

TRANSMISSION AND HOST RANGE DETERMINATION OF DOLICHOS MOSAIC VIRUS OF FIELD BEAN (*LABLAB PURPUREUS* L.)

H. M. RENUKA*, H. K. RAMAPPA AND B. S. PAVITHRA

Department of Plant Pathology,
University of agricultural sciences, GKVK, Bangalore - 560 065, Karnataka, INDIA
e-mail: hmrenu843@gmail.com

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*Corresponding
author

ABSTRACT

A preliminary work on Transmission and Host range determination of DMV was conducted. Dolichos mosaic virus (DMV) disease of field bean caused by a poty virus and it was readily sap transmissible. The percentage of sap transmission varied from 88.00 to 93.08. The symptoms like vein clearing, uneven leaf lamina, mosaic mottling, blistering, puckering of leaves appeared on test plants 8 -10 days after inoculation. The virus was transmissible by the two species of aphid viz., *Myzus persicae* Sulz. and *Aphis craccivora* Koch. to a maximum of 72.00 and 33.34 per cent respectively. The *Myzus persicae* Sulz was more efficient vector. The virus was to be seed transmissible. The level of seed transmission of the virus varied from 5.80 to 12.06 per cent, with an average of 10.30 per cent. The virus produced systemic symptoms on *Glycine max* L. *Phaseolus lunatus* L. *Phaseolus vulgaris* L. and *Vigna umbellata*. The DMV was transmitted through sap, Aphid and Seed and produced systemic symptoms on above three host plants.

INTRODUCTION

Lablab bean (*Lablab purpureus* L. Sweet) is a widely cultivated, highly drought tolerant legume vegetable crop grown in diverse environmental conditions worldwide. It is also called as *Dolichos* bean or hyacinth bean, Field bean (FAO, 2012). It belongs to the family Fabaceae and originated in India (Deka and Sarkar 1990). It is mainly cultivated as an inter crop with cereals and presently grown throughout the tropical parts of Asia, Africa, West Indies, China and India (Purseglove 1968). Within India, Field bean is cultivated to a large extent in Karnataka and adjoining districts of Tamil Nadu, Andhra Pradesh and Maharashtra. Karnataka contributes a major share, accounting for nearly 90 per cent in terms of both area and production in the country (Anon., 2012).

In Southern India, this crop is grown for fresh green pods used as vegetable, dry seeds for preparations of various dishes and the other plant parts as fodder for livestock. The green pods contain a small amount of vitamin A, C, proteins, iron and rich in calcium. The nutritive quality of Dolichos bean is better than that of French bean (Aykroyd, 1963). The leaf has crude protein of 21 to 38% and the seed contains 20 to 28% crude protein (Cook *et al.*, 2005). It is susceptible to several diseases caused by fungi, bacteria and viruses. Among viral diseases, Dolichos Mosaic Virus (DMV) belonging to poty virus (strain of bean common mosaic virus) causes mosaic disease with an yield loss from 30 – 40 per cent in field bean Udayshankar *et al.* (2011). Therefore the present experiment was carried out for Transmission and Host range determination of DMV of Field bean.

MATERIALS AND METHODS

The experiments were carried out in the insect proof conditions in the green house of Department of Plant Pathology, UAS, GKVK, Bengaluru. For transmission studies followed the procedure of Balgude *et al.* (2012)

Sap transmission

Young leaves showing characteristic mosaic symptoms were collected from infected Field bean plants. The leaves were then macerated in chilled mortar and pestle using potassium phosphate buffer (pH 7.0, 0.05M) at the rate of 1ml/gm of leaf tissue. The resultant pulp was squeezed through absorbent cotton and the extract thus obtained was used as standard inoculum. To the standard extract, celite (600 mesh) at the rate of 0.025 g/mL of the extract and 0.02% Mercaptoethanol was added. The inoculum was applied gently on the upper surface of the leaves with a small piece of absorbent cotton wool. The inoculated leaves were washed 1-2 minutes after inoculation to remove the excess of inoculum with a fine jet of distilled water from a squeeze bottle and plants were kept under observation for 15 - 20 days in the glass house followed the procedure of Capoor and Sawant (1984).

