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Research Article

Electron Microscopic and Histopathological Studies of the First Viral Infection Reported in *Ipomoea Cairica* (L.) Sweet

Neha Rauniyar, Deepa Srivastava*

Department of Botany, Deen Dayal Upadhyay Gorakhpur University, Gorakhpur, India

Abstract

Viral infections pose significant threats to plant health, impacting agriculture and medicinal plant production. This study focuses on diagnosing viral infections in *Ipomoea cairica* (L.) Sweet using advanced techniques such as Transmission Electron Microscopy (TEM) and Microtomy. Phenotypic symptoms, anatomical changes, and the confirmation of Gemini virus through TEM are investigated. Transmission studies reveal multiple vectors, including *Bemisia tabaci*, highlighting the importance of understanding viral spread mechanisms. Host range studies demonstrate widespread susceptibility among various plant families. The research emphasizes early diagnosis and management to mitigate economic losses and preserve medicinal metabolites. Future efforts will employ PCR and DNA sequencing for precise viral strain identification and targeted management strategies. The findings underscore the importance of early diagnosis and management of viral infections to safeguard plant health and preserve essential metabolites. Future research aims to utilize PCR and DNA sequencing for precise identification and targeted management of viral strains, enabling effective disease control strategies for *Ipomoea cairica* (L.) Sweet and similar plant species. This study underscores the necessity for proactive measures to protect plant species like *Ipomoea cairica* (L.) Sweet from viral infections, ensuring sustainable agriculture and medicinal plant production.

Keywords

Morphological, Histopathological Studies, Viral Infections, Electron Microscopy

1. Introduction

There is nothing so patient in the biosphere as a virus searching its host. We all live in a dancing matrix of viruses, they project rather like bees, from organism to organisms, from plant to insect vector to mammals, and back again in a cycling manner, tugging along pieces of its genome, strings of genes from that transplanting grafts of DNA, passing around heredity. It is often hard to fight the viruses as they evolve at a much faster rate. Since the identification of viruses as the causative agent to certain diseases in the last few decades has striven the scientists and researchers to clearly visualize their structure [1]. Electron microscopy with high resolving power has permitted the studies up to a nanometer scale, providing direct and clear images of the viruses leading to a beneficial help in diagnosis and research. Electron microscopic has validated and explored the new concept of virology in the Anthropocene age of the present world [2].

Insects/ Aphids, directly or indirectly act as a vector for the transmission of viral disease. Climate change and industrialization have led to widespread change in the feeding mechanisms of the vector leading to the evolution of viruses at its

*Corresponding author: drdeepasrivastav@gmail.com (Deepa Srivastava)

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peak. This has challenged the routine diagnosis mechanism through ELISA and PCR techniques, but with the discovery of Electron microscopy, it has been easier to determine the functional features along with the underlying mechanism of the virus prevailing in the natural environment as well as in the synthetic virological laboratories.

The first and the foremost observable character of viral infection is the change in the phenotype of the host, but further analysis depends on the confirmation tests required for viral infections. In Phyto pathological studies, the symptoms of viral infections can vary from single infection to mixed complex infections which could be the results of the different growing environments of the host, species dependency, and the feeding activity of vectors on the host plant.

Transmission electron microscopy (TEM) is the best initial step in the diagnosis of a viral infection [3] as it provides an open and clear view of the actual status and shape of viruses present or infecting the hosts. In the initial step of the pathogen, detection TEM requires a very minute amount of sample with high viral loads. One of the greatest advantages of TEM is its unbiased nature against RNA and DNA genome of the viral capsid or ribonucleoprotein complex [4]. Based on particle stability, structure, and size as observed under TEM it helps in immediate preliminary classification up to the level of the family. Thus, TEM is a decisive tool in the determination of the rest of the molecular biology approaches for further study of viral infections in plants.

The ill effect of viral infections not only harms the host phenotypically but also the anatomical and histological structure of the host changes to a larger extent as the result of these viral diseases. The microtome is one of the best and essential tools to analyze and study the anatomical structure of cells and tissues and the internal damage caused by the virus to the plant tissues which could be easily diagnosed and differentiated from the normal cell tissues visible through the thin sections released in the form of bands of embedded between the paraffin wax. Since the viral outbreaks are rigorous and have serious consequences so their identification is quite crucial as they may cause a large extent of damage to the plants in every term.

