

## Occurrence, transmission and genotype response of a filamentous virus associated with leaf mustard (*Brassica juncea* var. *rugosa*) in Manipur

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**ABSTRACT:** A mosaic disease of leaf mustard (*Brassica juncea* var. *rugosa*) was found widespread in different locations of Manipur (upto 90.50%) and prevalent in the market samples from different sources (upto 89.58 %). Disease incidence was low during October-November and high during February-March months. The causal virus was transmissible by sap (7.88%) and by three species of aphid (*Myzus persicae* Sulz, *Lipaphis erysimi* Kalt and *Brevicoryn brassicae* L.) but not through seeds. Electron microscopic examination revealed the virus to be flexuous rod shaped particles with an average particle length of 735nm. Out of six local genotypes tested, *Hangam-Lamtachabi* and *Hangam – Apakpi* were found to be resistant to the disease. This is the first report of the occurrence of the mosaic disease in leaf mustard in Manipur.

**Key words:** Leaf mustard (*Brassica juncea* var. *rugosa*), mosaic disease, aphid transmission, potyvirus

### INTRODUCTION

A mosaic disease of leaf mustard (*Brassica juncea* var. *rugosa* Roxb. Tsen and Lee) was found to be prevalent in the north-eastern region of India particularly Manipur where the crop is being grown extensively. In Manipur the crop is usually grown in *rabi* season (October-March) as the most important leafy vegetable crop. The crop suffers from many other fungal diseases like white rust, *Alternaria* leaf blight and foot rot. Reports on the mosaic disease of leaf mustard are very few. A mosaic disease of cultivated rat-tail or serpent radish (*Raphanus sativus* L. var. *caudatus* Alef.) was reported as early as 1924 by Kulkarni from Poona. Hino in 1933 reported a mosaic disease of radish on the cultivated variety Diakon from Japan. Tompkins (1939) observed a mosaic disease of radish in California which could cause severe mosaic disease on leaf mustard *Brassica juncea* L. Coss. Raychaudhuri and Pathanian (1955) reported that the causal organism of radish mosaic was able to develop the mosaic

symptom on some species of mustard, *Brassica nigra*, *rapa* and *alba*. Ahlawat and Chenulu (1984) found that a mosaic disease of radish caused by turnip mosaic virus could infect *Brassica juncea* L. variety Kalimpong local and Varuna. Zheng and Ke (1997) reported the wide spread occurrence of a mosaic disease of Brassicaceae vegetables in China. In leaf mustard, symptoms appeared in the form of vein clearing and vein banding on the young leaves followed by mosaic mottling and irregular chlorotic patches. Affected plants showed stunted growth and reduced size of leaves which appeared thickened leathery and brittle in texture. Distortion of leaf lamina and midrib were also sometimes observed. Occurrence of this mosaic disease of leaf mustard was found to be very high (above 90 %) in the later months of the crop season. In the present studies survey to assess the disease incidence, transmission, electron microscopy and screening of local genotypes against the causal virus were taken up.

## MATERIALS AND METHODS

### Survey

Surveys were conducted for the presence of the mosaic disease in field and on the marked samples. Field surveys were carried out at two different stages of plant growth *i.e.*, at early growth stage (October- November) and at late (bolting) stage (February-March) in the farmers' fields at seven different locations in Manipur, *viz.*, Mayang Imphal, Thoubal, Singjamei, Iroisemba, Lamphel, Mantripukhri and Lamsang in 1999-2000 and 2000-2001. The incidence of the virus was recorded through random sampling with 20 plants per plot (plot size ranges from 1.2m x 2m to 1.6m x 2.4m) and ten plots were observed at each location. Diseased samples were collected for further study. Market surveys were conducted in the main market with bundles as the unit of observation at seven days interval from 1<sup>st</sup> September, 2000 till the end of March, 2001 from three different sources *viz.*, Mayang Imphal, Thoubal and Samurou. Twenty bundles from each source were surveyed weekly and compiled for each month. The disease incidence was based on the visual observation of the characteristic symptoms.