Aphid transmission

Maintenance of aphid culture

Studies on the transmission of virus by insect vectors were made by raising virus free colonies of Aphids in cages in the green house. Two different Aphid species were multiplied from single viviparous wingless female insect on suitable host

*Aphis craccivora* Koch*Myzus persicae* Sulz**Plate 1: Maintenance of Aphid species for transmission studies**

plants as follows.

a. *Aphis craccivora* Koch. on Cowpea

b. *Myzus persicae* Sulz. on Raddish

Handling of aphids

The aphids were transferred from rearing cage onto infected leaves with the help of camel hair brush. At the time of collection, aphids were disturbed by gentle touching with camel hairbrush to ensure withdrawal of their stylet from plant tissue. Later, the aphids were collected in Petri plates covered with black cloth to provide dark condition to arrest the movement of aphids. While transferring aphids individually on to test plants, each aphid was disturbed by a gentle touch with camel hair brush and carefully lifted by its posterior end to avoid damage to the stylet.

Transmission

Apterous adult aphids were collected from the host plant in a Petriplate and starved for 2 hrs. The starved aphids were transferred on to infected leaves and allowed to feed for 30 min. After aphids were transferred to test plants at the rate of ten aphids per plant for 12 hours inoculation feeding. After, aphids were killed by spraying the plants with 0.2 per cent Monocrotophos. The inoculated plants were kept in the insect proof glass house for twenty days for symptom expression.

Seed transmission

Seeds collected from Field bean cv. HA-4 infected with Dolichos mosaic virus disease were sown in polyethylene bags at the rate of 2- 3 seeds per polyethylene bag and kept in the insect proof glasshouse. Plants raised from the seeds collected from healthy plants served as control. The plants were observed for disease symptoms up to a period of 4 weeks. The per cent seed germination and rate of seed transmission was recorded.

Host Range

Studies were undertaken to know the host range of the virus and to identify differential hosts which could help in identifying the virus. Tested the virus to infect *Vigna mungo* (L.) Hopper, *V. radiata* (L) Wilzek, *Arachis hypogaea*, *Macrotyloma*

uniflorum, *Cajanus cajana* (L.) Millsp, *Cicer arietinum* L., *Phaseolus lunatus*, *Vigna umbellata*, *Phaseolus vulgaris*, *Glycine max* (L.) Merr., *Vigna unguiculata* subsp. *Sesquipedalis*, *Vicia faba* L., *Crotolaria juncia* L. Host plants belonging to Leguminoceae family, *Cucumis sativus* L., *Benincosa hispida* Thumb., *Cucurbita moschata* Duchsne, *Memordica charantia* L. belonging to Cucurbitaceae family, *Nicotiana tobaccum* L., *Nicotiana glutinosa* L., *Nicotiana benthamiana*, *Lycopersicon esculantum* Mill., *Solanum nigrum*, *Capsicum annum* L. belonging to Solanaceae family, *Chenopodium amaranticolor* belonging to Chenopodiaceae family were evaluated.

Plants of each species were raised in Polyethylene bags. Plants were inoculated at primary leaf stage with standard extract of virus by mechanical sap inoculation as described earlier. In each plant species, ten plants were inoculated and one set of uninoculated plants were maintained as control. The inoculated plants were kept in the insect proof glass house and examined periodically for symptom expression. The symptoms expressed by the different plant species were recorded.

RESULTS AND DISCUSSION

The virus under study was found to be readily transmissible by mechanical sap inoculation. The percentage of sap transmission varied from 80 to 93.03 on field bean cv. HA-4, probably higher percentage of sap transmission due to the stable nature of the causal agent (Table. 1 and Figure.1). The results are in conformity with the reports by several workers such as Basavaraja and Keshava Murthy (1992) who reported sap transmissible nature of Dolichos mosaic virus infecting Field bean. Similarly Balgude *et al.*, (2012) reported that the soybean mosaic virus (SMV) was easily mechanically transmitted to test plants.

Broad bean mosaic virus was reported to be readily transmitted by sap inoculation by several workers viz., Azad *et al.*, (1961) from Shimla; Chowfla and Niriani (1975) from Delhi and Borges and De (1982) from Portugal. Capoor and Sawant (1984), Gay

Table 1: Sap transmissibility of Dolichos mosaic virus of Field bean

Set of Experiment	Plants inoculated	Plants infected	Percentage of transmission
1	20	19	95.00
2	15	14	93.00
3	18	16	88.00
4	15	15	100
5	15	14	93.00
Average	16.6	15	93.80

Table 2: Transmission of Dolichos mosaic virus of Field bean by aphids - *Myzus persicae* Sulz. and *Aphis craccivora* Koch.