Ipomoea cairica is a herbaceous perennial plant of the Convolvulaceae family [5]. Infection of many viruses over different species of *Ipomoea* has been well reported all over the worldwide such as sweet potato feathery motile virus (SPFMV, Potyvirus) which are known to be transmitted by aphids [6]. In the current study different species of *Ipomoea* have been diagnosed with viral infect symptoms such as leaf bending, crinkling of leaves, vein clearing, and mosaic appearance [7]. These infections can lead to a decrease in the normal growth of the plant along with the reduction in certain essential metabolites in the plants, decreasing its medicinal importance. Hence it is essential to diagnose the disease at the initial stage of infection with techniques such as Electron microscopy and Microtomy to confirm the presence of viral infection in *Ipomoea cairica* as a first report to viral infection in the same.

Through this paper author has tried to describe the technique used which confirms the presence of viral infection in *Ipomoea cairica*.

2. Material and Methods

2.1. Sample Collection

Both healthy and infected leaf samples of *Ipomoea cairica* were collected from the experimental plot designed for the purpose of research from Deen Dayal Upadhyay Gorakhpur University, Gorakhpur located at Latitude and longitude coordinates of 26.765844 and 83.364944 respectively in the month of July 2021. These samples were separated into two slots for both healthy and infected leaves among this slot 1 was used in studying the anatomical changes due to infection using Microtomy while slot 2 was used for carrying Transmission Electron Microscopy in order to confirm the viral infection and identify the type of pathogen mainly the Family of virus infecting the plant [8].

2.2. Phenotypic Symptoms of the Disease

Figure 1. *Demonstration of Infection based on Phenotypic symptoms of Ipomoea cairica (L.) Sweet.*

The symptoms observed while the field survey was Crinkled leaves, leaf bending, vein clearing, mosaic patches on leaves, and Necrosis (Figure 1). In some samples, the infection is not considerably prominent but stunted growth in plant was clearly observed. These symptomatic appearances are assumed to be viral infection due to the feeding activity of *Bemisia tabaci* on large scale. Although the phenotypic symptoms are not the confirmation of viral infection in plants regarding which TEM is carried out as a response to confirmation of first viral infection in *Ipomoea cairica* (L.) Sweet.

2.3. Histological Sectioning Using Microtome

For comparative analysis of anatomical changes which are result of infection in the sample plant, the healthy and the infected leaves were subjected to microtome [9]. The leaves were mounted in Paraffin Wax and sectioning were done by sharp edged blade of Microtome. The resulting sections were attained in the look of ribbons of wax with leaf sections embedded in it, on exposing these ribbons to hot water bath the paraffin wax melts leaving behind the thin sections of leaf's to be observed under the light microscope for further studies.

2.4. Transmission Electron Microscopy of Infected Sample

Fresh samples of infected leaves of sample plant collected from the experimental plot were washed and dried in blotting sheets and were sent for TEM for identification of the pathogen causing the infection to Division of Plant Pathology (Electron Microscopic Unit), IARI, PUSA, New Delhi. The isolate was investigated using JOEL JM 1011s TEM from Japan.

2.5. Transmission Studies of Viral Isolate

Young, infected leaves showing the prominent symptoms of viral infection were collected and washed thoroughly using running tap water, removing all the dirt and germs. Later the washed leaves were dried using blotting paper. The standard inoculum is prepared by grinding young, infected leaves in chilled 0.1M phosphate buffer at a pH of 7.0, containing 0.02M 2-mercaptoethanol while macerating. The buffer was added at the rate of 1 ml/gm of infected leaf tissue. The resulting pulp was squeezed by the use of 2 folds of sterile absorbent cotton and the liquid extracted was used as standard inoculum (SI) for further studies. The transmission studies were undertaken using mechanical transmission, seed transmission and Insect transmission [10].

2.6. Host Range Studies of Viral Isolate

The symptomatic plant of *Ipomoea cairica* (L.) Sweet was used as a source for inoculum. The viral inoculum was transferred to seedlings at the 4-6 leafy stage as described earlier. The plant with proper symptoms was used as a source of inoculum. The phenotypic symptoms were observed in nearly 35 host species tested during the experimentation. Further, the symptomless test plant was re-tested by inoculating back via the viral isolates to the healthy *Ipomoea cairica* (L.) Sweet. The plants were kept in a net house under observation. The observation is demonstrated through the table.

