

Contd..

Table 1.1 contd.

Viruses, synonyms and Cryptograms	Symptoms on *Tropaeolum majus* L.	Sap inoculation	Vectors	Shape of the virus particles	References
		II *Experimentally infected*			
A. *Ring spot group of Viruses*					
8. *Aster ring spot virus* *Annulus wellmanii* Anderson Tobacco Rattle virus R/1 2.3/5 E/E S/Ne	Plants: Stunted Leaves: mild chlorotic patterns of rings and spots bordering veins, crinkled & sometimes accompanied by necrosis	Sap	Nematodes	Rods	Anderson (1954)
9. *Tobacco broad ring spot virus* *Annulus apertus* Holmes x/x x/x x/x S/x	Leaves: Mild vermiculate-pattern and chlorosis	Sap	-	-	Johnson and Fulton (1942)
10. *Tobacco ring spot virus* *Annulus tabaci* Holmes R/1 1.8/42 S/S S/Ne	Symptoms not described (Also on *T. peregrinum*)	Sap	Nematodes. Also fea beetle, thrips, spider mite, grass-hoppers, aphids	Spherical	Price (1940)
11. *Tobacco ring spot virus 2* *Annulus zonatus* Holmes Tomato ring spot x/x x/41 S/S S/Ne	Symptomless carrier	Sap	Nematodes	Spherical	Brierley (1954)

Contd..

Table 1.1 contd.

Viruses, synonyms and Cryptograms	Symptoms on *Tropaeolum majus* L.	Sap inoculation	Vectors	Shape of the virus particles	References
12. **Tobacco streak virus** **Annulus orae** Holmes x/x x/x S/S S/x	Symptoms not described	Sap	-	Spherical	Fulton (1948)
13. **Tomato black ring virus** x/x x/x S/S S/Ne	Leaves: faint mosaic mottling with chlorotic rings and line-pattern	Sap	Nematodes	Spherical	Smith (1946)
B. Other Viruses					
14. **Asteryellows** **Chlorogenus callistephis** Holmes x/x x/x x/x S,I/Au	Plants: Stunted & bushy Leaves: chlorotic	-	Leaf hoppers	-	Severin & Freitag (1945)
15. **Tobacco mosaic** virus **Marmor tabaci** Holmes F/1 2/5 E/E S/x	No symptoms	Sap	-	Rods	Holmes (1946)
16. **Tomato aspermy** **Chrysanthemum** aspermy x/x x/x S/S S/Ap	Leaves: Mosaic mottling (only strain 1)	Sap	Aphids	Spherical	Hollings (1955)

Leguminosae and Tropaeolaceae, usually showing characteristic chlorotic and necrotic ring spot symptoms, capable of being sap-transmissible and also by M. persicae and A. fabae Scop. Schmelzer (1960) observed a similar disease at Ascherleben, East Germany and described it to be caused by ring mosaic virus, infecting several other host species belonging to 19 families and also having some additional aphid vectors. In India, Bhargava and Joshi (1959) described the ring spot disease of garden nasturtium from Nainital and found it to be transmitted by two additional aphids, namely A. gossypii Glover and Brevicoryne brassicae L. Bisht (1962) reported A. gossypii to be the most efficient vector. These reports indicate that all these viruses are either the same or strains of the same virus. This is a rare example of aphid transmitted ring spot group of viruses.

Another example of an aphid transmitted ring spot virus is that of cabbage black ring spot virus (Smith, 1950) occurring in nature on garden nasturtium and transmitted by M. persicae and B. brassicae. The virus produces mosaic mottling and flower colour breaking. It also differs from the above two viruses in causing clear and distinct mosaic mottling on Nicotiana glutinosa L.

Beet curly top (Severin and Freitag, 1933) and tomato spotted wilt (Pittman, 1934; Gardner and Whipple, 1934), characterized by stunting and general chlorosis, were found to occur in nature on garden nasturtium. The former is transmitted by leaf hoppers and the latter by thrips.

Smith (1952) reported garden nasturtium to be a susceptible host of cucumber mosaic virus and tomato spotted wilt. However, cucumber mosaic virus has been reported to occur on this host in nature in East Germany (Schmelzer, 1960).

All the remaining viruses listed in Table 1.1 have been reported to be infecting Tropaeolum majus or T. peregrinum L. on artificial inoculation as one of the hosts. With the exception of aster yellows, all these viruses are sap-transmissible (Severin and Freitag, 1945). Aster yellows is characterised by general chlorosis and stunting. Tobacco mosaic virus does not appear to multiply and cause symptoms on garden nasturtium as it remains confined to the inoculated leaves only (Holmes, 1946). Chrysanthemum aspermy virus (Hollings, 1955) is also transmitted by aphids, causing mottling and necrosis. Tobacco streak (Fulton, 1948) causes necrosis and spotting on the leaves and has no vectors.

Besides tropaeolum ring spot and cabbage black ring spot viruses, other ring spot group of viruses i.e. aster ring spot virus (Anderson, 1954), Tobacco broad ring spot virus (Johnson and Fulton, 1942), Tobacco ring spot virus (Price, 1940), tobacco ring spot virus No.2 (Brierley, 1954) and tomato black ring virus (Smith, 1946), are known to be infecting garden nasturtium. Amongst all of them tobacco ring spot virus is the most important. It has a wide host-range, in which are included some of the economically important hosts. The virus produces characteristic necrotic local lesions and systemic chlorotic and necrotic rings on many hosts

besides tobacco, on which the virus was first described (Frommeet al., 1927; Priode, 1928; Wingard, 1928 and Fenne, 1931). Moreover, it also causes pollen sterility in tobacco (Valleau, 1941). Besides sap-transmissibility, it is also transmitted through seeds of petunia (Henderson, 1931)and Lincoln variety of soybean (Desjardins et al., 1954) and through several vectors viz., nematodes, Xiphinema americana Cobb (Fulton, 1962; Sauer, 1966), differential grass-hopper, Melanoplus differentiales (Thos) (Dunleavy, 1957), tobacco flea-beetle, Eptrix hertipennis (Melsheimer) (Schuster, 1963), thrips, Thrips tabaci Lindeman (Valleau, 1951) and Frankliniella sp. (Bergeson et al., 1964), and red spider mite, Tetranychus sp. (Thomas, 1969). Valleau (1951) gave only circumstantial evidence in support of transmission by Thrips tabaci, whereas Smith and Brierley (1955) suggested that Myzus persicae may transmit it from gladiolus particularly when associated with bean yellow mosaic virus. Of all these agencies nematodes are considered to be the most efficient vectors in the glasshouse experiments (McGuire, 1964a,b; Teliz, 1967).

Although the disease syndrome of the virus under consideration resembles more closely with the ring spot group of viruses, none of the earlier reported viruses are known to suppress sexual development and induce sterility in garden nasturtium. Only Valleau (1932) reported different degrees of pollen sterility in tobacco as a result of tobacco ring spot virus infection. It was, therefore, considered necessary

to study the following aspects:

1. Characterization and identification of the causal virus,
2. The infectivity of floral parts of double tropaeolum plants,
3. Taxonomic affinity of susceptibility,
4. Stabilizing the infectivity of the virus in the extracted sap of double tropaeolum plants, and
5. Tissue culture studies for developing virus-free plants as also cultures of virus affected tissues.

II MATERIAL AND METHODS

2.1. Source material

The virus affected plants of double tropaeolum, Tropaeolum majus L., were originally collected from Throp's Nursery earlier known as Emme Villa Garden Nursery, Simla. According to the present owner, these plants are being maintained from the time of the former owner of this nursery, Mr. T.R. Thrope, and their original source is not known. The mode of their propagation has always been by 'cuttings', which are rooted into individual plant-lets in earthen pots. These plants are also being maintained at Raj Niwas gardens and at the I.A.R.I. Plant Pathological Research Station orchard in Simla as ornamentals.

2.2. Isolation of the virus culture

The causal virus was isolated from infected double tropaeolum plants through aphid transmission (Myzus persicae Sulz.) as also by sap inoculation on single garden nasturtium, (Tropaeolum majus L.). From aphid transmitted cultures on garden nasturtium plants, further isolations were attempted first by inoculating the turkish tobacco (Nicotiana tabacum L. cv. Xanthi) plants and then isolating single set of concentric rings produced on the inoculated leaves of turkish tobacco plants. The cultures for the experimental purposes were thus developed on turkish tobacco seedlings through serial

transfers by subsequent sap inoculations.

2.3. Maintenance of the culture

The experimental work was done mostly at the Department of Botany, Aligarh Muslim University, Aligarh and the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi during October-April over a period of four years. Since the remaining part of the year was too hot for the expression of visible disease syndrome, the virus was maintained at Simla on its original host or at Aligarh and Delhi on Crotalaria brownae on which it produced systemic chlorotic concentric rings. Some experiments were undertaken at the Instituut voor Plantenziektenkundig Onderzoek and Laboratorium voor Virologie (Landbouwhogeschool) Wageningen, the Netherlands, where the author spent some time on FAO-fellowship.

Attempts were also made to maintain the virus in tissue culture (Mishra and Raychaudhuri, 1967) by growing virus affected tissues on synthetic culture medium by repeated subculturing. The details of these methods are discussed separately. The virus culture was also maintained in desiccated leaf bits over calcium chloride ($CaCl_2$) in sealed tubes stored at temperature of 4-7°C as suggested by Bos (personal communication). This method comprised of the following steps: The turkish tobacco leaves showing severe disease syndrome were washed thoroughly in tap water immediately after plucking them. These leaves were then dried on a blotting paper sheet and chopped into fine small pieces

before desiccating them in a desiccator over $CaCl_2$ at 4°C for one week. The desiccated leaf-bits were stored in tubes with a little amount of $CaCl_2$ at 4-7°C after carefully plugging and sealing. The infectivity was periodically tested. The inoculum was prepared by soaking the desiccated leaf-bits for overnight in 0.5% solution of Sodium sulphate (Na_2SO_3), macerating and inoculating the turkish tobacco plants with a pinch of celite (John-Manville, U.S.A.).

2.4. Technique of bioassay

Many preparations were assayed for their infectivity by counting the local lesions produced on the inoculated leaves of Chenopodium amaranticolor Coste and Reyn with a 4x magnifying lens. Treatments were distributed on equal sized leaves using Latin square design, leaving out the lowermost two leaves. Infectivity was also assayed by counting the local lesions on the inoculated leaves of N. tabacum cv. Xanthi.

2.5. Mechanical transmission

Inoculum was prepared from turkish tobacco plants grown in insect-proof glass-house with the temperature ranging from 10 to 25°C. The leaves showing clear systemic chlorotic symptoms were soaked in 0.5% solution of Na_2SO_3 for at least two hours before macerating and straining through two layers of muslin cloth. One volume of freshly extracted crude sap was diluted with 9 volumes of 0.01M phosphate buffer at pH 7 and rubbed with a little pinch of

celite (John-Manville, USA) on the upper surface of the leaves with a swab of cheese-cloth saturated with the inoculum. The inoculated leaves were washed with a jet of cold tap-water after about 5 minutes and placed in shade for at least 24 hours on the glass-house bench. This technique has been used in all the experiments unless stated otherwise.

2.6. Biological transmission

2.6.1. Aphid transmission

Establishment of virus-free aphid colonies: The most commonly occurring species, viz. *Aphis gossypii* Glover, *A. craccivora* Koch, *Myzus persicae* Sulz. and *Macrosiphum pisi* (Kalt.) Baker, were collected from their respective natural hosts, viz. cotton, cowpea, chillies and pea. For raising virus-free cultures of these aphids, their robust and apterous adults were separately collected in pairs of petri dishes having moist blotting paper. The next day, tiny newly-borne nymphs were transferred to healthy succulent seedlings of garden nasturtium with a camel hair brush. These aphids were reared separately in individual cages in cool and shady site of the glass-house. All the transmission experiments were done with these aphid colonies.

Acquisition and transmission feeding: For acquisition feeding, the aphids were first starved for two hours for increasing the efficiency of transmission, before releasing them on the leaves of diseased plants showing clear symptoms, plucked and placed in a pair of petri dishes on a moistened

filter paper. These petri dishes were kept in a dark chamber for the required acquisition feeding period. At the end of the required acquisition feeding period, presumably viruliferous aphids, seen actually feeding at the time of the transfer, were gently disturbed and transferred carefully with a camel hair brush on to the test plants. Batches of ten aphids were transferred to each healthy test plant. The test plants were covered with a glass chimney, their open ends being covered with a muslin cloth, and kept in a dark chamber for a required transmission feeding period. Afterwards, the feeding aphids were removed and the plant was sprayed with 0.1% Ekatox spray so as to destroy the nymphs, if any. The test plants were kept under observation for 4-5 weeks. Seedlings fed with non-viruliferous aphids served as controls.

2.6.2. Nematode transmission

Turkish tobacco plants were transplanted in sterilised soil contained in 4" earthen pots, with one seedling per pot. Five days later, ten such seedlings were inoculated with the double tropaeolum virus. Two plants were kept uninoculated to serve as control. A week later approximately 50 adults of Xiphinema americanum* were seeded in the soil near the roots of these plants and left to feed on them for picking up the virus. After a period of two months, the nmmatodes were reisolated and again seeded in the pots having

---

* The nematode inoculum was obtained through the courtsey of Dr. C.L. Sethi, Division of Nematology, Indian Agricultural Research Institute, New Delhi and the experiment with this nematode was set up with his help.

7-10 day old healthy tobacco seedlings transplanted in sterilised soil in 4" earthen pots. After a period of a month these plants were uprooted and their roots were assayed for infectivity on turkish tobacco plants. The extracted sap from the roots of these plants was also serologically tested with TRSV-antiserum, earlier found to react with the virus.

Similar experiment was also set up with Xiphinema basiri inoculum. In this case approximately 200 adults were used for starting the experiments.

2.7. Soil transmission

Evidence of soil transmission was sought by conducting the following two experiments with glass-house and field soil in which infected plants were earlier growing.

Experiment I - Infested soil was collected from glass-house pots having infected turkish tobacco plants and field plots having infected garden nasturtium plants. After removing the plants along with their remifying roots, the soil samples were repotted without sterilization in 10" earthen pots for testing the viral-infestation of the soil. The seeds of turkish tobacco and C. amaranticolor plants were sown in individual pots for growing bait plants. After germination the plants were allowed to grow for one and half months before bioassaying their roots separately on turkish tobacco plants. Ten bait-plants in each sample were thus tested with equal number of plants left in the pots to grow for two more months for development of any disease syndrome. Identical

controls were maintained by sowing seeds in fresh and non-sterilized soil.

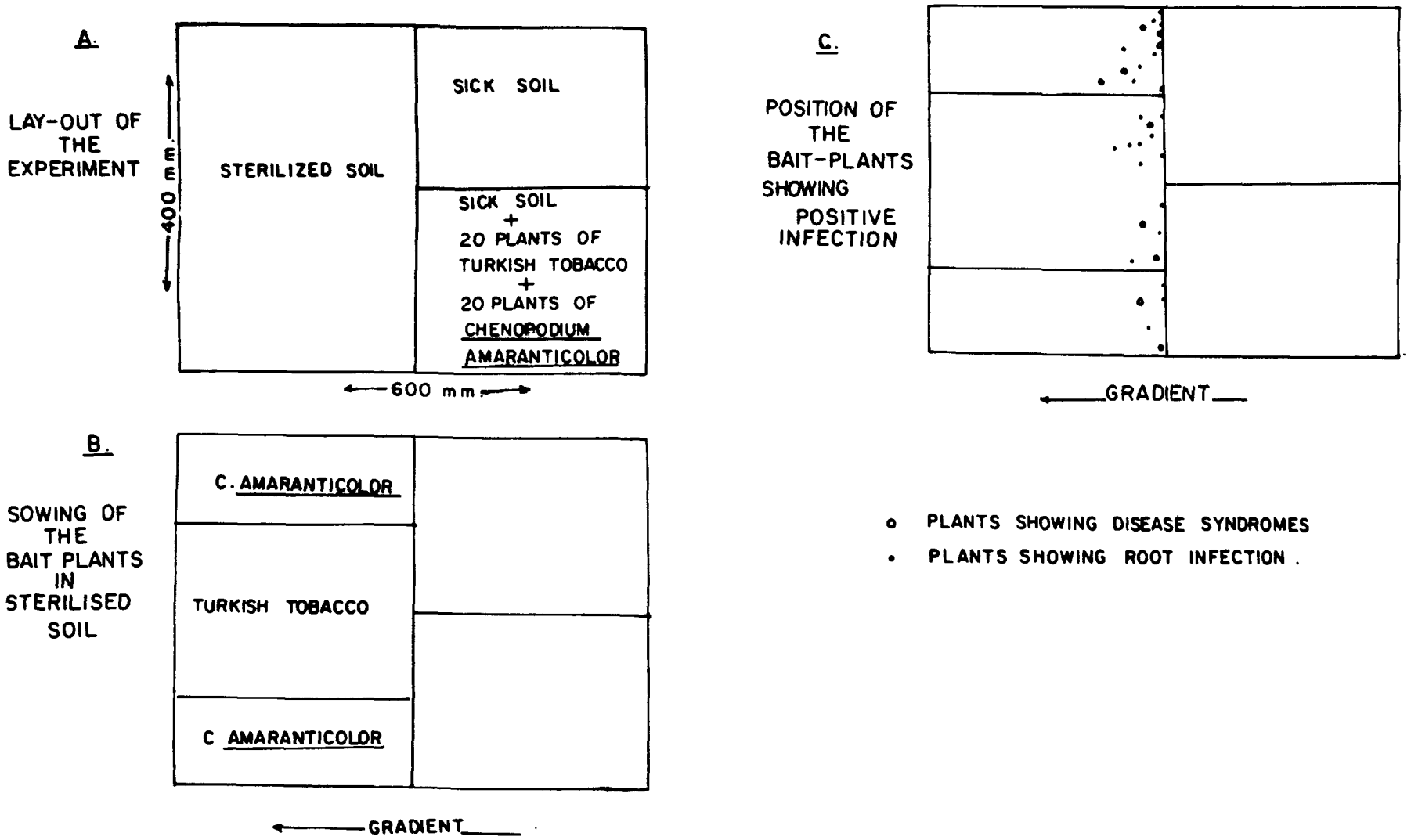
Experiment II - A rectangular wooden box 40 x 60 x 10 cms (Fig.2.1) was divided into two equal parts. One half being filled with sterilized soil and the other half was again divided into two. In one of these, sick soil was filled after removing the plants with remifying roots and in the remaining portion sick soil with 20 plants of turkish tobacco and C. amaranticolor (mixed up) were transferred. After setting up the tray, seeds of turkish tobacco and C. amaranticolor were sown in sterilized soil in the manner as illustrated in the figure. The tray was kept in such a way that the flow of water was always from infested to sterilized soil. Two months after germination, the bait plants were selected at random and their roots were bioassayed on turkish tobacco leaves. An equal number of plants were left in the tray for the disease syndrome to develop.

2.8. Host range studies

For host range studies 143 plant species as given in Appendix I, were tested. In each case the seedlings were raised in autoclaved soil in 6 inch seed-pans. They were later transplanted in 4 inch earthen pots with rich by manured soil in insect-proof glasshouse. The plants thus raised were inoculated 10-15 days after transplanting, when they were in stage of rapid growth. About 10 to 20 plants or more of each plant species were inoculated at a time by swabbing the upper surface of 3-5 youngest leaves dusted with

FIG. 2.1.

LAY—OUT OF THE EXPERIMENT ON SOIL TRANSMISSION OF THE VIRUS.



celite. The plant species not showing symptoms in the first instance were inoculated again to eliminate the possibility of escapes.

Susceptibility was judged either by the production of detectable disease syndrome in the inoculated or freshly opened young leaves or by testing the possibility of viral multiplication in the youngest leaves by inoculating their extract on another test plant. Generally the plants were kept under observation till the apparent symptoms were visible or upto 60 days after inoculation, when the plants, not showing any symptoms, were tested on the known host viz. turkish tobacco for the presence of virus. Some of the plants were kept for a year, when the symptoms were expressed. The test plants that failed to give any evidence of viral multiplication were considered to be immune. The following scale of susceptibility was formulated to ascertain the extent of ability of the host tissue to support viral multiplication. Expression of symptoms were taken as an indication of the degree of susceptibility and accordingly alloted scoring numbers in increasing order.

Scale of susceptibility

Grades of symptom expression	Presence or absence of viral multiplication	Scoring Nos. (susceptibility index)
1. Complete immunity	-	0
2. Subliminal infection	+	1
3. Localised necrotic reaction	+	2
4. Localised chlorotic reaction (Chlorotic concentric rings, etc.)	+	4

Contd..

Scale of susceptibility contd.

Grades of symptom expression	Presence or absence of viral multiplication	Scoring Nos. (susceptibility index)
5. Systemic necrosis or death of the plant	+	8
6. Systemic chlorosis including oak leaf pattern	+	16

Percentage of infection was not taken into consideration so as to avoid individual plant variations in a single plant species. Only the characteristic symptoms ultimately established, were taken into consideration. The grades of scoring thus obtained for each of the families of the tested plant species, were statistically analysed so as to ascertain any relationship of the taxonomic position of the families with the ability of the hosts to support viral reproduction and compare the host range of other similar viruses, like tobacco ringspot (Price, 1940) and tropaeolum ring mosaic (Schmelzer, 1960).

The families were categorised into five groups on the basis of maximum disease reaction exhibited by plant species tested under almost identical conditions. These groups were compared with each other at 1% and 5% level of significance (Snedecor, 1965).

Since one of the groups (Group V) represented plant species showing highest disease reaction, it was analysed

further for frequency of the occurrence of different host reactions within the group and within the families constituting it.

Out of 143 plant species tested, 21 hosts were common with those reported for tobacco ring spot virus (Price, 1940) and tropaeolum ring mosaic virus (Schmelzer, 1960). The host reactions as reported for these viruses were compared with those of the virus under study for any possible similarity or relationship, using analysis of variance (Snedecor, 1965).

2.9. Physical properties

For studying the physical properties of the virus, the inoculum was prepared from leaves of virus affected turkish tobacco plants. Standard extract of the infected plant material was prepared by macerating the weighed amount of leaves with 1 ml per gram of distilled water. To study thermal inactivation, two ml of standard extract was distributed in thin walled glass tubes of 10 mm diameter. These tubes were exposed, at different temperatures ranging from 40 to 90°C in a water-bath for a period of 10 minutes each. Immediately after cooling these tubes, inoculations were made on turkish tobacco plants. These test plants were kept in the insect-proof glass-house for a fortnight for further observations.

For investigating the tolerance of the virus to dilution, series of dilutions were prepared in test tubes by adding measured volume of sterile distilled water to the juice extracted from the diseased leaves of turkish tobacco

plants. Inoculations were made on the test plants as indicated above and with all the necessary precautions in order to avoid any possible contamination. The plants inoculated with undiluted extract served as control.

In order to study the longevity *in vitro*, standard extract was prepared in sterile distilled water and stored in a conical flask, plugged with a rubber stopper in the refrigerator at 8-10°C. and at room temperature of 19-27°C (in winter months). Inoculations were made with the extract on turkish tobacco plants immediately after its preparation and at different intervals thereafter. Longevity *in vivo* was also ascertained by periodically testing the infectivity of desiccated leaf material stored at 4-7°C as described earlier.

Optimum pH range for the infectivity of the virus was studied by using 0.01 M Glycine buffer and 0.1 M phosphate buffer separately. The extracted sap from the diseased leaves was added to different buffer solutions (pH ranging from 1.0 to 13.0) in the ratio of 1:1. The inoculations were made after an hour on half leaf of *Chenopodium amaranticolor*. Treatments were distributed according to Latin square design. The other half was inoculated with the clarified extract diluted with only distilled water. After the development of symptoms local lesions were counted. Number of lesions per 100 lesions of control were calculated for each treatment.

2.10. Cytopathology

Phloxine staining: For studying the presence of inclusion bodies, pealed epidermis from stems, petioles and underside of leaves were stained in a 1% solution of phloxine in water (Rubio-Huertos, 1950). Staining was also done with phloxine mixed with 1% solution of methylene blue in 1% saline for better contrast (Bos, 1969). The stained strips were finally washed in water and mounted in water or 1% glycerine for examination under ordinary light microscope.

Fluorescent staining: Leaves from healthy and diseased single as well as double tropaeolum plants were infiltrated with water by putting them in 250 ml flasks containing sterile water and connected to 'Speedivac' vaccum pump for 10 to 15 minutes. Epidermal peals were removed from the lower surface of leaf lamina and petiole and stained with Acridine Orange (AO) (lot 2013, Allied Chemicals, N.Y., USA) using the schedule followed by Hooker and Summanwar (1964). The epidermal peals were first fixed in 50% alcohol for 1 hour. After keeping the epidermal peals for 10 minutes in distilled water, these were stained with 0.01% AO prepared in M/15 phosphate buffer with a pH of 6.0 for 10 minutes. The final solution of AO i.e. 0.01%, was prepared by diluting 0.1% stock solution in distilled water with phosphate buffer. Subsequently, the epidermal peals were washed in M/15 phosphate buffer of 6.0 pH for 10 minutes and rinsed in double distilled water before destainihg with 0.1 M aqueous solution of $CaCl_2$

at pH 7.3 for 5 minutes. The epidermal peals after a short rinse in distilled water were washed in two changes of phosphate buffer and mounted in the same buffer.

These preparations were examined under fluorescent Carl Zeiss Zena microscope (No. O.G.I., Germany). Ultra-violet light for examination of the preparations was supplied by an HBO 200, Carl Zeiss Zena, air cooled, and high pressure mercury lamp. Light filters (Carl Zeiss, Nos. BG 12/2g and GG 3/2g) were used for transmitting the blue light. A yellow barrier filter (Carl Zeiss No. OG 1) was placed on the eye piece of the microscope. Black and white photographs were made with ORWO film 100 ASA exposed for 10, 15, 20, 25 seconds.

Nucleic acid identification by enzymatic treatment: The epidermal peals from diseased leaves were treated with ribonuclease (RNAse) enzyme from bovine pancrease (BDH, England) at 10°C for 3 hours. The RNAse was used at the concentration of 50 r/ml in 0.05 M phosphate buffer (pH 7.5). For control, epidermal peals were treated with only 0.05 M phosphate buffer (pH 7.5). The peals were rinsed with sterile distilled water after the treatment and stained with phloxine as above.

2.11. Purification

Selection of production host: A number of host plants viz. *Petunia hybrida* Vilm., *Datura stramonium* L. *Nicotiana glutinosa* and *N. tabacum* cv. Xanthi were inoculated with the virus for ascertaining the viral contents. The leaves of these plants showing characteristic symptoms were harvested 10 days after inoculation, weighed and macerated with a few

drops of 0.5% solution of Na_2SO_3 in distilled water so as to prepare a standard solution. The infectivity of the extracts was bioassayed.

Storage of harvested material: The leaves of the inoculated turkish tobacco showing severe symptoms, 7-10 days after inoculation, were harvested. They were then soaked in a small volume of 0.5% solution of Na_2SO_3 and stored in refrigerator at 0-4°C for further processing.

Extraction of sap: Fresh leaf material from 10-15 days old infected turkish tobacco was macerated in waring blender and homogenised with equal volume of buffer. Frozen material was first thawed before maceration and homogenization. The different buffers tried for stabilizing the infectivity of the extracted sap were, potassium phosphate buffer (0.05 M, 0.01 M and 0.1 M), sodium phosphate buffer (0.1 M), borate buffer (0.5 M) and sodium sulphite (0.5%) at different pH ranges, 6.7, 7.0 and 7.6 with or without 0.1% of thioglycolic acid or 0.05 M ascorbic acid. The sap thus obtained was pressed through cheese cloth to remove the fibrous plant material.

Clarification - The extracted sap was then clarified by mixing it with 1/4 volume of chloroform or 8.5% of butanol, or 1/4 volume of the mixture of equal volumes of chloroform and butanol by stirring it vigorously for about an hour or keeping it overnight at 4°C. The mixture of the sap with n-butanol kept at cool temperature for overnight was clarified

for removing the plant material by centrifuging at 12,000 rpm for 20 minutes. The emulsions formed with chloroform or with chloroform and butanol mixture were broken by low speed centrifugation at 3,000 rpm for 5 minutes and then the aqueous phase separated out and centrifuged at 12,000 rpm for 20 minutes as above.

Purification: Generally two alternate cycles of low speed (12,000 and 4,000 rpm for 20 and 30 minutes, respectively) and high speed (30,000 and 40,000 rpm for 120 and 90 minutes, respectively) ultracentrifugation of the clarified sap were employed for obtaining further purification. The final pellet was dissolved in 0.02M or 0.03M phosphate buffer at pH 7.0 and 7.6, respectively. The final pellet was also suspended in 0.01M ethylenediamine tetra-acetate (EDTA) at 7.0 pH in some of the purification schedules.

Infectivity of the crude plant sap was compared with suspension of first high speed centrifugation (30,000 rpm) pellet. In order to make the test comparable, the pellet was dissolved in 0.02M phosphate buffer equal to the volume of clarified sap that yielded the pellet and the crude extract was made with the equal volume of the same buffer.

The final virus suspension was clarified by another low speed centrifugation at 4,000 rpm for 10 minutes.

The finally purified virus suspension was used for electron microscopy density gradient centrifugation as also ultra violet absorption spectrum for analysing the preparations.

Sucrose density gradient centrifugation: Density gradient centrifugation was used for further purification and isolation of different components of the virus. The finally purified virus suspension was analysed by density gradient centrifugation in linear gradient containing 0.2 to 0.7 M sucrose in 0.02 M phosphate buffer, pH 7.0. Two ml of viral suspension was layered on sucrose gradient. These tubes were centrifuged for 150 min. at 22,500 rpm in a Spenco Model L ultracentrifuge using SW 25 rotor. After centrifugation, the tubes were examined in a vertical beam of light and the light scattering zones removed with a hypodermic syringe. The virus containing fractions thus separated were diluted with the same buffer and centrifuged at 45,000 rpm for 60 minutes. The pellets were dissolved in phosphate buffer and used for infectivity test and electron microscopy.

Agar gel filteration: Two 100 mm agar gel columns were prepared in two glass cylinders, one with 4 per cent and the other with 8 per cent agar preparations (autoclaved for 30 min. at 15 lb. pressure) and then grained by passing the chopped pieces of agar through 40 mesh but not through 60 mesh screen before putting in the tubes for making columns. After washing these columns with sufficient amount of 0.2 M sodium phosphate buffer of pH 7.0, clarified suspension of the virus was layered first on top of the column with 4 per cent agar. The filtered suspension was again layered on top

of the other column with 8 per cent of agar. The clarified suspension of the virus was earlier obtained by macerating 100 g of inoculum in 100 ml of buffer (0.2 M of sodium dihydrogen phosphate and 0.3 M sodium chloride adjusted to pH 6.8 and then passing the suspension through 6 to 8 mm thick celite cake after mixing the suspension with 10 per cent celite.

Ten ml samples of effluent fluid were collected until virus fraction, detectable by its light scattering properties was obtained. The eluted viral suspension was concentrated by acid precipitation.

2.12. Ultra violet absorption

The ultraviolet absorption spectra of different preparations as also different components were recorded using Hilger Intermediate quartz spectrograph with a Spekker photometer. Distilled water was used for comparable controls.

2.13. Electron microscopy

Electron microscopy was used for the examination of the purified material and leaf-dip preparations. The purified material to be examined by negative staining technique was mixed with an equal volume of 1 per cent phosphotungstic acid at pH 7.0 and deposited on the grid coated with a thin collodion film followed by carbon film. The preparations were examined in the Philips EM 100 and Siemens Elniskop-I electron microscopes.

Negative staining was also attempted to examine leaf-dip preparations. One square centimeter pieces of diseased leaf of Tropaeolum majus were first fixed with 5-10 per cent of glutaraldehyde for 2-3 hours and washed in distilled water. Fresh cut ends of infected tissues were then dipped for 2-3 seconds in a drop of 1 per cent phosphotungstic acid (PTA) adjusted to pH 7.0 with 0.1 N KOH, to which was added a small drop of detergent, 'tween 20', placed on a carbon coated grid. The drop was air dried. Preparations were also made from epidermal peals, cut-up, squeezed and the small drop thus obtained was mixed with 1.5 ml of PTA solution.

Some of these preparations both from purified material and leaf dip preparations were shadow casted with gold after the drop was air dried.

2.14. Serology

As the virus affecting double tropaeolum, resembled more closely with the tobacco ring spot virus, its two antisera were obtained, one from Laboratorium voor Bloombollenonderzook, Lisse through the courtsey of Dr. D.H.M. van Slogteren and the other from Instituut voor Plantenziek-tenkundig Onderzoek, Wageningen through the courtsey of Dr. D.Z. Maat. These antisera preserved with 50 per cent glycerine, were used after mixing with equal volume of normal saline (0.9 per cent aqueous solution of NaCl). Antigenicity of the double tropaeolum virus with tobacco ring

spot virus antisera was tested for ascertaining their relationship. The petals and leaves of the virus affected single and double tropaeolum plants along with the corresponding controls of healthy single tropaeolum plants were soaked in 0.5 per cent Na_2SO_3 aqueous solution for over night and macerated with 2 ml per gram of the same solution. The extracted sap after straining through two layers of muslin cloth, was diluted to 1:10 with normal saline and centrifuged at 4,000 rpm for 10 minutes. The supernatant, which contained antigen, was used for micro-precipitin reaction of van Slogteren or the ordinary precipitin reaction with different dilutions of the antisera. On the other hand, there was no need of clarifying the sap by centrifugation for Ochterlony double-diffusion tests. The details of the different reactions are as follows:

Micro-precipitin reaction: For micro-precipitin reaction (van Slogteren, 1955), the bottom of petri dishes were coated with a thin film of 'flexible collodion' (May and Baker) which served as hydrophyllic film preventing the minute droplets of antiserum-antigen mixture from spreading out while covering with the parafine oil. Small droplets of the antisera were put at the bottom of the petri dishes on the hydrophyllic film and mixed with equal sized droplets of plant extract from different sources as mentioned above. After thoroughly mixing the droplets, parafine oil was carefully layered on the droplets so that they are covered. This served to prevent the droplets to evaporate and stop

spontaneous reaction which might occur at the border of the droplets due to excess of evaporation. These petri-dishes were incubated at 37°C for reaction to take place. Observations were recorded after 15 - 20 minutes, either through magnifying lens or by naçked eyes.

Precipitin reaction: Various two-fold dilutions of the antisera were prepared in normal saline and 0.1 ml of each were pipetted in serological tubes. Subsequently, 0.1 ml of clarified plant extract having the antigen was laid over the antiserum. These tubes were placed half immersed in water bath at 40°C. Observations were recorded after 15 min., 30 min., 45 min., 1 hr., 2 hrs., 4 hrs., 8 hrs. and 16 hrs.

Gel-diffusion test: A uniform layer of 0.8 per cent agar, 0.5 cm thick was prepared in the petri dishes or on slides for such tests. The agar suspension was made by dissolving 800 mg of cleaned bacto agar in 100 ml of normal saline along with 1 ml of 0.5 per cent sodium azide. After solidifying, small holes were bored in the solidified agar layer with a cork-borer and filled with antiserum and antigen separately in the required manner. The plates and slides were incubated at 20°C. Reactions, in the form of translucent bands in the agar layer in between the reacting antigen and antiserum wells, were recorded.

2.15. Studies on inhibition of infectivity of the CPO strain of TMV with tropaeolum leaf extracts and its chemical stabilization

Since mechanical inoculation with the tropaeolum

leaf extract in distilled water failed to induce infection, the inhibitory effect of the tropaeolum leaf was investigated. Young green and old yellowish-green leaves of Tropaeolum majus were separately macerated to make standard extract. Two dilutions of the standard extracts i.e. 10^{-1} and 4×10^{-1} were mixed with an equal volume of infectious sap from TMV-CPO (Mathur et al., 1966) infected tobacco leaves and inoculated on a set of half leaves of its local lesion host, C. amaranticolor. The other half was inoculated with a comparable control i.e. infectious sap mixed with an equal volume of distilled water. The local lesions were counted and percentage of inhibition calculated.

For neutralizing the inhibitory principle present in tropaeolum leaves, the leaf extract was mixed with equal volume of 0.5 per cent solution of EDTA, Caffeic acid, Na_2SO_3 and water. In subsequent experiment single tropaeolum leaves were soaked in 0.5 per cent solutions of EDTA, Caffeic acid, Na_2SO_3 and water overnight, after which the leaves were macerated and the extract was mixed with equal volume of infectious TMV-CPO extract as above. Local lesion host was inoculated with the freshly prepared mixture as well as with the mixture stored for 72 hours at 5-7°C. Inoculum was also prepared from tropaeolum leaves frozen for 72 hours and later mixed with infectious TMV-CPO extract. C. amaranticolor plants were inoculated with the inocula prepared as above. Different treatments were distributed according to latin square design on half leaves of C. amaranticolor. The other

half of the leaves were inoculated with suitable control i.e. infectious sap diluted with equal amount of distilled water. Local lesions were counted and percentage of inhibition or stimulation of infectivity was calculated.

Similarly, virus affected double tropaeolum leaves were soaked overnight in EDTA, caffeic acid and Na_2SO_3 solutions at 5-7°C and macerated with each solution separately so as to make standard solution. The inoculum thus prepared was inoculated on the local lesion host C. amaranticolor and local lesions were counted.

2.16. Studies on inhibition of viral infectivity by coumarin and sodium salicylate

Effect of coumarin and sodium salicylate on the infectivity of the virus was investigated in vitro by mixing various dilutions with the inoculum obtained by macerating the leaves of turkish tobacco infected with CPO strain of TMV. In every case the treatments were distributed on the leaves of C. amaranticolor according to the latin square design using half leaf technique.

2.17. Infectivity of virus affected tobacco plants

Infectivity of different plant parts: Plants of N. glutinosa and N. tabacum cv. Xanthi were inoculated at 4 leaf stage. Fifteen days after inoculation, the inoculated leaf, next upper leaf and the youngest leaf were removed and macerated separately to prepare standard extract(s). C. amaranticolor leaves were inoculated for assaying the infectivity. Local lesions were counted and mean number of

lesions per leaf was calculated.

Infectivity at different levels: Infectivity of different leaf positions and the corresponding pieces of internodes (stem) was ascertained above and below the inoculation level in plants of N. tabacum cv. Xanthi. The plants were inoculated at 4 leaf stage, upper two leaves being inoculated, with the inoculum prepared from turkish tobacco plants infected with the virus. Thirty days after inoculation, the plants were uprooted along with their complete root system, and thoroughly washed in water. Leaves were plucked and numbered according to their positions above and below the inoculation level before preparing the inoculum for assaying the infectivity on C. amaranticolor. Internodes of the size of 10 mm. corresponding to each leaf position were cut, macerated and bioassayed. Besides, 2-5 mm. bits of meristem-tip were cut and bioassayed.

Infectivity of the underground parts of the same plant i.e. rootlets, main root and the portion just above the main root was compared by sampling 10 mm bits of these tissues.

Infectivity of leaves at different intervals after inoculation: A number of plants of N. tabacum cv. Xanthi were inoculated at four leaf stage with standard extract prepared from virus infected turkish tobacco plants. Different sets of plants were inoculated at different intervals. Thereafter all the sets of plants were harvested at the same time so as to have inocula from plants 7, 9, 11, 13, 14, 17 and 21 days after inoculation. The inocula were prepared

from these plants, separately, by macerating all the leaves showing clear symptoms to make 'standard extract' for bioassaying the infectivity on C. amaranticolor.

In another set of experiments, inoculated and uninoculated young leaves showing clear systemic symptoms were collected from plants inoculated at different intervals so as to have inocula from plants after 3 to 33 days of inoculation and bioassayed for infectivity. This experiment was done in two stages, in one the inoculum was taken from plants harvested 3, 10, 20 and 28 days after inoculation, whereas in the second case the period of harvesting was 8, 15, 25 and 33 days after inoculation. In both the cases the treatments were randomized on half leaves of C. amaranticolor.