### Virus isolate and test plant

The virus isolate collected from the field was maintained on purple pigmented local leaf mustard cultivar, *Hangam Amubi* plants by insect inoculation in insect proof cages. This cultivar was used as test plant in all the experiments. Test plants were raised in 30 cm diameter earthen pans containing well mixed soil and compost inside the insect proof nylon netted cages. Twenty to twenty five days old seedlings (3-4 leaf stage) was transplanted to 6.5 cm diameter pot filled with mixture of soil and manure with one plant per pot. Inoculation of the virus to the test plants were made at 4-5 leaf stage.

### Mechanical transmission

For mechanical transmission of the virus, young infected leaves of leaf mustard were macerated with sterile pestle and mortar in 0.1 M phosphate buffer (pH 7.0) @ 1ml/gm of fresh leaf tissue. Inoculation was carried out by leaf rubbing method using carborandum powder (600 mesh) as an abrasive.

### Aphid transmission

Three aphid species which colonized on leaf mustard plants *viz.*, *Myzus persicae* (Sulz), *Lipaphis erysimi* (Kalt) and *Brevicoryne brassicae* (L) were employed for the transmission of the causal virus. Culture of healthy colonies of these aphids were maintained separately on radish seedlings inside insect rearing cages. The aphids were given pre – acquisition starvation for 1 h, acquisition feeding for 10 min and inoculation feeding for 24 h under dark conditions. Five apterous aphids per plant were used for transmission studies. Thirty seedlings were inoculated at one time and the experiment was repeated five times for each aphid species separately. After inoculation, aphids were killed by spraying Dichlorovos (76 EC) and inoculated plants were observed for symptom development.

### Seed transmission

Seeds collected from virus infected plants were sown in seed pan inside insect proof nylon netted cages. Fifty seeds were sown at one time and the experiment was repeated five times. Seeds collected from healthy plants were also sown separately which served as control. Plants were kept under observation for symptom development upto bolting stage.

### Electron microscopy

Electron microscopy of the diseased leaves collected from the young infected plants maintained inside the cage by leaf dip method was carried out at the Virology Unit, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi. The grids were negatively stained with 2 % uranyl acetate (pH 4.5).

### Plant genotypic reactions

Screening of six genotypes of leaf mustard *viz.*, *Hangam Amubi*, *Hangam Ayatpi* (purple), *Hangam Ayatpi* (light green), *Hangam Angoubi*, *Hangam Apakpi* and *Hangam Lamtachabi* against mosaic disease was carried out in the field and laboratory. The field experiment was laid down using randomized block design in plots of 1.2 m x 2 m size with 15 plants per plot at 40 cm x 40 cm plant to plant and row to row spacing. Five plots were used for each genotype. Laboratory screening

**Table 1.** Natural incidence of mosaic disease on leaf mustard (*B. Juncea* var. *rugosa*) in farmers' fields in Manipur during rabi season<sup>#</sup>

Location	Percent incidence of mosaic disease					
	Early stage			Late stage		
	1999-2000	2000-01	Mean	1999-2000	2000-01	Mean
Mayang Imphal	25.30±6.28	58.50±2.47	41.90	88.00±2.13	93.00±2.38	90.50
Thoubal	19.50±2.63	32.50±4.48	26.00	87.50±2.00	89.00±3.05	88.25
Singjamei	14.50±4.68	35.00±2.41	22.75	85.50±2.58	90.50±3.02	88.00
Iroisemba	2.50±1.53	5.00±1.66	3.75	62.50±7.71	64.00±8.02	63.25
Lamsang	11.50±2.79	19.50±2.29	15.50	71.50±3.25	76.50±3.73	74.00
Mantripukhri	3.50±1.30	4.00±1.24	3.75	77.00±2.80	80.00±4.21	78.50
Lamphei	5.00±1.66	10.00±1.66	7.50	69.50±3.53	79.00±3.63	74.25
Mean	11.68	22.92	17.30	77.35	81.71	79.53