3. Results

3.1. Histological and Anatomical Effect

The virus reacts with different attributes of the host plant which result certain phenotypic and histological changes in plants such as leaf curling, narrowing of leaves, yellowing of leaves, stunted growth etc., the sample with reference to these symptoms were sectioned using Microtome and visualized under Light Microscope [11-15].

3.2. Histology of Healthy Leaves

The section of healthy leaves demonstrated the presence of Flat lamina, with barrel shaped epidermis on both upper and lower side which are arranged in single layer compactly with thick cuticle in upper epidermis as compared to lower epidermis. The clear distinction of cortex in to 3 zones is observed. Zone 1 is layer of few thick cells Hypodermis just below the epidermis. Below hypodermis is parenchyma cells spreading in 2-3 layers besides which are closely organized barrel shaped starch sheath cells. The vascular bundles are in order of continuous cylindrical fashion of Xylem and Phloem with collateral, conjoint, and open type of vascular bundle. The quantity of stomata is normal in both upper epidermises along with lower epidermises.

3.3. Histology of Infected Leaves

The section of diseased leaves demonstrates concave lamina which are the result of phenotypic symptoms as appeared in leaf sample. The epidermis is oval shaped with more compaction and smaller cell size. The cuticle in upper in addition to lower epidermis are thick when evaluated to the healthy leaves. No clear distinction between the three zones of cortex which is owing to the pathogenic mechanisms leading to the compaction among the hypodermal cell and the layer of parenchymal cells with no clear differentiation among the layers. The last zone of the cortex does not display the clear structure of starch sheath due to reduction in intercellular spaces. The stoma on the lower epidermis is more compacted in contrast to upper epidermis (Figure 2).

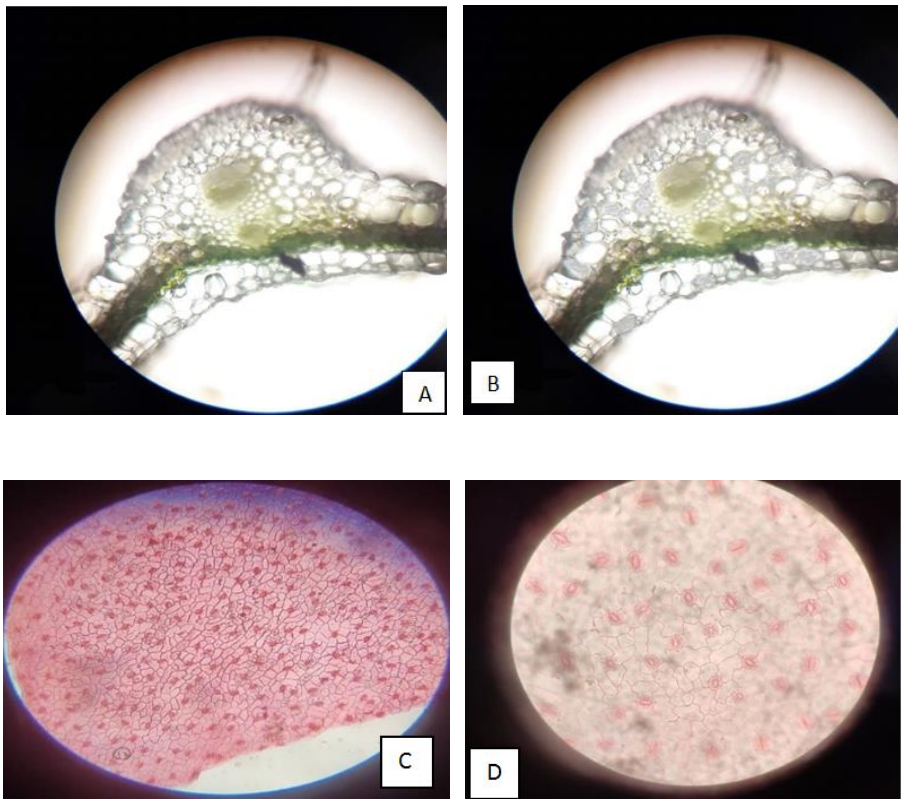

Figure 2. *Histological demonstration of Ipomoea cairica (L.) Sweet. (A) Section of infected Leaf. (B) Section of Infected leaf. (C) Numerous stomata in infected leaf. (D) Reduced stomata in healthy leaf.*

3.4. Transmission Electron Microscopy (TEM)

The Transmission Electron Microscopy (TEM) confirmed the incidence of twinned quasi-icosahedral particle as a validation to Gemini virus. This confirmation also led to report first infection of viral infection in *Ipomoea cairica* (L.) Sweet (Figure 3). For the further analysis of species of virus DNA isolation and purification was carried out in queue. The preservation of isolated DNA at -20^{o}C for carrying out further analysis in consideration with Gemini virus on Host plant.