2.18. Infectivity of different parts of single and double tropaeolum plants

Infectivity of different parts of single and double tropaeolum plants namely, leaves, stem, roots and flowers was estimated by preparing the standard extract from these tissues in 0.5 per cent Na_2SO_3 and inoculating the turkish tobacco plants with a little pinch of celite for bioassay.

2.19. Tissue culture studies

For tissue culture studies, several modifications of the media of White (1943), Murashige and Skoog (1962), Miller (1963), Quak (1957), Linsmaier and Skoog (1965) and Buys (1968) with respect to contents like auxins, kinin,

adenine, myoinositol and introduction of compounds like casein hydrolysate,coconut milk and bean cotyledon extract were tried (see Appendix II) for induction of undifferentiated and differentiated callus cultures from various tissue explants and organised meristem growth. These media were solidified with 0.8 per cent Difco Bacto agar. The pH of the media in each case was adjusted to 5.8 before dispensing them in corning culture tubes and sterilising by autoclaving at 10 psi for 15 minutes. The culture tubes and other glass wares were cleaned with teepol and washed in two changes of distilled water before use.

Before transplanting the different explants into the culture medium for inducing the desired growth pattern, they were sterilized by giving a quick rinse in 90 per cent ethanol followed by passing them through 0.1 per cent mercuric chloride ($HgCl_2$) and 30 per cent hydrogenperoxide (H_2O_2) for 1 minute each. After washing the explants in two changes of sterile distilled water, excess of moisture was soaked in sterile filter paper strips and planted on medium with one end embedded.

The cultures were maintained at $25^{\circ} \pm 5^{\circ}C$ under continuous fluorescent light and subcultured regularly after a growth period of 3-4 weeks.

The infectivity of the explants from infected plants and their subsequent cultures was estimated regularly at each transfer by bioassaying the 'standard' infectious sap, prepared from weighed tissue (macerated with 0.02 M phosphate

buffer at pH 7.0) and inoculated on the leaves of C. amaranticolor or turkish tobacco plants with a pinch of celite.

2.19.1. Undifferentiated and dedifferentiated callus cultures

Attempts were made to obtain callus culture from different tissues of healthy and virus affected single tropaeolum, turkish tobacco and N. glutinosa and virus affected double tropaeolum plants. The cultures obtained after establishment of callus growth were marked as P_1 and subsequent transfers as P_2, P_3, P_4 and so on. The culturing of different tissues were done separately in corning tubes (25 x 2.5 cm) as described below.

Embryos and seeds of single tropaeolum plants: For culturing seeds and embryos, the seeds were sterilized before and after soaking for overnight in sterile distilled water (Mishra et al., 1967). Some of the seeds were transferred directly into the medium while in some cases excised embryos were transferred. The growth pattern was noted after 30 or 45 days and subsequent transfers were made as and when required.

Roots, stem and flower-buds: Young unopened flower buds of single tropaeolum plants were transplanted into the medium after sterilization as described above. Ten mm bits of explants of shoots and roots of single and double tropaeolum, turkish tobacco and N. glutinosa plants were transplanted after sterilization as described by Raychaudhuri and Mishra (1962).

Leaf callus: For obtaining leaf callus, the leaves of infected tropaeolum, turkish tobacco and N. glutinosa plants showing clear symptoms as well as healthy leaves of these hosts were cut transversly along the mid-rib into 10 mm wide strips and sterilized. After soaking the excess of moisture from the leaf strips on sterile filter paper, they were further cut into 10 mm segments and transplanted with one end immersed into the medium.

2.19.2. Meristem-tip cultures of double tropaeolum

Axillary as well as terminal meristem-tips, having one meristem-dome enclosed in a pair of leaf-primordia and measuring approximately 0.1 to 0.5 mm, were dissected aseptically with a sterilised scalpel or the flattened end of a needle. The double tropaeolum shoots with axillary and terminal meristems were first sterilised in $HgCl_2$ and H_2O_2 as described above before planting into different culture media. About 8 to 10 meristem-tips were first planted into one tube (25 x 2.5 cm) with about 10 ml of solidified culture medium. After a period of 7 to 10 days, the explants showing meristematic activity were transferred to 7 x 1 cm tubes individually on the same medium. Care was taken in both the cases to submerge the meristems beneath the agar surface as it was found to be essential to induce the growth of the meristems. Subsequent transfers were made after initiation of rooting on a semi-solid medium (0.4% agar) without any auxin and kinin. A fully grown meristem with roots and leaves was finally transferred to the

sterilised soil mixed with sand and a little amount of cow-dung manure and watered daily with the nutrient medium having only mineral components till the plant-lets were large enough. The tube cultures were kept in cool room maintained at $25^{o} \pm 5^{o}C$ under 20 hrs illumination from two fluorescent tubes.

III RESULTS

3.1. Mechanical transmission (sap inoculation)

Inoculum from double tropaeolum leaves was prepared with distilled water as also with 0.5 per cent Na_2SO_3 solution and was applied on the dorsal surface of single tropaeolum and turkish tobacco leaves with or without celite or carborundum (about 600 mesh) as abrasives. Results are given in Table 3.1.

Table 3.1. Mechanical transmission of the virus

Inoculum prepared with	Test plants			
	Single Tropaeolum		Turkish tobacco	
	No. of plants inoculated	No. of plants showing symptoms	No. of plants inoculated	No. of plants showing symptoms
1. Distilled water	10	-	10	-
2. Celite	10	5	10	7
3. Carborundum (about 600 mesh)	10	-	10	-
4. 0.5% Na_2SO_3	10	7	10	9
5. 0.5% Na_2SO_3 and celite	10	9	10	10

These results indicate that the disease of double tropaeolum is transmissible by mechanical inoculation with the help of a mild abrasive like celite and a reducing agent

like Na_2SO_3. The visible symptoms of the disease on turkish tobacco appeared within 5 days in the form of chlorotic spots surrounded by concentric rings which tend to become systemic within 7-10 days (25-28°C). On Tropaeolum majus it took 7-10 days for the appearance of first visible symptoms in the form of mild vein-banding later on interspersed with concentric chlorotic rings. The inoculum with carborundum either added or dusted on the leaves did not produce any infection.

3.2. Biological transmission

3.2.1. Aphid transmission: Four aphid species, namely Aphis gossypii, A. craccivora, Myzus persicae and Macrosiphum pisi were tested for their ability to pick up the virus from three different hosts i.e. turkish tobacco, Datura stramonium and tropaeolum and to transmit on to tropaeolum, turkish tobacco and Nicotiana glutinosa. Before feeding the aphids on these hosts for acquiring the virus, pre-acquisition fasting for 2 hours was given. The acquisition feeding was for 30 minutes, whereas transmission feeding was for 19 hours. Ten aphids were fed on each plant and the data is presented in Table 3.2.

The virus was picked up from tropaeolum by the three aphid species only namely, Aphis gossypii, A. craccivora and Myzus persicae which could transmit the virus only to tropaeolum and turkish tobacco. A. gossypii and M. persicae were equally efficient vectors whereas A. craccivora had low percentage of transmission. The preliminary symptoms in case of aphid transmission, in general, were development of clear vein-banding

Table 3.2. Transmission of the virus by aphids

Source of the host	Aphids tried	Host tested		
		Tropaeolum	Tobacco	N. glutinosa
Garden nasturtium (Tropaeolum majus)	Aphis gossypii	7/10	5/10	0/10
	Myzus persicae	8/10	6/10	0/10
	A. craccivora	3/10	1/10	0/10
	Macrosiphum pisi	0/10	0/10	0/10
Nicotiana tabaccum var. Xanthi	A. gossypii	0/10	0/10	0/10
	M. persicae	0/10	0/10	0/10
	A. craccivora	0/10	0/10	0/10
	M. pisi	0/10	0/10	0/10
Datura stramonium	A. gossypii	0/10	0/10	0/10
	M. persicae	0/10	0/10	0/10
	A. craccivora	0/10	0/10	0/10
	M. pisi	0/10	0/10	0/10

1. Pre-acquisition fasting 2 hrs
2. Acquisition feeding 30 min.
3. Infection feeding 19 hrs
4. No. of aphids used 10 aphids/plant

on both tropaeolum and turkish tobacco leaves within 10-15 days after inoculation. Subsequent mechanical inoculations from the same hosts produced characteristic chlorotic spots and concentric rings on turkish tobacco and tropaeolum.

3.2.2. Nematode transmission: The results of nematode transmission are presented in Table 3.3.

The data indicates positive transmission with X. americanum to one plant only, detectable with antiserum reaction. The lack of infectivity of the plant roots may be due to presence of small amount of virus contents. The nematode X. americanum was unable to reproduce under the conditions of the experiments as is evident from the number of nematodes recovered from the inoculated pots. From none of these pots more than 25 adults besides a few juveniles, could be recovered. This probably explains the positive transmission of the virus in only one seedling out of ten inoculated. The results with X. basiri Siddiqui were negative.

3.3. Soil transmission

Experiment I

The roots of bait-plants grown in sick soil were bioassayed. Equal number of bait-plants were left to grow in the sick soil for the possible appearance of disease syndrome. The following are the results obtained (Table 3.4).

The data indicate mechanical or biological transmission of the virus through sick soil. Roots of bait-plants grown in sick soil from the two sources were almost equally infective.

Table 3.3. Nematode Transmission of the virus from tobacco to tobacco

Plant nos. for source feeding	Virus inoculation 5 days after transplanting	Nematodes seeded 7 days after plant inoculation with virus	Test plant nos. for transmission	No. of nematodes recovered	Bioassaying of the roots	Serological reaction
1		*X. americannum* =50	1	2	-	-
2		"	2	-	-	-
3		"	3	5	-	-
4		"	4	10	-	-
5		"	5	7	-	-
6		"	6	-	-	-
7		"	7	25	-	+
8		"	8	11	-	-
9		"	9	-	-	-
10		"	10	-	-	-
11 (control)	-	"	11	12	-	-
12 (control)	-	"	12	-	-	-
1		*X. basiri* = 200	1	90	-	-
2		"	2	107	-	-
3		"	3	-	-	-
4 (control)	-	"	4	87	-	-
5	-	"	5	111	-	-

However, the number of plants showing visible symptoms was less probably due to slow movement of the virus or meagre amount of the inoculum.

Table 3.4. Transmission of virus through soil as detected through bait-plant infections

Soil samples	Bait-plants	Bioassaying: No. of plants infected/no. of plants tested	Symptom appearance: No. of plants showing symptoms/ no. of plants left in the pots
1. From pots	1. Turkish tobaccoo roots	5/10	2/10
	2. *C.amaranticolor* roots	4/10	2/10
2. From field	1. Turkish tobacco roots	7/10	3/10
	2. *C.amaranticolor* roots	5/10	1/10
3. Control	1. Turkish tobacco roots	0/10	0/10
	2. *C.amaranticolor* roots	0/10	0/10

Experiment II

The roots of 10 bait-plants grown in the sterilized soil were collected at random and bioassayed. Equal number of plants, widely dispersed in the soil, were left to grow for observations regarding the possible appearance of disease

syndrome. The results are presented in Table 3.5 and Figure 2.1.

Table 3.5. Transmission of the virus through soil detected through bait-plant infection and development of disease syndrome

Position of the tested bait plants (in the box)	Bait-plants	No. of plants showing root infection (out of 10 random selections)	No. of plants showing disease syndromes (out of 10 random selections)
Against sick soil	A. Chenopodium	9	4
	B. Tobacco	7	1
Against sick plants and soil	A. Chenopodium	3	2
	B. Tobacco	3	2

The results indicate transmission of the virus from sick soil to the bait-plants growing in sterilized soil. The number of bait-plants showing infection was much less in the other half adjacent to the infected plants indicating possibility of some biological agency being involved which travel out freely into the sterilized soil in search of fresh bait-plants.

3.4. Host range studies

In all 143 plant species, belonging to 29 families of Angiosperms were screened for their susceptibility. Out of these 80 plant species were found to be susceptible and

the rest as immune. These are listed in Appendix I along with their reactions to two other viruses i.e. the tobacco ring spot (Price, 1940) and tropaeolum ring mosaic (Schmelzer, 1960). In this list identical and closely related plant species have been included as far as possible. The grades with respect to susceptibility to the three viruses are also mentioned along with each plant species.

3.4.1. Host plant reaction: The symptoms produced by the double tropaeolum virus on different hosts have been described below family/wise along with the susceptibility index (SI) of each host.

Family: Tropaeolaceae. Double, semidouble and single tropaeolum were found to be highly susceptible. Symptoms on the three cultivars are given separately.

Double tropaeolum (Tropaeolum majus L.) - (SI : 16)

The symptoms of viral infection were most evident on leaves, which showed light and dark green mottling with yellowish or yellowish green rings and line patterns and yellowish spots along the veins and vein-lets. Under favourable cold climatic conditions, the leaves showed crinkling and puckering (Figure 3.1.b).

The petals which were scarlet in colour sometimes showed colour breaking. The flowers showed complete sterility with indefinite number of petals (Figure 3.1c). It appears that sepals become modified eliminating the spur formation: whereas petals, though retaining the same orientation, develop

Fig.3.1. Symptoms on Tropaeolum majus L.

a) Leaves of tropaeolum plants showing mottling, curling and puckering, with rings and line-pattern, y = youngest leaf, H = healthy leaf, O = Older leaves.

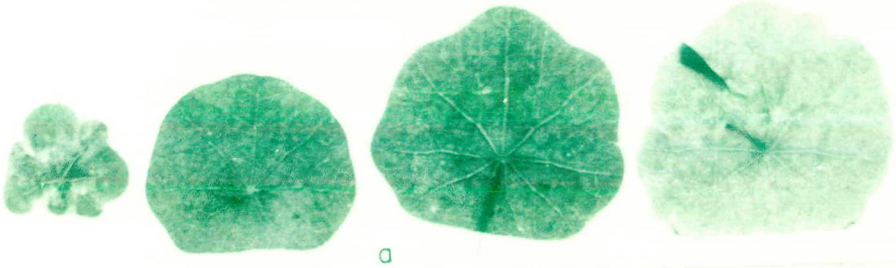
b) Leaves of double tropaeolum plant showing mottling, green vein-banding and pin-point chlorotic specks on younger leaf. Rings and line-pattern not clear.

c) Plant of double tropaeolum showing antholysed flower and severe curling and puckering of the younger leaves.

d) Two plants of single tropaeolum showing mild chlorotic symptoms.

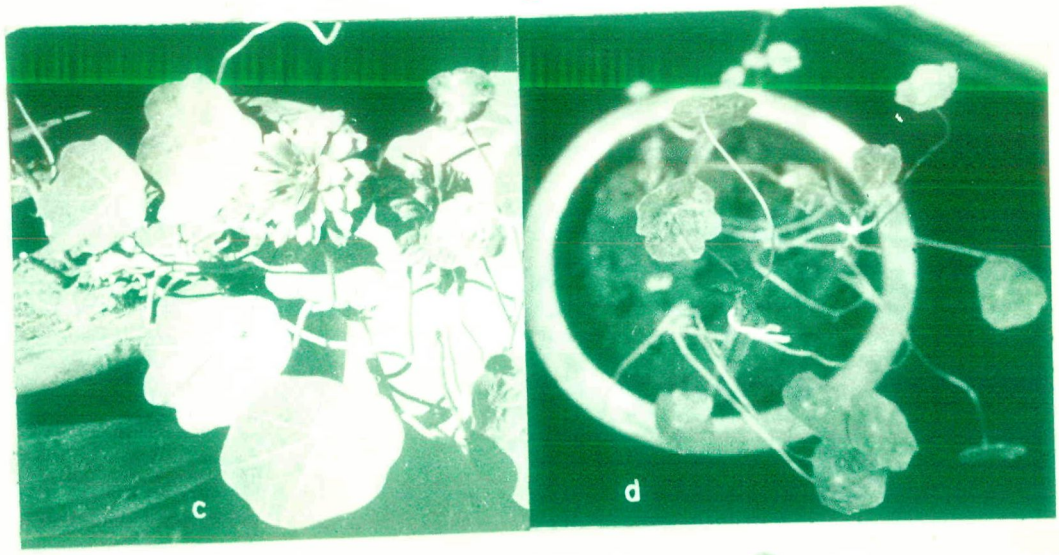
e) Flowers of (1) double (2) semi-double and (3) single tropaeolum, dessected out to show the structure of all the floral parts. Petals of double tropaeolum antholysed flower showing colour breaking.

f) A leaf of single tropaeolum showing vein-banding and chlorosis accompanied by faint rings.

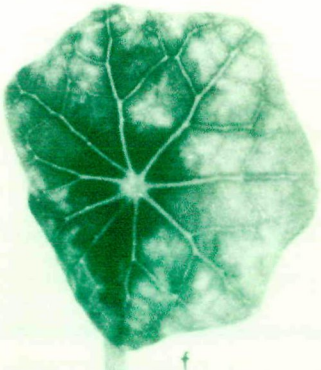


a

b



e



f

FIG. 3.1

indefinitely in number at the expense of androeceum and gynoeceum which are completely suppressed (Fig.3.1.e).

Semi double tropaeolum (Tropaeolum majus) - (SI : 16)

The leaf symptoms were like the ones described for double tropaeolum. In this case [illegible], there was no colour breaking of the flowers or any change in its morphology (Fig.3.1.e).

Single tropaeolum (Tropaeolum majus) - (SI : 16)

The symptoms on the leaves were similar to those described above. There was no colour breaking or any change in the morphology of the flower (Fig.3.1a,d,f).

Family Solanaceae: Out of 35 plant species belonging to this family, 26 were found to be highly susceptible (SI:16) whereas three showed immune reaction (SI:0). The remaining six showed different grades of susceptibility. Reactions of some of the plant species are given below.

Nicotiana tabacum L. cv. Harrison's Special (SI : 16)

Chlorotic spots of 1 mm diam. with broken rings appeared in 5-7 days of inoculation with a tendency to become systemic (Fig.3.2a).

N. tabacum L. cv. White Burley : (SI : 16)

Chlorotic spots developed in 5-7 days and the infection later became systemic inducing mild mosaic symptoms on young emerging leaves (Fig.3.2a).

Fig.3.2. Symptoms on some of the solanaceous hosts.

a) Leaves of (1) Nicotiana tabacum L cv. White Burley and (2) N. tabacum cv. Harrison's Special showing systemic mild mottling with scattered chlorotic patches.

b) Leaves of Lycopersicon esculentum Mill. showing systemic mild chlorosis and distortion of leaf lamina. h = healthy.

c) Leaf of Nicotiana rustica L. cv. moti showing systemic mosaic mottling and green vein-banding with occasional necrotic patches.

d) Leaves of Nicotiana glutinosa L. showing systemic concentric rings and distortion of lamina.

e) Leaves of Nicotiana sylvestris Spegaz & Commes showing mosaic mottling and distortion of leaf lamina. h = healthy.

FIG. 3.2

N. tabacum L. cv. Xanthi - (SI : 16)

Chlorotic lesions appeared within 5 days which were later surrounded by chlorotic concentric rings. The infection became systemic with chlorotic oak-leaf pattern, occasionally necrotic resulting in the distortion of the leaves (Fig.3.3). In older plants symptoms were masked.

Nicotiana glutinosa L. - (SI : 16)

Localised chlorotic or necrotic lesions on inoculated leaves developed within 3-5 days with systemic mottling and concentric rings developing in about a fortnight (Fig.3.2d).

Nicotiana rustica L. - (SI : 16)

Localised necrotic or chlorotic lesions, about 1 mm in diameter, subsequently becoming systemic (Fig.3.2c).

Nicotiana repanda Willd. - (SI : 16)

Plants developed systemic mild mottling.

Nicotiana sylvestris Spegaz. & Commes - (SI : 16)

Systemic mild mottling developed as in N. repanda (Fig.3.2e).

Nicotiana glauca Grah. - (SI : 16)

Concentric chlorotic rings were produced on the inoculated leaves subsequently becoming systemic in the plant.

Nicotiana longiflora Cav. - (SI : 4)

Concentric chlorotic rings were formed on the inoculated leaves only.

Fig.3.3. Symptoms on Nicotiana tabacum L. cv. xanthi

a) Systemic symptoms of oak-leaf and line-pattern with severe distortion and chlorosis on the youngest leaves (y) and faint symptoms on the older leaves.

b) Localised symptoms of concentric ring spots on the inoculated leaves accompanied by distortion.

c) Localised and systemic infection compared with healthy leaf (h). The symptoms being distortion, line-pattern and tattering on the leaf showing systemic infection (s) and ring spots are clear on the inoculated leaf (i).

d) Plant of turkish tobacco showing variations in the expression of symptoms on the same plant viz. from concentric rings to line-patterns. The symptoms are masked on some of the younger leaves.

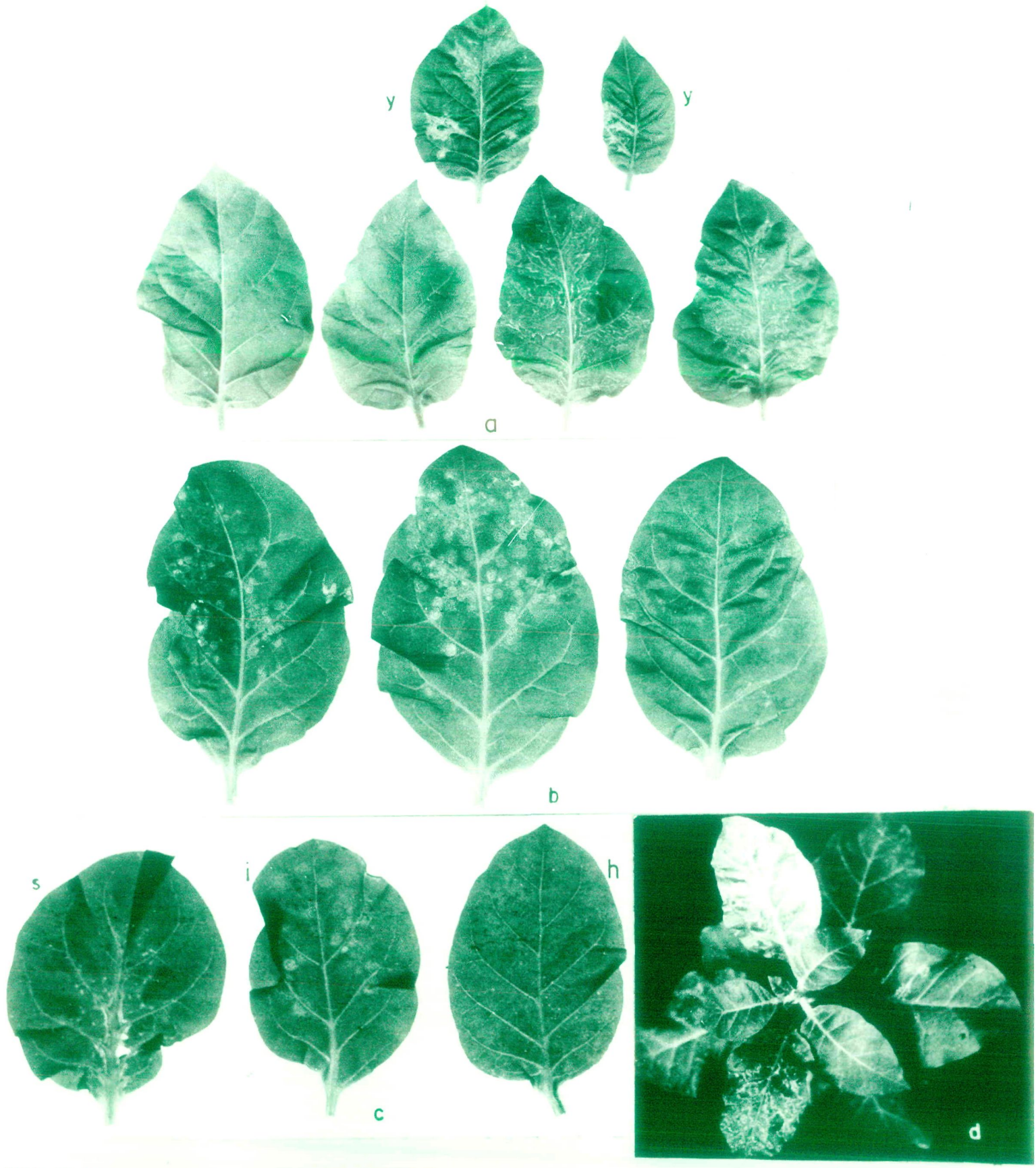


FIG. 3.3

Nicotiana debneyi Domin. - (SI : 8)

Inoculations resulted in general necrosis, wilting with ultimate death of the plants - a hypersensitive reaction.

Petunia hybrida Vilm. - (SI : 8)

Chlorotic or necrotic lesions developed within 5 days (Fig.3.4). Distinct chlorotic, systemic diffused rings, developed within 15-17 days. The plants ultimately died due to severe necrosis.

Solanum nigrum L. - (SI : 16)

Systemic mild flecks with vein-banding appeared within 5-7 days which got masked when the plants became older.

Solanum nodiflorum L. - (SI : 16)

Systemic mild flecks on the leaves (Fig.3.5h).

Solanum melongena L. - (SI : 16)

Vein-clearing symptoms appeared within a week, but systemic concentric rings developed only after about a period of six months.

Solanum capsicastrum L. - (SI : 16)

Systemic mild mosaic with diffused concentric rings and vein-banding developed on plants (Fig.3.4).

Solanum khasianum Clarke - (SI : 2)

Brown necrotic local lesions developed within 5 days (Fig.3.5f).

Fig. 3.4. Systemic symptoms on different hosts.

a) Leaves of Gomphrena globosa L. showing mottling and chlorotic patches.

b) Leaves of Capsicum annuum L. showing mottling and chlorotic specks.

c) Leaves of Solanum capsicastrum L., Lycopersicon pimpinellifolium (Just) Mill. and Physalis floridana Rydb. showing systemic mottling, vein-banding and chlorotic specks.

d) Leaf of Apium graveolens L. showing systemic mottling.

e) Leaf of Lupinus hartwegii Lindl. showing systemic mottling.

f) Leaves of Petunia hybrida Vilm. showing systemic necrotic patches.

FIG. 3.4

Fig.3.5. Localised and systemic symptoms on a number of hosts.

a) Leaf of *Chenopodium murale* L. showing numerous necrotic local lesions.

b) Leaf of *Chenopodium ambrasoides* showing necrotic and chlorotic pin-point local lesions.

c) Leaf of *Chenopodium amaranticolor* Coste and Reyn. showing necrotic local lesions with a chlorotic halo around them.

d) A leafy shoot of *Chenopodium amaranticolor* Coste & Reyn. showing systemic chlorotic patches and specks.

e) Leaves of *Salvia splendens* Kerr. showing necrotic localised irregular lesions.

f) Leaves of (1) *Solanum khasianum* and (2) *Amaranthus spinosus* L. showing necrotic local lesions.

g) Leaf of *Amaranthus caudatus* L. showing systemic chlorotic specks.

h) Leafy shoot of *Solanum nodiflorum* L. showing systemic chlorotic patches and specks.

i) Leaves of *Datura stramonium* L. showing systemic mottling and chlorotic patches.

j) Leaves of *Datura tatula* showing systemic mottling with vein-banding, line-pattern and chlorotic and necrotic blotches and lesions.

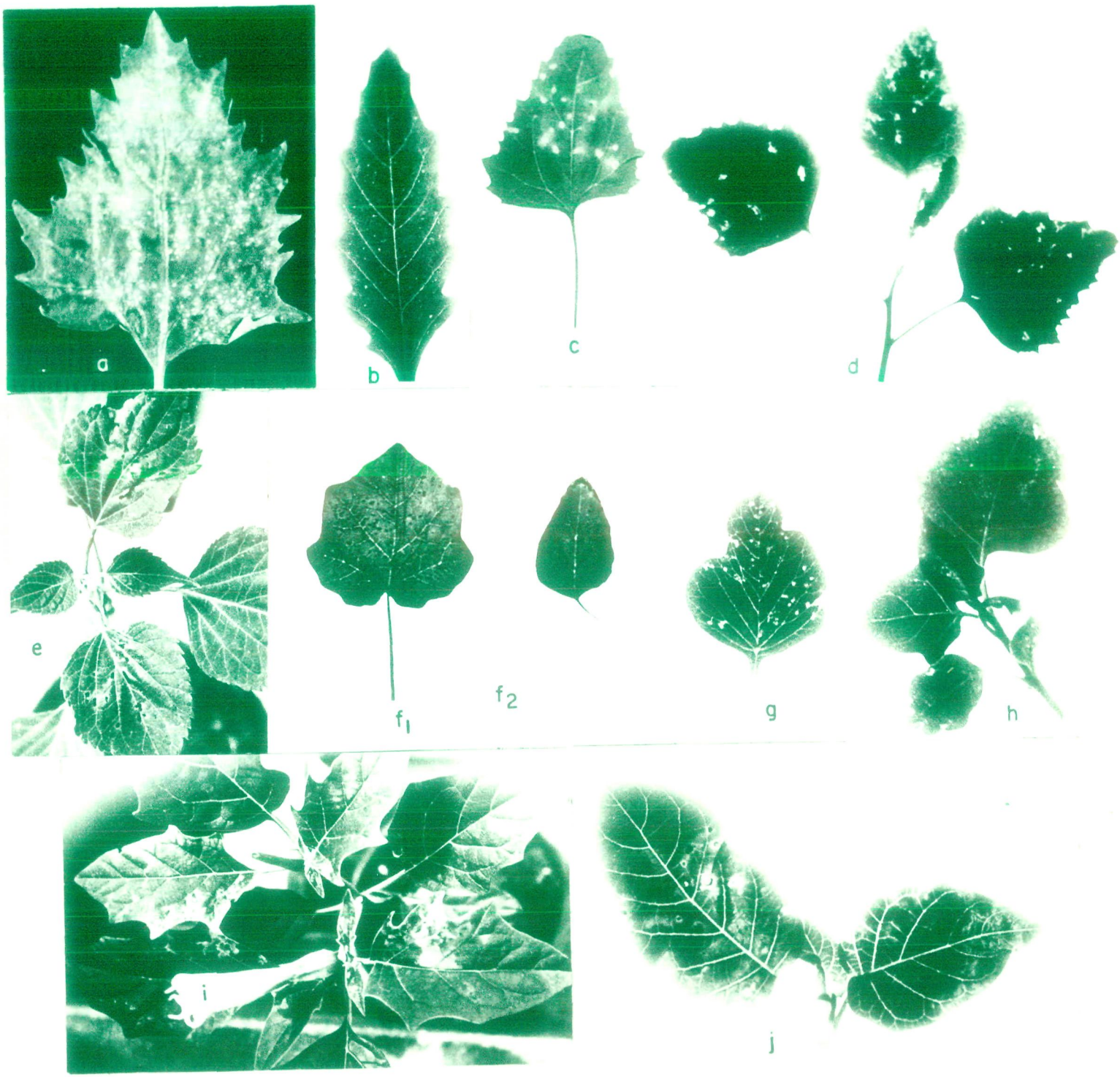


FIG. 3.5

Datura stramonium L. - (SI : 16)

Minute pin-point chlorotic lesions developed within 5 days leading to the development of chlorotic and necrotic concentric rings gradually spreading with systemic mottling (Fig.3.5i).

Datura tatula L. - (SI : 16)

Broad necrotic and chlorotic local lesions were produced on inoculated leaves later developing systemic mosaic and oak-leaf pattern (Fig.3.5).

Lycopersicon esculentum Mill. - (SI : 16)

A distinct mosaic mottling developed within 10 days, sometimes resulting in distortion of leaf-lamina also (Fig.3.2b).

Lycopersicon pimpinifolium (Just) Mill. - (SI : 16)

Mild mosaic symptoms (Fig.3.4).

Capsicum annuum L. - (SI : 16)

Systemic whitish green flecks of various sizes and shape, irregularly appeared on younger leaves. On the older leaves the symptoms were masked (Fig.3.4b).

Physalis ixiocarpa Brot. - (SI : 16)

Concentric chlorotic rings appeared with a tendency to become systemic.

Physalis floridana Rydb. - (SI : 16)

Whitish grey necrotic local lesions with brown hallo were followed by systemic mild mosaic symptoms (Fig.3.4).

Leguminosae

Out of 23 plant species tested, 12 were highly susceptible (SI 16) whereas 9 were showing complete immunity. One of the remaining two showed subliminal infection (SI 1) and the other produced only necrotic local lesions (SI 2).

Vigna sinensis Savi - (SI : 16)

Chlorotic local lesions, about 2 mm in diameter appeared with a brownish hallo on the inoculated leaves (Fig.3.6a). Within 10-15 days, the infection became systemic with the appearance of mosaic symptoms.

Phaseolus vulgaris L. - (SI : 16)

Small red coloured chlorotic local lesions developed on inoculated leaves, the infection becoming systemic within 15 days with the appearance of mosaic symptoms.

Dolichos lablab L. - (SI : 16)

Systemic light green mosaic mottling with white chlorotic flecks and vein clearing appeared in 5-7 days (Fig.3.6b). The whole plant later became bushy and severely stunted with signs of sterility with the advance of the disease as evidenced by reduced number of seeds in the pods. In the later stage of disease development only empty pods were formed.

Crotalaria juncea L. - (SI : 2)

Necrotic local lesions with reddish brown hallo developed within 4-5 days on inoculated leaves.

Fig.3.6. Symptoms on Leguminous hosts

a) Leaf of *Vigna sinensis* Savi ex Hassk. showing only necrotic local lesions.

b) Leaf of *Dolichos lablab* L. showing systemic chlorotic specks and patches.

c) Leaf of *Crotalaria brownae* showing chlorotic systemic round patches.

d) Leaf of *Melilotus indica* All. showing systemic chlorotic patches.

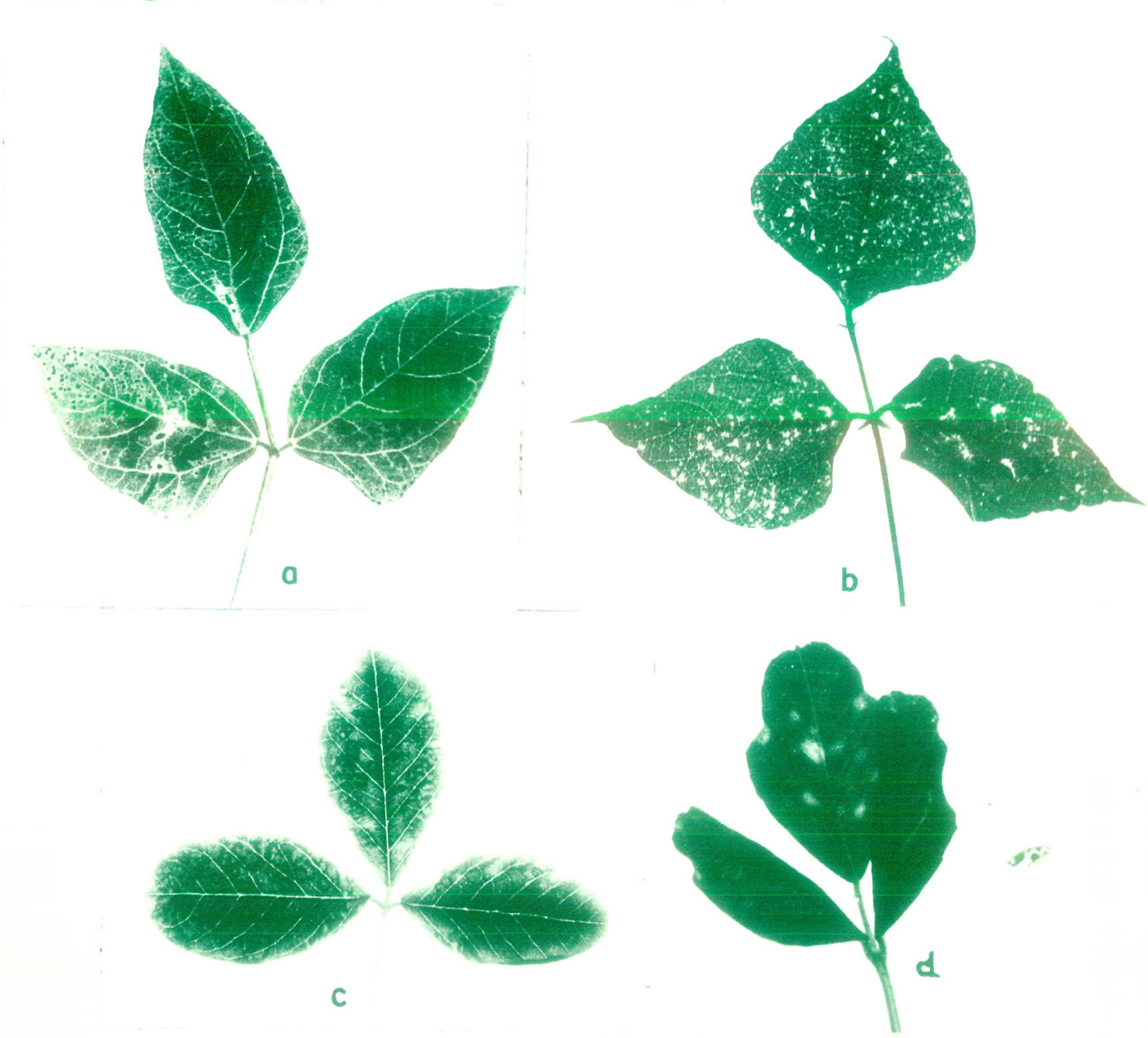


FIG. 3·6

Crotalaria brownei Bert. ex DC - (SI : 16)

Big, brown lesions of 2-3 mm diameter developed on inoculated leaves within 7 days of inoculation. The whole plant appeared bushy after sometime with signs of sterility as evidenced by complete absence of bearing. The systemic mosaic symptoms developed after 6 months which disappeared later in summer months i.e. April to July, reappearing in September and October when the temperature is condusive for the expression of symptoms (Fig.3.6c).

Vicia faba L. - (SI : 16)

Very few, broad local lesions followed by systemic mosaic mottling appeared on inoculated plants.

Trigonella foenum-graecum L. - (SI : 16)

Mild, chlorotic spots appeared on inoculated primary and secondary leaves within 4-5 days. Systemic mosaic symptoms with mild chlorotic concentric rings having a light centre developed in about 10 days time.

Trigonella corniculata L. - (SI : 16)

Symptoms same as above.

Trifolium alexandrinum L. - (SI : 16)

Systemic mild mosaic symptoms appeared in about 2 months time.

Melilotus indica All. - (SI : 16)

Chlorotic lesions about 2 mm in diameter were formed

on the inoculated leaves later developing systemic mosaic (Fig.3.6d).

Lupinus hartwegii Lidl. - (SI : 1)

Mild chlorosis developed on a few leaves which disappeared later (Fig.3.4e).

Amaranthaceae

Out of seven plant species tested only 2 gave maximum disease reaction (SI 16) whereas one was immune and the remaining 4 host species reacted by producing different intermediate reactions.

Gomphrena globosa L. - (SI : 4)

Numerous small, pin point local lesions appeared on inoculated leaves within 42 hours, later on developing into systemic flecking on young leaves (Fig.3.4a).

Amaranthus caudatus L. - (SI : 16)

Small pin point, circular, chlorotic local lesions were formed on inoculated leaves after about 5 days. Subsequently infection became systemic with younger leaves showing mild mottling in the form of flecks accompanied by curling down of the leaves (Fig.3.5g).

Amaranthus gangeticus L. - (SI : 8)

Necrotic local lesions which appeared on inoculated leaves became systemic with the appearance of mild chlorosis leading to ultimate necrosis.

Amaranthus leucarpus L. - (SI : 4)

Small, pin point, local lesions appeared within seven days. The chlorotic spots appeared on some of the younger leaves also.

Amaranthus spinosus L. - (SI : 2)

Necrotic pin point local lesions developed on the inoculated leaves only (Fig.3.5f).

Umbelliferae

Only two plant species were tested. Apium graveolens L. gave subliminal reaction (SI 1) whereas Daucus carota L. was found to be immune (SI 0).

Compositae

Out of 15 plant species tested only two were showing highest degree of susceptibility (SI 16), 10 were immune and the remaining 3 gave chlorotic or necrotic local infection.