Set of Experiment	<i>Myzus persicae</i>			<i>Aphis craccivora</i>		
	Plants inoculated	Plants infected	Transmission (%)	Plants inoculated	Plants infected	Transmission (%)
1	10	7	70.00	14	4	28.57
2	15	11	73.00	12	4	33.33
3	9	6	66.66	10	3	30.00
4	10	8	80.00	13	5	38.46
5	12	9	75.00	11	4	36.36
Average	11	8	72.00	12	4	33.34

Field bean cv. HA - 4; Number of aphids per plant - 10; Pre acquisition starvation – 2 hr; Acquisition access period – 30 min.

Table 3: Seed transmission of Dolichos mosaic virus of Field bean.

Set of Experiment	Number of seeds sown	No of seeds germinated	Number of plants infected	Percentage of transmission
1	75	60	7	11.66
2	80	58	7	12.06
3	60	51	3	5.80
4	55	43	5	11.62
5	90	77	8	10.38
Average	72	57.8	6	10.30



Figure 1: Transmission studies of Dolichos mosaic virus disease of field bean a) Symptoms on mechanically inoculated field bean plant b) Symptoms on field bean plant inoculated with viruliferous aphids (*Myzus persicae* Sulz) c) Field bean plants grown from infected seeds showing mosaic symptoms

(1972) with Lima bean mosaic virus which was mechanically transmitted by sap.

The DMV was found to be transmitted by two species of aphids namely *Myzus persicae* and *Aphis craccivora*. But the per cent transmission was varied. The aphid *Myzus persicae* transmitted the virus to a maximum of 72.00 per cent and *Aphis craccivora* which transmitted the virus to a maximum of 33.34 per cent. *Myzus persicae* was found to be more efficient vector than *Aphis craccivora* (Table. 2 and Figure.1). These results were similar to the reports of Vasudeva (1960) reported that three aphid spp. viz., *Myzus persicae* sulz. *Aphis luburni* and *Macrosiphoniella sanbornii* Gills, Chowfla and Niriani (1975) reported four Aphid spp viz., *Aphis craccivora*, *A. gossypii*, *Myzus persicae* and *Rhaphalosiphum maidis* Fitch

as vector of broad bean mosaic virus. Borges and De (1982) reported that Broad bean mosaic virus was successfully transmitted by *myzus persicae* and *Aphis fabae*.

Balgude *et al.*, 2012 reported that the soybean mosaic virus was aphids (*Myzus persicae* Sultz) transmitted from soybean to soybean (80%). Basavaraja and Keshavmurthy (1992) reported that the Dolichos Mosaic virus was transmissible by aphids-*Aphis craccivora* and *Myzus persicae* in a non-persistent manner. However in the present study both *Myzus persicae* and *Aphis craccivora* tested was found to transmit the virus in a non persistent manner.

The per cent seed transmission was varied from 5.8 to 12.06 with an average of 10.03 per cent. The plants developed symptoms like mosaic mottling after 20 - 25 days of sowing