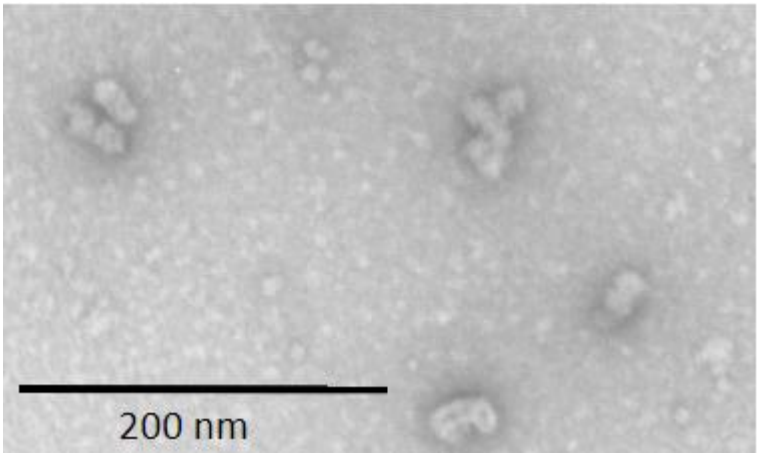

Figure 3. *Particles of Gemini virus stained in Uranyl acetate showing typical twinned quasi-isometric subunit. The bar represents 200nm.*

3.5. Transmission Studies

The result is illustrated in the tabular format according to [16, 17].

Table 1. Results of Mechanical transmission studies through different methods.

Method of Transmission	No. of plants inoculated	No. of plant infected	Incubation Period (Days)	Transmission rate
Bain's Method	10	5	20	50%
Matz's Method	10	4	20	40%
Combined method	10	6	20	60%

Table 2. Transmission of causal virus by insect vectors.

Aphids	Plant infected/ Plant exposed	% Transmission
Aphis craccivora	2/20	10%
Aphis gossypii	6/20	30%
Aphis nerii	2/20	10%
Bemisia tabaci	18/20	90%
Myzus persicae	2/20	10%

3.6. Host Ranges Studies of Viral Isolate

Table 3. Host range of the viral isolate of Ipomoea cairica (L.) Sweet.

S.No	Host Species	Family	Response-Positive (+) or Negative (-)	Symptoms
1.	*Abelmoschus esculentus* (Bhindi)	Malvaceae	+	Necrotic Spots
2.	*Ageratum Spp.* (Whiteweed)	Asteraceae	+	Leaf deformation
3.	*Benincasa hipsida* (Wax gourd)	Cucurbitaceae	+	Leaf deformation
4.	*Cucumis melo* (Melon)	Cucurbitaceae	+	Vein clearing
5.	*Cucumis sativa* (Cucumber)	Cucurbitaceae	+	Vein clearing
6.	*Capsicum annum* (Chillies)	Solanaceae	+	Leaf deformation
7.	*Carica papaya* (Papaya)	Cairicaceae	+	Chlorotic spots and leaf deformation
8.	*Citrullus lanatus* (Watermelon)	Cucurbitaceae	+	Mild Mosaic
9.	*Cucumis pepo* (Pumpkin)	Cucurbitaceae	+	Vein clearing
10.	*Cyamopsis tetragonoloba* (Guar)	Fabaceae	+	Blistering of leaf lamina
11.	*Catharanthus roseus* (Sadabahar)	Apocynaceae	+	Mild Mosaic, colour breaking petals
12.	*Chenopodium album* (Baconweed)	Amaranthaceae	+	Systemic lethal necrosis
13.	*Coccinia grandis* (Ivy gourd)	Cucurbitaceae	+	Leaf deformation
14.	*Cucucurbita maxima* (Winter squash)	Cucurbitaceae	+	Vein clearing
15.	*Calatropis procera* (Madaar)	Apocynaceae	+	Systemic lethal necrosis
16.	*Convolvulus arevensis* (bindweed)	Convolvulaceae	+	Mild mosaic
17.	*Cucurbita vulgaris* (Squash)	Cucurbitaceae	+	Vein clearing
18.	*Chrysanthemum indicum* (Chrysanthemum)	Asteraceae	+	Mild mosaic