Zinnia elegans Jacq. - (SI : 16)

Systemic specks with vein-banding symptoms developed on the leaves in about 8-10 days.

Chrysanthemum sp. - (SI : 4)

Numerous small local lesions developed within 7 days of inoculation.

Dahlia sp. - (SI : 4)

Pin point, chlorotic, local lesions appeared on inoculated leaves.

Helianthus annuus L. - (SI : 2)

Small pin-point, brown local lesions appearing within 7 days after inoculating the leaves. These lesions were first chlorotic, but later turned necrotic within 15 days of inoculation.

Chenopodiaceae

Out of 7 plant species only one was immune, whereas others mostly showed necrotic local lesions. Only Chenopodium murale L. reacted by hypersensitive reaction (SI 8).

Chenopodium amaranticolor - (SI : 2)

Chlorotic or necrotic pin point local lesions on inoculated leaves appeared within 7 days (Fig.3.5e). The infection tended to become systemic under warmer conditions (Fig.3.5d).

Chenopodium ambrasoides L. - (SI : 2)

Small necrotic local lesions with brownish hallo, 1-2 mm in size developed on the inoculated leaves (Fig.3.5b).

Chenopodium quinoa Willd. - (SI : 2)

Chlorotic pin point lesions appeared on the leaves which gradually turned brown and necrotic. The lesions enlarged to coalesce with each other.

Chenopodium murale L. - (SI : 8)

Necrotic local lesions became enlarged to coalesce with each other (Fig.3.5a).

Beta vulgaris L. - (SI : 2)

Reddish localised lesions with brownish hallo appeared on the leaves. The centre of the lesions was first chlorotic finally turning necrotic.

Labiatae

Only 5 plant species were tested. Out of these 2 were immune, 2 were showing subliminal infection and only one gave necrotic lesions.

Salvia splendens Kerr. - (SI : 2)

Angular, necrotic, local lesions of various sizes with chocolate coloured margins appeared on inoculated leaves alone (Fig.3.5a).

Hyptis suaveolens Poit. - (SI : 1)

Only light systemic chlorosis of the leaves which disappeared later.

3.4.2. Effect of general climatic conditions on host reactions

The host reactions described in the preceding section were observed in winter months i.e. from November to March, when the temperature in the glass-house ranges from 10° to 30°C. During the summer months i.e. from May onwards, when the temperature was above 30°C, the plants failed to exhibit characteristic symptoms presumably due to very low level of virus contents. However, the symptoms reappeared from September onwards when the temperature again started coming down.

3.4.3. Distribution of susceptibility in the families of Angiosperms: On the basis of maximum disease reaction exhibited by the plant species within different families, the following five categories were made:

I. having families with immune plant species (Susceptibility index = 0)

II. having families with plant species showing subliminal infection as the maximum disease reaction (SI = 1)

III. having families with plant species showing necrotic local lesions as the maximum disease reaction (SI = 2)

IV. having families with plant species showing hypersensitivity as the maximum disease reaction (SI = 8)

V. having families with plant species showing systemic mosaic mottling and other systemic symptoms as the maximum disease reaction (SI = 16)

The distribution of families of Angiosperms in each category is given in Table 3.6.

The families having plant species with systemic infection were considered most susceptible, indicating maximum support for the viral multiplication. No family as a whole indicated SI 4 as the maximum disease reaction presumably, it may be an intermediary step in the development of localised necrotic reaction i.e. SI 2 which characterises one of the above groups. Although a large number of plant species having different disease reactions occur in a particular family, the maximum disease reaction provides a

Table 3.6. Distribution of susceptibility in the families of Angiosperms and catagorization on the basis of maximum disease reaction

Group		Family	No. of species/ var. tested	No. of species/ var. with positive reaction	Maximum disease reaction	Remarks *
I	1.	Apocynaceae	1	0	0	
	2.	Caryophyllaceae	1	0	0	
	3.	Cruciferae	6	0	0	
	4.	Euphorbiaceae	2	0	0	
	5.	Malvaceae	4	0	0	
	6.	Onagraceae	1	0	0	
	7.	Papaveraceae	2	0	0	
	8.	Polygonaceae	1	0	0	
	9.	Portulaccaceae	1	0	0	
	10.	Pedaliaceae	1	0	0	
	11.	Gramineae	2	0	0	
	12.	Irridaceae	3	0	0	
II	13.	Cucurbitaceae	11	2	1	2
	14.	Moraceae	1	1	1	1
	15.	Umbelliferae	2	1	1	1
III	16.	Labiatae	5	3	2	1
	17.	Oxalidaceae	1	1	2	1
	18.	Violaceae	1	1	2	1
IV	19.	Balsaminaceae	1	1	8	1
	20.	Chenopodiaceae	7	6	8	1
V	21.	Amaranthaceae	7	6	16	2
	22.	Compositae	15	6	16	2
	23.	Leguminosae	23	14	16	12
	24.	Polemoniaceae	1	1	16	1
	25.	Ranunculaceae	1	1	16	1
	26.	Scrophulariaceae	3	3	16	3
	27.	Solanaceae	35	32	16	26
	28.	Tropaeolaceae	2	2	16	2
	29.	Verbenaceae	2	1	16	1
			29	143	82	

* No. of species/ var. showing maximum disease reaction.

clear basis for such a categorization as it shows the extent of susceptibility exhibited by the families.

Significance of variation of different groups of host reaction

The five groups were analysed and compared by group analysis and testing the significance of variation by F test. The results are in Table 3.7.

The table indicates that different groups not only differ significantly in their disease reactions but also in the existence of high degree of positive correlation between maximum disease reaction and number of host species showing positive reaction in each group. Variance test indicates that each group differs significantly from the other.

Frequency analysis of different disease reactions in Group V

Table 3.8. Frequency analysis of Group V

Disease reaction	No. of species included	Per cent
1	3	4.54
2	5	7.58
4	6	9.09
8	4	6.06
16	48	72.73
Total	66	100.00

Table 3.7. Significance of variance of different groups of families on their reactions to the virus

Group	Maximum disease reaction (x)	No. of families	No. of species tested	No. of species with positive reaction	Positive reaction per cent (y)	Group average	Variance	Test of significance
I	0	12	25	0	0.00	0.00	0.0000	
II	1	3	14	4	28.57	1.00	0.0000	
III	2	3	7	5	71.43	1.60	0.3000	F.(IV,III) = 28.572** F.(V,III) = 105.042**
IV	8	2	8	7	87.50	3.71	8.5716	F.(V,IV) = 3.676*
V	16	9	79	66	83.54	12.68	31.5126	

Correlation coefficient between x and y = 0.7261

* Significance at 5% level

**Significance at 1% level

There was a wide variation in the susceptibility of plant species of Group V as indicated in the table. This indicates that susceptibility is related to the host species. However, the number of host species showing the type reaction is predominant.

Frequency analysis of disease reactions in the individual families of Group V

Further analysis of the nine families constituting Group V is given in Table 3.9.

At the family level too, there was a wide variation of susceptibility amongst host species indicating random distribution. However, on the basis of percentage of plants showing maximum disease reaction, Amaranthaceae and Compositae were found to be closer to each other as both of them include one-third of the number (33.33%) of host species exhibiting such reaction. On the other hand, rest of the families namely Leguminosae, Solanaceae, Scrophulariaceae, Tropaeolacae, Verbenaceae, Polemoniaceae and Ranunculaceae were found to have more than 66% of plant species showing maximum disease reaction. Amongst these Leguminosae and Solanaceae definitely indicate high degree of susceptibility. With regard to other families no definite opinion could be formulated as the number of plant species tested under each was too less.

Table 3.9. Frequency analysis of the families of Group V

Families	Disease reaction	No. of species included	Per cent
Amaranthaceae	2	1	16.66
	4	2	33.33
	8	1	16.66
	16	2	33.33
	Total	6	
Compositae	2	2	33.33
	4	2	33.33
	16	2	33.33
	Total	6	
Leguminosae	1	2	14.28
	2	1	7.14
	16	11	78.57
	Total	14	
Solanaceae	1	1	3.12
	2	1	3.12
	4	1	3.12
	8	3	9.37
	16	26	81.25
	Total	32	
Scrophulariaceae	4	1	33.33
	16	2	66.66
	Total	3	
Tropaeolaceae	16	2	100
Verbenaecae	16	1	100
Polemoniacae	16	1	100
Ranunculaceae	16	1	
		66	

3.4.4. Comparision of host reactions of double tropaeolum virus with those of tobacco ring spot and tropaeolum ring spot viruses: In the list of host plants as shown in Appendix I 21 plant species tested during the present investigations were common with these reported for tobacco ring spot virus (TRSV) (Price, 1940) and tropaeolum ring spot or ring mosaic virus (NRSV) (Schmelzer, 1960). The susceptibility indices of the hosts of TRSV and NRSV as reported in the literature, are tabulated in Table 3.10 along with those of the virus (DNRSV) under study.

No significant difference in the host reactions of the three viruses were observed except with regard to Impatiens balsamina L., Spinacea oleracea L., Callistephus chinensis Nees and Delphenium cultorum Voss. which are immune to one or the other virus.

The difference in the mean host reactions of these viruses are not significant statistically. However, the little difference in the mean host reaction of TRSV with those of the other two may be considered at the level of strain differentiation, thereby indicating that the other two viruses i.e. NRSV and the virus under study may be strain(s) of TRSV. The mean host reaction of NRSV and that of the virus from double tropaeolum is almost the same.

Table 3.10. Comparision of host-reactions of the virus with Tropaeolum ring spot and tobacco ring spot viruses

Sl. No.	Host species	Host reactions with				
		TRSV	NRSV	DNRSV	Total	Mean
1.	*Amaranthus candatus*	2	16	16	34	11.33
2.	*Impatiens balsamina*	8	0	8	16	5.33
3.	*Beta vulgaris*	16	16	2	34	11.33
4.	*Chenopodium album*	2	8	2	12	4.00
5.	*Spinacea aleracea*	16	16	0	32	10.66
6.	*Callistephus chinensis*	16	16	0	32	10.66
7.	*Zinnia elegans*	16	16	16	48	16.00
8.	*Phaseolus vulgaris*	8	2	16	26	8.67
9.	*Vicia faba*	8	16	16	40	13.35
10.	*Vigna sinensis*	8	16	16	40	13.33
11.	*Delphenium cultorum*	0	16	16	32	10.66
12.	*Datura stfamonium*	8	2	16	26	8.67
13.	*Lycopersicon esculentum*	16	16	16	48	16.00
14.	*Nicandra physaloides*	2	16	16	34	11.33
15.	*Nicotiana glutinosa*	16	2	16	34	11.33
16.	*N. rustica*	16	16	16	48	16.00
17.	*N. sylvestris*	8	16	16	40	13.33
18.	*Petunia hybrida*	16	16	8	40	13.33
19.	*Physalis angulata*	8	16	16	40	13.33
20.	*Solanum nigrum*	2	16	16	34	11.33
21.	*Tropaeolum majus*	16	16	16	48	16.00
	Total	208	270	260	738	
	Mean	9.90	12.86	12.38		

Analysis of Variance

Source	D.F.	S.S.	M.S.S.	F.
Host	20	628.19	31.41	0.82 N.S.
Virus	2	105.52	52.76	1.37 N.S.
Error	40	1537.15	38.43	
Total	62	2270.86		

3.5. Physical properties

Thermal-inactivation

In the first experiment, samples were treated at temperatures of 40, 50, 60, 70 and 80°C for 10 minutes whereas in the next two experiments the difference in temperatures was narrowed to 2°C, starting from 50 to 70°C. The results of these experiments are given in Table 3.11.

Table 3.11. Thermal-inactivation of virus *in vitro*

Temperature (°C)	No. of plants infected/10 inoculated			Total no. of plants infected/ inoculated	% Plant infection
	Expt I	Expt II	Expt III		
40	8	-	-	8/10	80.0
50	7	8	7	22/30	73.2
52	-	8	7	15/20	75.0
54	-	8	8	16/20	80.0
56	-	7	8	15/20	75.0
58	-	6	7	13/10	65.0
60	7	6	6	19/30	63.3
62	-	0	0	0/20	0
64	-	0	0	0/20	0
66	-	0	0	0/20	0
68	-	0		0/10	0
70	0	0		0/20	0
80	0			0/10	0
Control	8	8	9	25/30	83.3

It was observed that the virus was not inactivated in the standard extract exposed to 60°C for 10 minutes but was rendered inocuous when heated to 62°C for the same period. The trend of inactivation has been shown in Fig.3.7.

Tolerance to dilution

Nine dilutions ranging from 1:10 to 1:20,000 were tried and ten turkish tobacco plants per dilution were inoculated. The results of the experiments which were repeated thrice are given in the Table 3.12.

Table 3.12. Tolerance to dilution of the virus

Plant extract dilution	No. of plants infected/10 inoculated			Total no. of plants infected/ inoculated	% Plant infection
	Expt I	Expt II	Expt III		
10^{-1}	8	7	5	20/30	66.6
10^{-2}	7	6	5	18/30	60.0
10^{-3}	6	5	6	17/30	56.6
$5x10^{-4}$	-	4	5	9/20	45.0
10^{-4}	3	2	2	7/30	23.3
$1.5x10^{-5}$	-	0	0	0/20	0
$2x10^{-5}$	-	0	0	0/20	0
$5x10^{-5}$	-	0	0	0/20	0
10^{-5}	0	-	-	0/10	0
Control	8	7	6	21/30	70.0

The results presented in the table indicate that the virus remained infective upto the dilution of 1:10,000 and rendered non-infectious when diluted to 1:15,000.

Aging in vitro (Longevity)

The results of the experiments on the effect of aging in vitro are presented in the table 3.13.

Table 3.13. Effect of aging (Longevity) in vitro, on the infectivity of the virus

Period of storage (hrs)	Room temperature (19-27°C)		Frigidaire temperature (8-10°C)	
	No.of plants infected/10 plants inoculated	% Plant infected	No. of plants infected/10 plants inoculated	% Plant infected
24	5	50	6	60
48	1	10	7	70
75	0	0	5	50
96	0	0	5	50
168	0	0	4	40
240	0	0	5	50
480	0	0	0	0
Control	7	70	8	80

It was observed that the standard extract retained infectivity even after storing for 168 hours (8 days) but not after 240 hours (10 days) at 8 to 10°C whereas at 19 to 27°C (room temperature during January) the plant extract became non-infectious when tested after 75 hours of storage.

Aging in vivo (maintenance of virus cultures in dried leaves of tobacco

The infectivity of desiccated leaves of virus infected turkish tobacco plants kept in sealed tubes over $CaCl_2$ at 4-8°C was estimated at regular intervals. Turkish tobacco was used as test plants for assaying the infectivity and the results are presented in Table 3.14.

Table 3.14. Effect of aging (Longevity) *in vivo* on the infectivity of the dried leaves of virus affected tobacco plants

Periods of storage (months)	No. of lesions per leaf of tobacco					Total number of lesions	Mean no. of lesions/ leaf
	1	2	3	4	5		
6	25	12	37	29	17	120	24.0
10	10	9	20	22	12	73	14.6
11	5	4	10	11	6	36	7.2
Control (source material)	78	20	40	50	67	255	51.0

The above data indicate retention of infectivity in the dried leaves even after 11 months. The infectivity here is represented by the number of local lesions produced on turkish tobacco leaves. During summer months when the final test was done the expression of symptoms was much suppressed, which may be the reasons for the lesser number of local lesions.

According to personal communication from Dr. L. Bos.

with whom one set of the culture was deposited, the virus was also recovered from the dried preserved material even after 12 months.

Effect of pH on the infectivity of the sap

1. With 0.1 M Phosphate Buffer (0.1 M $Na_2HPO_4 12.H_2O$ + 0.1 M KH_2PO_4)

The different pH ranges, tested for their effect of infectivity of virus-affected plant sap, were 8.3, 8.0, 7.0, 6.0, 5.0 and 4.5 and the results on the infectivity at different pH ranges are presented in Table 3.15.

Table 3.15. pH range of infectivity : Effect of phosphate buffer at different pH. on the infectivity of the extracted sap of the virus affected turkish tobacco

pH	Infectivity (No. of local lesions/half leaf) c/t.							No.of lesions in treatment/100 lesions in control
	1	2	3	4	5	6	Total	
8.3	14/8	5/2	5/2	11/4	25/10	6/3	66/29	43.93
8.0	6/2	6/2	1/0	5/5	4/3	3/2	25/14	56.00
7.0	3/4	7/2	1/1	2/1	3/1	2/3	18/12	66.66
6.0	2/1	1/5	2/10	1/3	11/20	3/4	20/43	215.00
5.0	2/2	7/7	1/1	1/2	2/4	7/4	20/20	100.00
4.5	5/4	3/6	2/5	9/4	10/5	1/1	30/25	83.33

c - Control (inoculum mixed with equal volume of distilled water.

t - Treatment (inoculum mixed with equal volume of buffer)

These results indicated that infectivity in phosphate buffer ranged from pH 4.5 to 8.0 with the optimum at pH 6.0. It remains quite infective at lower pH i.e. even upto 4.5, however, reduced considerably at higher pH i.e. 8.3 (Fig.3.7).

2. With 0.1 M Sorenson's Glycine buffer (0.01 M Glycine, 0.1N NaOH, 0.1N HCl)

This buffer was used so as to have a wide range of pH variations i.e. from 1.0 to 13.6 for investigating the variability in the different pH ranges. The results thus obtained are presented in Table 3.16.

In Sorenson's Glycine buffer infectivity was observed even at pH 1.0 and 9.3, the optimum being at 6.0. At the acidic range of 1.0 and alkaline range of 9.3 and above, the infectivity was very low and indicated inconsistant development of local lesions in different replicates. However, phosphate buffer was twice as effective at the optimum pH of 6.0 (Fig.3.7).

3.6. Cytopathology

Light microscopy: Epidermal strips from healthy and infected leaves of Tropaeolum and tobacco when stained with phloxin and methyl green, did not indicate any difference and no inclusion bodies could be detected.

Fluorescent microscopy: In both infected and healthy cells, nucleis exclusive of nucleoli fluoresced yellowish-green colour, whereas cytoplasm and nucleoli fluoresced red

FIG 37

PH-RANGE OF INFECTIVITY IN PHOSPHATE & GLYCINE BUFFERS

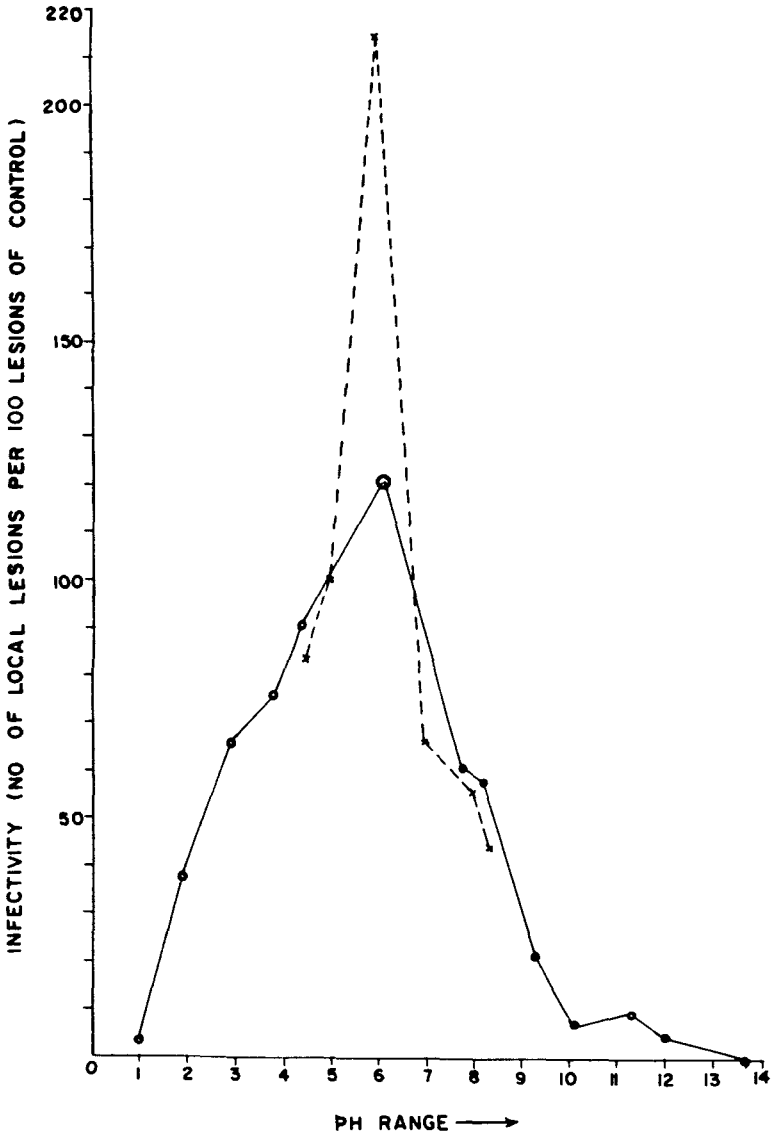
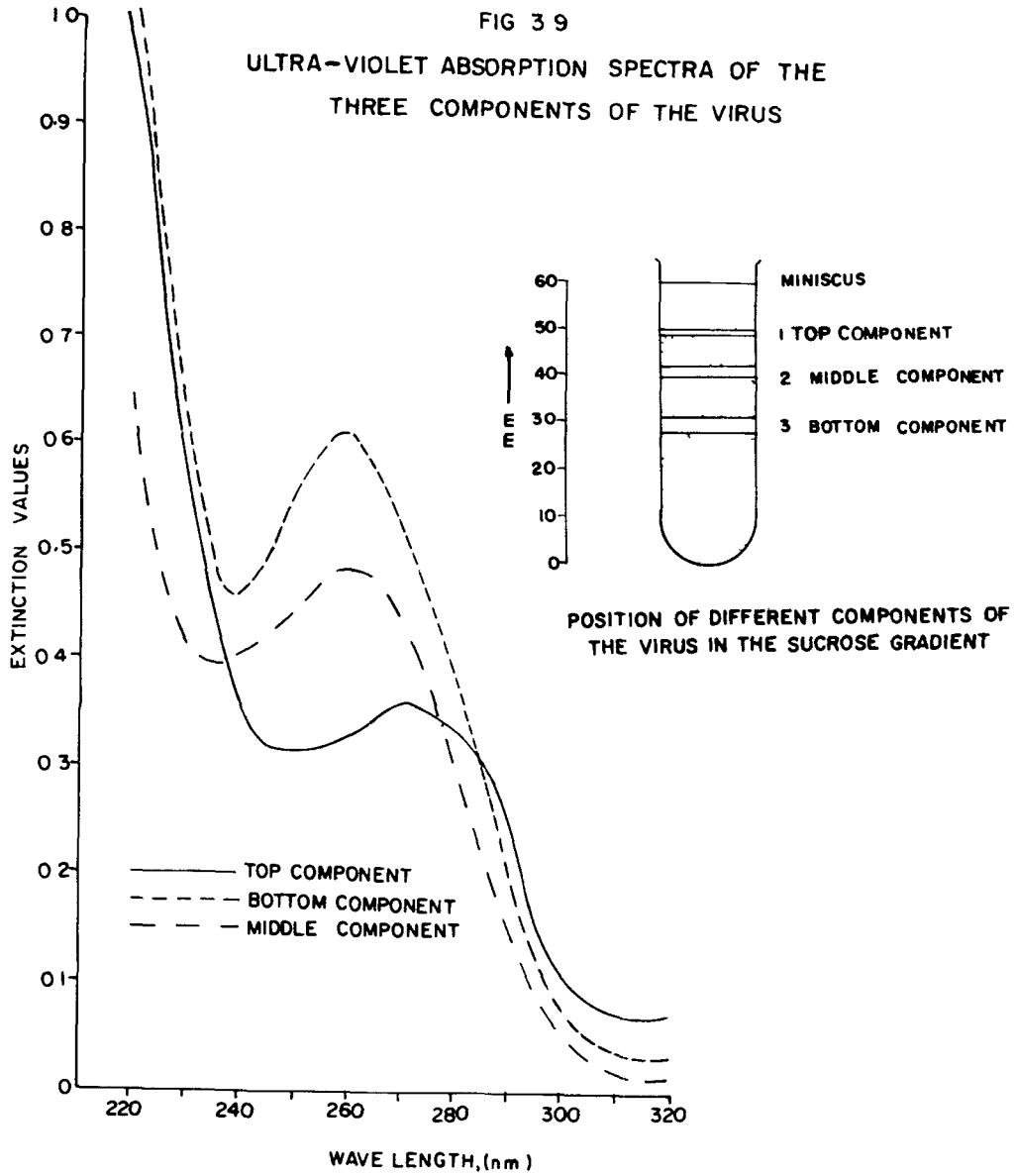


FIG 39

ULTRA-VIOLET ABSORPTION SPECTRA OF THE THREE COMPONENTS OF THE VIRUS



POSITION OF DIFFERENT COMPONENTS OF THE VIRUS IN THE SUCROSE GRADIENT

Table 3.16. pH range of infectivity : Effect of Glycine buffer. at different pH on the infectivity of extracted sap of the virus affected turkish tobacco

pH	Infectivity (No. of local lesions/ half leaf) - t/c						No. of lesions in treatment/ 100 lesions in control
	1	2	3	4	5	Total	
1. (1.0)	0/22	0/20	0/15	3/20	0/24	3/101	2.97
2. (1.9)	3/15	3/20	5/10	17/18	1/12	29/75	37.33
3. (2.9)	2/4	10/26	12/12	17/32	22/22	63/96	65.62
4. (3.9)	-	6/8	6/8	18/22	10/15	40/53	75.47
5. (4.4)	13/15	10/15	18/20	10/32	27/15	78/97	80.41
6. (6.1)	5/5	8/14	35/25	10/7	22/15	80/66	121.21
7. (7.8)	-	-	7/14	22/32	11/20	40/66	60.45
8. (8.2)	6/12	6/6	10/25	15/22	13/20	50/85	58.81
9. (9.3)	4/5	6/16	5/10	-	15/12	30/43	21.42
10.(10.1)	-	0/4	0/10	1/25	3/17	4/56	7.14
11.(11.3)	5/22	0/8	0/12	2/19	0/11	7/72	9.72
12.(12.0)	0/12	0/4	0/4	2/24	-	2/44	4.54
13.(13.6)	-	0/11	0/17	0/16	0/2	0/46	0

t - Treatment (Inoculum mixed with equal volume of buffer).

c - Control (Inoculum mixed with equal volume of distilled water.

and orange red colour, the latter being only faintly tinted. The virus infected tissues were characterised by the presence of inclusion bodies emitting distinct deep orange-red fluorescence. The inclusion bodies were either occurring singly or sometimes in groups of more than one aggregates adjacent to the nuclei, but mostly lying in one corner of the cell (Fig.3.8b). These inclusions could not be distinguished under the light microscope and only with difficulty under phase contrast. In the later case they appeared as hyaline bodies. These inclusion bodies in some cases, were found to envelop the nucleus (Fig.3.8c&d) in the form of a zone of uniform density. All these structures were not found in any of the preparations from healthy plants (Fig.3.8a).

The identity of the red fluorescing inclusions was confirmed by treating the epidermal peals with RNAse enzyme. In the RNAse treated epidermal peals when stained with AO, the red fluorescence was partially removed as its intensity was much less compared to untreated ones. This indicates that the red fluorescence in the cytoplasmic material was due to viral RNA which is absent in the healthy tropaeolum plants.

3.7. Purification

1. Selection of production host: The infectivity counts of the four hosts indicative of viral contents, were as given in Table 3.17.

Fig.3.8. Fluorescent microscopy of tropaeolum leaf epidermis from healthy and virus affected plants

a) Healthy plant leaf epidermis showing fluorescing nuclei.

b) Leaf epidermis from virus affected plants showing presence of fluorescing inclusions (arrow) at the perifery of the cytoplasm and sometimes more often adjacent to the nuclei.

c) Leaf tissues of the virus affected plants showing fluorescing material (arrow) enclosing the nucleus.

d) Fluorescing envelop around the nucleus photographed under oil-immersion lense.

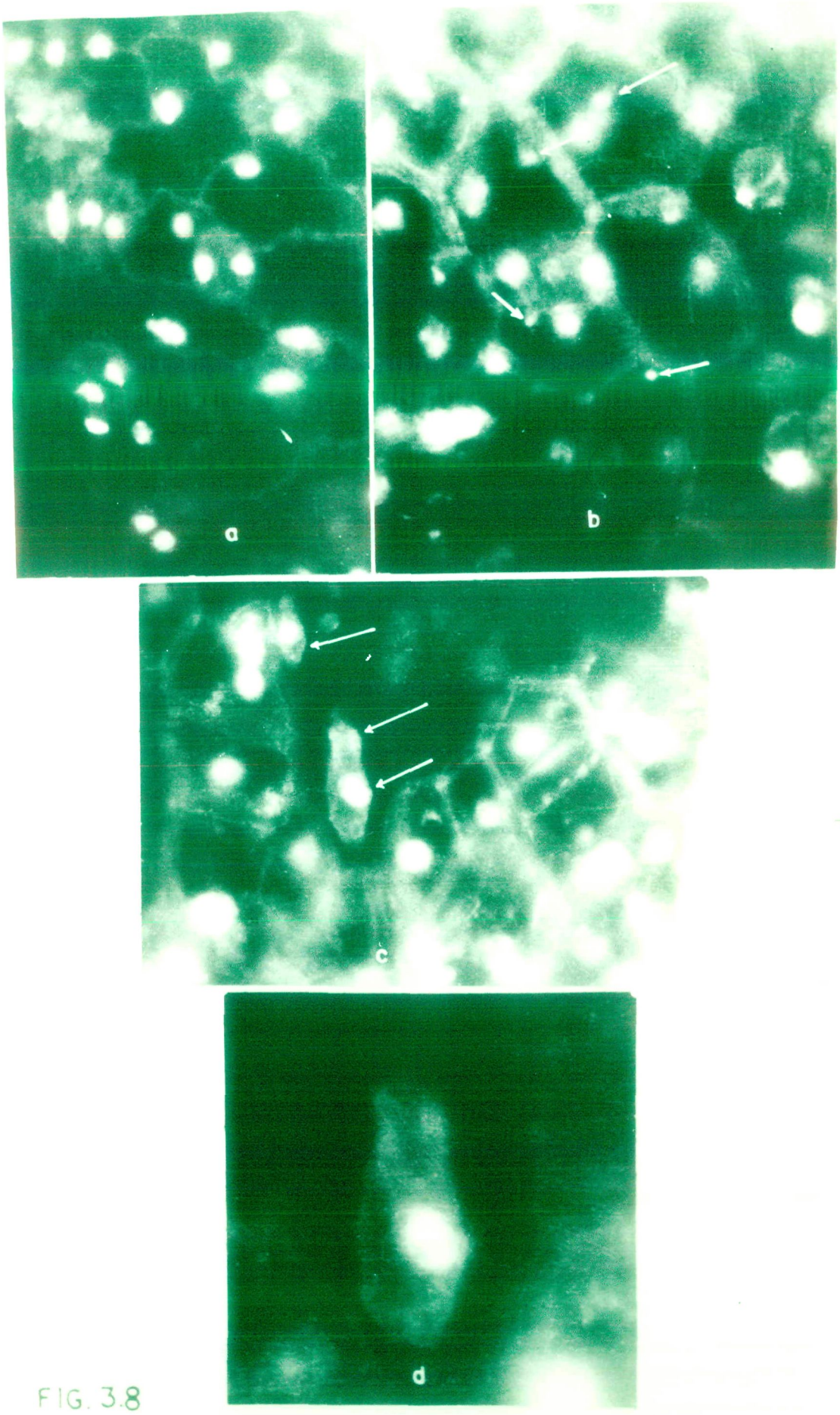


FIG. 3.8

Table 3.17. Infectivity of different production hosts

Production hosts	No. of local lesions/ leaf				Total		Mean
	1	2	3	4			
1. Petunia hybrida	29	59	16	20	124	270	33.75
	20	60	51	15	146		
2. Datura stramonium	29	40	25	8	102	206	25.75
	17	29	38	20	104		
3. Nicotiana glutinosa	48	112	12	32	204	415	51,87
	109	38	49	15	211		
4. N. tabacum cv. Xanthi	20	64	48	16	148	282	35.25
	27	29	58	20	134		

N. glutinosa, though had the highest virus titre, was not suitable as a production host, because it was difficult to develop inoculum from these plants rapidly. N. tabacum cv. Xanthi was preferred because of rapidity with which large quantity of infectious sap could be obtained with high viral titre.

Effect of buffers and chemicals used in purification cycle on the infectivity: The results obtained with different buffer systems and the chemicals used for clarification and purification are summarised in the Table 3.18.

Table 3.18. Effect of chemicals, used in various purification schedules, on the infectivity of final preparations

Juice extraction	Clarification	Buffers for dissolving pellets	Colour of the pellets	Infectivity*	
				Crude extract	Suspended pellet
1.Homogenized in equal volume of buffer at pH 7.6					
(a)0.05 M potassium phosphate buffer and 0.1%TGA	:8.5% n - Butanol	0.03 M K phosphate buffer (7.0)	Brownish white	146	50
(b)0.5 M Borate buffer and 0.1% TGA	:8.5% n - Butanol	0.01 M EDTA (7.0)	Brown	175	62
2.Homogenized in equal volume of buffer at pH 6.8					
(a)0.1 M potassium phosphate buffer and Ascorbic acid (0.05 M)	:0.25 volume of 1:1 mixture of n-butanol & chloroform	0.02 M K phosphate buffer (7.0)	Brownish grey	191	49
(b)0.1 M sodium phosphate buffer and Ascorbic acid(0.05 M)	:0.25 volume of 1:1 mixture of n-butanol & chloroform	0.02 M. Na phosphate buffer (7.0)	Brownish green	139	67
3.Homogenized in equal volume of buffer at pH 7.0					
(a)0.5% Na_2SO_3	:0.25 volume of 1:1 mixture of n-butanol & chloroform	0.02 M Na phosphate buffer (7.0)	Greenish	148	46
(b)0.01% potassium phosphate buffer	:20% chloroform	0.01 M EDTA (7.0)	Greaish brown	200	52

*Average no. of lesions/leaf.

The infectivity of the crude plant extract was compared with the first high speed pellets of all the preparations. It was observed that the loss of infectivity was high in all the preparations probably because in each case n-butanol or chloroform or mixture of both were used for removing plant material. The loss of infectivity was less when ascorbic acid was used instead of thioglycolic acid (TGA) but the difference in the two was not significant.

Use of Na_2SO_3, was as effective as phosphate buffer in the retention of viral infectivity, however, the use of chloroform or n-butanol or their mixture did not give a clear brownish while pellet. Some amount of greenish colour was indicative of contaminating plant material.

Source of inoculum: Since earlier studies showed greater infectivity, the roots were also used as the source of inoculum for purifying the virus along with the shoots showing systemic infection and leaves showing clear local lesions, obtained from 10-15 days old N. tabacum cv. Xanthi plants.

For purification procedures n-butanol method was used with 0.05 M potassium phosphate buffer at pH 7.0 and 0.1 per cent TGA. The final pellet was dissolved in 0.02 M phosphate buffer at pH 7.0. The final preparations were centrifuged on sucrose density gradient for further purification and their infectivity was also assayed (Table 3.19).

Table 3.19. Purification from different source material processed by n-butanol method

Source of inoculum	Weight (gm)	PO_4 buffer volume (ml)	Total volume (ml)	Vol BuOH (ml)	Pellet	Infectivity No.of local lesions/leaf	Zones in density gradient (thickness in mm)
1.Leaves showing localised symptoms	40	170	200	17.0	Greyish white 4 mm + 0.5	52	3.0
2.Shoots showing systemic symptoms	135	170	300	25.5	Greenish white 3.5 mm + 0.5	63	3.5
3.Roots	21	100	125	9.73	Dirty white 5 mm + 0.5	49	5.0

*Volume after extraction

The roots appeared to yield least amount of debris or contaminating material in the purified preparations as was evidenced by the dirty white colour of the pellet and broadest zone in the sucrose gradient. The infectivity, however, was maximum in the preparations from shoots showing clear systemic symptoms, though not much different from the other two samples.

Sucrose density gradient centrifugation

Gradient tubes were prepared from 0.2 to 0.7 M sucrose

buffered in 0.02M phosphate buffer at pH 7.0 formed into a linear gradient by use of an apparatus described by Stace-Smith.(1965). Two millilitres of the purified virus was layered on top of 24 ml of gradients and run in SW 25 rotar at 22,500 rpm for 150 minutes. After centrifugation, the tubes were examined with a verticle beam of light and the three light scattering zones designated as top, bottom and middle, were removed with a bent hypodermic syringe.

In the gradient column measuring 60 mm, the three scattering zones were resolved generally at the following positions (Fig.3.9).

1. Top component 49 - 50 mm
2. Middle component 40 - 42 mm
3. Bottom component 28 - 31 mm

In some preparations there was trailing of opalescence below the bottom component which generally contained pellet at the bottom of the tube. In still another preparation, the top and the middle components were more diffused and therefore, difficult to detect with certainty. Such cases of incomplete resolution of all the different components of the virus were due to high proportion of contaminating plant material. In most of the cases the bottom component formed a distinct and broad zone almost half way down the column.

The three components after removing from the column, were tested for the infectivity and for ultra-violet absorption spectra. For electron microscopy, the sucrose was

removed by repelleting the virus by centrifuging the suspension, after diluting in 0.02 M phosphate buffer (pH 7.0), at 40,000 rpm for 90 minutes, Table 3.20.

Table 3.20. Properties of different components of the virus, obtained on sucrose gradient

Components	Position on the sucrose gradient (mm from the bottom)*	UV absorption		Infectivity
		260:280 ratio	280:250 ratio	
1. Top	4.9 - 5.0	(33/34) .97	(34/32) 1.06	-
2. Middle	4.0 - 4.2	(48/31) 1.55	(31/45) 0.68	⊥
3. Bottom	2.8 - 3.1	(61/37) 1.64	(37/56) 0.66	+++

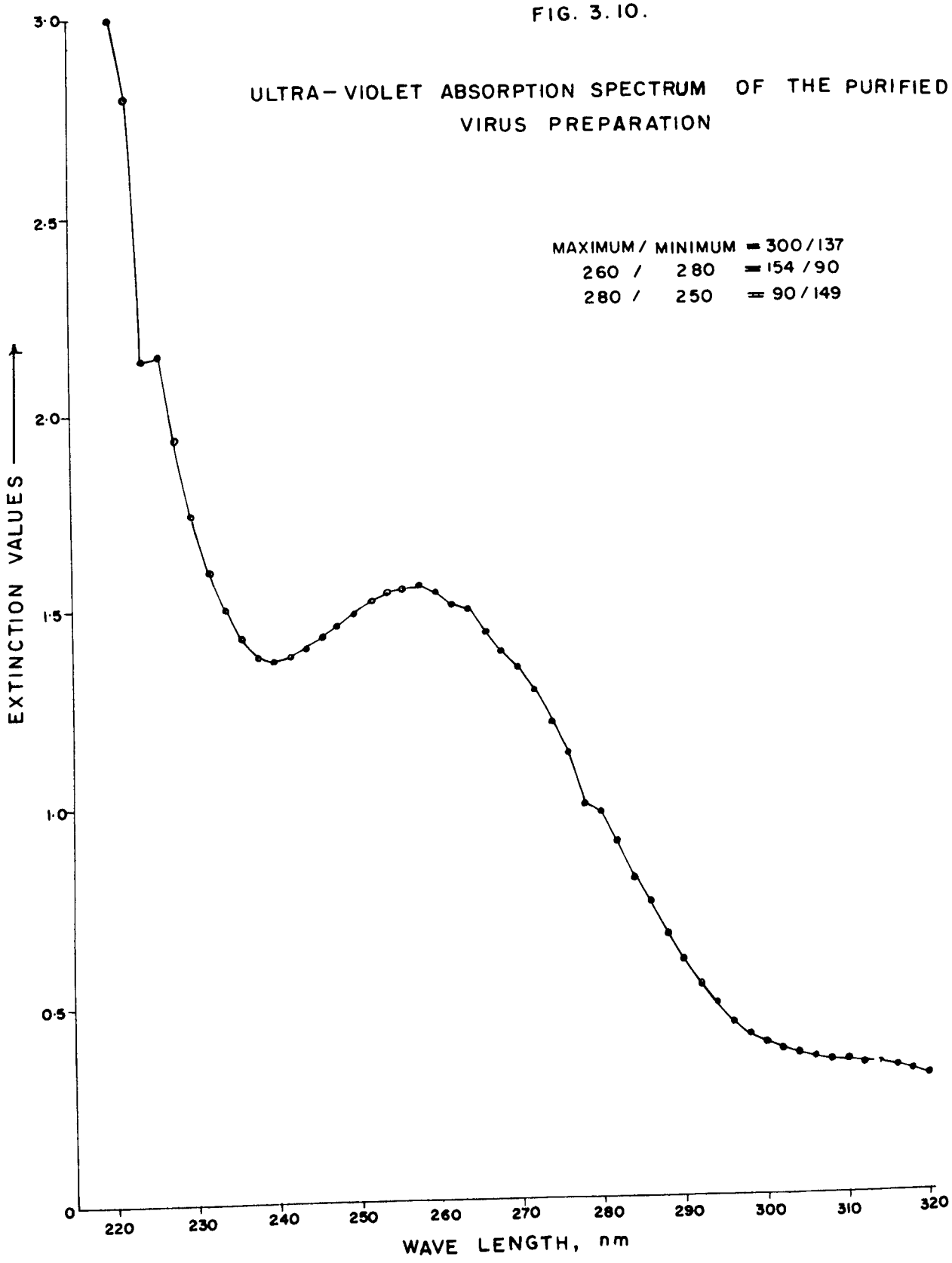
* The miniscus is at 6 mm
\- No infectivity
⊥ Slightly infective producing local lesions one in one of the leaves.
+++ Highly infectious (producing average local lesions of 98/leaf)

3.8. Ultra violet absorption spectra

Ultra-violet absorption spectra of the three components are shown in Fig.3.9 indicating transaction from nucleoprotein in the bottom to protein in the top component. The maximum absorption in the top component has shifted to 270 nm due to the predominance of protein in the preparation, the minimum absorption being at 250 nm, a characteristic of protein. The 280/250 ratio which is more than 1.0 in this case is indicative of the same. The 260/280 ratio in the protein

FIG. 3.10.