\* Mean±SE

<sup>#</sup> Weather conditions during the rabi season: temperature 19.30°C relative humidity (RH) 76.61%, rainfall 2.25 mm for 1999-2000 and temperature 17.26°C, RH 78.61%, rainfall 1.39 mm for 2000-2001

**Table 2.** Trend of mosaic virus disease incidence among the market samples of leaf mustard (*B. Juncea* var. *rugosa*) coming from various sources to Imphal main market during different months of 2000-01

Months	Per cent incidence of mosaic disease			
	Mayang Imphal # #	Shamurou # #	Thoubal # #	Mean
Sept., 2000	10.00±10.00	0.00±0.00	0.00±0.00	3.33±3.33
Oct., 2000	22.50±8.53	5.00±5.00	7.50±7.50	11.67±7.01
Nov., 2000	26.25±3.14	8.75±8.75	15.00±6.45	16.67±6.11
Dec., 2000	47.50±9.24	15.00±6.12	22.50±4.78	28.33±6.71
Jan., 2001	53.75±4.73	45.00±6.12	33.75±15.25	44.16±8.70
Feb., 2001	95.00±2.04	95.00±2.88	58.75±19.61	82.91±8.17
Mar., 2001	100.00±0.00	97.50±2.50	71.25±4.26	89.58±2.2

was carried out by inoculating the plants by *M. persicae* in cages. Thirty plants of each genotype were inoculated. Incubation period and percent infection for each genotype were counted and graded following the method of Gupta and Chowfla (1990).

## RESULTS AND DISCUSSION

### Survey

Survey of the different farmers' fields during rabi, 1999-2000 and 2000-01 revealed that in both the years, the incidence of the mosaic disease of leaf mustard was highest at Mayang Imphal both in the early and late crop stages (Table 1). During 1999-2000, disease incidence at Mayang Imphal in the early and late stages were 25.30 and 88.00% respectively with overall means of 41.90 and 90.50

% respectively. It was followed by Thoubal with 19.50 and 87.50%, respectively (mean of 26.00 and 88.25 %, respectively). During 2000-01 also, mosaic incidence was found to be highest at Mayang Imphal (58.50 and 93.00%, respectively) followed by Singjamei (35.00 and 90.50% respectively). In all the locations, disease incidence was lower in the early stage than the later stage of the plant growth. The overall disease incidence was found to be high in 2000-01 (mean infection of 22.92 % and 81.71% at early and late stages respectively) than in 1999-2000 (11.68% and 77.35%, respectively).

In the market survey, mean mosaic incidence was minimum during September month (3.33%) which gradually increased upto 82.91 and 89.58% in February and March, respectively (Table 2). Infection percentage was highest with Mayang Imphal source exhibiting 100% infection followed