S.No	Host Species	Family	Response-Positive (+) or Negative (-)	Symptoms
19.	*Crossandra infundibulum* (Firecracker)	Acanthaceae	+	Blistering of leaf lamina
20.	*Daucus carota* (Wild carrot)	Apiaceae	+	Necrotic local lesion
21.	*Glycine max* (Soyabean)	Fabaceae	+	Leaf deformation
22.	*Gossypium hirsutum* (Cotton)	Malvaceae	+	Chlorotic Spots
23.	*Hibiscus cannabinus* (Kenaf)	Malvaceae	+	Blistering of leaf lamina, Mild mosaic
24.	*Jatropha spp.* (Physic nut)	Euphorbiaceae	+	Vein clearing and bending
25.	*Jasminum multiflora* (Star jasmine)	Oleaceae	+	Necrosis
26.	*Lagenaria siceraria* (Bottle gourd)	Cucurbitaceae	+	Vein clearing
27.	*Luffa cyclindrica* (Sponge gourd)	Cucurbitaceae	+	Vein clearing
28.	*Lycopersicon esculentum* (Tomato)	Solanaceae	+	Mild mosaic
28.	*Momordica charantia* (Bitter gourd)	Cucurbitaceae	+	Vein clearing
29.	*Papaver somnifera* (Poppy)	Papaveraceae	+	Blistering of leaf lamina
30.	*Solanum nigrum* (Black night shade)	Solanaceae	+	Mild mosaic
31.	*Solanum melongena* (Eggplant)	Solanaceae	+	Mild mosaic
32.	*Solanum tuberosum* (Potato)	Solanaceae	+	Mild mosaic
33.	*Sauropus androgynus* (Star gooseberry)	Phyllanthaceae	+	Vein clearing
34.	*Trichosanthes cucumerina* (Snakegourd)	Cucurbitaceae	+	Leaf deformation
35.	*Triticum aestivum* (Wheat)	Poaceae	-	No Symptoms
36.	*Tagetus erectus* (Marigold)	Asteraceae	-	No Symptoms
37.	*Cynodon dactylon* (Scutch grass)	Poaceae	-	No symptoms
38.	*Lantana indica* (West Indian Lantana)	Verbenaceae	-	No Symptoms
39.	*Pennisetum pedicillatum* (Grassweed)	Poaceae	-	No symptoms
40.	*Zea mays* (Maize)	Poaceae	-	No Symptoms

4. Discussion and Conclusion

Medicinal plants are rich sources of certain phytometabolites which are used by many pharmaceutics in day-to-day life. These essential components are widely used in drug industries for several medicines. The disease caused due to several pathogens be it either bacteria, fungi or viruses has great impact on the lifecycle and metabolisms of the plant. Certain pharmabolites shows great reduction in their quality as well as quantity due to the effect pathogens. *Ipomoea cairica* (L.) Sweet a prominent member of Convolvulaceae family is well known for its medicinal efficiencies since ancient times such as leaves are very good source of decoction, people in some are use cooked leaves and seeds for treating rashes and fever in their daily life.

The presence of viral infectivity in plants modifies the cellular constitutions which in response leads to morphological deviation of these plants and an extent of great economical loss because of these symptoms [18]. The virus-related infection in plants play very important role as the whole physiology of the herb is affected, limiting the growth, yield of essential pharmabolites. So, there is an urge of identification of these viruses regarding healthy management of the disease, conserving the essential metabolites of the plant. The viruses not only effect the plant phenotypically, but it causes the great impact on whole machinery of plant.

In the current study the result determined the existence of Gemini viruses confirmed through TEM, further transmission studies the viral isolate demonstrated that the confirmed virus is transmitted through mechanical, seed and insect transmission. The study revealed that the virus has wide range of host especially for the families like Solanaceae and Cucurbitaceae etc.

In future studies the plant sample would be subjected to

PCR for the documentation of members of Gemini virus, later the sequencing of the filtered DNA sample of the affected leaf sample which would lead to identification of exact species of virus infecting the sample plant so that there could be the enhancement in proper management of these viruses and reducing it's on the host plant i.e., *Ipomoea cairica* (L.) sweet.

Abbreviations

ELISA: Enzyme Linked Immuno-sorbant Assay
PCR: Polymerase Chain Reaction
TEM: Transmission Electron Microscopy
SI: Standard Inoculum
IARI: Indian Agricultural Research Institute

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Conflicts of Interest

All authors declare no conflicts of interest.

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