ULTRA-VIOLET ABSORPTION SPECTRUM OF THE PURIFIED VIRUS PREPARATION



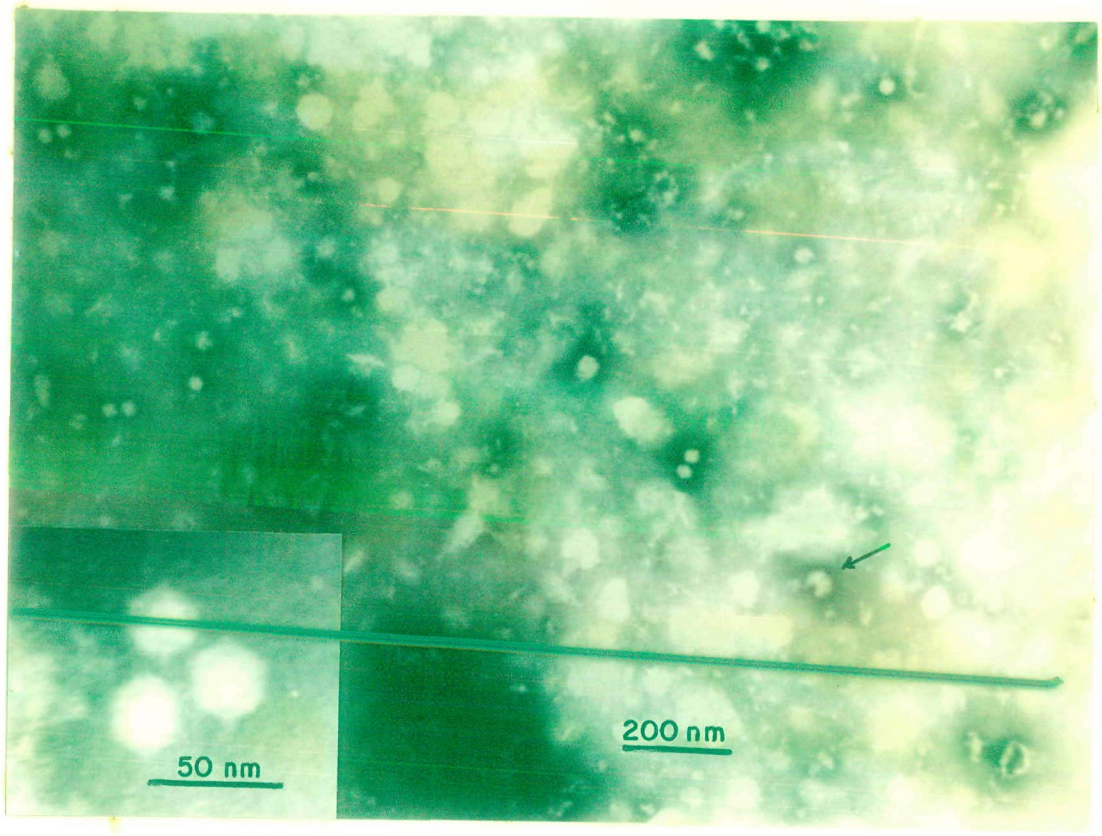
preparations is usually of the order of 0.50 to 0.66 whereas it was 0.97 in the top component thereby indicating trace contamination of RNA in the top component. As the preparation was not infective, it may be contaminated with plant RNA (Table 320). The lines indicating middle and bottom components are consistent with the shape of viral nucleoprotein absorption (Fig.3.9).

For detail study of the ultra-violet absorption spectra of this virus, a purified preparation, obtained by n-butanol method was used. The final pellet was dissolved in 0.02M phosphate buffer at pH 7.0. The U-V absorption spectrum as shown in Fig.3.12 demonstrates a typical nucleoprotein spectrum with minimum absorption at 240 nm and maximum at 260 nm. The 260 : 280 ratio in this case was 1.71 whereas it varied from 1.58 to 1.82 in several other preparations. A small bump at 280 nm is similar to tryptophane bump which is indicative of the presence of protein. The peak at 260 nm represents a purine band. One bump of weak intensity was also observed at 264 nm which may be assigned to vibrational frequency of purines of nucleic acid of the virus. A strong bump at 226 nm remains unexplained and may be due to contaminating plant materials (Fig.3.10).

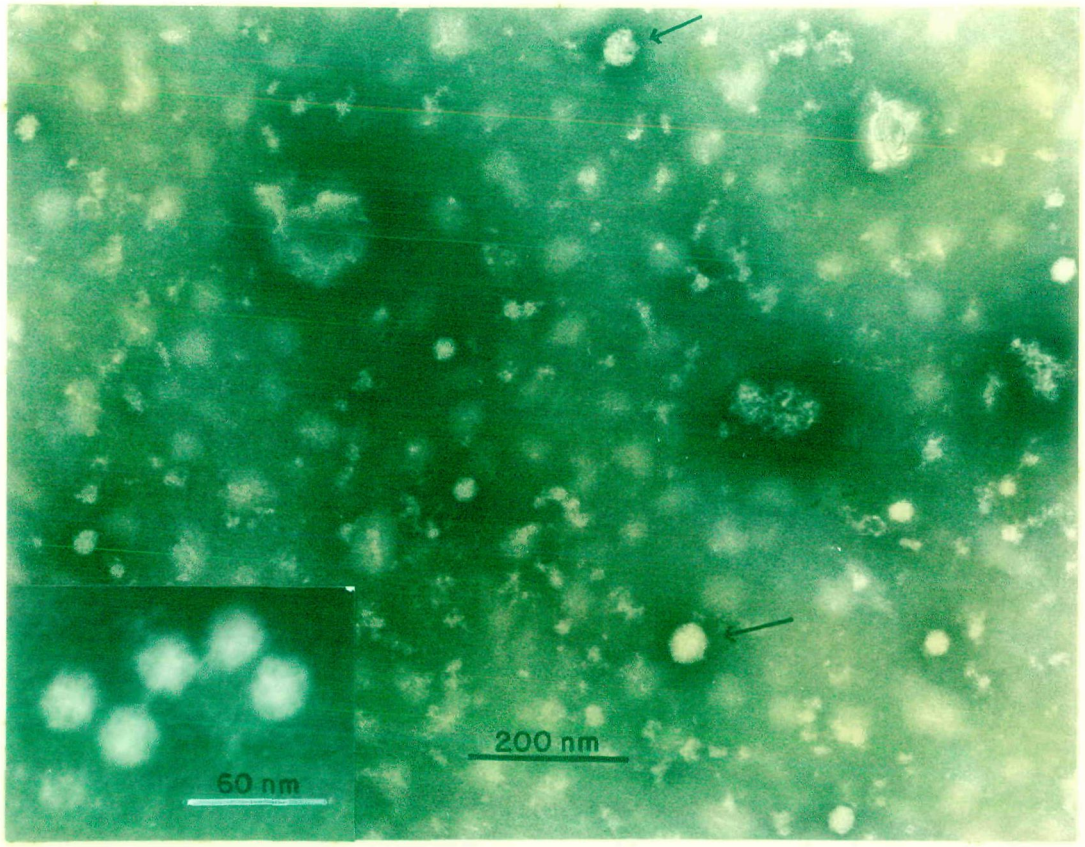
3.9. Electron microscopy

The material for electron microscopy was prepared by both leaf-dip method as well as by using purified virus preparations obtained by n-butanol method, agar-column method and using borate buffer which gave partially purified material.

Fig. 3.11. Electron-micrographs of the purified virus preperation (Butanol method), negatively stained with 2% PTA, showing polyhedral virus particles.Particles disintigrating (arrow).
A. Magnification X 73,000. Inset: Three virus particles- magnification X 360,000.
B. Magnification X 107,000. Inset: Five virus particles- magnification X 320,000.



A



B

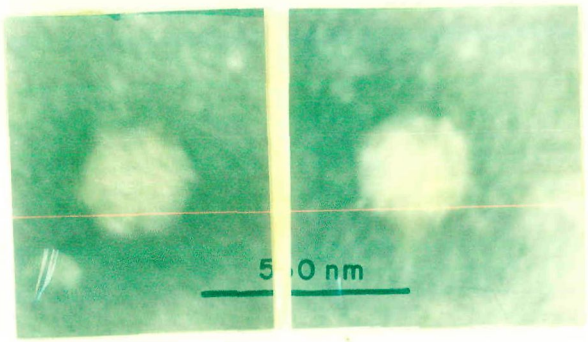
FIG. 3.11

Fig. 3.11. Electron-micrographs of the virus preperations.

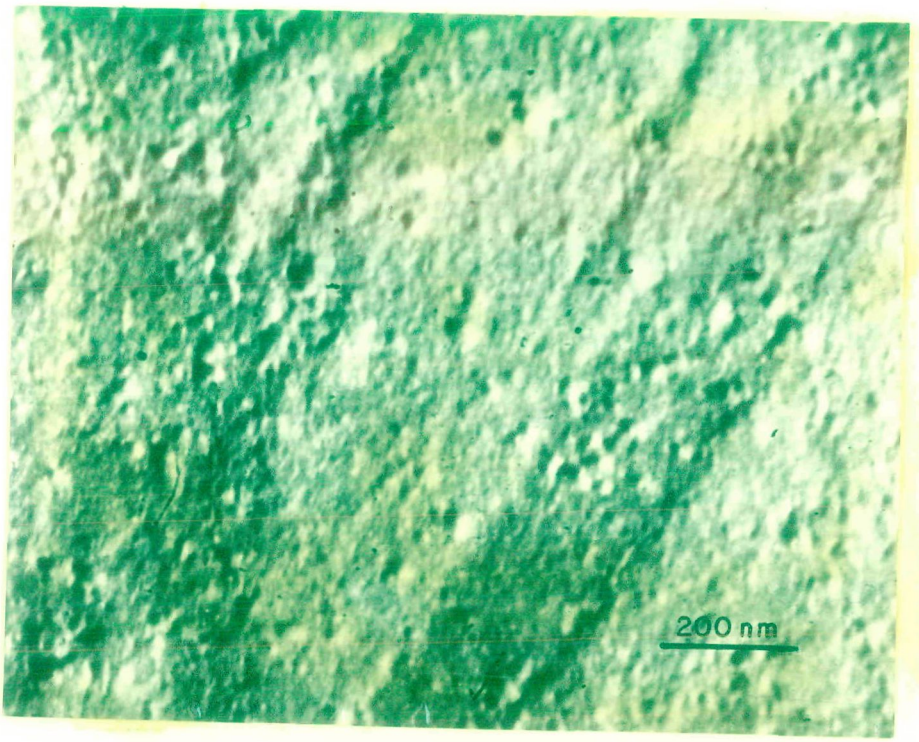
C. Two virus particles from a purified virus preperation (butanol method), negatively stained and showing capsomeres of approximately 0.08 nm diameter. Magnification X 500,000.

D. Leaf-dip preperation showing shadow casted spherical virus particles. Magnification X 93,000.

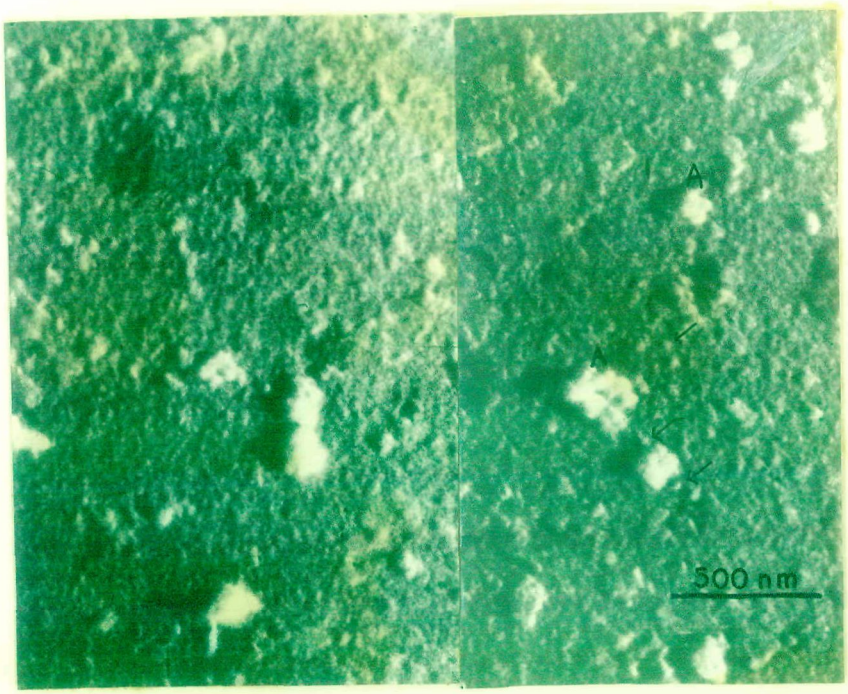
E. Preperation of purified virus (agar gel filteration method) showing shadow casted aggregates (A) as well as individual particles (arrow). Magnification X 39,000.



C



D



E

FIG. 3.11

of the cases the preparations were either shadow-casted or negatively stained.

The electron micrograph (Fig.3.11) is from dip-preparations indicating spherical virus particles measuring 30 nm average diameter.

The electron micrograph (Fig.3.11) is from the purified preparation processed by agar column method indicating spherical virus particles measuring 32 nm average diameter. In most of these cases the particles are aggregated.

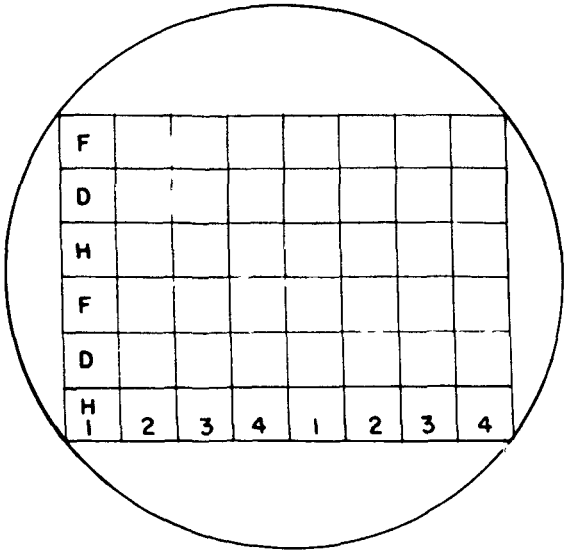
Fig.3.11 is the electron micrograph of virus particles from the preparations made by n-butanol method, negatively stained with 2.0 per cent phosphotungstic acid (pH 7.0). These preparations contained polyhedral particles measuring 29 nm average diameter and having 6 corners (hexagonal) as seen from one side. Some empty particles were also evident showing the protein ring and lacking RNA core. A certain amount of damage of the particles was noticed at places as a result of denaturing of protein shell probably due to use of PTA for negative staining (Serjeant, 1967). In some of the particles protein units were evident, measuring approximately 0.8 nm average diameter.

3.10. Serological relationship

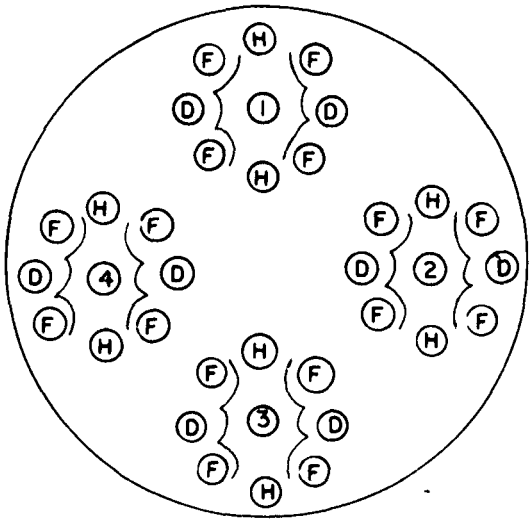
Micro-precipitin reaction: Dilutions of the two antisera of TRSV, when mixed with the sap extracted from flower petals and leaves of virus affected double and single tropaeolum and leaves of healthy tropaeolum plants (Fig.3.12) gave precipitin reactions as given in the Table 3.21.

FIG. 3.12

LAY-OUT OF MICRO-PRECIPITIN TEST.



LAY-OUT OF GEL-DIFFUSION TEST.



ANTIGEN- F = FROM FLOWER PETALS.
D = FROM DISEASED LEAVES.
H = FROM HEALTHY LEAVES.

ANTISERUM- 1 = FROM LISSE, DILUTED TO 1/8
2 = " " " " 1/16
3 = " WAGENINGEN, " " 1/8
4 = " " " " 1/16

Table 3.21. Microprecipitin reaction of the virus with TRSV antisera

Source of antigen	Precipitin reaction at different antisera dilutions			
	Antiserum from Lisse		Antiserum from Wageningen	
	1/8	1/16	1/8	1/16
I. Virus affected double tropaeolum plants				
1. Flower petals	++++	+++	++++	+++
2. Leaves	+++	+++	+++	++
II. Virus affected single tropaeolum plants				
3. Leaves	++	++	++	++
III. Healthy single tropaeolum plants				
4. Leaves	-	-	-	-

The above data indicated the presence of the same or similar antigen, in the petals and leaves of virus affected single and double tropaeolum plants. Negative results with the healthy leaves eliminated any chance for spontaneous reaction specific for the tropaeolum plants. The precipitate was heavy when extracted sap of petals was used thereby indicating high titre of virus in these tissues.

Precipitin reaction: Five dilutions of antiserum of TRSV obtained from Lisse were tested for precipitin reaction with respect to the extracted sap obtained from the virus affected double tropaeolum leaves. The data is presented in Table 3.22.

Table 3.22. Precipitin reaction of the virus with the TRSV antiserum (Lisse)

Antiserum dilution	Precipitin reaction at different durations						
	15 min.	30min.	45min.	1 hr.	2hrs	4hrs.	8hrs.
1/8	+	++	+++	++++	++++	++++	++++
1/16	+	++	++	+++	+++	+++	+++
1/32	-	+	++	++	+++	+++	+++
1/64	-	+	++	++	+++	+++	+++
1/128	-	+	++	++	++	++	++
1/256	-	-	+	++	++	++	++

The strong precipitin reaction at higher concentrations and some amount of precipitation even at 1/128 dilution further confirmed the above results, thereby indicating intimate relationship between the two viruses, namely TRSV and DNRSV.

Gel-diffusion test: Gel-diffusion tests were made with both the antisera of TRSV and the extracts from the virus affected tissues. The reactions observed were as represented diagramatically in Fig.3.12.

The formation of transluscent reaction bands in between the wells having antisera and those having extracted sap from virus affected tissues indicating a close relationship between the two viruses. Such bands were absent in between the wells having healthy sap and the antisera.

3.11. Stabilization of infectivity of the virus infected leaf extract of tropaeolum plants

Presence of inhibitors in the tropaeolum leaves:

The infectivity of TMV was found to be affected when infectious sap was mixed with equal volume of single tropaeolum leaf extract. The data obtained are presented in Table 3.23.

The maximum inhibition of 86.61 per cent was obtained with 1:40 diluted extract from old yellow leaves of tropaeolum. The inhibition was less with preparations diluted to 1:10 as also with those obtained from fresh green leaves.

Neutralization of tropaeolum leaf inhibitor

Inoculation with the TMV-tropaeolum leaf extract mixture, after neutralizing the inhibitors in tropaeolum leaves by the process mentioned in 'Materials and Methods', gave the results indicated in Table 3.24.

EDTA, Caffeic acid and Na_2SO_3 treatments gave enhanced infection, not only by neutralizing the viral inhibitors present in the leaves, but also by stimulating the viral infectivity. Caffeic acid when mixed with the leaf extract, stimulated the viral infectivity to the maximum of 80 per cent. By soaking the leaves of tropaeolum and allowing them to be

Table 3.23. Inhibition of infectivity of the CPO strain of TMV by leaf extract of *Tropaeolum majus* L.

Dilution of leaf extract	Leaf age	No. of lesions per half leaf (control/treatment)						% inhibition of infectivity	Leaf age	No. of lesions per half leaf (control/treatment)						% inhibition of infectivity
		1	2	3	4	5	Total			1	2	3	4	5	Total	
S : 10	green fresh leaf	30/15	24/7	-	-	41/18	95/40	57.89	old yellow leaf	25/6	27/11	30/5	24/5	-	106/27	74.52
S : 40	"	20/10	6/0	34/21	55/25	30/15	145/71	51.03	"	53/8	32/0	28/5	14/4	-	127/17	86.61

Table 3.24. Stabilizing the infectivity of the CPO strain of TMV by neutralizing tropaeolum leaf inhibitors

Tropaeolum leaf extract + chemical	Infectivity (no. of lesions/half leaf) Treatment/control Replications					Percentage of inhibition	Remarks	No.of lesions/ 100 lesions of control
	1	2	3	4	Total			
1. With water	4/2	3/5	23/32	18/50	48/89	46.07		53.93
2. With 0.5% EDTA	7/11	8/42	0/3	3/8	18/64	71.88		28.12
3. With 0.5% Na_2SO_3	1/5	19/8	15/8	3/6	38/27	-40.74	Stimulation	140.74
4."0.5% caffeic acid	20/6	7/3	7/6	2/5	36/20	-80.00	"	180.00
Leaves soaked overnight in solution of chemicals								
1. With water	14/17	10/13	49/64	33/21	106/115	7.82		92.18
2. With 0.5% EDTA	6/3	7/0	56/31	43/16	112/50	-124.00	Stimulation	224.00
3. With 0.5% Na_2SO_3	1/14	3/9	36/21	56/32	96/76	-26.31	"	126.31
4. With 0.5% caffeic acid	5/12	4/12	29/14	69/22	107/60	-78.33	"	178.33
Solution stored for 72 hrs								
1. In water	5/6	17/20	15/9	25/28	62/63	1.58		98.42
2. In 0.5% EDTA	68/18	19/11	13/20	3/0	103/49	-110.20	Stimulation	210.20
3. In 0.5% Na_2SO_3	26/25	17/15	3/1	55/50	101/91	-9.92	"	109.92
4. In 0.5% caffeic acid	80/21	23/19	10/10	5/5	118/55	-114.54	"	214.54
5. Frozen with water	1/0	57/47	30/36	4/10	92/93	1.07		98.93

in contact with the leaf extract for 72 hours at cool temperature, further stimulated the viral infectivity to 114.54 per cent. Similarly, EDTA soaking also enhanced the viral infectivity to 124 per cent and Na_2SO_3 stimulated viral infectivity to 78.33 per cent but none of these compounds seem to exhibit any appreciable change in their activity when kept for 72 hours at cool temperature. Water soaking and freezing of the leaves improved the local lesion formation. Fig.3.13 illustrates these improvements in the stability of viral infectivity by use of these chemicals.

Inhibition of double tropaeolum virus in vitro by coumarin and phenol: Since coumarins and phenols constitute important groups of plant virus inhibitors present in the plant systems the effect of such compounds on the infectivity of the virus infecting double tropaeolum plants was estimated by mixing the inoculum with different concentrations of these compounds. The data are given in Table 3.25.

Inhibition of infectivity of the virus was to the extent 74.54 per cent in the case of coumarin at the level of 1 ppm. At higher concentrations, inhibition percentage was reduced. With regard to sodium salicylate, the percentage of inhibition was not much affected by diluting the chemical. The maximum percentage of inhibition was found to the extent of 50.8 when sodium salicylate was used at the level of 10 ppm (Fig.3.14).

FIG. 3.13.

STABILIZATION OF THE INFECTIVITY OF THE CPO STRAIN OF TMV BY NEUTRALIZATION OF INHBITORS IN TROPAEOLUM LEAVES.

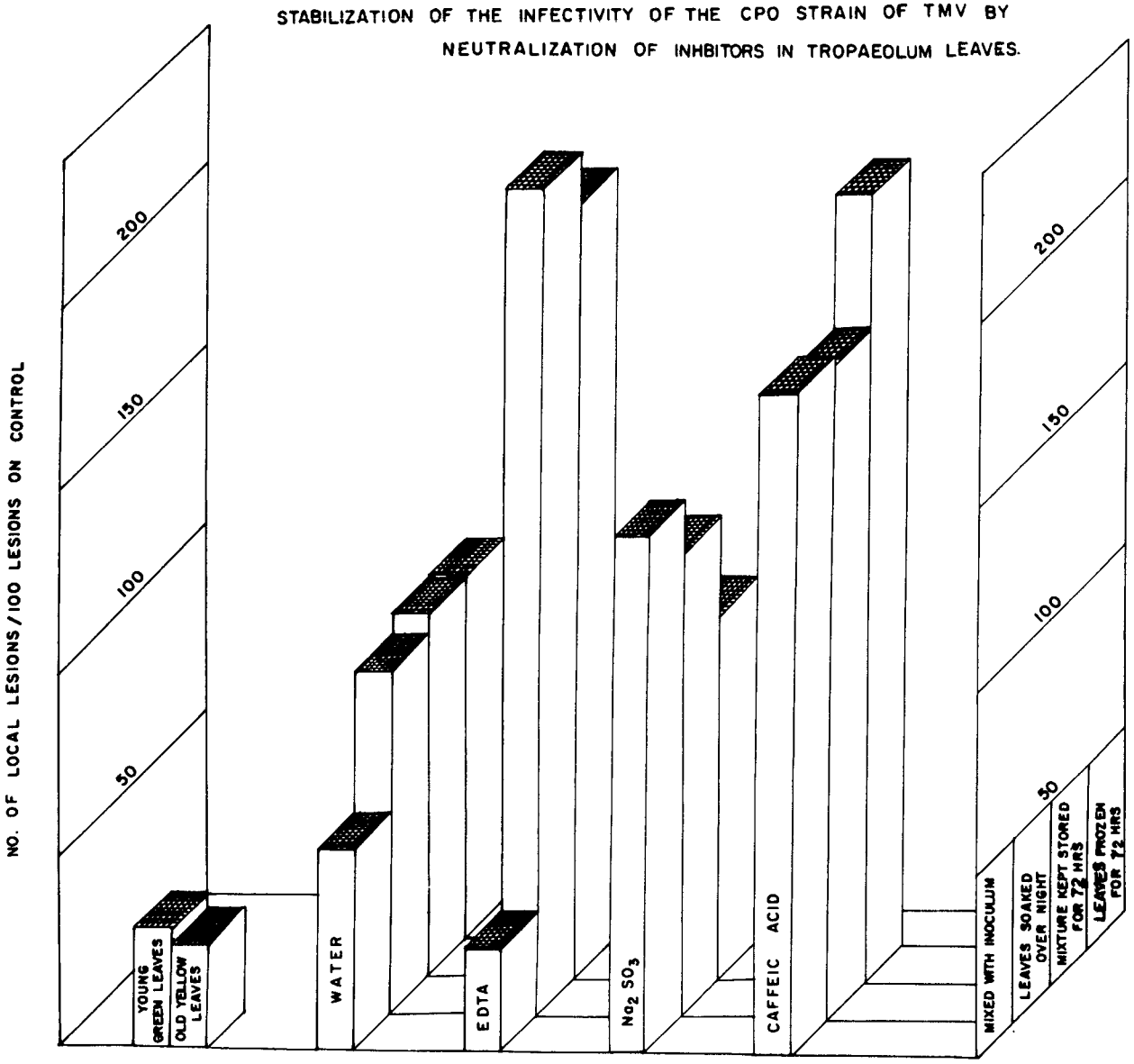


Table 3.25. Inhibition of the infectivity of the Tropaeolum virus *in vitro*, by coumarin and sodium salicylate

ppm	Dilutions									
	1	2	3	4	5	6	7	8	Total c/t	%inhibition
I. Coumarin										
1	27/11	105/21	75/24	20/10	47/13	62/6	26/19	49/1	411/105	74.45
10	55/32	102/20	38/20	20/2	33/9	43/28	12/9	32/13	335/133	60.29
100	46/36	68/61	28/19	11/2	39/10	70/34	21/12	63/63	346/237	31.50
500	62/48	136/114	49/93	34/30	38/13	56/30	15/6	28/24	418/358	14.35
II. Sodium salicylate										
1	25/19	106/70	56/42	28/19	42/24	47/28	29/12	78/37	411/251	38.92
10	70/40	97/55	83/31	21/12	40/15	37/18	18/9	-	366/180	50.81
100	76/46	98/46	67/54	48/17	48/17	67/22	24/8	41/30	439/240	45.33
500	49/37	52/54	65/27	32/12	46/21	54/19	29/16	-	327/186	43.11

FIG. 3.15.

STABILIZATION OF THE INFECTIVITY OF THE VIRUS IN TROPAEOLUM LEAVES

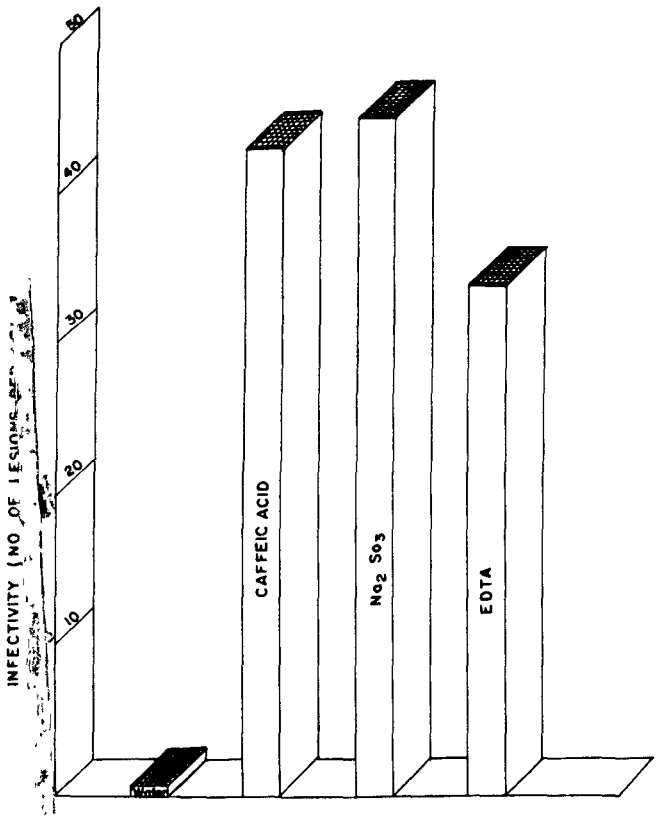


FIG 3 14

INHIBITION OF THE VIRUS IN VITRO BY CHEMICALS COUMARIN AND SODIUM SALICYLATE

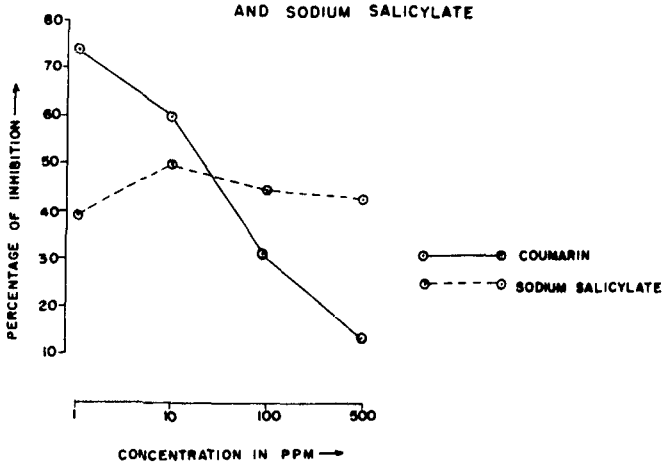
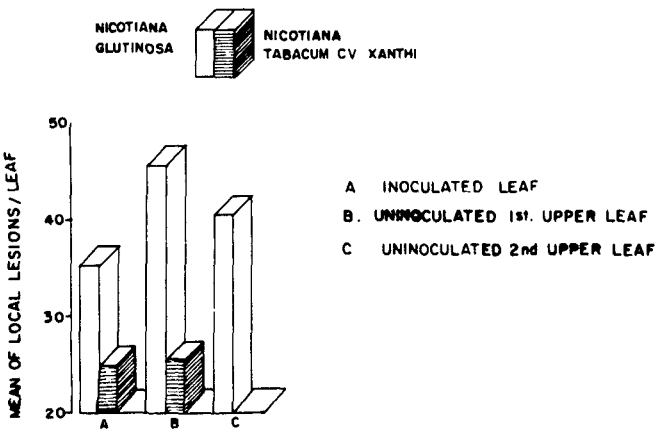


FIG. 3 16

INFECTIVITY OF INOCULATED & UNINOCULATED LEAVES OF NICOTIANA GLUTINOSA & N TABACUM CV. XANTHI



Chemical stablization of the infectivity of leaves from virus affected double tropaeolum plants: The infectivity of double tropaeolum leaves, treated with EDTA, caffeic acid and Na_2SO_3 solutions separately was assayed on turkish tobacco plants and the number of local lesions was compared with that of control indicated in Table 3.26.

Table 3.26. Stablizing the infectivity of the virus infecting double tropaeolum leaves

	Infectivity No. of lesions/leaf				Mean
	1	2	3	4	
EDTA	37	29	28	42	34.00
Caffeic acid	41	45	37	51	43.50
Na_2SO_3	40	49	29	64	45.50
Water	0	1	0	2	0.75

These results are similar to the ones obtained in the earlier experiments with TMV. Sodium sulphite was found to be the most effective followed by caffeic acid and EDTA (Fig.3.15).

3.12. Distribution of infectivity in different parts of tobacco plants infected with the virus

Infectivity of the inoculated and uninoculated leaves of Nicotiana tabacum cv xanthi and N. glutinosa: The infectivity of the inoculated and uninoculated younger leaves, showing characteristic disease syndrome, was compared and the data are presented in Table 3.27.

Table 3.27. Infectivity of inoculated and uninoculated leaves of *Nicotiana glutinosa* L. and *N. tabacum* cv. Xanthi

Plant species and the leaf position bioassayed	No. of local lesions per leaf						
	1	2	3	4	5	Total	Mean
I. *Nicotiana glutinosa*							
1. Inoculated leaf	40	70	16	20	30	176	35.2
2. 2nd upper leaf	47	78	16	27	35	203	40.6
3. Youngest leaf	48	122	12	32	15	229	45.8
II. *Nicotiana tabacum* cv. Xanthi							
1. Inoculated leaf	20	64	10	10	20	124	24.8
2. Youngest upper leaf	30	24	30	18	25	127	25.4

The infectivity of N. glutinosa leaves was always more than that of the turkish tobacco leaves. The inoculated leaves in both the plant species were found to have less infectivity than the uninoculated younger leaves.(Fig.3.16).

Infectivity of leaves at different leaf-positions and corresponding internodes above and below inoculation level in N. tabacum var. xanthi: The data on virus contents, as indicated by the infectivity, in different leaves and corresponding internodes above and below the inoculated leaves are presented in Table 3.28. The lower two leaves having smooth surface were not inoculated.

The viral contents of the two uninoculated leaves below the inoculated ones (inoculation level) were very low. The infectivity increased in the leaves above the inoculation level and was maximum in the third leaf from the top. In the internode bits, the infectivity increased above the inoculation level and was maximum just below the meristem tip. The meristem tip consisting of two pairs of primordial leaves and the corresponding meristem dome did not show any infectivity. (Fig.3.17).

Infectivity of different components of the root system: Similarly the infectivity of the root system was also estimated and data are presented in Table 3.29.