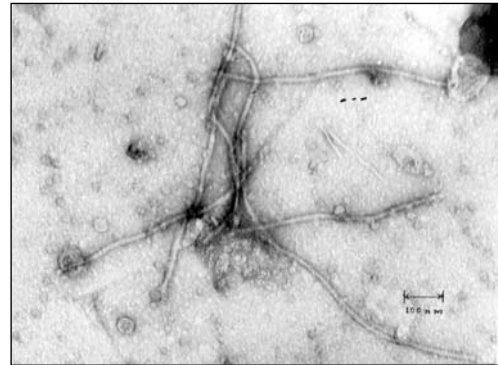


**Fig. 1a.** Infected leaves showing distortion of leaf lamina and mosaic

by Samurou (97.50%) and Thoubal sources (71.25%). Symptoms of the samples collected varied from mosaic mottling to irregular chlorotic patches and leaves were thickened, leathery and brittle in texture. Distortion of leaf lamina and midrib curvature were also observed (Fig. 1a). Variations in the mosaic incidence at different locations could be due to the variations in the colonization of the insect vectors at different locations and the increase in disease incidence from first year to the second year might be due to the environmental conditions favouring the development of vector population or might be due to the increased source of inoculum or lack of management practices to control the vector. Ahlawat and Chenulu (1984) reported varied incidence levels of mosaic disease in radish and *Brassica juncea*, Verma *et al.*, (1968) in Rocket-salad and by Zheng and Ke (1997) in radishes and Indian mustard. In both the field and market surveys, the disease incidence was found to be low in the early stages of plant growth and higher in later stages which might be due to low population of the insect vectors during early plant growth stage. Ahlawat and Chenulu (1982) recorded high incidence of radish mosaic during February and March and lowest during November to January.

#### Mechanical transmission

The causal virus could be transmitted mechanically from diseased to healthy leaf mustard plants although the percent transmission was low (5.66 to 10.4%). Initial symptoms of the disease appeared on inoculated plants in 17-28 days after inoculation. Low mechanical transmission of the



**Fig. 1b.** Electron micrograph of the leaf mustard mosaic virus

virus could be due to the nature of the causal virus or may be due to the non use of antioxidant and celite powder while extracting the virus from the infected tissue.

#### Aphid transmission

Among the three aphid species studied, *M.persicae* was found to be the effective vector for the transmission of the disease (80.66%) followed by *B.brassicae* (51.72%) and *L..erysimi* (29.59%) (Table 3). Inoculated plants expressed symptoms in 7 to 8 days after inoculation.

#### Seed transmission

The causal virus was not transmitted through seeds. Seeds from infected plants showed lower seed germination (88.0%) as compared with seeds from healthy plants (96.0%). Raychaudhuri and Pathanian (1955) reported that radish mosaic disease was not transmitted through seeds. Ahlawat and Chenulu (1984) found that radish seeds from TuMV diseased plants showed poor germination and the virus was transmitted through *Brassica rugosa* (0.8%) and *Cardamine impatiens* (1.4%) seed but not through radish seeds.

#### Electron microscopy

Leaf dip preparations from infected leaf mustard when examined under electron microscopy revealed the presence of flexuous rod shaped particles with average length of 735 nm indicating that virus might belong to the genus, *potyvirus* (Fig. 1b). Mosaic virus infecting leaf mustard (Haq *et al.*, 1994) and *Turnip mosaic virus* (TuMV) infecting radish (Ahlawat

**Table 3.** Transmission of mosaic disease by different aphid species in leaf mustard (*B. Juncea* var. *rugosa*)

Vector	Percent plants producing disease symptom *	
	Range	Mean+S.E.
<i>Myzus persicae</i>	63.33–86.66	80.66±1.57
<i>Lipaphis erysimi</i>	26.66–33.33	29.59±1.13
<i>Brevicoryne brassicae</i>	49.33–55.99	51.72±1.23

\* Acquisition access period (AAP) for 10 min.  
Inoculation access period (IAP) for 24 h  
Number of aphids/plants = 5  
30 plants inoculated at one time and 5 times.

**Table 4.** Response of different local genotypes of leaf mustard (*B. Juncea* var. *rugosa*) to mosaic disease under laboratory and field conditions

Genotypes	Percent symptomatic plants*		Incubation period (days) (Laboratory)	Disease grading**
	Laboratory	Field		
Hangam Amubi	96.66 (82.51)	98.66 (87.01)	11.55 ± 0.80	HS
Hangam Ayatpi (Purple)	94.99 (80.90)	95.99 (82.72)	11.80 ± 0.81	HS
Hangam Ayatpi (light green)	84.99 (67.77)	82.66 (65.63)	12.80 ± 0.85	HS
Hangam Angoubi	86.66 (68.90)	83.99 (66.78)	13.10 ± 0.81	HS
Hangam Apakpi	16.66 (23.98)	19.99 (26.43)	19.90 ± 1.12	R
Hangam Lamtachabi	9.66 (17.79)	11.99 (19.54)	20.11 ± 1.28	R
S.E.(m) ±	5.14	4.06		
C.D. at 5 %	10.96	8.48		

\* Figures in italics are arc sine transformed values. \*\* Mean ± S.E. HS - Highly susceptible; R - Resistant

and Chenulu, 1984) also were reported to have similar particle morphology.

