

CYTOPATHOLOGICALLY STUDY OF *JATROPHA CURCAS* INFECTED WITH JATROPHA MOSAIC DISEASE

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Abstract: Plant of *Jatropha curcas* infected with Jatropha mosaic disease by the whitefly (*Bemisia tabaci*), were studied cytopathologically. Plant leaves with typical symptoms exhibited the nucleopathic effects that are the characteristic of known whitefly-transmitted geminiviruses. Electron-dense fibrillar bodies occurred at an early stage of infection and were closely associated with virus like particles at a late stage of infection. Cytoplasmin inclusions which have membrane bound oval bodies containing granular or fibrillar material were also observed. Presence of fibrillar bodies suggested that Jatropha mosaic disease caused by a whitefly-transmitted geminivirus.

Keywords: Electronmicroscopy, cytopathology, *Jatropha curcas*

INTRODUCTION

Jatropha curcas L. is a major commercial biodiesel fuel plant. *Jatropha curcas* L. belongs to family Euphorbiaceae and is a drought-resistant bush or small tree with spreading branches. It is popularly cultivated in the tropics as a living fence (Heller, 1992). The tree is of significant economic importance for its numerous industrial and medicinal uses. The oil extracted from *Jatropha* seeds is being used as a biofuel for diesel engines thus *Jatropha* has a great potential to contribute to the renewable energy sources. In India the area under the cultivation of jatropha is increasing in recent years with the ever increasing demand for fossil fuels that are exhausting at a rapid rate.

Jatropha plant usually found affected by a mosaic disease. JMD was first reported on *Jatropha* Plant in Puerto Rico (Bird, 1957). In India JMD was first reported from Karnataka state, south India in (2004) by (Rangaswamy *et al.*, 2005; Aswatha Narayana *et al.*, 2006). JMD also susceptible to *J. multifida*, *J. podogrica*, *J. gossypiifolia*, *Croton lobatus* and can be transmitted by whitefly (*Bemisia tabaci*) and not by seeds and mechanical inoculation. The JMD has been presumed to be caused by a virus based on its virus-like foliar inclusion. This type occurrence of

disease, symptoms and association of the disease with a whitefly vector suggested that it could be caused by a member of the geminiviruses.

MATERIAL AND METHOD

To determine the incidences of mosaic disease of *Jatropha curcas* six months survey carried out *i.e.*, from September 2008 to February 2009 in certain *Jatropha curcas* growing area of Western U.P. which include Baghpat, Meerut, Muzaffarnagar, Bijnor, Saharanpur. This period is found to be very suitable to grow this virus. The disease incidence at each place was assessed by counting the number of infected plants out of the total planted in a 25 m. long live fence as given in Table-1

The culture of JMV was initially collected from *Jatropha* field at Agricultural research farm, J.V. College, Baraut, district Baghpat. The affected plants showed leaf mosaic, reduced size, distorting of leaves and stunting of whole plants. The period of infection begins when the plants are about 8 weeks old and growing rapidly. The first symptoms in all cases appear in the young leaves which develop small greenish-yellow areas, often not more than a millimeter or two in diameter, occasionally circular spots limited in out line.

Table 1. (%age of incidence of JMD at different *Jatropha* cultivated area.)

District	Area	Total No. of plants studies	No. of infected plants	% of incidence of JMD
Baghpat	Baraut	20	11	55
	Baoli	23	12	52
	Kisahnpur	14	6	42
Meerut	Paulikhas	25	16	64
	Khatoli	22	10	45
	Mawana	17	6	35
Muzaffarnagar	Hind	36	11	31
	Shamli	8	2	25
Saharanpur	Nanota	13	2	15
	Biharigarh	15	4	27

Bijnor	Najibabad	20	8	40
	Dhampur	10	3	30

Virus Transmission: For virus transmission experiments were designed in the experimental agricultural field of J.V. College, Baraut, for this adult *B. tabaci* was collected from the other infected plants. These white fly were released from the infected *Jatropha* plants for 24 hrs for virus

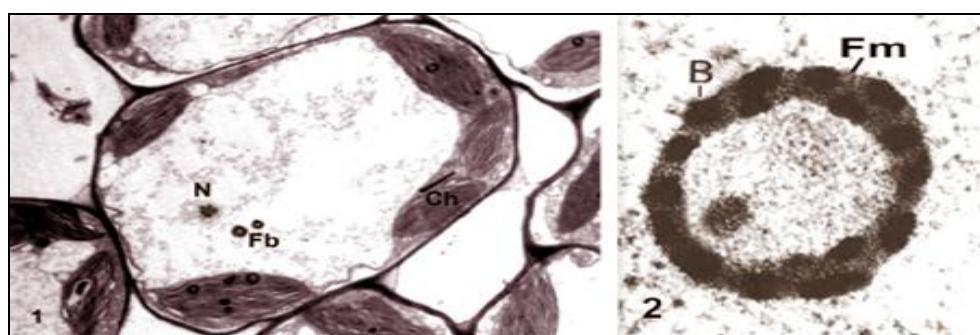
acquisition, which were then transferred to healthy *Jatropha* seedling at 4-6 leaf stage. The individual *Jatropha* seedling were inoculated by 25 viruliferous white flies for 48 hrs and plant were then sprayed with an insecticide and maintained in insect proof cages for symptoms development.



White fly infected leaves of *Jatropha* mosaic disease

Now we took the leaves of *Jatropha curcas* plant as a sample which having the typical *Jatropha* mosaic symptoms. These samples were fixed for 2 hrs. at room temperature in a modified Karnovsky's fixative (11) consisting of 2% glutaraldehyde and 2% paraformaldehyde in 0.05M sodium cacodylate buffer, pH-7.2. After several rinses with the same buffer solution, the tissues were postfixed in 1%

OsO₄ for 2 hrs, prestained in bulk overnight in 0.5% aqueous uranylacetate at 4°C and dehydrated in an ethanol series before they were embedded in Spurr's medium, sectioned with a diamond knife. Thin sections were stained with 2% uranyl acetate followed by lead citrate and examined with an electron microscope.



1. A general view of vascular bundle cell nuclei of *Jatropha curcas* infected with *Jatropha* mosaic virus, having Fibrillar bodies (Fb), Nucleus (N), Chloroplast (Ch.)

2. A ring shaped fibrillar body with a large central cavity. The ring is made of highly electron-dense beads (B) deposited in a less electron-dense fibrillar matrix (Fm).

RESULT AND DISCUSSION

Cytopathologically study shows that virus like particles (*i.e.*, intranuclear fibrillar bodies) which were similar to those characteristic of whitefly-transmitted geminivirus occurred in cells of specimens taken from infected leaves of *Jatropha curcas*. These structures were found in sieve elements and associated phloem parenchyma cells of vascular bundles. These fibrillar bodies were circular in shape and different in size. Many of them were

ring-shaped with a large central cavity containing a substance that is different from the nucleoplasm. This ring is made of highly electron-dense beads embedded in a less electron-dense matrix of fibrillar bodies. These beads are aligned in a row throughout the entire ring with the even spaces between them producing circular strings of beads.

The above study shows that fibrillar bodies in the nuclei of phloem-associated parenchyma cells and sieve elements are very similar to those induced by other whitefly-transmitted geminivirus. Since

fibrillar bodies precede the appearance of virus particles and are associated with virus particles when they appear, it was suggested that fibrillar bodies are the site of virus assembly. Therefore, it is suggested that the agent of JMD, which is transmitted by the whitefly *B. tabaci* is a virus that belongs to the geminivirus group.

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