Table 3.28. Infectivity of leaves and internodes at different levels, above and below inoculation level

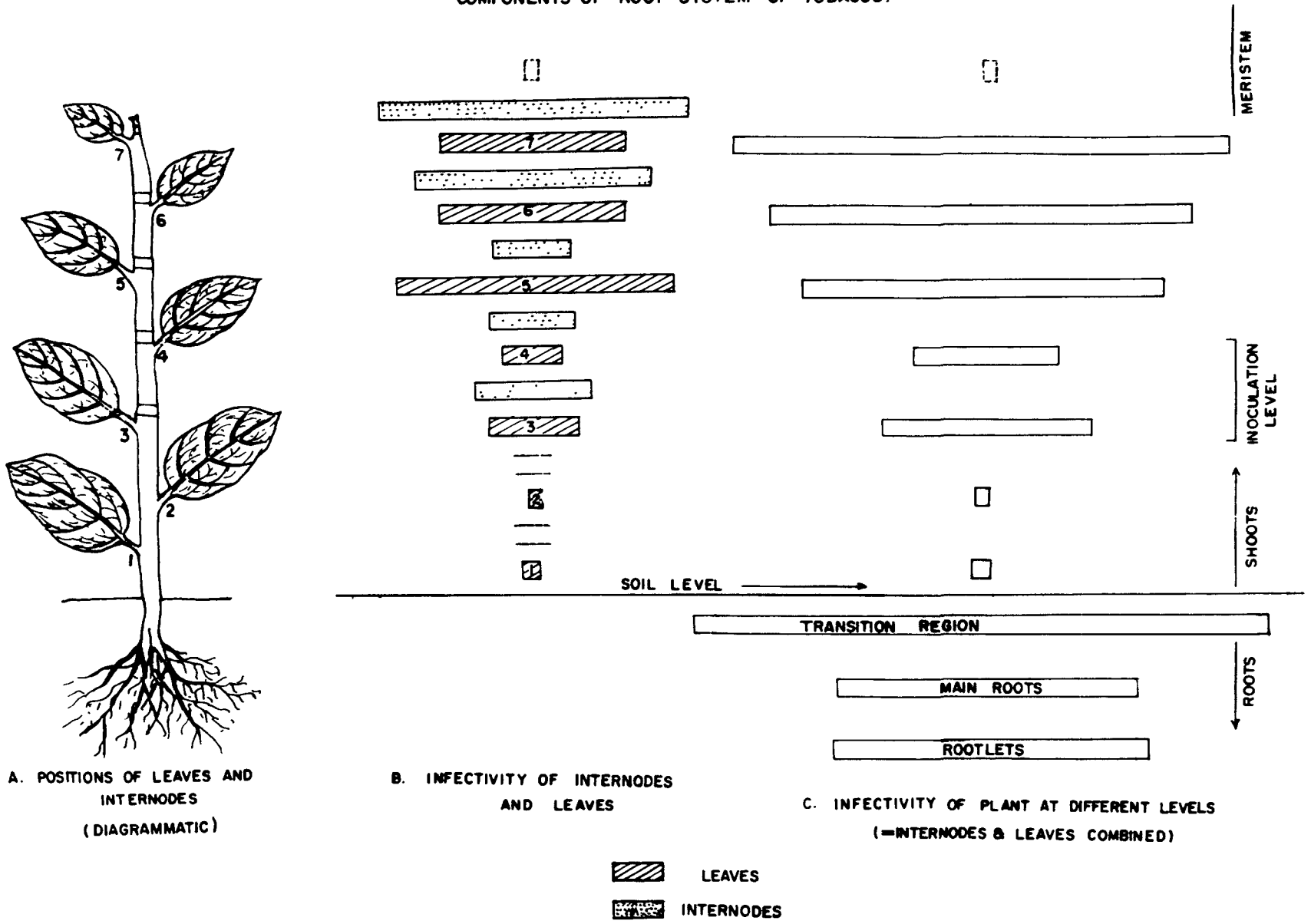
Leaf position from base	Dilution of standard extract	Infectivity (No. of lesions/leaf) Replication I							Replication II							Total of II replications	Mean No. of lesions	Mean at S: 10
		1	2	3	4	5	6	7	1	2	3	4	5	6	7			
1	S	34	61	72	67	-	64	40	-	103	165	103	92	106	98	1005	83.7	8.37
2	S	94	95	94	52	35	78	-	61	81	58	46	46	107	-	847	70.5	7.05
3	S:10	151	44	49	48	23	32	34	83	9	30	22	48	73	60	706	50.42	50.42
4	S:10	17	102	15	30	62	33	21	46	6	19	18	10	31	32	442	31.57	31.57
5	S:30	36	70	143	41	97	6	29	40	10	63	44	21	54	72	726	51.85	155.55
6	S:10	154	170	251	93	69	33	22	93	105	40	110	60	99	192	1491	106.50	106.50
7	S:20	35	75	86	82	32	37	5	26	41	80	60	48	66	65	738	52.71	105.42
Internodes position from base																		
1	S:10	43	105	128	42	71	88	-	-	29	45	22	89	84	-	746	67.89	67.89
2	S:10	-	56	66	61	85	58	29	91	18	32	36	26	29	38	625	47.69	47.69
3	S:10	74	57	24	133	8	68	31	56	11	30	52	13	29	64	650	46.42	46.42
4	S:20	21	27	142	18	82	7	5	3	-	42	16	5	11	21	400	30.76	61.52
5	S:10	-	-	-	-	-	-	-	6	-	42	15	13	19	10	105	17.50	175.00
Meristem	S:100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3.29. Infectivity of different components of root system of the virus affected turkish tobacco plants

Different root components	Dilution of standard extract	Infectivity (No. of local lesions/leaf)								
		1	2	3	4	5	6	Total	Mean	Mean at S:10
1. Just below the soil level (Transition region)	S:20	133	221	172	219	-	69	814	162.8	325.6
2. Main roots	S:10	194	379	5	194	184	66	1022	170.3	170.3
3. Rootlets	S:10	258	163	24	279	180	151	1055	175.8	175.8

FIG 3.17.

INFECTIVITY OF LEAVES & INTERNODE AT DIFFERENT LEAF POSITIONS BELOW & ABOVE INOCULATION—LEVEL & DIFFERENT COMPONENTS OF ROOT SYSTEM OF TOBACCO.



A. POSITIONS OF LEAVES AND INTERNODES (DIAGRAMMATIC)

B. INFECTIVITY OF INTERNODES AND LEAVES

C. INFECTIVITY OF PLANT AT DIFFERENT LEVELS (=INTERNODES & LEAVES COMBINED)

Generally, the infectivity was higher in the roots system, optimum being just below the soil level i.e. portion of the stem just above the main root, but reduced in the main root and root-lets (Fig.3.17). Fig.3.17 also illustrates infectivity of the plant at different levels i.e. root and shoots (consists of leaves and stem). It increases above and below the inoculation level but is altogether absent in the meristems.

Infectivity of different tissues of the stem: Tissues i.e. pith , conductive tissue and cortex alongwith the epidermis were dissected out from infected turkish tobacco plants and bioassayed for estimation of viral concentration. The data are presented in Table 3.30.

The viral contents were highest in the conductive tissue and least in the pith (Fig.3.18).

Infectivity of turkish tobacco plants at different intervals after inoculation: The data on infectivity of the whole plant at different intervals are presented in Table 3.31.

The data presented in the table indicated steep rise in the viral concentration upto 9 days after inoculation, thereafter, it decreased slowly and after 21 days the concentration was very much less (Fig.3.19).

Table 3.30. Infectivity of different component tissues of stem of virus affected turkish tobacco plants.

Different tissue components of stem	Dilution of standard extract	Infectivity (No. of local lesions/leaf)							
		1	2	3	4	5	6	Total	Mean
1. Pith	S:10	221	-	61	43	88	39	452	90.4
2. Conductive tissue	S:10	278	177	115	-	159	101	830	166.0
3. Cortex and epidermis	S:10	-	226	164	174	129	84	777	155.4

Table 3.31. Infectivity of the turkish tobacco plants at different intervals after inoculation

Intervals after inoculation (in days)	Dilution of standard extract	Infectivity (No. of lesions/leaf)					
		1	2	3	Total	Mean	Mean at S:10
7	S:10	140	310	350	800	266.0	266.0
9	S:50	101	299	65	465	155.0	775.0
11	S:50	104	292	14	410	136.6	683.0
13	S:10	165	270	234	669	223.0	223.0
14	S:50	31	206	15	252	84.0	420.0
17	S:50	70	47	24	141	47.0	235.0
21	S:50	22	23	7	52	17.6	88.0

Infectivity of old inoculated and younger uninoculated leaves at different intervals after inoculation: The results presented in Table 3.32 indicate identical trend in conformity with the earlier experiment. In the uninoculated younger leaves, the infectivity was maximum on 8th and 10th day after inoculation, thereafter it declined sharply till 15th day and 20th day respectively in the two experiments. The lower level of infectivity of the old inoculated leaves as compared to the young uninoculated ones persisted all along the period after inoculation (Fig.3.20).

Table 3.32. Infectivity of old inoculated and younger uninoculated leaves of turkish tobacco plants infected with the virus at different intervals after inoculation

Days after inoculation	Dilution of standard extract	Old inoculated leaves								younger uninoculated leaves							
		Infectivity (No.of lesions/half leaf)								Infectivity (No. of lesions/half leaf)							
		1	2	3	4	5	6	Total	Mean	1	2	3	4	5	6	Total	Mean
Experiment I																	
3		75	43	22	-	-	-	140	46.6	145	76	90	-	-	-	311	103.6
10	S: 50	71	91	63	-	-	-	225	75.0	99	118	103	-	-	-	320	106.6
20		82	92	130	-	-	-	304	101.3	97	152	135	-	-	-	384	128.0
28		90	97	-	-	-	-	187	93.5	122	137	-	-	-	-	259	129.5
Experiment II																	
8		308	119	157	-	-	-	584	194.6	692	162	287	-	-	-	1141	280.3
15	S:10	218	222	120	212	134	143	1049	174.8	215	307	97	182	105	264	1170	195.0
25		34	32	236	-	234	408	944	188.8	26	30	255	-	295	438	1044	208.8
33		78	147	166	208	-	58	657	131.4	70	119	303	307	-	72	871	174.2

FIG 3 18

INFECTIVITY OF DIFFERENT COMPONENTS OF STEM TISSUE OF VIRUSAFFECTED TOBACCO PLANTS

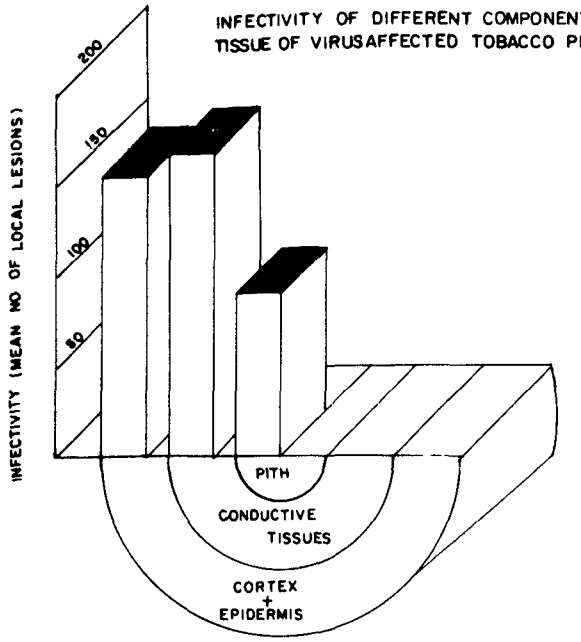


FIG 3 19

INFECTIVITY OF TOBACCO PLANTS AT DIFFERENT INTERVALS AFTER INOCULATION

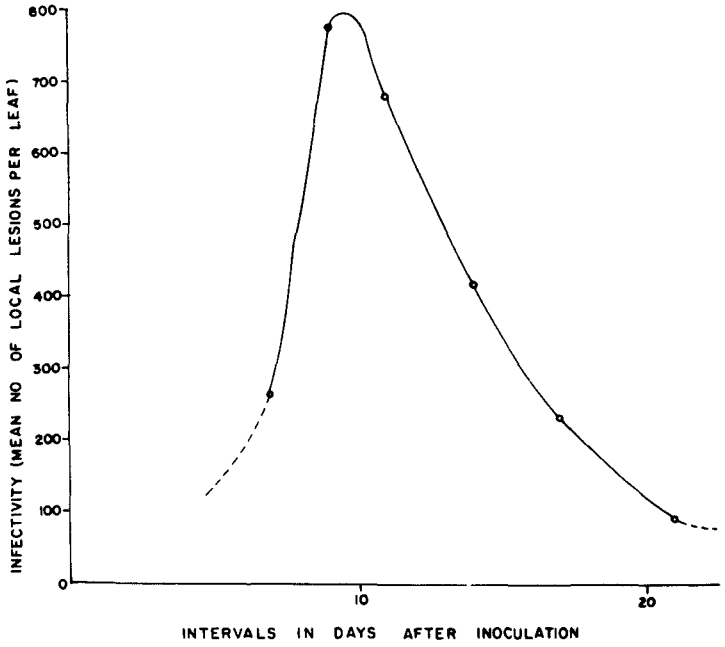
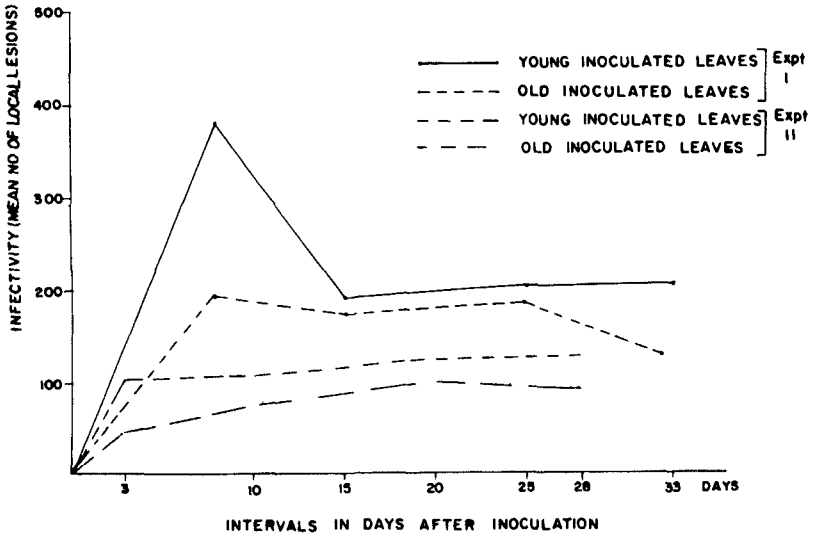


FIG 3 20

INFECTIVITY OF INOCULATED & UNINOCULATED LEAVES OF TOBACCO PLANTS AT DIFFERENT INTERVALS AFTER INOCULATION



3.13. Distribution of infectivity in different parts of tropaeolum cultivars

Various plant parts namely, stem, leaves, roots and flower parts of single and double tropaeolum and also floral parts of semi-double tropaeolum were assayed on turkish tobacco plants and local lesions were counted for ascertaining the relative infectivity. The results are given in Table 3.33.

It appears that the infectivity was more in the double tropaeolum plant parts. Floral parts of double tropaeolum were highly infectious having highest infectivity in sepals and petals. On the other hand, floral parts of single and semi-double tropaeolum were non-infectious, although other parts showed infectivity.

3.14. Tissue culture studies

3.14.1. Callus cultures: Attempts were made to obtain callus cultures from tropaeolum, turkish tobacco and *N. glutinosa* explants.

3.14.1.1. Tropaeolum explants

From embryos and seeds of single tropaeolum: Embryos and seeds of single tropaeolum plants were planted, ten, each, into different media, namely, A, B_1, C_1, C_2, C_3, C_4, D_3, K_1 and MS (see Appendix II). The qualitative and quantitative estimation of growth response of these tissues as influenced by different doses of NAA, kinetin, adenine, myoinositol and casein hydrolysate, was recorded after a period of 30 days and the data are presented in Table 3.34.

Table 3.33. Infectivity of different tissues of single, semi-double and double tropaeolum plants

Plants	Tissues	Infectivity (No. of lesions/leaf)							Symptoms
		1	2	3	4	5	Total	Average	
Single tropaeolum	1. Leaves (old yellow)	5	7	8	4	6	30	6.00	:Chlorotic patches with mild concentring rings
	Leaves (young green)	10	12	1	10	9	42	8.40	
	2. Stem	2	3	2	4	4	15	3.00	
	3. Roots	8	9	7	8	9	41	8.20	
	4. Floral parts								
	a. Sepals & petals	0	0	0	0	0	0	0	
	b. Androeceum and gynoceceum	0	0	0	0	0	0	0	
Semi-double tropaeolum	Floral parts								
	a. Sepals & petals	1	0	0	0	0	0	0.2	:Same as above
	b. Androeceum and gynoceceum	0	0	0	0	0	0	0	
Double tropaeolum	1. Leaves (young green)	10	12	11	10	9	52	10.40	:Chlorotic patches with concentric rings, puckering and curling
	Leaves (old yellow)	8	7	9	5	8	37	7.40	
	2. Stem	5	7	8	6	5	31	6.20	
	3. Roots	12	15	12	11	13	63	12.60	
	4. Floral parts								
	a. Sepals	10	15	12	19	20	76	15.20	
	b. Petals	20	25	18	19	21	103	20.60	:Colour breaking

Table 3.34. Growth pattern of tropaeolum embryos and seeds as affected by different doses of auxin, kinetin, casein hydrolysate and myoinositol

Media	Essential constituents of the media (mg/l)		Growth pattern	
			Embryos	Seeds
A.	NAA K	2.0 -	Cr ++10 Cp ++7 Rh +++10 S +	R + 10
B_1	NAA K CH	2.0 - 1.0 g/l	Rh +++10 S ++++10	R ++++10 S ++++ 9
C_1	NAA K	1.0 1.0	C +++10	-
C_2	NAA K	0.5 0.2	C ++++10	-
C_3	NAA K	1.0 0.2	C ++10 Rh+++8 S ++7	R +++10 S ++5
C_4	NAA K	1.5 0.2	C ++10 Cp++6 Rh+++7 S ++5	-
D_3	NAA K CH Ino	1.5 1.0 400 0.5	C ++10 R +++9 S +++10	-
K	NAA K CH Ino Ad	1.0 0.5 200 1.0 5.0	Cp ++10 R ++8 S +++10	

Abbreviations used are given in the Appendix.

The embryos grew best on B_1 (Fig.3.21a) and MS media giving out balanced growth of roots and shoots. C_1 medium induced callusing, while C_2 medium having NAA-Kinetin in the ratio of 0.5:0.2 mg/l supported better callus growth. By increasing the doses of NAA to 1.0 (C_3) or 1.5 (C_4) mg/l, root development was further increased (Fig.3.21.6). The growth was more uniform with kinetin at the level of 1.0 or 0.5 mg/l i.e. on D_3 and K_1 media. The MS medium with 100 mg/l of myoinositol and 1.0 mg/l of NAA in place of IAA, induced a good balanced growth of the embryos. Adenine incorporated in K and MS media at the level of 5.0 mg/l, supported caulogenic growth i.e. better shoot development.

Seeds, on the other hand, grew best even on media like A and B_1 which lacked kinetin, thereby indicating that kinetin and other compounds had no appreciable effect on the growth pattern.

From other tissues of tropaeolum: Different plant parts namely, meristem, stem, leaves, flowerbud, sepals and petals from both virus-affected and virus-free plants of single tropaeolum as also virus affected double tropaeolum plants were planted on a number of media as given in Appendix II. The qualitative and quantitative estimation of growth response of these tissues, as influenced by different doses of auxins, kinins, adenine, myoinositol, casein hydrolysate, coconut milk etc. was made after a growth period of 30 days and recorded in the following tables (3.35 to 3.38). As

Fig.3.21. Tissue culture of tropaeolum tissues

a) Growth of tropaeolum embryos on (1) A medium (with 2.0 mg/l NAA and no kinetin, (2) B_1 medium (with 2.0 mg/l NAA, no kinetin and 1.0 gm/l casein hydrolysate) and (3) C_1 medium (with 1.0 mg/l NAA and 1.0 mg/l kinetin.

b) Growth of tropaeolum embryos on media with different proportions of auxin and kinin (1) on C_1 medium (with NAA:Kinetin as 1:1), (2) on medium C_2 (with NAA : Kinetin as 0.5:0.2), (3) on medium C_3 with NAA:Kinetin as 1.0:0.2 and (4) on medium C_4 with NAA:Kinetin as 1.5:0.2.

c) Growth of stem explants inducing callus formation 1) on K_2 medium, 2) on D_3 medium and 3) on C_1 medium: all the cultures are 3-weeks old. 4) on WD medium and 5) on E medium; all the cultures are 8-weeks old in P_2 transfers.

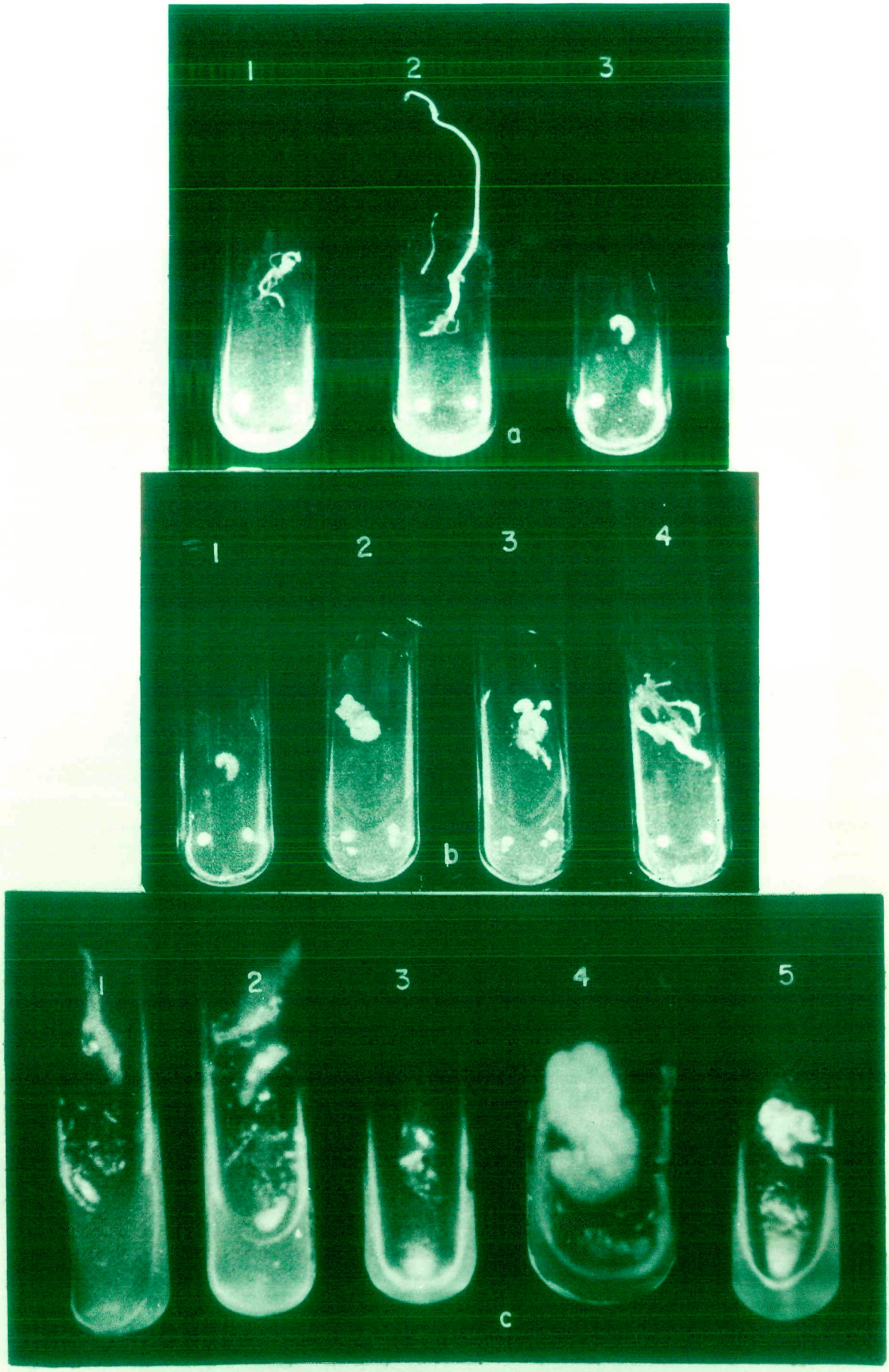


FIG. 3.21

there was no difference in the growth response of virus-affected and virus-free tissues as also those of single and double tropaeolum plants, only general growth pattern of these tissues were recorded.

Presence of kinetin was essential for any type of growth of tropaeolum explants as there was no growth of these tissues on the media, which did not have any kinetin (i.e. A, B_1 and B_2). In the media C_1, C_2, D_3, E and F, the inclusion of kinetin from 0.5 to 1.0 mg/l induced meristematic activity besides callus formation. Presence of casein hydrolysate and coconut milk further increased the callus formation as in D_3 and E media (Fig.3.21c), whereas 2,4-D at 2.0 mg/l had no effect on callusing.

In media with NAA, kinetin, adenine, myoinositol and casein hydrolysate at the concentrations of 1.0, 0.5, 5.0, 1.0 and 200 mg/l, respectively (K_1 medium), the growth of the meristem was not appreciably influenced with the addition of compounds like choline chloride, ascorbic acid, fumaric acid, succinic acid and riboflavin in different proportions (K_4 and K_5 media). The former three compounds when added at the concentration of 0.25 mg/l each (K_2 medium) induced callus formation (Fig.3.21c). The concentration of these compounds, when increased to 0.5 mg/l each, the root development seemed to be better manifested. Addition of 2,4-D and coconut milk only slightly increased the callus growth (L medium), whereas 2,4-D and GA induced some rhizogenic tendency as evidenced by root development, besides accompanied by a little bit of shoot growth.

Table 3.35. Growth pattern of tropaeolum explants as affected by different doses of auxin, kinetin, casein hydrolysate and coconut milk

Media	Essential constituents mg/l		Growth pattern					Remarks
			Meristems	Stem	Leaves	Flower buds	Sepals and petals	
A	NAA K	2.0 -	-	-	-	-	-	No growth
B_1	NAA K CH	2.0 - 1000	m	m	-	-	-	Slight meristematic activity only
B_2	NAA K CH	2.0 - 2000	m	m	-	-	-	-do-
C_1	NAA K	1.0 1.0	M + C +	C ++	-	C ++	-	Slight brown callus died after P_2 transfer
C_2	NAA K	0.5 0.2	M + C ++	C ++	-	C ++	-	-do-
D_3	NAA K CH	1.5 1.0 400	M ++ C +++	C ++	-	C ++	-	Whitish compact callus growth
E	NAA K CH CM	1.5 0.5 200 150 ml	M ++ C +++	C ++	-	C ++	-	-do-
F	NAA K CH CM 2,4-D	1.5 0.5 200 150 ml 2.0	M + C ++	C ++	m	C ++	m	-do-

Abbreviations used are given in the Appendix.

Table 3.36. Growth-pattern of tropaeolum explants as affected by accessory growth factors

Media	Essential constituents (mg/l)		Growth pattern					Remarks
			Meristem	Stem	Leaves	Flower buds	Sepals and petals	
K_1	NAA K Ad Ino. CH	1.0 0.5 5.0 1.0 200	M + C ++ R ++	C +	C +	m	m	Partial growth of meristem with callus formation
K_2	NAA K Ad Ind CH Cocl+ As+F	1.0 0.5 5.0 1.0 200 0.25each	M + C +++ R +	C ++	C +++	Cv ++	-	-do- (Increase in callus growth).
K_3	NAA K Ad Ano CH Cocl+ As + F	1.00 0.5 5.0 1.0 200 0.5each	M + C ++ R +++	C ++	C ++	m	-	Increase in root growth
K_4	NAA K Ad Ino CH Cocl+ As+F Succ+ Ribf	1.00 0.5 5.0 1.0 200 0.25each 0.25each	M + C ++ R +	C ++	C ++	Cv ++	-	Growth in general reduced
K_5	NAA K Ad Ino CH Cocl+ As+F Succ.+ Ribf	1.00 0.5 5.0 1.0 200 0.25each 0.5each	M + C ++ R +	-	-	m	-	Growth suppressed

Contd...

Table 3.36 contd.

Media	Essential constituents (mg/l)		Growth pattern: Meristem	Stem	Leaves	Flower buds	Sepals and petals	Remarks
L	NAA	1.0	M+					
	K	0.5		C++	-	-	-	Callus growth only
	Ad.	5.0	C+++					
	Ino	1.0						
	CH	200						
	2,4-D	2.0	R+					
	CM	100						
O	NAA	1.0						
	K	0.5	M++	C++	-	-	-	Slight shoot growth, besides callus but roots are more pronounced
	Ad	5.0	C++					
	Ino	1.0						
	CH	200	R++					
	GA	1.0						

Abbreviations used are given in the Appendix.

Table 3.37. Growth pattern of tropaeolum explants as affected by different doses of auxin, kinetin, adenine and myoinositol

Media	Essential constituents (mg/l)		Growth pattern: Meristem	Stem	Leaves	Flower buds	Sepals and petals	Remarks
MS	IAA	2.0	C+++	C++			C++	Meristem tip & stem gives dedifferentiated growth but rooting predominates
	K	0.4	S+++	R+++	m	C++		
	Ad	5.0	R+++					
	Ino	100						
MS_1	IAA	2.0						
	K	0.4	-	C+	m	-	-	No growth
	Ad	12.5						
	Ino	100						

contd...

Table 3.37 contd.

Media	Essential constituents (mg/l)		Growth pattern: Meristem	Stem	Leaves	Flower buds	Sepals and petals	Remarks
MS_2	IAA	2.0						
	K	1.4	C++	C++	m	-	C++	Small amount of callus and dedifferentiated growth
	Ad	5.0	S+++	R+				
	Ino	100	R+++					
MS_3	IAA	2.0	C++	C++	C+	C+	C+	Increase in callus growth, no roots
	K	1.4	S++	R+				
	Ad	7.5						
	Iho	100						
MS_4	IAA	2.0	C+	C++				No dedifferentiation, callus turns brown in P_2 transfer
	K	1.0		R+	m	-	-	
	Ad	12.5						
	Ino	100						
MS_5	NAA	1.5						
	K	1.0	-	C+	-	-	-	No growth
	Ad	7.5						
	Ino	125						
MS_6	IAA	1.5						
	K	0.75	C+	C+	-	-	-	Increase in caulogenesis, differentiation of meristem
	Ad	7.5	S++++	m				
	Ino	100	R++					
MD	IAA	2.0						
	K	0.4	-	-	not tried			No growth
	Ad	7.5						
	Ino	100						
	2,4-D	2.0						
MC	IAA	2.0						Callus growth enhanced but turns brown in P_3 transfer
	K	0.4	C++	m	m	not tried		
	Ad	7.5						
	Ino	100						
	2,4-D	4.0						
	CM	150 ml						

Abbreviations used are given in the Appendix.

On Murashige and Skoog's medium with IAA at 2.0, kinetin at 0.4, adenine at 5.0, myoinositol at 100 mg/l (MS medium), the root development as well as callus formation and shoot growth in the meristem explants was more pronounced. Stem explants gave rise to callus formation and root development (Fig.3.22c). Increasing the adenine contents to 12.5 mg/l as in MS_1 and MS_4 media, was harmful as there was no growth. On the other hand, the increase in the kinetin contents to 1.4 mg/l along with adenine at the level of 5.0 mg/l, the balance of growth was tilted towards caulogenesis as no root formation was observed in the meristem explants (MS_2 medium) (Fig.3.22b). The caulogenic growth was further improved by increasing the dose of adenine to 7.5 mg/l (MS_3 medium). It appears that adenine was complementary to kinetin in supporting caulogenic growth. The increase in the myoinositol level from 100 mg/l to 125 mg/l like that of adenine was also detrimental to the growth (MS_5 medium). The ideal medium for the well organised growth of meristem was MS_6 which was incorporated with 1.5 mg/l of IAA, 0.75mg/l of kinetin, 7.5 mg/l of adenine and 100 mg/l of myoinositol. The addition of 2,4-D (MD medium) or coconut milk (MC medium) had no appreciable effect on the induction of non-differentiated callus growth.

Table 3.39. Growth pattern of tropaeolum explants as affected by Benzyl amino purine, kinetin, adenine and myoinositol

Media	Essential constituents (mg/l)		Growth pattern					Remarks
			Meristem	Stem	Leaves	Flower buds	Sepals and petals	
EaM	NAA BAP Ino Ad	5.0 0.2 - -	C+ S+ R++++	C+ R+++	m	m	m	Rooting predominates at the expense of shoot growth and callus formation
WD	NAA K Ino Ad	1.0 0.05 100 5.0	C+++ R++	C+++ R++	C+++	C+++	C+++	Callus predominates with complete suppression of shoots
WK	NAA K Ino Ad	1.0 0.5 100 7.0	C+ S++++ R++	C+	m	m	m	Dedifferentiated growth with more response towards caulogenesis

WK medium with NAA-1.0 mg/l, kinetin 0.5 mg/l, adenine 7.0 mg/l and myoinositol 100 mg/l supported organised dedifferentiated growth of meristem explants. Profuse callus growth was obtained in all the types of explants, when kinetin was reduced to 0.05 mg/l (WD medium) (Fig.3.21c). Even the leaves, sepals and petals gave profuse callus formation (Fig.3.22a). Kinetin can also be substituted with BAP (benzyl

amino purine), which was equally effective. The EaM medium incorporated with 5.0 mg/l of NAA and 0.2 mg/l of BAP indnced heavy rooting at the expense of caulogenic growth (Fig.3.22d).

In general, the response of meristem tissue, which consists of a region having actively multiplying cells with least number of other non-active cells, was most pronounced in producing callus cultures as compared to other tissues. On the other hand, stem and leaves were least responsive. Only the leaves which were very young (next to primordial leaves) gave some callus formation (Fig.3.22a). Incipient flower-buds or petals also responded to callus formation but not as good as meristem explants. The callus obtained from meristem and young leaf explants remained actively growing for a period of one and half years i.e. upto 16 transfers on MS medium. The rate of growth was very slow in these tissues on MS medium but increased when transferred to MS_6 or EaM media. Callus growth obtained from other tissues, however, turned brown after about 5-6 months. The data on infectivity of callus cultures from different explants and transfers are presented in Table 3.39.

Infectivity of leaf callus cultures was lost after second transfer whereas, meristem and stem callus became non-infectious after first transfer.

Fig.3.22. Leaf callus and meristem-tip cultures of double tropaeolum.

a) Leaf callus on WD medium

b) Meristem-tip culture on MS_2 medium showing callusing and caulogenesis.

c) A portion of stem next to meristem-tip on MS medium, giving strong rhizogenic response.

d) Meristem-tip culture on EaM medium showing strong rhizogenic response.

e) Meristem growth on MS_6 and WK media. 1) and 2) on MS_6 medium the former being only explant whereas the latter showing 6 week's growth. 3) a 6 weeks' growth showing strong caulogenesis with large number of plant-lets coming out.

f) Meristem-tip culture on MS medium in semi-solid medium after 8 weeks growth on second transfer.

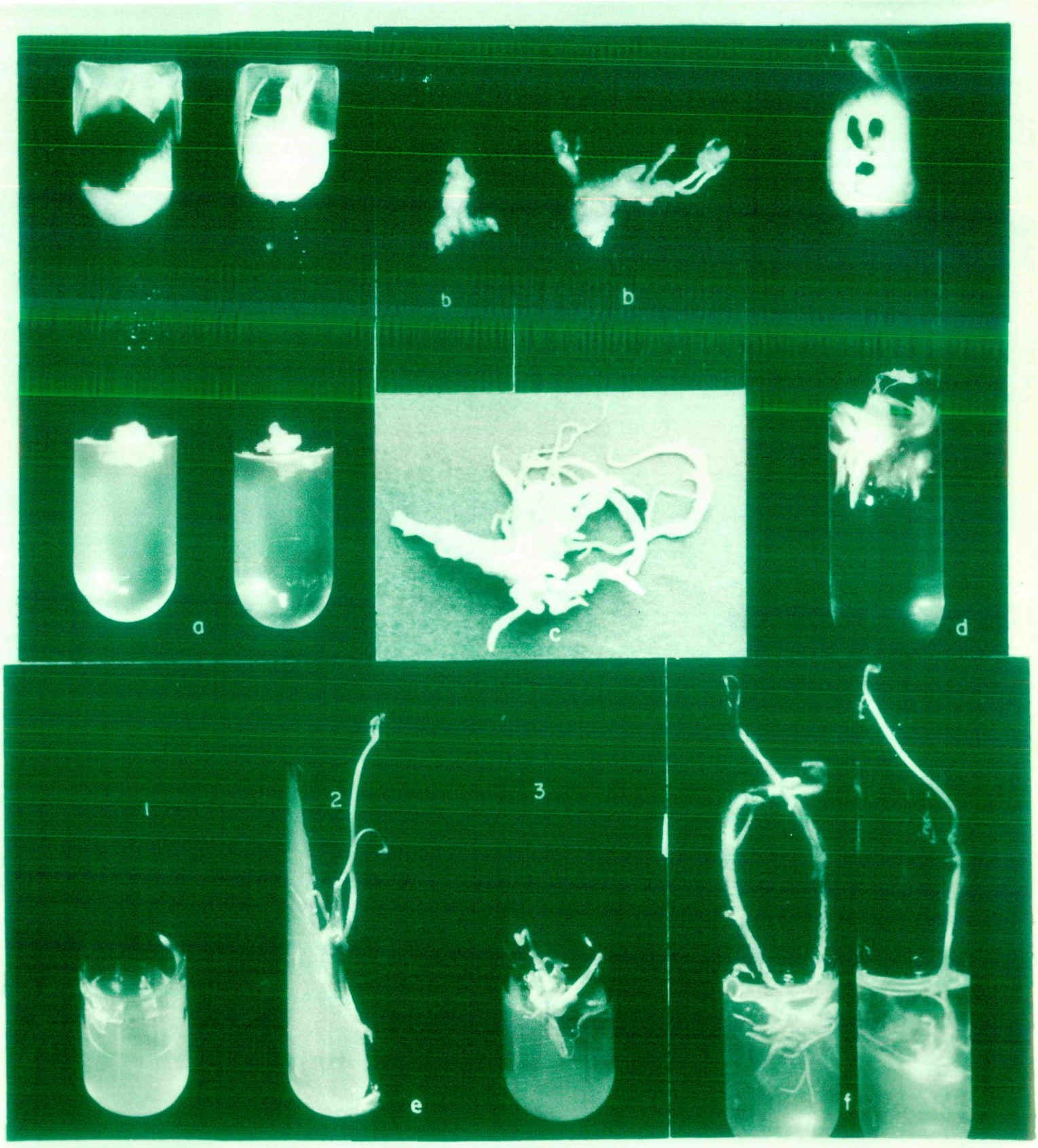


FIG. 3.22

Table 3.39. Infectivity ofcallus cultures obtained from different tissue-explants of double tropaeolum plants

Tissue explants	Infectivity during subsequent transfer of cultures													
	Passage 1	2	3	4	5	6	7	8	9	10	12	16	18	20
1. Meristem-tip callus	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2. Leaf callus	+	+	-	-	-	-	-	-	-	-	-	Tissue died		
3. Stem callus	+	-	-	-	Tissue died									

3.14.1.2. Tobacco explants: The stem and leaf tissues of Nicotiana tabacum cv. Xanthi and N. glutinosa were planted in different media tabulated in Appendix II for obtaining non-differentiated and de-differentiated callus cultures.

Table 3.40. Growth pattern of stem and leaf explants of turkish tobacco and N. glutinosa L. as affected by auxin, kinin, adenine, myoinositol and accessory growth factors

Media	Essential constituents (mg/l)		Turkish tobacco		N. glutinosa		Remarks
			Stem	Leaves	Stem	Leaves	
F	NAA	1.5	-	m	m	m	Slight meristematic activity
	K	0.5					
	Ad	-					
	Ino	-					
	CH	200					
	CM	150 ml					
	2,4-D	2.0					

contd...

Table 3.40 contd.

Media	Essential constituents (mg/l)		Turkish tobacco		*N. glutinosa*		Remarks
			Stem	Leaves	Stem	Leaves	
K_2	NAA	1.0					
	K	0.5					
	Ad	5.0	C++	Cv++	C++	-	Callus on leaf veins
	Ino	1.0					
	CH	200					
	CM	-					
	2,4-D	-					
	Cocl+ As+FA	0.25each					
K_3	NAA	1.0					
	K	0.5					
	Ad	5.0	-	m	-	m	Slight merismatic activity + proliferation of cells
	Ino	1.0					
	CH	200					
	Cocl+ As+FA	0.5 each					
K_4	NAA	1.0					
	K	0.5					
	Ad	5.0	-	C++ m	-	-	Slight callusing & proliferation of cells
	Ino	1.0					
	CH	200					
	Cocl+ As+FA	0.25 each					
	Succ A+ Rf	0.25 each					
K_5	NAA	1.0					
	K	0.5					
	Ad	5.0					
	Ino	1.0					
	CH	200	-	-	-	-	No growth
	Cocl+ As+FA	0.25 each					
	Succ A+ Rf	0.5 each					
MaM	NAA	5.0	C+++	C+++	C++++	C++++	Differentiation of callus growth, but predominance of roots.
	BAP	0.2	S++	S++	R++	R++	
	Ad	-	R++	R+++			
	Ino	-					

contd..

Table 3.40 contd.