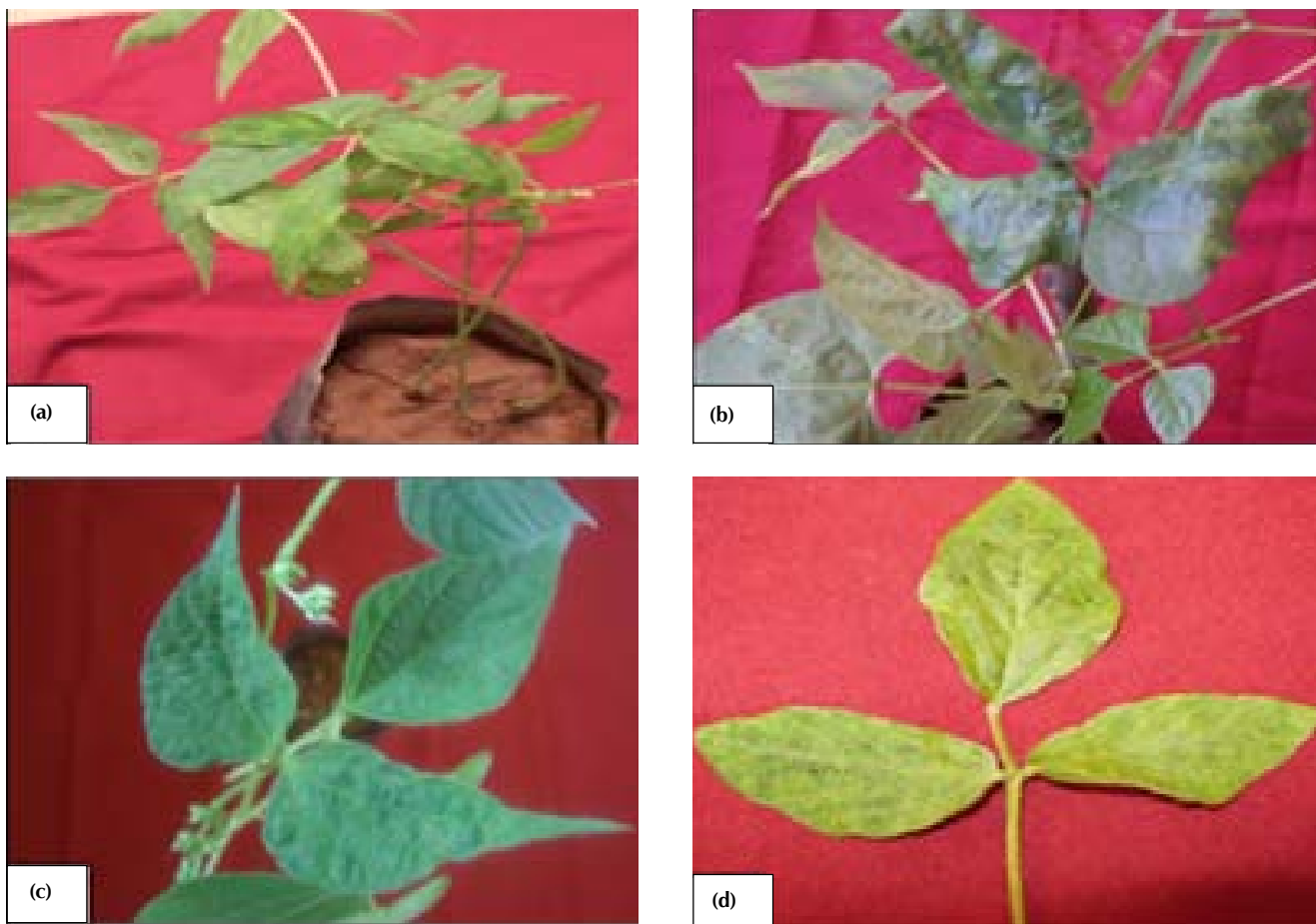


Figure 2: Plants showing different kinds of symptoms upon mechanical inoculation with DMV under green house condition (a) Rice bean showing severe mosaic symptoms (b) Lima bean showing puckering and mosaic symptoms (c) French bean showing systemic mosaic (d) Soybean with light and dark green patches

(Table 3 and Fig.1). The results are in conformity with the reports of Sawant and Capoor (1983) reported lima bean mosaic virus found to be seed borne. The seed transmission ranged from two to six per cent. Robert *et al.* (1991), Khan *et al.* (1993), Mink *et al.* (1994) reported that Bean common mosaic virus spread in nature by seed borne inoculum. Udayshankar *et al.* (2011) reported that bean common mosaic virus infecting *lablab purpureus* L. was seed transmissible at the rate of 6.5% was determined based on symptoms.

In host range studies, 24 host plants species belonging to four families namely Leguminaceae, Cucurbitaceae, Solanaceae and Chenopodiaceae, were inoculated by sap. The virus produced systemic symptoms with light green mosaic mottling on leaves of *Glycine max* L. and vein clearing and severe mosaic mottling of leaves on *Phaseolus lunatus*, *P. vulgaris*, and Rice bean (*vigna umbellata*) belonging to the family Leguminaceae (Fig. 2). Plant species belonging to other families were not infected. These results are in conformity with the findings of Basavaraja and Keshava Murthy (1992) reported that three leguminous plants *Glycine max*, *Phaseolus lunatus*, and *Vicia faba* producing systemic symptoms related to the Dolichos mosaic virus. Pudashini *et al.*, 2013 reported that *Bean common mosaic necrosis virus* (BCMNV) Infecting Sweet

Bean also showed systemic mosaic symptoms on *Phaseolus vulgaris* cv. Aron and *Glycine max* cv. Togenkyou, *Psophocarpus tetragonolobus* and *Vigna unguiculata*.

Host range studies of bean common mosaic virus on 19 different plant species belonging to five families was reported by Bhadamurthy and Bhat (2009) Out of five family bean common mosaic virus could infect *C. amaranticolor*; *N. benthamiana* and *V. planifolia*. *V. unguiculata* (cvs. C-52, C-152, Kanakamani and Lola). Allum and El-Kady (1966) reported that the broad bean mosaic virus infected only members of family Leguminaceae, Producing systemic symptoms on Egyptian lupin, Garden pea, and *Trigonella feonum graecum*. However, the virus in the present study was found to be restricted to four plant species of the family leguminaceae.

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