### Screening of genotypes

Among the six local genotypes of leaf mustard screened against the disease, *Hangam Lamtachabi* and *Hangam Apakpi* were found resistant to mosaic disease (Table 4). In the laboratory trial, the infection percentage in *Hangam Lamtachabi* and *Hangam Apakpi* were 9.66 and 16.66%, respectively with incubation period of 15-25 and 15–27 days, respectively. In the field trial also, infection percentage on these two genotypes were found to be low (11.99 and 19.99%) respectively. Other genotypes were found to be highly susceptible and their incubation periods were also short. Li et al. (1992) have also found that out of the 658 accessions of Indian mustard varieties (*Brassica*

*juncea*) two accessions were resistant and ten moderately resistant to TuMV.

The present study revealed that mosaic disease of leaf mustard occurring in Manipur has a viral etiology. Based on the present study, the virus associated with leafy mustard appears to belong to the genus *Potyvirus*. However, further studies on serology and genome sequence are needed to identify the exact taxonomic status of the virus inciting mosaic disease in leaf mustard. This is the first report of occurrence of the disease in Manipur. As the disease has become increasingly important, the reaction of local genotypes studied

here will be helpful in the management of the disease.

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### REFERENCES

- Ahlawat Y.S. and Chenulu, V.V. 1982. Losses due to radish mosaic caused by a strain of *Turnip mosaic Virus* and its control. *Indian Phytopath.* **35**: 225-260.
- Ahlawat, Y.S. and Chenulu, V.V. 1984. Radish mosaic: a new disease caused by *Turnip Mosaic Virus* in India *Trop. Agric.* **61**: 188-192.

- Gupta, Y. and Chowfla, S.C.** 1990. Screening of French bean germplasm for resistance to bean common mosaic virus. *Indian Phytopath.* **43**: 434-435.
- Haq, Q.M.R., Srivastava, K.M., Raizada, R.K., Singh, B.P., Jain, R.K., Mishra, A. and Shukla, D.D.** 1994. Biological, serological and coat protein properties of a strain of *Turnip Mosaic Virus* causing a mosaic disease of *Brassica campestris* and *Brassica juncea* in India. *J. Phytopathology* **140**: 55-64.
- Hino, I.** 1933. List of plants susceptible to mosaic and mosaic like disease. *Miyazaki Col. Mag. And Forestry Bull.* **97**: pp. 111.
- Kulkarni, G.S.** 1924. Mosaic and other related disease of crops in the Bombay Presidency. *Poona Agric. Col. Mag.* **16**: 6-12
- Li, X.Y., Wang, B. and Yu, J.L.** 1992. Identification of leaf mustard germplasm to *Turnip Mosaic Virus*. *Plant Protection* **18**: 6-8.
- Raychaudhuri, S.P. and Pathanian, P.S.** 1955. A mosaic disease of radish (*Raphanus sativus* L. ). *Indian Phytopath.* **8**: 99-104 .
- Tompkins, C.M.** 1939. A mosaic disease of radish in California. *J. Agril. Res.* **58**: 119-130.
- Verma, G.S., Verma, H.N. and Hajela, O.P.** 1968. A severe mosaic disease of rocket salad (*Eruca sativa* Mill ). *Indian J. Agric. Sci.* **39**: 865-869.
- Zheng, S. and Ke, C.** 1997. Study on the mosaic disease of Cruciferous vegetables in Fuzhou. *J. Fujian Academy of Agricultural Sciences* **12**: 24-30.

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