Media	Essential constituents(mg/l)		Turkish tobacco		N.glutinosa		Remarks
			Stem	Leaves	Stem	Leaves	
MS	IAA	2.0					
	K	0.4	C++++	C++++	C++++	C++++	Differentiation of callus growth, predominance of shoots
	Ad	5.0	S+	S+++			
	Ino	100		R++			
WK	NAA	1.0	C+++	C+++	C+++	C+++	Differentiation of callus, shoots predominate
	K	0.5	S+	S++++			
	Ad	7.0	R++				
	Ino	100		R+			

F-medium initiated only slight meristematic activity of the explants, though this medium had casein hydrolysate, coconut milk and 2,4-D known to induce callus formation. Callus formation of explants was observed on K_2, K_3, EaM, MS and WK media (Fig.3.23d). These media essentially had adenine, myoinositol and different ratios of auxin and kinetin. On K_2 medium slight callus growth was observed on veins and veinlets but remained only incepient. Since no callusing was observed on K_4 and K_5 medium, it may be inferred that the addition of succinic acid and riboflavin is not condusive for the growth of these tissues especially inducing callus formation. Initiation of good callus growth on MS, EaM and WK media indicated the need of adenine, myoinositol and the right proportion of auxin and kinetin not only for the initiation of good callus growth but also

Fig.3.23. Tissue culture of turkish tobacco and N. glutinosa plants.

a) Callus cultures of virus affected (1 and 2) and healthy (3 and 4) leaf explants of N. glutinosa plants, on MS medium.

b) Leaf-explants of virus affected turkish tobacco plants (1 and 2) leaf-explant giving out bunches of callus cells, 3) Leaf-explant regenerating into a plant-let (arrow). The explant is aetiolated.

c) Cultures of undifferentiated callus from (1) stem, (2) leaf and (3 & 4) differentiated callus cultures from leaf explants of virus affected turkish tobacco plant. The former two on MS medium and later two on MS_6 medium. They are all 4, 4 and 6 week's old. The last tube is also showing formation of few roots as well.

d) Virus affected callus cultures of turkish tobacco plants 1) dedifferentiating culture with rhizogenic response showing a root with a tuft or root hair (arrow) on MS medium. 2) a caulogenic response with shoots growing out on WK medium. 3) undedifferentiating callus on MS medium. This being an actively multiplying young culture whereas i) is an old culture freshly transferred on MS medium - 3rd transfer. 4) A proliferating tropaeolum meristem-culture.

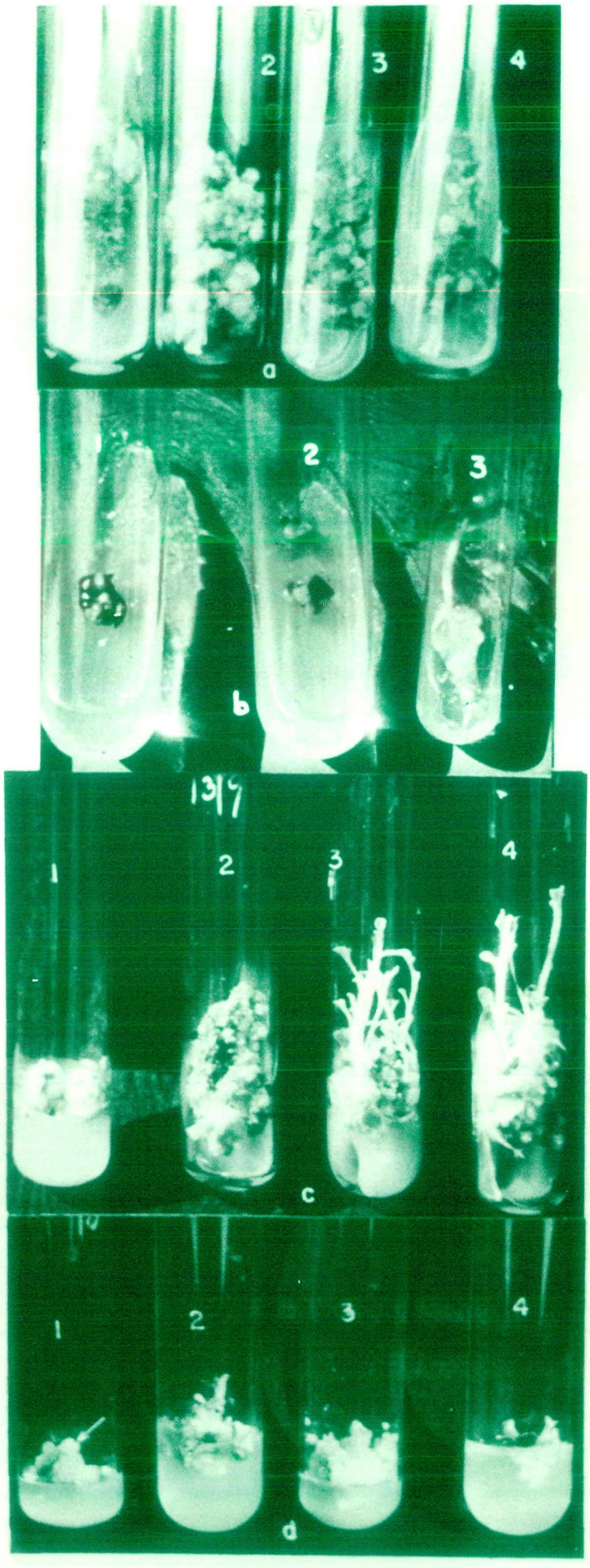


FIG 3.23

for de-differentiation of the undifferentiated callus cells. De-differentiation was more pronounced in leaf callus and that too only in turkish tobacco (Fig.3.23c). There was no de-differentiation in either stem or leaf callus of N. glutinosa plants (Fig.3.23a).

During the process of de-differentiation, roots and shoots were formed according to the composition of the medium. Rooting was most pronounced on EaM medium which had high auxin contents (5.0 mg/l of IAA) (Fig.3.23d). On the other hand, caulogenesis was most pronounced on WK medium which was incorporated with high adenine (7.0 mg/l) and kinetin (0.5 mg/l) contents (Fig.3.23d). Both seemed to be additive in inducing caulogenesis.

Leaf explants were easily induced to produce callus growth on any of the callus inducing medium. They regenerate to produce shoots directly on MS_6 medium (Fig.3.23b).

Infectivity of the source explants and callus cultures:
The infectivity of the non-differentiated and de-differentiated cultures and their source tissues from N. tabacum cv. Xanthi are given in Table 3.41. As the stem consists of pith, cortex, epidermis and conductive tissue which are included in the explants, the infectivity of these tissues was estimated individually. The infectivity data of the callus cultures obtained from these explants after their being established on MS medium, are given in the Table 3.41.

Table 3.41. Infectivity of callus cultures and the source explants of virus infected turkish tobacco plants

Explants	Source Tissues Infectivity		Cultivated Tissues Non-differential callus infectivity		De-differentiated callus infectivity	
	Total No.of local lesions/ leaf	Mean	Total No. of local lesions/ leaf	Mean	Total No. of local lesions/ leaf	Mean
I Stem 1. Pith	231/4	57.75	3/5	0.6	-	-
2. Cortex & Epidermis	777/5	155.40	-	No growth of tissues		
3. Conductive tissue	830/5	166.00	-	No growth of tissues		
II Leaf	1266/7	180.85	830/5	166.00	846/5	168.78

The infectivity of the non-differentiated callus cultures was reduced to the average of 0.6 lesions/leaf even before the first transfer as compared with the source tissue i.e. pith (57.5 lesions/leaf) whereas the infectivity of the leaf callus was very high (166 lesions/leaf) though less than the original tissue (180.85 lesions/leaf). It was closer to the infectivity of the original tissue in the de-differentiated callus culture (168.78 lesions/leaf) (Fig.3.24).

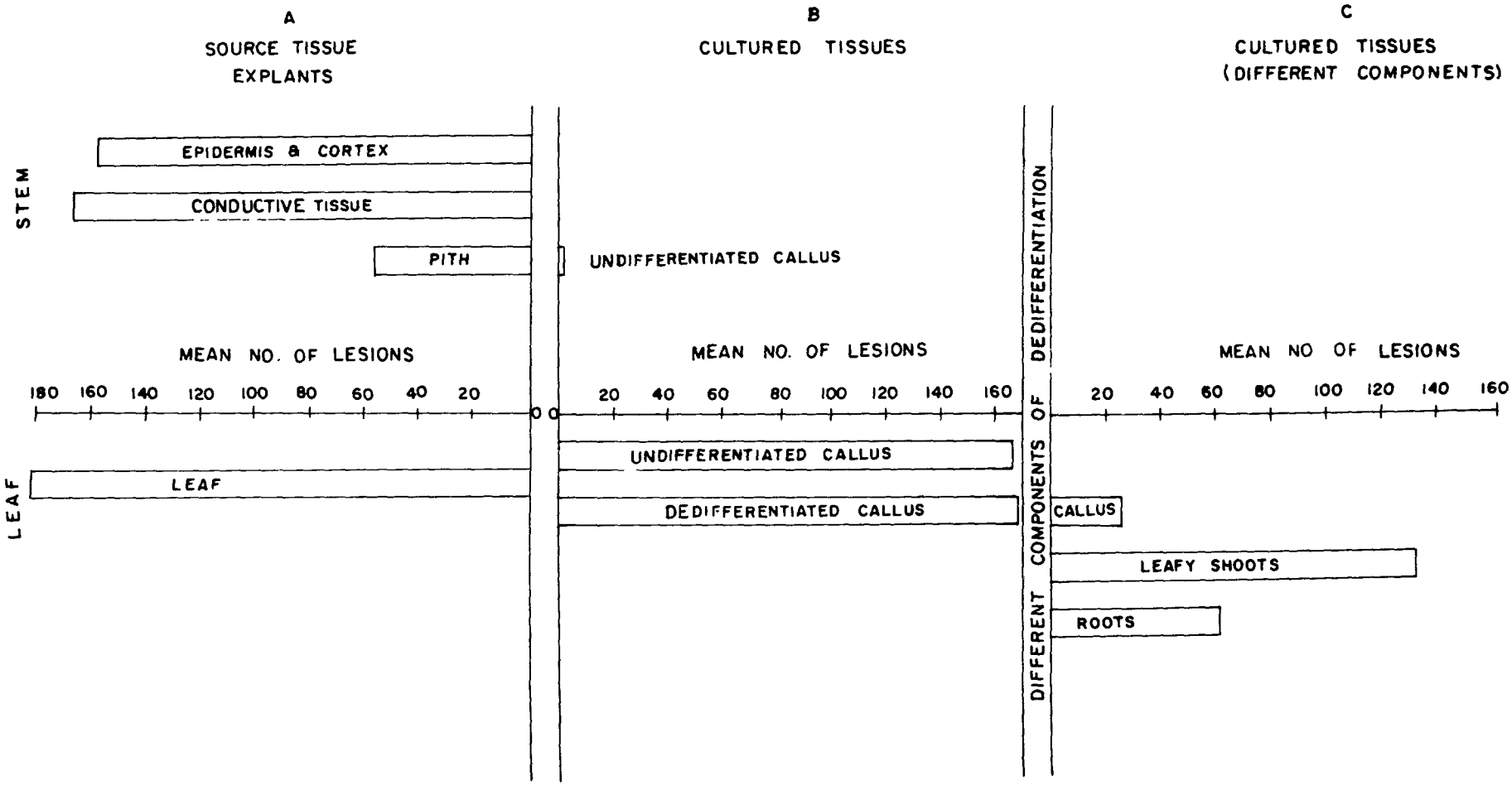
Effect of morphogenetic de-differentiation on the infectivity of the cultures: The de-differentiated tissues of shoots, roots and non-differentiated callus grown from leaf callus were separated and bioassayed for the estimation of infectivity. The results are given in Table 3.42.

Table 3.42. Effect of morphogenetic de-differentiation of the virus affected leaf callus on the infectivity

De-differentiated callus	Total no. of local lesions on leaves	Total	Mean no. of local lesions on leaves
1. Undifferentiated callus	1. 10 2. 39 3. 26	75	25
2. De-differentiated shoots	1. 202 2. - 3. 196	398	132.6
3. De-differentiated roots	1. 39 2. 55 3. 89	183	61.0

FIG. 3·24.

INFECTIVITY OF DIFFERENT CULTURES OBTAINED FROM DIFFERENT SOURCE EXPLANTS



The results indicated increase in the infectivity of de-differentiated shoots and roots compared to non-differentiated callus cultures (Figs. 3.24 and 3.25a). The shoots showed highest degree of infectivity, but were not uniformally infective as some of the shoots remained virus free when assayed individually.

Effect of histogenetic de-differentiation on the infectivity of the cultures: The histogenetic de-differentiation introduced into normal callus cultures (a loose mass of cells) was distinguishable by its compact growth. Sections through these tissues indicated the presence of thick walled cells with lignified cell walls appearing to be differentiating into vascular elements. The following are the results on the infectivity of the two types of the tissues.

Table 3.43. Effect of histogenetic de-differentiation on the infectivity of the callus cultures

Differentiation callus growth	Total no. of local lesions on 6 half leaves	Total	Mean no. of local lesions on 6 half leaves
Loose callus growth	1. 6 2. 5 3. 10	21	7.0
Compact callus growth	1. 7 2. 11 3. 4	22	7.3

It is evident that virus infectivity was not affected by the histogenetic dedifferentiation.

Fig.3.25. A. Undifferentiated and dedifferentiated callus culture of turkish tobacco plants infected with the virus.

B. Turkish tobacco leaf-callus (undifferentiated) grown in continuous light showing green colour indicating chlorophyl production.

A

B

FIG. 3.25

Effect of continuous light and darkness on the infectivity of callus culture: Two sets of non-differentiated cultures of leaf-callus were grown under two different conditions of light - one in total darkness and the other under continuous fluorescent light of 80 ft candles. Virus infectivity was bioassayed after four weeks of growth period and results are presented in Table 3.44.

Table 3.44. Effect of continuous light and darkness on the infectivity of callus cultures

Callus culture growth in	Total no.of lesions on 6 half leaves	Total	Mean lesions per six half leaves	Type of callus growth
1.Continuous darkness	1. 17 2. 6 3. 10	33	11.0	Whitish compact growth
2.Continuous light	1. 5 2. 18 3. 11	34	11.3	Green loose growth

The callus grown in continuous light developed green colour whereas the one grown in darkness failed to do so (Fig.3.25b). But the virus infectivity was not at all affected in either case.

Retention of infectivity of callus cultures (Maintenance of virus in callus cultures): The non-differentiated and dedifferentiated leaf callus cultures from virus affected leaves of turkish tobacco were subcultured at regular intervals and bioassayed for the infectivity during subsequent transfer. The results are presented in Table 3.45.

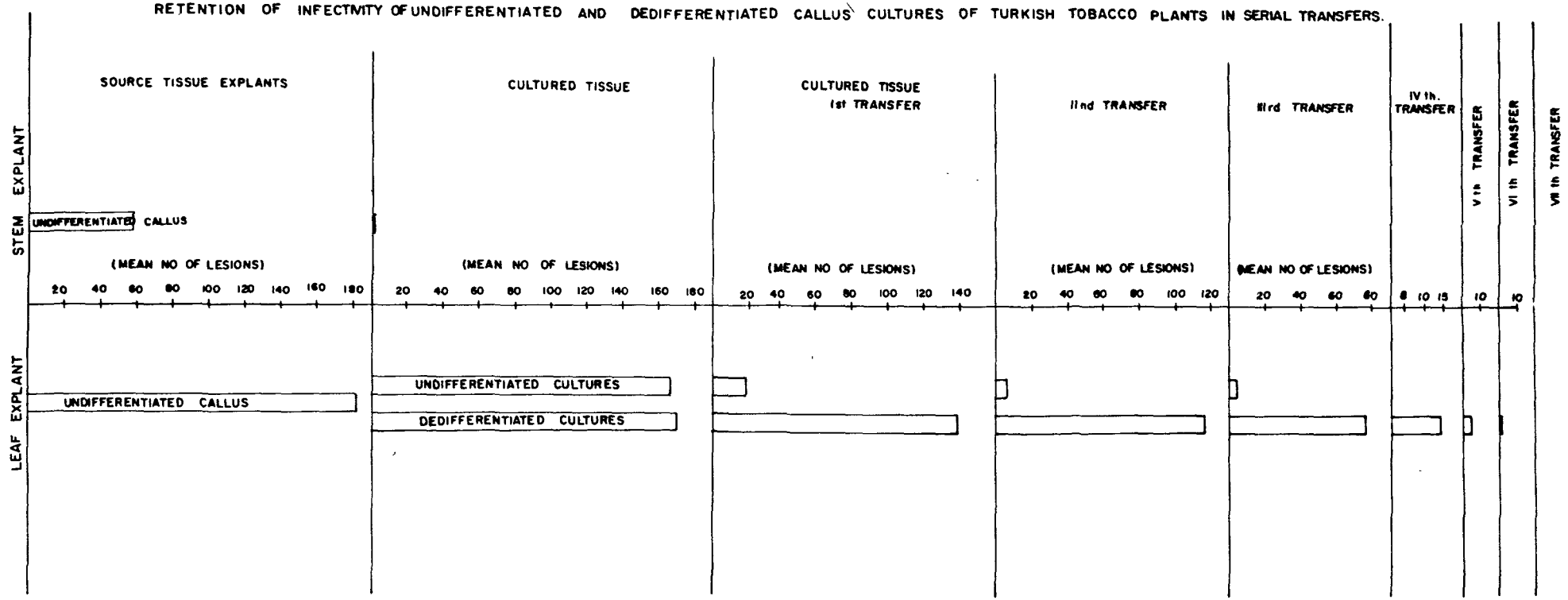
Table 3.45. Retention of infectivity in the dedifferentiated and un-differentiated callus cultures from virus affected turkish tobacco plants

Callus cultures		Infectivity (No. of lesions/leaves) during Serial transfers at 4 weekly interval									
		ST 1	2	3	4	5	6	7	8	10	12
1.Non-differentiated callus	1.	11	4	5	-	-	-	-	-	-	-
	2.	19	2	8	-	-	-	-	-	-	-
	3.	27	10	1	-	-	-	-	-	-	-
Mean		57/3 =19.0	16/3 =5.3	14/3 =4.6	-	-	-	-	-	-	-
2.De-differentiated callus	1.	197	150	100	60	4	1	-	-	-	-
	2.	107	100	50	15	-	-	-	-	-	-
	3.	111	98	70	11	5	-	-	-	-	-
Mean		415/3 =138.3	348/3 =116.0	220/3 =73.3	86/3 =28.6	9/3 =3.0	1/3 =0.3				

The non-differentiated callus cultures retained the infectivity upto 3rd transfer while in case of dedifferentiated shoots the infectivity was retained even after 5th transfer (5 months) but was reduced at every transfer (Fig.3.26).

FIG. 3.26.

RETENTION OF INFECTIVITY OF UNDIFFERENTIATED AND DEDIFFERENTIATED CALLUS CULTURES OF TURKISH TOBACCO PLANTS IN SERIAL TRANSFERS.

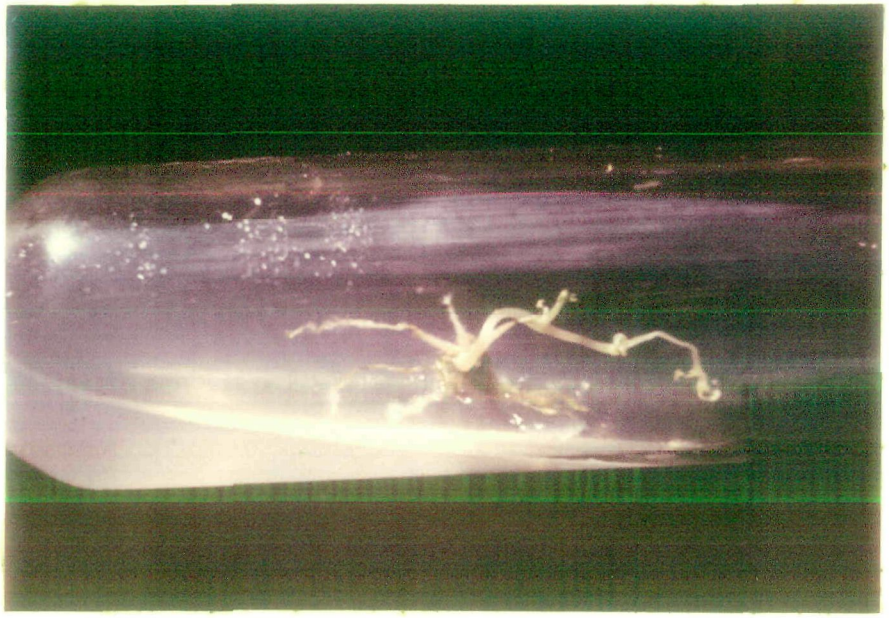


3.14.2. Meristem culture of Tropaeolum:

Meristem-tips consisting of meristem-dome and one pair of leaf primordia measuring approximately 0.5 mm were planted on different media as already described. A suitable proportion of auxin-kinin ratio along with adenine and myoinositol was required for obtaining organised growth. A well organised caulogenic growth was observed on MS 6 and WK media. Besides 7.5 mg adenine and 100 mg myoinositol, MS 6 medium contained 1.5 mg of IAA and 0.75 mg of kinetin whereas WK medium contained 1.0 mg of NAA and 0.5 mg of kinetin per litre. This proportion of auxin-kinin ratio favoured strong caulogenic response, inducing proliferating shoots with complete suppression of roots (Fig.3.27b and 3.23c). Root development predominated at the expense of shoot growth and callus formation (Fig.3.22d). EaM medium was incorporated with 5.0 mg of NAA and 0.2 mg of BAP per litre. MS medium having 2.0 mg of IAA and 0.4 mg of kinetin per litre, induced de-differentiated growth of roots, callus and stem in which callusing and root development predominated. Similarly, roots were more pronounced on K series of media which had 1.0 mg of NAA and 0.5 mg per litre of kinetin but reduced amounts of adenine and myoinositol (5.0 and 1.0 mg/l, respectively). This evidently seems to be the cause of the lack of proper response as adenine is known to be complementary to kinetin in its caulogenic response and myoinositol is complementary to auxin in rhizogenic response. In this series, addition of choline chloride, ascorbic acid

Fig.3.27. A. Meristem-tip culture of double tropaeolum

B. A proliferating meristem-tip culture.



A

B

FIG. 3.27

and folic acid at 0.5 mg/l each (K_3 medium) only induced increase in root growth. Addition of 2,4-D, casein-hydrolysate and coconut milk (L-medium) induced callusing only, though the callus growth stopped within 5 months. Inclusion of 1.0 mg/l of GA (O medium) induced slight shoot root growth, stimulus being more towards rhizogenesis.

The induction of proliferating growth (on MS 6 or WK media) from a single meristem unit consisting of meristem dome and a pair of leaf-primordia, was a strong caulogenic response, further evidenced by complete absence of root growth. The shoot-lets, thus developed, were separated out and transferred on MS medium which induced root-growth within 4-5 weeks (Fig.3.22). Reducing the agar percentage to 0.4 for making semi-solid medium resulted in well developed roots and it was easier to free the agar remanents for further transfering them to soil. The shoot-lets after separating out when transferred to MS 6 or WK medium, further evidenced proliferating caulogenic growth (Fig.3.22e).

The plant-lets growing with roots on MS medium (0.4 per cent agar) were removed from culture tubes and washed thoroughly in tap water, taking care not to injure roots while removing the interspersed agar fragments.

The plant-lets thus removed, were planted in sterilized mixture of soil and compost in 4" plastic pots. They were watered regularly with 1/10 strength of Knop's solution for a week. The plant-lets were covered with small tubes to prevent

excess of evaporation of moisture. The tubes were periodically removed from time to time to acclimatise the plant-lets with the outside environment till they get established in the soil.

These cultures were kept at cool temperature ranging from 27°C +2°C with continuous light from fluorescent tubes at 80 foot candle intensity along with a red bulb to provide infra-red range of wave length.

Three representative samples of fully developed cultures from K_3, L, O, MS 6 and MS media were bioassayed for infectivity counts. All the cultures were found to be virus-free as no local lesions or systemic symptoms could be observed on inoculated tobacco plants.

IV. DISCUSSION

1. Characterization of the virus

Double tropaeolum plants (Tropaeolum majus L.), exhibiting abnormal floral morphology leading to complete antholysis and suppression of sexual organogenesis, are grown as ornamentals in and around Simla. These plants are found invariably affected by a viral infection, evidenced by the association of such disease syndrome as chlorotic and necrotic concentric rings accompanied by severe mottling, curling and puckering of the leaves. The bright scarlet colour of antholysed flowers shows breaking which gives it a distinctive position as one of the coicest collection for ornamental interest. As these plants do not bear seed, they are propagated by cuttings, thereby, perpetuating the infection with each planting. The association of floral abnormality and its perpetuation with the virus like infection was interesting and needed to be investigated with a view to identify and characterise the virus.

In India, Bhargava and Joshi (1959) described for the first time a similar viral infection of garden nasturtium (Tropaeolum majus L.) from Nainital. However, these plants did not exhibit abnormal floral morphology. The virus infecting this host was considered to be the same as tropaeolum ring spot virus (NRSV) described by Smith (1950). Schmelzer (1960) worked out its host range in detail and preferred to call it as ring mosaic virus, probably explaining more explicitly the extent of disease syndrome, exhibited by this virus on

tropaeolum. This virus is considered to be one of the two rare examples of a sap-inoculable and aphid transmitted ring spot viruses. The most important of the ring spot group of viruses, namely, tobacco ring spot (TRSV) has also been reported to be infecting the garden nasturtium plants (Price, 1940) producing similar disease syndrome and is known to have a very wide-host range. This virus is interesting in having diverse types of vectors for its spread and causing variable disease syndrome. As is evident from the published work, the tobacco ring spot virus differs from tropaeolum ring spot virus in not being transmitted by aphids (though there have been reports by Smith and Brierley ,(1955) about its transmission by Myzus persicae from gladiolus when in association with another virus and by Shyama Rani et al. (1969) about its transmission by M. persicae and A. gossypii).

Host range studies of double tropaeolum virus (DNRSV), during present investigations, indicated similarity in the reactions of certain hosts already reported by other workers to be the common hosts of TRSV and NRSV. These hosts are: Nicotiana tabacum, N. glutinosa, N. rustica, Lycopersicon esculentum, Zinnia elegans, and Tropaeolum majus. Some of these hosts have the characteristic ability to recover from severity of disease syndrome, though still retaining the virus. In the present studies, this is more evident in hosts such as N. tabacum and N. glutinosa but not in T. majus. Price (1936) has shown that this recovery accompanied by decrease in virus contents is comparable to the acquired immunity

observed in the animals. In the studies on the turkish tobacco plants inoculated with the double tropaeolum virus a wide variation in the expression of symptoms on different leaves of the same plant was observed as shown in Fig.3.3. The localised symptoms consist of chlorotic or necrotic lesions with concentric rings on inoculated leaves whereas the systemic symptoms on the younger and uninoculated leaves varied from severe chlorotic and necrotic symptoms to faint oak-leaf pattern. Finally, the leaves completely free from any necrotic or chlorotic symptoms appeared just before initiation of flowering in the plants. The new shoots, when developed have milder symptoms. The concentration of the virus in the plant as such is reduced considerably by 21st day of inoculation after reaching a brief peak near about on 9th day. Though this reduction is observed both in old inoculated and young uninoculated leaves, the infectivity of younger uninoculated leaves always remains higher as compared to old inoculated leaves. The symptom expression in some of these younger leaves is milder to such an extent that they exhibit faint oak-leaf pattern which may even disappear altogether, showing an apparent recovery. With respect to TRSV, Wingard (1928) pointed out that the leaves that recovered were not always entirely free from disease syndrome and they were thicker and leathery in texture. Fulton (1949) found that the virus contents of the leaves of Havana 38 and xanthia tobacco affected by streak disease, was as high as or even higher than that in the actual diseased leaves.

It has been reported that in case of TRSV the recovered leaves do not develop lesions with one or the other strain of the tobacco ring spot virus on reinoculation (Wingard, 1928; Price, 1932, 1936) but do develop symptoms when inoculated with unrelated viruses (Price, 1936). In the present studies also, reinoculation of such leaves failed to produce characteristic symptoms as if the plants have become immune. Besides the leaves of the same age on healthy plant also fail to produce any characteristic symptoms on inoculation, thereby indicating that severity of disease syndrome depends on the age of the plant at the time of infection.

Conversly, some of the host plants like celery and Crotalaria brownei, etc. indicated visible expression of symptoms only after a considerable lapse of time from the inoculation date. In the case of brinjal and Dolichos lablab, symptoms are better expressed after a brief period of recovery in the summer months, when the temperature remains invariably above 30°C. This can be compared with the phenomena of heat-reactivation, reported by Follmann (1961) in the case of some isolates of tobacco ring spot virus.

Out of the 143 plant species tested for host-range studies with double tropaeolum virus, 21 species have also been reported to be the hosts of the other two viruses namely, TRSV and NRSV. Except for Phaseolus vulgaris and Datura stramonium which have altogether different reactions to all the three viruses, other hosts have reacted similarly with TRSV or NRSV. The reactions of Impatiens balsamina, Chenopodium album

and Nicotiana glutinosa with DNRSV were similar to those with TRSV, whereas, the reactions of Amaranthus caudatus, Vicia faba, Vigna sinensis, Delphenium cultorum, Nicandra physaloides, Nicotiana rustica, Physalis angulata and Solanum nigrum were the same as with NRSV. Four of the hosts namely, Beta vulgaris, Spinacea oleracea, Callistephus chinensis and Petunia hybrida are reported to have similar reactions to the infections with TRSV and NRSV but they are different in their reactions with DNRSV altogether. Statistically, when analysed, TRSV, NRSV and DNRSV are found to be closer to each other since the variation in their 'mean host-reactions' is not significant. There is some variation in the mean host-reaction of TRSV with the other two, however, the difference is not significant thereby indicating a close relationship. It may, however, be pointed out that some of the reactions as given in the Table 3.10, are of low degree of susceptibility such as 2 or 4 which are indicative of chlorotic or necrotic local lesions. In the present studies some such reactions when further investigated developed into syndrome such as systemic oak-leaf pattern, flecks and necrosis indicating a high degree of susceptibility (S.I.Nos. 8 or 16) especially if the plants are kept for a longer duration. In view of these observations, some of the hosts of TRSV, if investigated further may raise the value of 'mean host reaction' and bring it nearer to the other two viruses. Solanum nigrum, Amaranthus caudatus etc. are the hosts which may indicate higher degree of susceptibility. Therefore, with the majority of the hosts indicating close host

reaction, all the three viruses might be having closer mean host reactions. On the basis of host reactions, DNRSV and NRSV appear to be closely related to TRSV.

The virus (DNRSV) was studied extensively for its physical properties. The thermal-inactivation point for the virus was between 60 and 62°C, dilution-end-point between 10^{-4} to $15x10^{-3}$, longevity *in vitro* at 19-27°C upto 48 hours but not 75 hours; and at 8-10°C upto 168 hours but not 240 hours, and optimum pH for infectivity as 6, i.e. towards acidic level. The infectivity in both phosphate and glycine buffers was identical. The thermal-inactivation point (TIP), reported herein is less than the one reported by Schmelzer (1960) for tropaeolum ring mosaic virus (i.e. 66-68°C), but higher than the one reported by Smith (1950) for tropaeolum ring spot virus (55-60°C) and tobacco ring spot virus (55-60°C) according to Text book by Smith (1957). Similarly, dilution-end-point ranged slightly above than reported for these two viruses, whereas longevity of this virus was almost the same as recorded by Schmelzer (1960) for tropaeolum ring mosaic virus. In the present studies, the virus could be maintained for 11 months in dried leaves at 4-7°C on $CaCl_2$ though the infectivity was reduced, whereas Schmelzer reported the longevity of his virus to be only 8 days in dried leaves.

However, these studies do not reveal any major differences in the physical properties with those reported for NRSV and TRSV. Whatever existing differences are, may be attributed to the influence of original hosts and environmental factors.

Transmission studies indicated that the virus affecting double tropaeolum was transmitted successfully by three aphids namely, Aphis gossypii, A. craccivora and Myzus persicae in a non-persistent manner to tropaeolum and turkish tobacco plants after being picked up from tropaeolum. Both A. gossypii and M. persicae were equally efficient, whereas A. craccivora had low percentage of transmissibility. Nevertheless, it happens to be an additional vector not reported so far. All these aphids were unable to pick up the virus from turkish tobacco and other hosts. With respect to broad bean wilt virus, Stubbs (1960), reported A. craccivora to be less efficient vector than M. persicae, as it could pick up virus only if the feeding-host source was rich in virus concentration. In addition to M. persicae, A. fabae (Smith 1949a,b), M. persicae, Macrosiphon solanifolii (Ashm.) (M. euphorbae) and Acyrothosiphon onobrychis (B.d.F.) (A. pisum) (Schmelzer, 1960), two additional aphids namely, A. gossypii and Brevicoryne brassicae have been reported to be the vectors of tropaeolum ring spot virus in India (Bhargava and Joshi, 1959 and Bisht, 1962). Smith and Brierley (1955) pointed out that M. persicae can also transmit tobacco ring spot virus from gladiolus, particularly when associated with bean yellow mosaic virus. Similarly, Shyama Rani et al. (1969) showed transmission of TRSV isolated from petunia by M. persicae and A. gossypii. In the present studies the aphids used in transmission studies failed to pick up virus from tobacco though they could transmit the virus to it producing symptoms

of vein banding instead of ring spots. This indicates that transmissibility of a virus through vector also depends on the nature of source plant.

Further experiments on soil transmission indicated positive results especially in the case of tobacco plants, from which aphids failed to pick up the virus and also in Chenopodium, though a local lesion host, wherein the virus has a tendency to become systemic. Exceptionally high concentration of the virus in the roots of tobacco plants observed during the course of present studies may be serving as a ready source of inoculum for its transmission through soil. Experiments were carefully planned by growing tobacco plants infected with the virus in the sterilized soil so as to provide enough inoculum for making sick soil. The virus was detected in some cases in the roots of 'bait plants' seeded later in the sick-soil without any sick plants. In a few plants, the virus multiplied sufficiently to become systemic and produced visible systemic disease syndrome on the leaves. Transmission experiments with Xiphinema americanum were successful only in one case where infection was detected in the roots. Tobacco ring spot virus on the other hand is transmitted by a variety of vectors including aphids such as M. persicae. Therefore, the distinction in the ability of different vectors to pick up virus seems to be 'host-influenced'. DNRSV, having, aphids and nematodes as the vectors, seemsto be nearer to TRSV.

Intracellular bodies, characteristic of numerous virus

diseases of plants, have been studied by various workers. Wood (1933) reported occurrence of such inclusion bodies in the cytoplasm of the cells in the ring spot formed on Havana seed-leaf and leaves of turkish tobacco, N. glutinosa, N. rustica and petunia induced by TRSV infection. In the present studies, such inclusion bodies were not observed in the leaf cells of N. tabacum cv. xanthi. But indistinguishable hyaline bodies of different shapes and sizes were observed under phase-contrast microscope in the cells of ring spots affected leaves of double and single tropaeolum plants, generally associated with the nucleus. When stained with a fluorescent dye, acredine orange, the virus-infected tissues showed characteristic deep orange-red fluorescent inclusions singly or in groups associated with the nucleus and sometimes enveloping the nucleus, indicating their RNA nature. From the account of Wood (1933) such bodies bear a definite relationship with the ring spot virus in the diseased area. No such inclusions have been reported to be occurring with any of the other ring spot group of viruses.

In order to affirm and compare the finer structure of the virus with other similar viruses, it was purified by various methods using different buffers and differential centrifugation of which butanol method described by Hollings (1965) was found to be the most suitable. In this technique phosphate buffer of 0.5M at pH 7.0 was used, with one cycle of low and high speed centrifugation.

The present study happens to be the first attempt to

purify the virus infecting tropaeolum. The closely similar virus, viz. TRSV has been a subject of thorough investigations since the early attempts of Stanley and Wyckoff (1937) to purify it. Steere (1956), using butanol and chloroform method, purified TRSV from several hosts and characterised it. He obtained uniform polyhedral (hexagonal) particles with average diameter of 26 nm (24-28 nm) from shadow casted material. The electron microscopic studies of DNRSV during the present investigations have also revealed distinct polyhedral (hexagonal) particles of average diameter of 29 nm (26-32 nm) on being negatively stained. Some of the particles in these preparations were exceptionally bigger due to denaturing of protein shells, probably an effect of 2% PTA (Sergeant, 1967). TRSV, isolated and purified from cucumber was reported to have particles of 25 nm in diameter by Stace Smith et al. (1965) and 28 nm by Corbett and Roberts (1962). Both these measurements were recorded with the negatively stained purified preparations. Spherical particles of approximately 26 nm were detected by Bawden and Nixon (1951) in the electron micrographs of their TRSV preparations. Desjardins et al. (1953) purified the virus by differential centrifugation and electrophoretic fractionation and reported infectivity to be associated with particles of 22 nm diameter. These differences in the size of virons associated with TRSV seem to be due to different purification procedures or the different types of host plants used for producing the inoculum. In the studies conducted with DNRSV, the shadow-casted material

was of 30-32 nm. This very well illustrated the range of variability one can come across due to different procedures of purification used. On the basis of size and shape of particles also, the presant virus appears similar to TRSV.

Following Steere's method of purification, Schneider and Diener (1966) obtained three components of TRSV on the sucrose gradient by ultra-centrifugation, confirming the findings of Stace Smith et al. (1965). These findings are contradictory to Steere's observations who found only two components. However, according to Stace Smith et al. Steere's preparation may have been more stable and, therefore, the top component was missing. In fact, State Smith (1966) purified tomato ring spot virus also and obtained only two components on the sucrose gradient, and considered it to be the only difference with the otherwise similar TRSV.

The DNRSV studied, herein, also resolved into three components on sucrose gradient as in the case of TRSV. The UV-absorption studies and infectivity tests indicated bottom component supposedly containing RNA and being infectious whereas middle and top components as non-infectious. The maximum absorption of top component has shifted to 270 nm due to predominance of proteins in the preparation. The 280:260 ratio is also indicative of the same, and this reveals trace contamination of RNA, which may be plant RNA. The middle component indicated a further shift to the peak towards 260 nm indicating more RNA in the components whereas the curve depicting the absorption of the bottom component is in

consistence with the viral nucleoprotein absorbency giving high proportion of 260/280 ratio.

The UV-absorption spectra of viruses are usually characterised by a prominent peak at 260 nm which has been attributed to the purines of the nucleic acid. The absorption of protein, on the other hand, shows a strong peak at 280 nm. The absorption spectra of the purified samples of the virus was studied for UV absorption under the spectrophotometer which gave typical nucleoprotein spectrum having maximum absorption at 260 nm and minimum at 240 nm. The average 260:280 nm ratio was 1.71 (ranging from 1.58 to 1.82) which was quite high indicating high proportion of RNA in the virus. In fact, the TRSV is known to have 34.4 per cent nucleic acid as estimated by phosphorus analysis (Steere, 1956). Basing the calculation on 260:280 nm ratio of the extinction values of the sample of present virus, the nucleic acid composition was about 36.91 per cent which seems to be very close to Steere's observations. Stanley's (1937) preparations were reported to contain 40 per cent of the nucleic acid whereas Stace Smith et al. (1965) reported RNA contents of the bottom components to be 42 per cent.

A small bump at 280 nm is similar to tryptophan bump indicating its presence in the protein structure of the present virus.

With respect to serological relationship, the results with the different antisera of TRSV, obtained from Lisse &

Wageningen, gave strong reaction, indicating similarity in the antigens of TRSV and DNRSV i.e. having similar proteins shells.

Considering the information gathered during the present investigation, the characterization of the virus affecting double tropaeolum may be summed up in the following cryptogram (Cryptogram 1).

R/* */36.9(?) s/s S/Ap, Ne (?).....(1)

The type of nucleic acid was ascertained indirectly by studies with fluorescent dyes which stained the inclusions associated with virus infection as fluorescing orange red colour, typical of RNA which was further proved by their dissolution with RNA-ase. These inclusions are, therefore, aggregations of virus particles. Strandedness of RNA and molecular weight of the virus remain undefined. RNA in the virus preparations was calculated on the basis of ratio of extinction values at 260:280 nm and found to be 36.9 per cent. This finding is close to the data obtained from sedimentation coefficient, phosphorus contents or by direct method. The shape of the capsids associated with the viral infection and their core, as observed by electron microscope with negative staining was clearly observed to be spherical (hexagonal). The last pair gives the information that virus affects the seed-plants and transmitted by aphids. Nematodes may act as additional vectors. The question marks in the parenthesis are for the figures to be confirmed. This cryptogram may be compared with

that of ring spot group of viruses which is as follows (Cryptogram 2):

R/1 1.8/42 S/S S/Ne (Ap.M.).....(2)

This is according to the CMI List of Plant Virus Names (Martyn, 1968) and represents a group of viruses which includes arabis mosaic virus, raspberry ring spot virus, strawberry latent ring spot virus, tomato black ring virus and tomato ring spot virus. They only differ in the figures in the 2nd pair. TRSV which is the type virus has many vectors included in the parenthesis. With respect to DNRSV, the percentage of RNA is less which may be due to many factors including the techniques involved. With regard to last figure of the terminal, the emphasis here is more on aphids. In this respect it is more similar to broad bean vascular wilt virus (Cryptogram 3):

Broad bean wilt virus:

R/1 */35 S/S S/Ap.....(3)

The virus affecting double tropaeolum, differs entirely from broad bean wilt virus in symptomatology and to some extent from tobacco ring spot virus in having not so many vectors. Since the vector transmissibility ortheir specificity for virus transmission is dependent on the hosts concerned (shown under present study), it may not be a suitable character to be depended upon for establishing the relationship. Therefore, due to its similarity in symptom expressions, host range, physical properties, structure and function of its capsids, the virus affecting double tropaeolum may be considered to be closer to TRSV. In other respects such as

serological relationship also which are important for specific strain identification, DNRSV gave positive reaction with the antisera of TRSV. Considering the efficiency of aphid vectors in the transmission of DNRSV as compared to TRSV as also differences in some of the individual host reactions DNRSV could be a strain of TRSV affecting tropaeolum plants. Also, the tropaeolum ring spot viruses as reported by Schmelzer (1960), Smith (1950) and in India by Bhargava and Joshi (1959) may also be similar or same as DNRSV.

As there is no provision in the cryptograms of Gibbs et al. (1966) for including the consideration of serological relationship, it may not suitably describe the difference in the viruses as is evident from the present investigations.

4.2. Floral abnormality

Double tropaeolum differs from both semi-double and single tropaeolum plants in having completely different floral morphology (Appendix III). There is a drastic change in the floral parts viz. elimination of spur of superior sepal, formation of indefinite number of bright coloured peals instead of normal 5 and complete suppression of stamens and carpels instead of 9 and 3 in the single tropaeolum plants respectively. These changes in the floral structure of double tropaeolum have not been reported earlier and are different from changes in the flora morphology found in the semi-double flowers which is a genetical variation. This results in breaking of each of the three

lower petals (2 + 6) and addition of one unit each in stamen and carpel. This type of orderly change seems to be lacking in the case of double tropaeolum . Bos (1957), in fact reported breaking-down of the flowers to phylloid structure as a result of incomplete antholysis, since there was still some production of seeds. The cause of this is now ascribed to be pathogenic (mycoplasma origin). It is a complete antholysis in the case of double tropaelum. These changes also affect the infectivity of the floral parts, compared to the single tropaelum plants. The flowers, especially petals of double tropaeolum plants were highly infectious. The manifestation of symptoms on the leaves of double tropaeolum plants were also more severe as evidenced by curling and puckering of the leaves. There was also evidence of breaking of the colour in the petals which was not evidenced in the case of single tropaeolum. The same observation i.e. absence of the breaking of the flower colour in the case of tropaeolum ring spot virus has also been reported by Smith (1949a,b). It may, therefore, be inferred that the virus here is more systemically distributed into the double tropaeolum plant system than in the single tropaeolum plants. The infectivity of the floral parts such as petals, has been observed in prunus necrotic ring spot and prune dwarf viruses isolated from sour, sweet and flowering cherry plants and an unidentified virus from apple petals (Tremaine et al., 1964). A latent virus has also been isolated from cherry flowers (Milbrath, 1953). McWhorter (1953), working with cucumber mosaic virus in

gladiolus and a latent virus complex in cherry, found that the inoculum from flowers was 4 to 17 times more infectious than from leaves. This indirectly indicates that the inhibitors in floral parts were less than in the leaves, and same seems to be the condition in the double tropaeolum plants. Indefinite and continuous vegetative propagation of double tropaeolum might have saturated these plants with virus infection. The possibility of further manifestation of the effect of the viral saturation on the morphogenesis of the floral parts was investigated by developing virus free plants through meristem culture. The virus-free plants thus developed did not flower at all. However, with regard to another host, Dolichos lablab there has been clear indication of induction of gradual sterility with the advance of disease as evidenced by the reduction and ultimate suppression of the seed formation.

4.3. Stablization of infectivity of DNRSV in tropaeolum plant

Since the inoculum prepared from the tropaeolum leaves failed to infect most of the hosts, leaf extract was considered to be having some inhibitors, inactivating the virus. The data presented in Table 3.1 indicates that the use of celite and Na_2SO_3 is necessary for successful infection. The treatment, especially with Na_2SO_3, stablizes the viral infectivity probably by checking the oxidation of the polyphenols in the leaf extracts known to be present in large amounts in tropaeolum plant (as myrosin and tropaeolin).

The leaf extract of T. majus (single) was tested for its effect on the infectivity of the CPO strain of TMV and found to inhibit the viral infectivity by about 58 per cent when the extract was prepared from green leaves. On the other hand, the old yellow leaves gave still higher inhibition i.e. upto 87 per cent. This may also be the reason for the higher concentfation of the virus in the young leaves of infected plants. A number of other viruses have also been reported to lose their infectivity in various tissue extracts. Evidence has multiplied indicating the inactivation of these viruses due to oxidation of phenolic compounds in the extracted sap. Bald and Samuel (1934) found rapid inactivation of tomato spotted wilt virus which could be prevented for several hours by mixing the extracted sap with Na_2SO_3 solution. Fulton (1949, 52) observed similar stablizing effect of reducing agents on tobacco streak and rose mosaic viruses. A virus inactivating system from tobacco has been reported by Bawden and Pirie (1957) apparently evolving free oxygen. Hamptom and Fulton (1961) argued that the rapid loss of infectivity of the viruses is due to in vitro inactivation of the viruses, by oxidized polyphenols of the hosts upon homogenization of the tissues. The results obtained during the present investigations indicate the probable presence of similar polyphenol oxidase system in the leaves of T. majus, being more in the older leaves than in the younger ones. The presence of phenolic inhibitors in tropaeolum leaves has an important implication so far as transmissibility of this virus is concerned

since the properties of donner plants and the suscept rather than the virus itself are responsible for the failure of the virus to infect a host. Much earlier, a mosaic virus affecting Phytolacca decandra L. was reported not being transmitted to tobacco and cucumber by sap inoculation but to cucumber by aphids. This was considered to be due to the presence of virus inhibitor in the plant system. (Allard, 1914, 1918; Dolittle and Walker, 1925). However, in recent years, it has been possible to transmit mechanically some of the viruses supposed to be transmitted only by grafting, by using stabilizers of plant virus infectivity.

Attempts were, therefore, made to test the relative effectiveness of Na_2SO_3, caffeic acid and EDTA for stablizing the infectivity of the CPO strain of TMV, in vitro, by treating the tropaeolum leaves in their solutions before extraction and testing against the virus. These chemicals were applied either by soaking the leaves in these solution for 12 hours under frigidair temperature (10-12°C) or the extract was kept frozen for 72 hours after extracting in the same solutions or by just mixing the leaf extract and the solutions in equal volumes. The corresponding controls were in distilled waters. The results indicate that mixing of the leaf extract with Na_2SO_3 and caffeic acid stimulated the virus infectivity by neutralizing the inhibitory system present in these plants. EDTA, however, has no effect when it was mixed with leaf extract but it stimulates the infectivity to the extent of 210 per cent when

the leaves were soaked in its solution prior to the extraction. It was necessary to saturate the leaf tissue before the extracted sap had the chance to get oxidised after coming into contact with atmospheric oxygen. With other chemicals, the soaking of the leaves did not improve the infectivity appreciably. In the case of caffeic acid, stimulation of viral infectivity was further increased by freezing the soaked-leaf extract for 72 hours. Caffeic acid, which itself is a phenol, is also serving here as a reducing agent, generally known to stop oxidation of polyphenols present in the extracted sap by getting themselves oxidised. These polyphenols have a tendency to get oxidised rapidly when exposed to polyphenol oxidase system and form such compounds as O-quincne or O-dihydrophenol which are both injurious to plant as well as inhibitory to viral infectivity (Hampton and Fulton, 1961). Such inactivating host-virus system was reported to be stronger in the case of tobacco ring spot virus than TMV-infected or healthy plants of tobacco (Bawden and Pirie, 1957). These findings explain the stimulatory effect of such treatments as with Na_2SO_3 which help in causing effective infection with DNRSV by establizing the infectivity after neutralizing the oxidation of polyphenols.

1.4. Taxonomic affinity of susceptibility

From the stand point of taxonomic affinities, Holmes (1928) while working with the host range of tobacco mosaic virus suggested an orderly distribution in nature of substances

and conditions essential for viral multiplication. On the other hand, with respect to some other viruses, Price (1940) found that the ability of hosts to support virus multiplication in its tissue is specific for the host species individually and has little or no relationship with the taxonomic position of the family. During the present studies susceptibility was categorised into different degrees depending on the expression of different disease syndromes and indicated as 'susceptibility index' in increasing numbers (viz. 0, 1, 2, 4, 8 and 16). The numerical data thus obtained was analysed statistically. The evidence presented in Table 3.9 is indicative of random distribution of susceptibility in different families of Angiosperms. The five groups designated on the basis of maximum disease reaction not only differ significantly in their disease reactions but also in the existence of high degree of positive correlation with maximum disease reaction and number of host species showing positive reaction in each group. However, these groups have increasing number of host species showing variations in the degree of susceptibility also. Group V with maximum 'susceptibility index', has widest variations in the degree of susceptibility among the species and varieties of different families constituting it. This confirms Price's view so far as this virus is concerned. Frequency analysis of different disease reactions in the families included in Group V, which includes most susceptible host species, indicated that the families like Solanaceae and

Leguminosae have maximum number of above 75 per cent of plant species showing highest degree of susceptibility (viz. systemic chlorosis: S.INo.16). Families like Amaranthaceae and Compositae include only one third of the number (33.33%) of the host species exhibiting such reaction, besides having a large number of host plant species showing necrotic and chlorotic localised infection (S.I.No.2 and 4, respectively) - lesser degree of susceptibility. Since families viz. Scrophulariaceae, Tropaeolaceae, Verbenaceae, Polemoniaceae and Ranunculaceae have less number of plant species tested in the present studies, their comparison is not possible. The families Solanaceae and Leguminoceae which include a large number of most susceptible host species,also have a large number of genera susceptible to many other plant viruses also. The relative position of these two families in the phylogenetic arrangement of the orders of Angiosperms, as given by Hutchinson in 'The Families of Flowering Plants' is far dispersed in the two columns viz. LIGNOSAE and HERBACEAE. Leguminosae is quite lower in the phylogenetic sequence in the fundamentally woody group of dicotyledons (Appendix 4) i.e. Lignosae, whereas Solanaceae lies high up in the column representing Herbaceae. Both these columns are representing parallel evolution from one distantly related 'pro-angiosperms'. The position of other susceptible families is more or less irregularly dispersed in Lignosae and Herbaceae. In the Herbaceae, however, they generally lie at the top of the colum, representing more evolved groups. It would be difficult to associate susceptibility here with the evolution as

susceptibility is indicated by the most primitive order of this group also viz. Ranales. Probably the susceptibility to viral infection is associated in some form right from pro-angiosperms. Also, the criterion of evolution in this phylogenetic system, viz. evolution of the flower, correlation with anatomical structure, cytology, pollen structure, growth form and geographical distribution, seems to be inadequate for ascertaining the evolution of susceptibility in various families of angiosperms.

4.5. Tissue culture studies

The tissue culture technique has been employed for plant virus studies connected with the raising of virus-free plants and virus infected tissue-cultures. The latter are employed also for studying nutritional and other requirements for virus multiplication *in vivo* as also maintenance of virus cultures. These have been discussed by Mishra *et al.* (1964), Raychaudhuri (1966) and Kassanis (1967). Attempts were, therefore, made to raise virus affected and healthy callus cultures of *Tropaeolum majus* (single and double), *Nicotiana tabacum* cv. Xanthi and *N. glutinosa*.

The explants of embryos, leaves, stem, meristem-tips, sepals and petals of virus affected and healthy *Tropaeolum majus* plants (both single and double) were used for investigating their growth response with respect to growth promoting substances like, auxins, kinins, myoinositol and adenine.

Kinetin was not found to be necessary for the normal

growth of the embryos as they grew well on the medium without it. Its inclusion in other media such as K and MS, enhanced caulogenic growth i.e. good growth of shoots. Callus growth was induced by inclusion of kinetin alongwith NAA in the ratio of 0.2 and 0.5 mg/l, respectively. By further increasing NAA to 1.0 or 1.5 mg/l, the growth response tilted towards rhizogenesis i.e. root-formation. Seeds grew well without any growth-promoting chemicals, being nursed by cotyledons enclosing the embryos.

For other tissues, mainly meristem-tips, a well organised caulogenic growth was observed on MS 6 and WK media. MS 6 medium contained 1.5 mg of IAA, 0.75 mg of kinetin, 7.5 mg of adenine and 100 mg of myoinositol per litre, whereas WK medium, contained 1.0 mg of NAA, 0.5 mg of kinetin, 7.5 mg of adenine and 100 mg of myoinositol per litre. These proportions of auxin and kinin (i.e. 1.5:0.75 and 1.0:0.5 in MS6 and WK, respectively) seem to have favoured strong caulogenic response inducing large number of shoots formed from one single meristem-tip explant. This is important as these media can be employed for obtaining more than one plantlets from one single meristem-tip as has been obtained by Mishra and Quak (in press) with respect to carnations and Ben-Jaacov & Langhans (1970) with respect to Chrysanthemum. On the other hand, rhizogenic response of tropaeolum meristem-tips or stem explants was induced by seeding them on EaM medium, with NAA, at 5.0 and kinetin being replaced by BAP at 0.2 mg/l. The root-growth was enhanced at the expense of shoot growth, completely suppressing the growing

point. A somewhat similar response was obtained on K medium, which had 1.0 mg of NAA and 0.5 mg of kinetin per litre, and reduced amount of adenine and myoinositol i.e. at 5.0 and 1.0 mg/l, respectively. It seems that reduced adenine contents in the medium was also responsible for induction of rhizogenesis at a kinetin level which otherwise had supported caulogenic growth in other media. In fact, kinetin is known to depend for its caulogenic response on the presence of right amount of adenine (Gautheret, 1966), whereas myoinositol is complimentary to auxins in their rhizogenic response as indicated for the carnation explants (Mishra and Quak, in press). However, in this medium myoinositol level was not of any consequence. Other media in this series, wherein cholin chloride, ascorbic acid and folic acid at 0.5 mg/l were added (K 3 medium), also induced only root growth. Profuse callus growth was induced with 2.0 mg of IAA and 0.4 mg of kinetin per litre, as on MS medium, though root formation was also observed in the stem and meristem-tip explants. Leaves and other explants, however, did not respond to any of the media for inducing roots or shoots formation. Profuse callus growth was obtained from meristem-tip and freshly opened young leaf explants, indicating probably the importance of the presence of least number of non-multiplying and non-active cells for inducing profuse and long lasting growth of calli. These callus cultures remained actively growing for over one and half years, by regular 4 week subculturing on MS medium. Callus cultures obtained from incipient buds, sepals, petals

and stem, however, had slow rate of growth, and turned brown with senescence within a few weeks. There was no difference in the growth response of healthy and diseased single tropaeolum and diseased double tropaeolum explants. Pillai and Hilderbrandt (1969) developed geranium callus from stem-tips and internodes on their C and D media having coconut milk and 2,4-D (Hildebrandt, 1962) as also on MS medium (Murashige and Skoog, 1962) with 10 mg of kinetin and 6.1 mg per litre of NAA.

The infectivity of these tissues was lost during subsequent transfers of callus cultures indicating a sharp drop in the infectivity of virus affected tissues, which is in sharp contrast to the results obtained with respect to a number of other viruses (Morel, 1948; Augier de Montgremier et al., 1948; Raychaudhuri and Mishra, 1965; Reinert, 1966; Mishra and Raychaudhuri, 1968).

Meristem-tip, being virus-free region of the fast and actively growing shoots, developed into virus-free plants or plantlets on MS 6 medium. No infectivity was detectable when shoots and plantlets were tested. The meristem-tips of tropaeolum which consist of actively dividing cells of meristem dome along with one pair of leaf primordia gave out profuse growth only under anaerobic conditions i.e. when the meristem-tip explants were immersed into the solid medium. The explants seeded on the surface of the solidified medium or in the semi-solid medium did not grow.

This was also observed by Ball (1946) who found that the meristem-tips consumed less oxygen per unit area than the other adjacent tissues and therefore, grow well under the surface of agar. The tissue measuring 0.4-0.43 nm consists of meristem-dome and three younger foliar primordia. The other portion below this region were not responsive to normal culture media and needed coconut milk and some accessory chemicals to suppliment it.

Stem and leaf-bits from healthy and virus-affected turkish tobacco and N. glutinosa plants were planted on a set of different media. Callus growth was obtained from these explants on the medium incorporated with the required proportion of auxins, kinins, adenine and myoinositol along with organic and inorganic constituents as in MS, EaM and WK media. Coconut-milk was not required for inducing the leaf cells to callus as was reported by Steeves et al. (1957) for obtaining callus from sunflower and Nicotiana leaves. Under the present studies, prolonged culturing of the leaf-explants for two subsequent transfers at 3-week intervals on K 2 medium, induced slight callusing of the leaf cells. The callus growth was more pronounced and rapid on MS medium. Further transfer on MS or WK medium at the above interval induced dedifferentiation of callus growth i.e. formation of large number of shoots and some roots. Only few roots were differentiated in the case of N. glutinosa leaf-callus on EaM medium, whereas on other media, the callus cultures remained undifferentiated. As pointed earlier, the EaM medium

was incorporated with 5.0 mg/l of NAA, which might probably be responsible for the rhizogenic dedifferentiation in an otherwise strongly undifferentiated N. glutinosa leaf-callus. With regard to turkish tobacco, dedifferentiation was also observed in the callus cultures obtained from stem, but was more pronounced in leaf-callus. The shoot differentiation in callus cultures was more abundant on MS 6 and WK media, a response which is not diffdrent from that observed in the case of tropaeolum explants indicating an entirely caulogenic response. Gupta et al. (1966) reported regeneration of N. tabacum leaves i.e. formation of shoots directly from leaf explants on the medium incorporated with coconut-milk and supplemented with kinetin and low concentration of IAA. According to them, coconut-milk is essential for the leaves to give out shoots directly. In the present studies, coconut-milk was not necessary for direct regeneration and production of shoots from leaf-cells which was observed on MS 6 medium within 4 weeks of planting. The roots were generally induced on the same medium after the cultures were a little older, indicating the phenomenon of 'organic correlation', i.e. bud induced root development as explained by Gauthret (1966) while analysing factor affecting dedifferentiation of plant tissues grown in vitro. It has been shown by Skoog and Miller (1957) that organogenetic development depends upon the ratio of the concentration of IAA and kinetin (a kinin) incorporated into the medium, a higher ratio initiating root formation and

a lower ratio inducing formation of shoots. The callus growth is induced by the ratio which is in between the two. In the above two media, namely, MS 6 and WK, the auxin : kinin ratio being 1.5:1.0 and 1.0:0.5, respectively, which seems to be suitable for a good caulogenic growth. Addition of 7.5 mg/l of adenine has further complemented this growth-pattern. The similar trend was also observed with respect to tropaeolum explants as discussed above.

There was no difference in the growth response of healthy and virus affected cultures of these tissues. However, the callus cultures obtained from leaf cells were more infective than the ones obtained from stem which were almost noninfective. This appears to be depending on the infectivity of the source tissues as is evident from Fig.3.26. Pith region which is essentially the source tissue for developing the callus from the stem, was found to be least infective. These results are in conformity with the observations reported by Raychaudhuri and Mishra (1965) with respect to a number of other viruses. The effect of induced organogenesis on the infectivity was also investigated. The infectivity of callus cultures increased considerably in the dedifferentiated leafy shoots. The roots were less infective and the undifferentiated callus cells were least. These tissues were obtained from the mixed culture, which consisted of leafy shoots, roots and undifferentiated callus cells. The increase in the infectivity of dedifferentiated tissues indicates a possible relationship between organogenetic

phenomenon and virus multiplication which is in conformity with the results on TMV reported by Mishra and Raychaudhuri (1968). It appears that caulogenic growth supported virus multiplication more than the rhizogenic or callus growth. Kinetin, an important constituent of the tissue culture media inducing shoot-growth is also known to be intimately connected with the nucleic acid metabolism and protein synthesis (Osborne, 1962). It might, therefore, be presumed that kinetin may also be sustaining viral synthesis *in vivo* in the dedifferentiated leafy-shoots. It is, however, interesting to note that kinetin is inhibitory to viral infectivity when used at 30 ppm and applied at the site of infection. At other doses and with other methods of application, it stimulates the local lesion formation (Mukherjee *et al.*, 1967). Milo and Sahai Srivastava (1969) working with TMV infected callus cells originated from pith tissues, reported increase in virus contents in the tissues grown on high cytokinin doses but strong inhibition of the virus multiplication when low doses of cytokinin were used. They also indicated that the concentration of cytokinins (low doses) inducing profuse growth and rapid multiplication of callus cells alone restricted the movement of the virus thereby reducing the infectivity of the cells.

The lower concentration of virus in the callus cells has been explained by many earlier workers. Augier de Montgremier and Morel (1948) considered that the concentration of the virus in callus cultures is affected by rapidity of tissue proliferation. Kassanis (1956) attributed it to the low

protein contents of the callus cells. Hensen and Hildebrandt (1966) assayed individual cells of different tobaccoes and found that only 40 per cent of the cells of the callus cultures contained virus. These reports imply that there is unequal distribution of virus in constituent cells of the actively proliferating normal callus cultures. This is further confirmed by the present observations wherein some of the leafy-shoots, developed on dedifferentiation of undifferentiated callus cultures, were virus-free. These leafy-shoots presumably developed from the callus cells, which were virus-free. Svobodova (1966) has also utilised this method for developing virus-free tobacco plants. In the present studies also the virus-free shoots developed into healthy tobacco plant-lets by subsequent rooting.

It was also observed during the present studies that the production of chlorophyll in the normal callus cultures as a result of their continued growth in light and induction of histogenetic dedifferentiation have no effect on the infectivity of these cultures.

These cultures were maintained in serial transfers at 4 weeks' interval and infectivity was checked periodically. The infectivity was gradually lost during subsequent transfers in the non-differentiated as well as dedifferentiated cultures. The loss of infectivity in dedifferentiated cultures, which have many leafy shoots is interesting as some of these when tested individually, indicated maximum infectivity.

The loss in infectivity may, therefore, be due to gradual elimination of such shoots in subsequent transfers due to uneven distribution of the virus or slow rate of the movement of the virus in the actively multiplying callus cultures. The infectivity of the tropaeolum callus cultures was reduced likewise rather sharply during subsequent transfers thereby indicating that the undifferentiated callus cultures do not support the virus multiplication. However, there have been many other examples of successful maintenance of viruses in tobacco callus cultures for a considerable period. Morel (1948) while working with the cultivation of tumuor induced by Agrobacterium tumefaciens (Smith and Dowsn.) Conn. obtained callus tissues of tobacco infected with TMV, PVX and PVY. These tissues remained infective when cultured serially. Earlier, White (1934) grew tomato root-tips infected with TMV and tomato aucuba mosaic virus and found that they remained infective when propogated serially. Augier de Montgremier et al. (1948) similarly, grew callus from tobacco plant infected with TMV and CMV and found these two viruses to retain their virulence for over a year. Raychaudhuri and Mishra (1962, 1965) succeeded in cultivating four different viruses, namely, TMV, SMV, PVX and ChMV in normal tobacco callus, and maintaining them for over two years. Reinert (1966) could also keep tobacco etch virus in tobacco callus for 6 months. On the other hand, there have been examples of some viruses which could not be maintained in the callus culture of their respective hosts. Reinert found that

infectivity of tobacco ring spot, tomato ring spot and cucumber mosaic virus decreased during subsequent subculturing of tobacco calli. The same has been reported with respect to cowpea mosaic virus infecting cowpea callus and sunflower mosaic virus infection tobacco callus (Mishra and Raychaudhuri, 1968; Phatak, 1968). The infectivity of these calli was reported to be lost during subsequent transfers.

From the results obtained during the present investigations, it is clear that the double tropaeolum virus cannot be maintained in the undifferentiated tropaeolum callus and undifferentiated and dedifferentiated calli of turkish tobacco plants. It appears that the dedifferentiated cultures have better prospects for being used for maintaining the viruses in cultures of their respective hosts for a reasonably long duration. Undifferentiated callus cultures are unable to remain infective indefinitely at least with respect to some of the viruses.

SUMMARY

1. Double tropaeolum plants (*Tropaeolum majus* L.) exhibiting abnormal floral morphology leading to complete antholysis and suppression of sexual organogenesis, is grown as ornamental in and around Simla and is found to be invariably affected by a viral infection as evidenced by the association of disease syndrome like chlorotic and necrotic concentric rings, mottling, curling and puckering of the leaves. The bright scarlet colour of the antholysed flowers shows breaking which gives it a distinctive position as one of the choicest collection of ornamental interest.

2. Detailed investigations were undertaken to identify the causal virus which was found to be sap-inoculable although with difficulty and only when mixed with celite and a reducing chemical like Na_2SO_3.

3. The virus was transmitted through aphids, viz. *Aphis gossypii*, *A. craccivora* and *Myzus persicae* to tropaeolum and turkish tobacco plants in a non-persistant manner from tropaeolum plants. The aphid *A. craccivora* was found to be an additional vector not reported so far for this virus. However, the aphids were unable to pick up the virus from the tobacco. From tobacco the virus was not transmitted through aphid but was readily transmissible through soil and there was strong indications of its being transmitted through nematodes like *Xiphinema americanum* as well.

4. The virus was found to have thermal-inactivation between 60 to 62°C, dilution-end point between 10^{-4} to 15×10^{-3} longevity *in vitro* at 19-27°C between 48 and 75 hrs and at 8-10°C between 168 and 240 hrs. The optimum pH for infectivity was 6.0. The infectivity was maintained in dried leaves at 4-7°C for more than 11 months.

5. Host-range studies of the virus indicated susceptibility of a wide range of plant species belonging to the families: Cucurbitaceae, Moraceae, Umbelliferae, Oxalidaceae, Labiatae, Violaceae, Balsaminaceae, Chenopodiaceae, Amaranthaceae, Compositae, Leguminosae, Polemoniaceae, Ranunculaceae, Scrophulariaceae, Solanaceae, Tropaeolaceae and Verbenaceae. Different hosts showed different degree of susceptibility and produced symptoms varying from minute pin-point necrotic lesions, chlorotic rings, systemic mosaic mottling oak leaf pattern to systemic necrosis causing ultimate death. Statistical analysis of these families, divided into five categories depending on maximum disease reaction, indicated random distribution of susceptibility in different families of Angiosperms. However, out of the families mentioned above, the last nine included large number of most susceptible plant species. The families, like Solanaceae and Leguminosae, were found to have maximum number of host species showing highest degree of susceptibility. In the system of evolution as suggested by Hutchinson in his book 'The families of flowering plants, the families with susceptible plant species are irregularly distributed

indicating thereby that susceptibility cannot be correlated with the evolutionary trend in Angiosperms.

6. Comparing the host-range of this virus with those of tobacco ring spot virus (TRSV) and tropaeolum ring spot virus (NRSV), Phaseolus vulgaris and Datura stramonium could be considered as differential hosts as they gave different reactions with respect to all the three viruses. Rest of the nineteen common hosts were having similar reactions with either of the two viruses. Statistically, the 'mean host reaction' of the virus was not significantly different from NRSV and TRSV indicating a close relationship.

7. Fluorescing inclusions were observed in virus affected tropaeolum leaves, indicating their RNA nature. Their RNA nature was further confirmed by disappearance of these inclusions bodies from infected leaves when treated with RNAse.

8. The virus was purified by various methods. Hollings' butanol method using 0.5M phosphate buffer was found to be most satisfactory and yielded preparations with polyhedral (Hexagonal) particles of 29 nm average diameter. The purified virus preparation was resolved into three components on sucrose gradient. Ultra-violet absorption of bottom component was in consistence with the nucleoprotein absorbency giving high value of 260/280 ratio. The average value of 260/280 nm ratio of the purified virus preparation was 1.71, indicating high proportion of RNA, which was calculated to be 36.91 per cent,

based on the extinction values at 260 and 280 nm. The other components especially the top one, were deficient in RNA.

9. Positive precipitin-reactions and gel-diffusion tests with antisera of TRSV obtained from Lisse and Wageningen indicated closer relationship of the two viruses.

10. Considering similarities in symptom expression, host-range, physical properties, structure and function of the virus particles and serological relationship, the virus is considered to be closer to TRSV. However, efficiency of aphid vectors in the transmission of DNRSV as against TRSV, as also the differences in some of the individual host reactions suggests that DNRSV could be a strain of TRSV affecting tropaeolum plants and transmitted by aphids.

11. The petals of the double tropaeolum flowers were highly infectious. The infectivity was altogether absent in the petals of the flowers of infected single tropaeolum plants and very much reduced in the leaves of single and double tropaeolum plants, probably due to the presence of phenolic compounds which inhibited the infectivity. The inhibitory effect was neutralised by soaking the leaves in reducing chemicals like Na_2SO_3, caffeic acid or EDTA. Coumarin and sodium salicylate were found to inhibit the infectivity of the virus <u>in vitro</u>.

12. A comparison of different parts of virus infected tobacco plants indicated higher infectivity of the leaves

showing systemic disease syndrome than the ones actually inoculated. The roots were also highly infectious. The infectivity of the inoculated tobacco plants was maximum around 9th day of inoculation.

13. Callus cultures were obtained from tropaeolum tissues, especially meristems and very young leaves on the media incorporated with IAA at 2.0 mg/l and kinetin at 0.4 mg/l and other normal requirements like myoinositol at 100 mg/l and adenine 5 mg/l. These callus cultures remained actively growing for over one and half years. However, infectivity of these tissues was quickly lost during subsequent transfers. With respect to turkish tobacco and N. glutinosa explants also, the infectivity was lost in the callus cultures during subsequent transfers. The leaf-callus was found to be more infective than the callus developed from stem. The dedifferentiated cultures were having infectious as well as non-infectious shoots, but the infectivity of these cultures, as a whole, was more than that of the undifferentiated callus cultures. The infectivity of even these cultures was gradually lost during subsequent transfers. The histogenetic dedifferentiation had no effect on the infectivity. Production of chlorophyll in the callus as evidenced by the formation of green callus as a result of continuous light also had no effect on the infectivity of callus cultures.

14. Meristems of tropaeolum grew well on the medium with reduced amount of auxin and increased amounts of kinetin.

Incorporation of adenine at 7.0 mg/l and myoinositol at 100 mg/l, induced strong caulogenic growth and proliferating meristem cultures. This technique is useful for developing many plants from a single meristem. The meristem-tips require anaerobic conditions for induction of growth, as they grew only when immersed into the medium.

REFERENCES

Allard, H.A. 1914. The mosaic disease of tobacco. U.S. Dept. Agric. Bull. No. 40, 33 pp.

Allard, H.A. 1918. The mosaic disease of Phytolacca decandra. Phytopathology, 8: 51-54.

Anderson, C.W. 1954. The Aster ringspot virosis of Central Florida. Phytopathology, 44: 87-92.

Augier de Montgremier, H.P. and G. Morel 1948. Sur la diminution de la teneur en virus (Marmor tabaci Holmes) de tissus de tabac cultives in vitro. C.R. Acad.Sci., 227: 688-89.

Augier de Montgremier, H.P., P. Limasset and G. Morel 1948. Sur le mainten d'une maladie a virus complex dans des tissus de tabac cultives in vitro. C.R. Acad. Sci., 227: 606-608.

Bald, J.G. and G. Samuel 1934. Some factors affecting the inactivation rate of the virus of tomato spotted wilt. Ann. appl. Biol.,21: 179-90.

Ball, E. 1946. Development in sterile culture of stem-tips and subjacent regions of Tropaeolum majus L. and Lupinus albus L. Am. J. Bot., 33: 301-18.

Bawden, F.C. and H.L. Nixon 1951. The application of electron microscopy to the study of plant viruses in unpurified plant extracts. J. gen. Microbiol., 5: 104-109.

Bawden, F.C. and N.W. Pirie 1957. A virus-inactivating system from tobacco leaves. J. gen. Microbiol. 16: 696-710.

Ben-Jaacov, J. and R.W. Langhans 1970. Symposium Production of Healthy Plants by Therapeutic and other methods and their maintenance and use. 8th Int. Hort. Congr., Tel Aviv, Israel.

Bergeson, G.B., K.L. Athow, F.A. Laviolette and Sister Mary Thomasine 1964. Transmission, movement and vector relationships of tobacco ringspot virus in soybean. Phytopathology,54: 723-28.

Bhargava, K.S. and R.D. Joshi 1959. Observations on a virus disease of garden Nasturtium (Tropaeolum majus L.) occurring in Nainital. Jour. Indian Bot. Soc., 38: 379-82.

Bisht, N.S. 1962. Aphis gossypii Glov. as vector of Nasturtium ringspot virus. Curr. Sci., 31: 23-24.

Bos, L. 1957. Heksenbezemverschijnseleneen pathologisch-morfologisch onderzoek Medel. Landbh ogesch Wageningen, 57: 1-79.

Bos, L. 1969. Inclusion bodies of bean yellow mosaic virus, some less known closely related viruses and beet mosaic virus. Neth. J. Pl. Path., 75: 137-43.

Brierley, P. 1954. Symptoms in the florists' Hydrangea caused by tomato ringspot virus and an unidentified sap-transmissible virus. Phytopathology, 44: 696-99.

Buys, C. 1968. La culture de meristems et son application commerciale en vue de L'obtention de plants sains d'veillets. Collogue dans' Les Nonveautes recentes dans la multiplication des plants'. Gambloux Nov. 1968, p.71.

Corbett, M.K. and D.A. Roberts 1962. A rapid method for purifying tobacco ringspot virus and its morphology as determined by electron microscopy and negative staining. Phytopathology, 52: 902-905.

Desjardins, P.R., R.L..Latterell and J.E. Mitchell 1954. Seed transmission of tobacco ring-spot virus in Lincoln variety of soybean. Phytopathology, 44: 86.

Desjardins, P.R., C.A. Senseney and G.E. Hess 1953. Further studies on the electron microscopy of purified tobacco ringspot virus. Phytopathology, 43: 687-90.

Doolittle, S.P. and M.N. Walker 1925. Further studies on the overwintering and dissemination of cucurbit mosaic. J. agric. Res., 31: 1-58.

Dunleavy, J.M. 1957. The grass-hopper as a vector of tobacco ringspot virus in soybean. Phytopathology 47: 681-82.

Fenne, S.B. 1931. Field studies of Burley tobacco in Washington County, Virginia. Phytopathology, 21: 891-99.

Follmann, G. 1961. Hitzereaktivierung und Konzentrationsverhaltnisse von Ringfleckenviren der Tabakgruppe. Phytopath. Z., 41: 79-88.

Fromme, F.D., S.A. Wingard and C.N. Priode 1927. Ringspot of tobacco : an infectious disease of unknown cause. Phytopathology, 17: 321-28.

Fulton, J.P. 1962. Transmission of tobacco ringspot virus by Xiphinema americanum. Phytopathology, 52: 375.

Fulton, R.W. 1948. Hosts of tobacco streak virus. Phytopathology, 38: 421-28.

Fulton, R.W. 1949. Virus concentration in plants acquiring tolerance to tobacco streak. Phytopathology, 39: 231-43.

Fulton, R.W. 1952. Mechanical transmission and properties of rose mosaic virus. Phytopathology, 42: 413-16.

Gardner, M.W. and O.C. Whipple 1934. Spotted wilt of tomatoes and its transmission by thrips. Phytopathology, 24:1136.

Gautheret, R.J. 1966. Factors affecting differentiation of plant tissues grown in vitro. In Cell differentiation and morphogenesis. North. Holland Publishing Comp. Amsterdam, pp.55.

Gibbs, A., B.D. Harrison, D.H. Watson and P. Wildy 1966. What's in a virus name? Nature (Lond.), 209: 450-54.

Gooding, G.V.(Jr.) and F.A. Todd 1967. Virus diseases of burley tobacco in North Carolina. Plant Dis. Reptr., 51: 409.

Gupta, G.R.P., S. Guha and S.C. Maheshwari 1966. Differentiation of buds from leaves of Nicotiana tabacum Linn. in sterile culture. Phytomorphology, 16: 175-82.

Hampton, R.E. and R.W. Fulton 1961. The relation of polyphenol oxidase to instability in vitro of Prune dwarf and sour cherry necrotic ringspot viruses. Virology, 13: 44-52.

Henderson, R.G. 1931. Transmission of tobacco ringspot by seed of Petunia. Phytopathology, 21: 225-29.

Hensen, A.J. and A.C. Hildebrandt 1966. The distribution of tobacco mosaic virus in plant callus cultures. Virology, 28: 15-21.

Hildebrandt, A.C. 1962. Tissue and single cell cultures of higher plants as a basic experimental method. In 'Moderne Methoden Pflanzenanalyse' (edited by H.F. Linskens and M.V. Tracey), Springer-Verlag, Berlin.

Hollings, M. 1955. Investigation of chrysanthemum viruses 1. Aspermy flower distortion. Ann. appl. Biol., 43: 102.

Hollings, M. 1965. Anemone necrosis, a disease caused by a strain of tobacco ringspot virus. Ann. appl. Biol., 55: 447-57.

Holmes, F.O. 1938. Taxonomic relationships of plants susceptible to infection by tobacco mosaic virus. Phytopathology, 28: 58-66.

Holmes, F.O. 1946. A comparison of experimental host-range of tobacco etch and tobacco mosaic viruses. Phytopathology, 36: 643-59.

Hooker, W.J. and A.S. Summanwar 1964. Intracellular acridine orange fluorescence in plant virus infections. Exptl. Cell Res. 33: 609-12.

Hutchinson, J. 1959. The families of flowering plants. Vol. I, Dicotyledons, 2nd Ed. Clerendon Press, Oxford.

Jensen, D.D. 1950. Nasturtium mosaic, a virus disease of Tropaeolum majus L. in California. Phytopathology, 40: 967.

Johnson, J. and R.W. Fulton 1942. The broad ringspot virus. Phytopathology, 32: 605-12.

Kassanis, B. 1957. The multiplication of tobacco mosaic virus in cultures of tumorous tobacco tissue. Virology, 4: 5-13.

Kassanis, B. 1967. Plant tissue culture. Methods in Virology Vol. I. Acad. Press, pp. 537-66.

Linsmaier, E.M. and F. Skoog 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plantarum, 18: 100-27.

Martyn, E.B. 1968. Plant virus names: an annotated list of names and synonyms of plant viruses and diseases. C.M.I., Kew, England.

Mathur, S.B., M.D. Mishra and V.P. Tewari 1966. A new strain of tobacco mosaic virus affecting chilli pepper variety Puri Orange. Plant Dis. Reptr., 50: 619-22.

McGuire, J.M. 1964a. Efficiency of Xiphinema americanum as a vector of tobacco ringspot virus. Phytopathology, 54: 799-801.

McGuire, J.M. 1964b. Serial transfer of Xiphinema americanum as a tool for studying transmission of tobacco ringspot virus. Phytopathology, 54: 900.

McWhorter, F.P. 1953. The utility of flower tissues for making inocula for difficult virus isolations. Phytopathology, 43: 479.

Milbrath, J.A. 1953. Transmission of components of the stone-fruit latent virus complex to cowpea and cucumber from cherry flower petals. Phytopathology, 43: 479.

Miller, C.O. 1963. Kinetin and kinin like compounds. In 'Moderne Methoden Pflanzenanalyse' (edited by H.F. Linsken and M.V. Tracey), Springer-Verlag, Berlin, p.194.

Milo, G.E. and B.I. Sahai-Srivastava 1969. Effect of cytokinins on tobacco mosaic virus production in tobacco pith tissue and cultures. Virology, 39: 621-23.

Mishra, M.D. and S.P. Raychaudhuri 1967. Possibilities of a type-culture collection of plant viruses in tissue culture. Proc. 8th An. Congr. Assoc. Microbiol. India, p. 8.

Mishra, M.D. and S.P. Raychaudhuri 1968. Effect of morphogenesis on the infectivity of virus affected callus cultures. Proc. Seminar Plant Morphogenesis, Dept. Botany, Delhi Univ., Delhi, 49-52.

Mishra, M.D., S.P. Raychaudhuri and K. J. Chandra 1967. Detecting viral infection in seeds through embryo culture. Proc. Internl. Seed Testing Assoc., 32: 617-24.

Mishra, M.D., S.P. Raychaudhuri and H.C. Phatak 1964. Plant tissue culture - a tool for studying host-virus relationship. Bull. Ind. Phytopath Soc. 2: 18-34.

Morel, G. 1948. Recherches sur la culture associee de parasites obligatoires et de tissus vegetaux. Ann. epiphyt. n.s.,14: 123-34.

*Moriondo, F. 1958. Nuove probabili virosi di piante ornamentali (New probable viruses of ornamental plants). Reprinted from Riv. Ortoflorofruttic ital. 43: 5-6.

Mukherjee, A.K., L.C. Soans and M. Chessin 1967. Effect of kinetin and actinomycin D on the susceptibility of Nicotiana glutinosa L. to infection by tobacco mosaic virus. Nature (Lond.), 216: 1344-45.

Murashige, T. and F. Skoog 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum, 15: 473-97.

Osborne, D.J. 1962. Effect of kinetin on protein and nucleic acid metabolism in xanthium leaves during senescence. Plant Physiology, 37: 595.

Phatak, H.C. 1968. Studies on the effect of some chemicals and radiation on cowpea mosaic virus multiplication. Ph.D. thesis, University of Indore.

Pillai, S.K. and A.C. Hildebrandt 1969. Induced differentiation of geranium plants from undifferentiated callus in vitro. Am. J. Bot., 56: 52-58.

Pittman, H.A. 1934. Virus diseases of plants with particular reference to the spotted or bronzy wilt disease of tomatoes. J. Dept. Agric. W. Austr. Ser. 2, 11: 123-40.

Price, W.C. 1932. Acquired immunity to ringspot in Nicotiana. Contr. . Boyce Thompson Inst. 4: 359-403.

Price, W.C. 1936a. Virus concentration in relation to acquired immunity from tobacco ringspot. Phytopathology, 26: 503-509.

Price, W.C. 1936b. Specificity of acquired immunity from tobacco ringspot diseases. Phytopathology, 26: 665-67.

Price, W.C. 1940. Comparative host ranges of six plant viruses. Am. J. Bot., 27: 530-41.

Priode, C.N. 1928. Further studies in the ringspot disease of tobacco. Am. J. Bot., 15: 88-93.

Quak, F. 1957. Meristeemcultuur, gecombineerd met warmte-behandling voor het verkrijgen van virusvrije anjerplanten. Tijdschr. PlZieht, 63: 13-14.

Raychaudhuri, S.P. 1966. Plant viruses in tissue culture. Adv. Virus Res., 12: 175-206.

Raychaudhuri, S.P. and M.D. Mishra 1962. Cultivation of a mosaic virus isolated from chilli. Indian Phytopath. 15: 185-87.

Raychaudhuri, S.P. and M.D. Mishra 1965. Cultivation of some plant viruses in callus tissue cultures. Indian Phytopath. 18: 50-53.

Reinert, R.A. 1966. Virus activity and growth of infected and healthy callus tissue of Nicotiana tabacum grown in vitro. Phytopathology 56: 731-33.

*Rubio-Huertos, M. 1950. Estudios sobre inclusiones intracellulares prioducidas por virus en las plantas. Microbiologia esp. 3: 207-32.

Sauer, N.I. 1966. Simultaneous association of strains of tobacco ringspot virus within Xiphinema americanum. Phytopathology, 56: 862-63.

Schmelzer, K. 1960. Untersuchungen uber das Ringmosaik-virus der Kapuzinerkresse. Z. Pflkrankh, 67: 193-210.

Schneider, I.R. and T.O. Diener 1966. The correlation between the proportions of virus-related products and the infectious component during the synthesis of tobacco ringspot virus. Virology, 29: 92-99.

Schuster, M.F. 1963. Flea beetle transmission of tobacco ringspot virus in the Lower Rio Grande Valley. Plant Dis. Reptr., 47: 510-11.

Serjeant, E.P. 1967. Some properties of cocksfoot mottle virus. Ann. appl. Biol.,59: 31-38.

Severin, H.H.P. and J.H. Freitag 1933. List of ornamental flowering plants naturally infected with curly top or yellows diseases in California. Plant Dis. Reptr. 17: 1-2.

Severin, H.H.P. and J.H. Freitag 1945. Additional ornamental flowering plants naturally infected with California aster yellows. Hilgardia . . . 16: 599-618.

Shyama Rani, H.N. Verma and G.S. Verma 1969. A virus disease of Petunia hybrida. Plant Dis. Reptr. 53: 903-907.

Silberschmidt, K. 1953. Studies on a mosaic of nasturtium occurring in Brazil. Phytopathology, 43: 304-308.

Skoog, F. and C. Miller 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Soc. Exptl. Biol., 11: 118 - 31.

van Slogteren, D.H.M. 1955. Serological micro-reactions with plant viruses under paraffin oil. Proc. II Conf. Potato Virus Diseases Lisse-Wageningen.

Smith, F.F. and P. Brierley 1955. Aphid transmission of tobacco ringspot virus in gladiolus. Plant Dis. Reptr. 39: 35.

Smith, K.M. 1946. Tomato black ring: a new virus disease. Parasitology, 37: 126-30.

Smith, K.M. 1949a. A new virus disease of tropaeolum and other plants. Gard.Chron., 125: 160.

Smith, K.M. 1949b. Viruses and virus diseases. Jour. Roy. Hort.Soc., 74: 482-91, 521-28.

Smith, K.M. 1950. Some new virus diseases of ornamental plants. Jour. Roy. Hort. Soc., 75: 350-53.

Smith, K.M. 1952. Some garden plants susceptible to infection with cucumber mosaic virus. Jour. Roy. Hort. Soc., 77: 19-21.

Smith, K.M. 1955. Some recent work on plant viruses. Sci. Hort., 11: 98-103.

Smith, K.M. 1957. A text book of plant virus diseases. 2nd Ed. Little, Brown & Company, Boston.

Snedecor, G.W. 1965. Statistical methods: The Iowa State University Press, Ames. Iowa, U.S.A.

Stace Smith, R. 1965. A simple apparatus for preparing sucrose density gradient tubes. Phytopathology, 55: 1031.

Stace Smith, R. 1966. Purification and properties of tomato ringspot virus and an RNA deficient component. Virology, 29: 240-47.

Stace Smith, R., M.E. Reichmann and N.S. Wright 1965. Purification and properties of tobacco ringspot virus and two RNA deficient components. Virology, 25: 487-94.

Stanley, W.M. 1939. The isolation and properties of tobacco ringspot virus. Jour. Biol. Chem. 129: 405-28.

Stanley, W.M.and R.W.G. Wyckoff 1937. The isolation of tobacco ringspot and other virus proteins by ultra-centrifugation. Science, 85: 181-83.

Steere, R.L. 1956. Purification and properties of tobacco ringspot virus. Phytopathology, 46: 60-69.

Steeves, T.A., H.P. Gabriel and M.W. Steeves 1957. Growth in sterile culture of excised leaves of flowering plants. Science, 126: 350-51.

Stubbs, L.L. 1960. Aphid transmission of broad bean wilt virus and comparative transmission efficiency of three vector species. Austr. J. agric. Res. 2: 734-41.

Svobodova, J. 1966. Elimination of virus by means of callus tissue cultures. In 'Viruses of Plants' (edited by A.B.R. Beemster and J. Dijkstra), John Wiley & Sons, New York.

Teliz, D. 1967. Effects of nematode extraction method, soil mixture, and nematode numbers on the transmission of tobacco ringspot virus by Xiphinema americanum. Nematologica 13: 177-85.

Thomas, C.E. 1969. Transmission of tobacco ringspot virus by Tetranychus sp. Phytopathology, 59: 633-36.

Tremaine, J.H., W.R. Allen and R.S. Willison 1964. The extraction of viruses from fruit tree petals. Plant Dis. Reptr., 48: 82-85.

Valleau, W.D. 1932. Seed transmission and sterility studies of two strains of tobacco ringspot. Kentucky Agric. Exp. Sta. Bull. 327: 43-80.

Valleau, W.D. 1941. Experimental production of symptoms in so-called recovered ringspot tobacco plants and its bearing on acquired immunity. Phytopathology, 31: 522-33.

Valleau, W.D. 1951. Tobacco ringspot virus : the cause of eggplant yellows. Phytopathology, 41: 209-12.

White, P.R. 1934. Multiplication of the viruses of tobacco and aucuba mosaic virus in growing excised tomato root tips. Phytopathology, 24: 1003-11.

White, P.R. 1943. A hand book of plant tissue culture. Ronald Press Co. Lancaster.

Wingard, S.A. 1928. Hosts and symptoms of ringspot, a virus disease of plants. Jour. agric. Res., 37: 127-53.

Wood, M.W. 1933. Intracellular bodies associated with ringspot. Contr. Boyce Thomp. Inst., 5: 419-34.

*Original not seen.

...

APPENDIX I

Comparative host-range of TRSV, NRSV and DNRSV

Family	Botanical name	TRSV	DNRSV	NRSV
DICOTYLEDONS				
AMARANTHACEAE				
	Amaranthus caudatus L.	2	16	16
	A. paniculatus L.	2	-	-
	A. retroflexus L.	-	-	16
	A. gangeticus L.	-	8	-
	A. spinosus L.	-	2	-
	A. blitum L.	-	16	-
	A. leucarpus L.	-	4	-
	Gomphrena globosa L.	-	4	16
	Achyranthes aspera L.	-	0	-
APOCYANACEAE				
	Vinca rosea L.	8	0	-
BALSAMINACEAE				
	Impatiens balsamina L.	8	8	0
CARYOPHYLLACEAE				
	Dianthus plumerius L.	8	-	-
	D. barbatus L.	0	-	0
	D. caryophyllus L.	-	0	0
	D. chinensis L.	-	-	0
	Lychnis alba Mill.	8	-	-
	Silene orientalis Mill.	-	-	16
	Stellaria media (L.) Vill.	-	-	16
CHENOPODIACEAE				
	Beta vulgaris L.	4	2	16
	Chenopodium album L.	2	2	8
	C. foetidum Schrod.	-	-	8
	C. murale L.	-	8	-
	C. ambrasoides L.	-	2	0
	C. bromes-henericus L.	-	-	0
	C. quinoa Willd.	-	2	16
	C. amaranticolor Coste & Reyn.	2	2	-
	Spinacea oleracea L.	8	0	16

contd..

APPENDIX I CONTD.

Family	Botanical name	TRSV	DNRSV	NRSV
COMPOSITAE				
	Artemisia absinthium L.	-	-	0
	Ambrosia clatior L.	2	-	-
	Aster laevis L.	2	0	-
	Bidens discoides (T&G) Britton	4	0	-
	Calandula officinalis L.	16	0	-
	Callistephus chinensis Nees.	4	0	16
	Coreopsis tinctorius Nortt.	-	2	16
	Cosmos bipinnatus Cav.	-	-	16
	Centaurea moschata L.	8	-	-
	C. cyanus L.	-	0	0
	Chrysanthemum lucanthemum L.	-	-	0
	C. sp.	8	4	-
	Cichorium endivia L.	8	-	0
	Dimorphotheca aurantiaca DC.	-	-	16
	Erigeron canadensis L.			
	Helianthus annuus L.	8	2	-
	Lactuca sativa L.	8	0	-
	L. serriola L.	2	-	-
	Sonchus oleraceus L.	8	0	-
	S. arvensis L.	-	0	-
	Tagetes patula L.	-	-	0
	T. erecta L.	2	0	-
	T. signeta Berth.	-	-	0
	Zinnia elegans Jacq.	16	16	16
	Senecio cruentus DC.	-	0	-
	Dahlia hybrida Hort.	-	4	-
	Brachycombe iberidifolia Benth.	-	16	-
CRUCIFERAE				
	Brassica rapa L.	-	0	-
	Brassica oleracea L.			
	cv. Capitata L.	-	0	-
	cv. botrytis L.	-	0	-
	cv. caulorapa Pasq.	-	0	-
	B. campestris L.			
	var. Sarson	-	0	-
	B. pekinensis (Lur.)Rupr.	-	-	0
	Barbarea vulgaris R.Br.	2	-	0
	Erysimum allionii Grignan	-	-	0

Contd..

APPENDIX I CONTD.

Family	Botanical name	TRSV	DNRSV	NRSV
	Malconica maritima RBr	-	-	16
	Raphanus sativus L.	-	0	-
CUCURBITACEAE				
	Citrullus vulgaris Schrad.	8	0	-
	C. vulgaris Schrad. var. fistulosus (stocks) Duthie	8	0	-
	Cucumis sativus L.	16	0	-
	C. melo L.	16	0	-
	C. melo var. utilissimus Duthie & Fulton	-	0	-
	Cucurbita pepo L.	16	1	0
	C. moschata Duchesne	16	-	-
	Lagenaria leucantha Rusby	16	-	-
	L. siceraria Standl.	-	0	-
	Luffa cylindrica Roem	16	0	-
	L. acutangula Roxb.	-	0	-
	Momordica charantia L.	-	0	-
	Trichosanthes anguina L.	-	1	-
EUPHORBIACEAE				
	Euphorbia hirta L.	-	0	-
	Ricinus communis L.	8	0	-
LABIATEAE				
	Coleus blumei Benth.	8	0	-
	Lavandula obrotenoides Lam.	-	-	0
	Nepta cataria L.	-	-	16
	Ocimum basilicum L.	-	1	16
	O. canum Sims.	-	-	0
	O. sanctum L.	-	0	-
	Hyptis suaveolens Poit.	-	1	-
	Salvia splendens Kerr.	8	2	-
	S. terinaca Benth.	-	-	0
	Stachys grandiflora Benth.	-	-	0
	Physostegia viginiana Benth.	8	-	-
LEGUMINOSAE				
	Crotalaria juncea L.	-	2	-
	C. brownei Bert. ex DC.	-	16	-
	Dolichos lablab L.	8	16	-
	Glycine max Merr.	8	-	-
	Lathyrus odoratus (L.) DC.	4	0	-

contd..

APPENDIX I CONTD.

Family	Botanical name	TRSV	DNRSV	NRSV
	L. ochrus (L) DC.	-	-	16
	L. sativus L.	-	0	-
	Lupinus albus L.	8	-	-
	L. hartwegii Lindl.	-	1	-
	Cajanus cajan Spreng.	-	0	-
	Cyamopsis tetragonoloba (L) Taub.	-	1	-
	Arachis hypogaea L.	-	0	-
	Melilotus indicus (L) All.	-	16	4
	M. alba Desr.	16	16	-
	M. officinalis Lam.			
	Medicago sativa L.	-	-	0
	M. orbicularis (L) All.	-	-	4
	M. seutellata (L) All.	-	-	16
	M. terbinata Willd.	-	-	0
	Phaseolus angularis Wright	8	-	-
	P. aureus Roxb.			
	P. calcaratus Roxb.	8	-	-
	P. limensis Macf.	2	-	-
	P. lathyroides L.	-	16	-
	P. multiflora Willd.	-	16	-
	P. lunatus L.	2	0	-
	P. mungo L.	8	0	-
	P. radiatus L.	-	0	-
	P. vulgaris L.	8	16	2
	Pisum sativum L.	8	0	-
	Stizolobium sp. Vog.	8	-	0
	Trigonella foenum-graecum L.	-	16	-
	T. caerules Ser. in DC.	-	-	16
	T. corniculata L.	-	16	-
	Trifolium incarnatum L.	16	-	-
	T. alexandrinum L.	-	16	-
	T. pratense L.	0	0	-
	T. repens L.	0	-	-
	Vicia faba L.	8	16	16
	V. sativa L.	8	-	-
	V. articulata Hornem.	-	-	0
	Vigna sinensis Savi	2	16	16
	V. sesquipedalis (L.)Fruwirth	8	-	-
MALVACEAE				
	Abelmoschus esculentus W&A.	2	0	-
	Hibiscus manihot.L.	-	0	-
	Althaea rosea L.	0	0	-
	Lavatera trimestris L.	8	-	-
	Malva neglecta Wallr.	-	-	0

contd..

APPENDIX I CONTD.

Family	Botanical name	TRSV	DNRSV	NRSV
MORACEAE				
	Ficus carica L.	-	-	0
	F. religiosa L.	-	1	-
ONAGRACEAE				
	Clarkia elegans Dougl	8	0	-
	Godetia amoena Lilja	8	-	-
	Oenthera elutei A. Nelson	-	-	0
Oxalidaceae				
	Oxalis corniculata L.	0	2	-
PAPAVARACEAE				
	Eschscholtzia californica Cham.	8	0	-
	Papaver orientale L.	0	0	0
	P. glaucum Boyce ex. Helder	-	-	0
POLEMONIACEAE				
	Gilia capitata Dougl.	8	-	-
	Phlox drummondii Hook.	0	16	-
	Polemonium coeruleum L.	-	-	0
POLYGONACEAE				
	Polygonum hydropiper L.	2	-	-
	Rumex crispus L.	8	-	0
	R. acetosa L.	-	0	0
	Rheum rhaponticum L.	8	-	-
PORTULACACEAE				
	Portulaca grandiflora Hook.	8	-	-
	P. oleracea L.	-	0	0
PEDALIACEAE				
	Sesamum indicum L.	0	0	-
RANUNCULACEAE				
	Aquilegia caerulea James	8	-	-
	Delphinium cultorum Vass.	0	16	16
	Adonis aestivalis L.	-	-	16

Contd...

APPENDIX I CONTD.

Family	Botanical name	TRSV	DNRSV	NRSV
SCROPHULARIACEAE				
	Antirrhinum majus L.	2	4	0
	Linaria masedomica Griseb.	8	-	-
	L. bipartita Willd.	-	16	16
	Collinsia bicolor Berth.	-	-	16
	Digitalis alpinus L.	-	-	16
	Mimulus moschatus Doughl.	8	-	-
	M. guttatus DC.	-	-	16
	Nemesia strumosa Benth.	8	16	-
	Verbascum phoeniceum L.	8	-	-
	V. thapsiforme Schrad.	-	-	2
	Veronica longifolia L.	8	-	-
	V. vicana L.	-	-	0
	Digitalis grandiflora Lam.	-	-	0
	D. purpurea L.	-	-	0
SOLONACEAE				
	Capsicum annuum L.	-	16	4
	C. frutescens L.	0	16	-
	Datura stramonium L.	8	16	2
	D. metel L.	8	16	-
	D. tatula L.	-	16	-
	Hyoscyamus albus L.	8	-	-
	H. niger L.	8	-	-
	Lycopersicon esculentum Mill.	2	16	16
	L. glandulosum C.H. Mull	-	16	-
	L. hirsutum Hamp et. Bompl.	-	-	0
	L. pimpinellifolium (Jusl) Mill.	-	16	16
	Nicandra physaloides (L)	2	16	16
	Nicotiana acquminata Grah.	2	-	-
	N. clevelandii A. Gray	8	-	-
	N. glauca Grah.	-	16	-
	N. glutinosa L.	8	16	2
	N. longsdorffii Weinn.	8	8	-
	N. longiflora Cav.	8	4	-
	N. paniculata L.	8	-	-
	N. plumbeginifolia Viv.	8	-	-
	N. quadrivalvis Pursh.	8	-	-
	N. repanda Willd.	8	16	-
	N. rustica L.	16	16	16
	N. rustica var. Moti	-	16	-
	N. sanderae W. Wats.	8	-	-
	N. suaveolens Lehm.	8	-	-

Contd...

APPENDIX I CONTD.

Family	Botanical name	TRSV	DNRSV	NRSV
	N. sylvestris Spegaz. & Commes	8	16	16
	N. tabacum L.	16	-	16
	N. tabacum var. White burley	-	16	-
	N. tabacum var. Harrison's Special	-	16	-
	N. tabacum var. xanthi	16	16	-
	N. tomentosa Reiz & Pav.	4	-	-
	N. trigonophylla Donn.	8	-	-
	N. debneyi Domin.	-	8	-
	N. multivalvis	8	-	-
	Petunia hybrida Vilm.	16	8	16
	Physalis angulata L.	8	16	16
	P. peruviana L.	2	16	-
	P. floridana Rydb.	-	16	16
	P. ixocarpa Brot.	-	16	-
	Solanum carolinense L.	8	-	-
	S. melongena L.	16	16	-
	S. nigrum L.	2	16	-
	S. nodiflorum L.	2	16	-
	S. capsicastrum L.	-	16	-
	S. xanthocarpum Schrad. & Wendl.	-	0	-
	S. sisymbrifolium Lam.	-	-	16
	S. khasianum Clarke	-	2	-
	S. pseudocapsicum L.	4	-	-
	S. stoloniferum Schlecht & Bonche	-	0	-
	S. citrolifolium Willd ex. Roem.	-	0	-
	S. tuberosum L.	2	1	-
	Salpiglossis sinuata Ruiz & Pav.	8	-	-
TROPAEOLACEAE				
	Tropaeolum majus L.(single)	16	16	16
	T. majus (double) L.	-	16	-
	T. peregrinum L.	16	-	-
UMBELLIFERAE				
	Anthriscus cerefolium Hoffm.	8	-	0
	Apium graveolens L.	-	1	0

contd...

APPENDIX I CONTD.

Family	Botanical name	TRSV	DNRSV	NRSV
	Daucus carota L. var. sativa DC.	0	0	-
	Trachymene caerulea R. Grah.	8	-	-
VERBENACEAE				
	Lantana camara L.	0	0	-
	Verbena venosa Gill & Hook.	16	-	-
	V. officinalis L.	-	-	16
	V. hybrida Viss.	-	16	-
VIOLACEAE				
	Viola cornuta L.	2	-	-
	V. tricolor L.	2	-	-
	V. tricolor var. Hortensis	-	2	-
MONOCOTYLEDONS				
GRAMINEAE				
	Triticum vulgare Vill.	-	0	-
	Oryza sativa L.	-	0	-
IRIDACEAE				
	Gladiolus sp. (Tourn.) L.	-	0	-
	Iris xiphium L.	-	0	-
	Mesembryanthemum Dill. ex L.	-	0	-

Note: - represents plant species not tested for the particular virus, whereas 0 indicates immune reaction. Numbers from 1 to 16 indicate different degrees of susceptibility (Susceptibility indices).

APPENDIX II. Recipes of various media used in the tissue culture studies

White (1934, 43)

Chemicals	Modifications											
	A	B_1	B_2	C_1	C_2	C_3	C_4	D_1	D_2	D_3	E	F
$Ca(NO_3)_2$	300mg	300mg	300mg	300mg	300mg	300mg	300mg	300mg	300mg	300mg	300mg	300mg
NaH_2PO_4	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16,5	16.5	16.5	16.5	16.5
KNO_3	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0
$NaSO_4$	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
KCl	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0
$MgSO_4 7H_2O$	720.0	720.0	720.0	720.0	720.0	720.0	720.0	720.0	720.0	720.0	720.0	720.0
$MnSO_4 4H_2O$	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
$ZnSO_4$	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
H_3BO_3	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
$Fe_2(SO_4)_3$	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
$CuSO_4 5H_2O$	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
$Na_2MoO_4 2H_2O$	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Glycine	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Nicotinic acid	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	0.5
Thiamine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.1
Pyredoxine	1.0	1..0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.1
Biotin	2.0	2.0	2.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Calcium pentothenate	10.0	10.0	10.0	5.0	0.5	0.5	0.5	5.0	0.5	5. 0	5.0	0.5
Inositol	-	-	-	-	-	-	-	0.1	0.5	0.5	0.5	0.5
Adenine	-	-	-	-	-	-	-	-	-	-	-	-
NAA	2 .0	2.0	2.0	1.0	0.5	1.0	1.5	1.5	1.5	1.5	1.5	1.5
Kinetin	-	-	-	1.0	0.2	0.2	0.2	1.0	1.0	1.0	1.0	0.5
2,4-D	-	-	-	-	-	-	-	-	-	-	-	2.0
Caseinhydrolysate	-	1.0gm	2.0gm	-	-	-	-	20.0	200.0	400.0	200.0	200.0
Coconut milk	-	-	-	-	-	-	-	-	-	-	150 ml	150 ml
Cotyledon extract	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	20.0gm	20.0gm	20.0gm	20.0gm	20.0gm	20.0gm	20.0gm	20.0gm	20.0gm	20.0gm	20.0gm	20.0gm
Agar	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0

Contd..

APPENDIX II contd.

(Quak, 1957)

Chemicals	Modifications		
	K	L	O
$Ca(NO_3)_2 4H_2O$	500 mg	500 mg	500 mg
KNO_3	125	125	125
$MgSO_4 7H_2O$	125	125	125
KH_2PO_4	125	125	125
$MnSO_4 4H_2O$	1.0	1.0	1.0
$NiCl_2$	0.0025	0.0025	0.0025
H_2SO_4	Traces	Traces	Traces
$CoCl_2$	0.0025	0.0025	0.0025
$ZnSO_4$	0.005	0.005	0.005
$CuSO_4$	0.005	0.005	0.005
$Fe_2(SO_4)_3$	0.0025	0.0025	0.0025
Kl	2.5	2.5	2.5
Nicotinamide	0.01	0.01	0.01
Pyredoxine	0.01	0.01	0.01
Thiamine	0.01	0.01	0.01
Biotin	0.1	0.1	0.1
Cystein	1.0	1.0	1.0
Calcium pantothenate	0.01	0.01	0.01
Inositol	1.0	1.0	1.0
Adenine	5.0	5.0	5.0
NAA	1.0	1.0	1.0
2,4-D	-	2.0	-
G.A.	-	-	1.0
Kinetin	0.5	0.5	0.5
Caseinhydrolysate	200	200	200
Coconut milk	-	100	-
Sucrose	20 gm	20 gm	20 gm
Agar	8	8	8

Contd..

APPENDIX II CONTD.

(Quak, 1957)

Chemicals	Modifications				
	K	K_2	K_3	K_4	K_5
$Ca(NO_3)_2$	500 mg	500 mg	500 mg	500 mg	500 mg
KNO_3	125	125	125	125	125
$MgSO_4 7H_2O$	125	125	125	125	125
KH_2PO_4	125	125	125	125	125
$MnSO_4 4H_2O$	1.0	1.0	1.0	1.0	1.0
$NiCl_2$	0.0025	0.0025	0.0025	0.0025	0.0025
H_2SO_4	Traces	Traces	Traces	Traces	Traces
$CoCl_2$	0.0025	0.0025	0.0025	0.0025	0.0025
$ZnSO_4$	0.005	0.005	0.005	0.005	0.005
$CuSO_4$	0.005	0.005	0.005	0.005	0.005
$Fe_2(SO_4)_3$	0.0025	0.0025	0.0025	0.0025	0.0025
KI	2.5	2.5	2.5	2.5	2.5
Nicotinamide	0.01	0.01	0.01	0.01	0.01
Pyredoxine	0.01	0.01	0.01	0.01	0.01
Thiamine	0.01	0.01	0.01	0.01	0.01
Biotin	0.1	0.1	0.1	0.1	0.1
Cystein	1.0	1.0	1.0	1.0	1.0
Calciumpantothanate	0.01	0.01	0.01	0.01	0.01
Inositol	1.0	1.0	1.0	1.0	1.0
Adenine	5.0	5.0	5.0	5.0	5.0
Choline chloride	-	0.25	0.5	0.25	0.25
Ascorbic acid	-	0.25	0.5	0.25	0.25
Folic acid	-	0.25	0.5	0.25	0.25
Succinic acid	-	-	-	0.25	0.5
Riboflavin	-	-	-	0.25	0.5
Kinetin	0.5	0.5	0.5	0.5	0.5
NAA	1.0	1.0	1.0	1.0	1.0
Caseinhydrolysate	200	200	200	200	200
Sucrose	20 gm	20 gm	20 gm	20 gm	20 gm
Agar	8.0	8.0	8.0	8.0	8.0

Contd..

APPENDIX II CONTD.

(Murashige & Skoog, 1962)

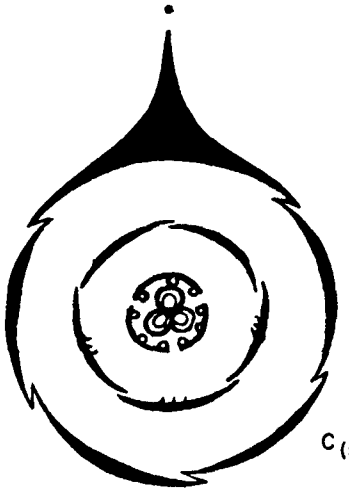
Chemicals	Modifications								
	MS	MS 1	MS 2	MS 3	MS 4	MS 5	MS 6	MD	MC
NH_4NO_3	1650mg	1650mg	1650mg	1650mg	1650mg	1650mg	1650mg	1650mg	1650mg
KNO_3	1900	1900	1900	1900	1900	1900	1900	1900	1900
$CaCl_2 2H_2O$	440	440	440	440	440	440	440	440	440
$MgSO_4 7H_2O$	370	370	370	370	370	370	370	370	370
KH_2PO_4	170	170	170	170	170	170	170	170	170
H_3BO_3	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2
$MnSO_4 4H_2O$	22.3	22.3	22.3	22.3	22.3	22.3	22.3	22.3	22.3
$ZnSO_4 4H_2O$	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6
$Na_2MoO_4 2H_2O$	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
KI	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
$CuSO_4 5H_2O$	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
$CoCl_2 6H_2O$	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
$FeSO_4 7H_2O$	27.8	27.8	27.8	27.8	27.8	27.8	27.8	27.8	27.8
NaEDTA (Triplex)	37.3	37.3	37.3	37.3	37.3	37.3	37.3	37.3	37.3
Glycine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Nicotinic acid	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Pyredoxine HCl	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Thiamine HCl	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cystein HCl	1.0	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Ascorbic acid	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
PABA	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Adenine	5.0	12.5	5.0	7.25	12.5	7.0	7.5	7.5	7.5
Myoinositol	100	100	100	100	100	125	100	100	100
IAA	2.0	2.0	2.0	2.0	2.0	-	1.5	2.0	2.0
NAA	-	-	-	-	-	1.5	-	-	-
Kinetin	0.4	0.4	1.4	1.4	1.0	1.0	0.75	0.4	0.4
2,4-D	-	-	-	-	-	-	-	2.0	4.0
CM	-	-	-	-	-	-	-	-	150 ml
Sucrose	30.0gm	30.0gm	30.0gm	30.0gm	30.0gm	30.0gm	30.0gm	30.0gm	30.0gm
Agar	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0

contd..

APPENDIX II CONTD.

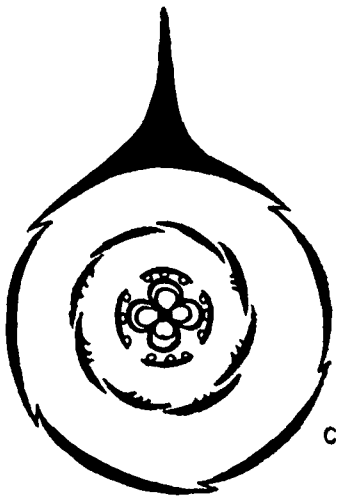
(Miller, 1963) modification		(Buys, 1969) modifications		
Chemical	EaM	Chemical	WK	WD
NH_4NO_3	1000 mg	$(NH_4)_2SO_4$	1650 mg	1650 mg
KNO_3	1000	KNO_3	1900	1900 mg
$Ca(NO_3)_24H_2O$	500	$CaCl_22H_2O$	440	440
$MgSO_47H_2O$	35	$MgSO_47H_2O$	370	370
KH_2PO_4	300	KH_2PO_4	170	170
H_3BO_3	6.2	H_3BO_3	16.2	16.2
$MnSO_44H_2O$	22.3	$MnSO_44H_2O$	16.9	16.9
$ZnSO_44H_2O$	8.6	$ZnSO_42H_2O$	8.6	8.6
$NaMoO_42H_2O$	0.25	$(NH_4)_6Mo_7O_{24}$	0.176	0.176
KCl	65.0			
KI	0.83	KI	0.83	0.83
$CuSO_45H_2O$	0.025	$CuSO_45H_2O$	0.025	0.025
$CoCl_26H_2O$	0.025	$CoCl_26H_2O$	0.025	0.025
		$FeSO_47H_2O$	270	270
NaEDTA (Triplex)	32.0	Na EDTA (Triplex)	37.3	37.3
		Cystein	1.0	1.0
Glycine	2.0	Glycine	1.0	1.0
Nicotinic acid	0.5	Nicotinamide	1.0	1.0
Pyredoxine HCl	0.1	Pyredoxine HCl	1.0	1.0
Thiamine HCl	0.1	Thiamine	1.0	1.0
		Myoinositol	100.0	100.0
		Adenine	7.0	5.0
BAP	0.2	Kinetin	0.5	0.05
NAA	5.0	NAA	1.0	1.0
Sucrose	30.0 gm	Sucrose	20.0 gm	20.0 gm
Agar (Bacto Defco)	7.0	Agar (Bacto Defco)	8.0	8.0

SINGLE



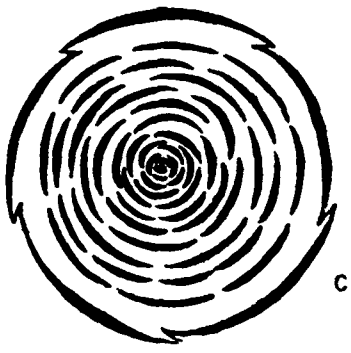
$C_{(5)}\,K_5\,A_{(3)+(3)+(3)}\,\underline{G_{(3)}}$

SEMI-DOUBLE



$C_{(5)}\,K_8\,A_{(3)+(3)+(3)+(3)}\,\underline{G_{(4)}}$

DOUBLE

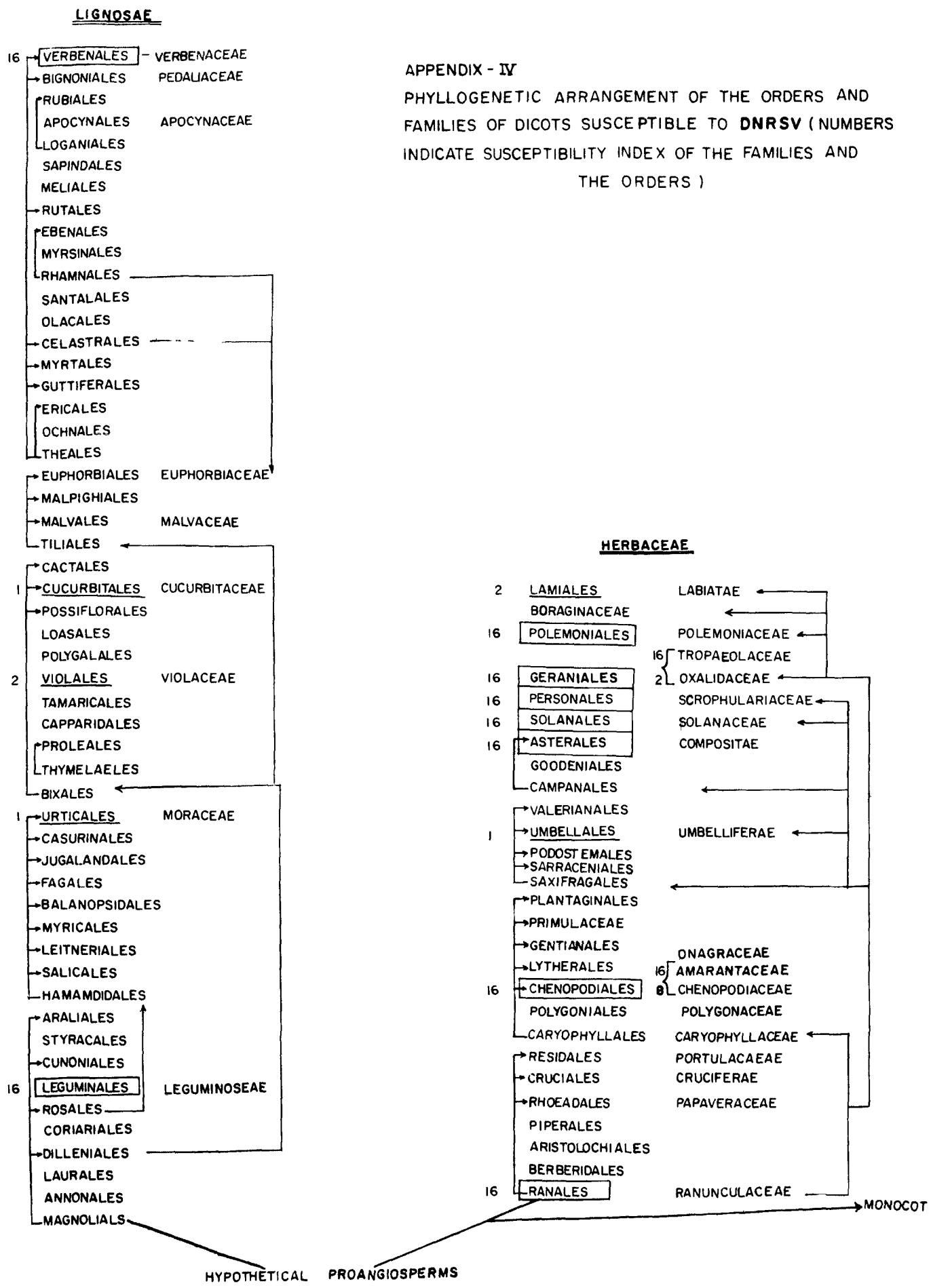


$C_{(5)}\,K_{\mathcal{L}}\,A\text{-}G\text{-}$

APPENDIX III FLORAL DIAGRAMS OF THE THREE CULTIVARS OF *TROPAEOLUM MAJUS*

APPENDIX - IV

PHYLLOGENETIC ARRANGEMENT OF THE ORDERS AND FAMILIES OF DICOTS SUSCEPTIBLE TO **DNRSV** (NUMBERS INDICATE SUSCEPTIBILITY INDEX OF THE FAMILIES AND THE ORDERS)



APPENDIX V

List of Abbreviations used

Chemicals -	Ad:	Adenine
	As:	Ascorbic acid
	BAP:	Benzyl amino purine
	CH:	Casein hydrolysate
	CM:	Coconut milk
	CoCl:	Choline chloride
	FA:	Folic acid
	Ino:	Myoinositol
	IAA:	Indol acetic acid
	K:	Kinetin
	NAA:	L-Naphthyl acetic acid
	Rf:	Riboflavin
	Succ.A:	Succinic acid
	2,4-D:	2,4-dichlorophenoxy acetic acid
	PABA:	para-aminobenzoic acid
	GA:	Gibberellic acid
Tissue culture-	C:	Callus growth in general
	Cp:	Callus from plumule
	Cr:	Callus from radicle
	Cv:	Callus from veins of leaves
	S:	Shoots
	R:	Roots
	Rh:	Root with root hairs
	m:	Meristematic activity, by way of proliferation of cells.
	M:	Growth of meristem-tip.

Grades of growth of tissue cultures:

+:	Insignificant
++:	Slight
+++:	Moderate
++++:	Profuse

Viruses-	TRSV:	Tobacco ring spot virus
	NRSV:	Tropaeolum ring spot virus
	DNRSV:	Double tropaeolum virus
	TMV:	Tobacco mosaic virus
	SMV:	Sann-hemp mosaic virus
	ChMV:	Chilli mosaic virus
	PVX:	Potato virus X
	PVY:	Potato virus Y
	CMV:	Cucumber mosaic virus.