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Biological Evidence on Host Range of Yellow Mosaic Disease of Greengram [*Vigna radiata* (L.) Wilczek]

H. Deepa[1*], M.R. Govindappa[2], Sunil Kulakarni[3], M. Kenganal[1] and S.A. Biradar[4]

[1]Department of Plant Pathology, College of Agriculture, Raichur-584104, Karnataka, India
[2]Krishi Vigyan Kendra, Hagari, Ballari -583111, Karnataka, India
[3]Krishi Vigyan Kendra, Janawada Road, Bidar-585401, Karnataka, India
[4]All India Co-ordinated Research Project on Linseed, MARS, University of Agricultural Sciences, Raichur-584104, Karnataka, India
**Corresponding author*

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A B S T R A C T

The study was conducted to know the alternate hosts of Mungbean Yellow Mosaic Virus (MYMV) disease on greengram by cross inoculated with virus on several crops and weed hosts under laboratory through insect vector whitefly (*Bemisia tabaci*). The results revealed that the causal virus of the disease was successfully transmitted from greengram to greengram (*Vigna radiata*), *Nicotiana benthamiana*, Blackgram (*V. mungo*), Horsegram (*Macrotyloma uniflorum*), Pigeonpea (*Cajanus cajana*) Soybean (*Glycine max*), Cowpea (*V. ungiculata*) and weed hosts *viz*., *Acalypha indica*, *Malvestrunm coromandelium*, *Croton bonplandianum*, *Euphorbia geniculata*, *Alternenthera sessile* and *Phyllanthus madraspatensis*, while *Parthenium hysterophorus* did not show any symptoms. The study indicated that, these host plants served as potential alternate hosts and major source of virus inoculum for MYMV disease during the off season.

Introduction

Greengram or mungbean [*Vigna radiata* (L.) Wilczek] is a vital crop grown throughout Asia, Australia, West Indies, South and North America, tropical and subtropical Africa. India accounts for 54 per cent of the world production and covers 65 per cent of the world acreage. In India, greengram is grown in an area of 34.4 lakh ha with production and productivity of 15 lakh tones and 407 kg/ha, respectively. In Karnataka, major greengram growing districts are Koppal, Kalburagi, Bidar, Dharwad, Gadag, Belgaum, Chitradurga, Haveri, Shivamogga, Vijayapura, Bellary and Davangere, occupies an area of 5.28 lakh ha with production and productivity of 1.08 lakh tones and 205 kg/ha, respectively (Anon., 2014).

The crop has been found suffering from many diseases which included fungi and viral diseases *viz*., leaf spot, powdery mildew, damping off, wilt, rust, scab, anthracnose, Yellow Mosaic Disease, Leaf Crinkle Virus, Alfa Alfa Mosaic Virus, Bean Common

Mosaic Virus, Cucumber Mosaic Virus and Mosaic Mottle Virus. Among viral diseases, Mungbean Yellow Mosaic Virus (MYMV) is the most destructive in Indian subcontinent and adjacent areas of South-East Asia, causing upto 100 per cent yield losses. It was first reported by Nariani (1960) at IARI (Indian Agricultural Research Institute), New Delhi with 20 to 30 per cent incidence. Apart from India several reports revealed for the occurrence and severity of Yellow Mosaic Virus (YMV) disease incidence across Sri Lanka, Pakistan, Bangladesh, New Guinea, Philippines, Thailand and Pakistan (Honda *et al.,* 1983; Chenulu and Verma, 1988; Malik and Bashir, 1992; Jones, 2003 and Ahmad and Harwood, 1973).

It was noticed that the crop infected at early stages, exhibited yellow mosaic and complete yellowing of all the leaves with puckering symptoms (Salam, 2005). Virus causes irregular green and yellow patches on older leaves and complete yellowing of younger leaves. Infected plants produce fewer flowers and pods, pods often remain small contain few seeds that are malformed and discoloured that affecting yields qualitatively and quantitatively (Nene, 1973 and Dhingra and Chenulu, 1985).

Globally, whitefly transmitted geminiviruses (Geminiviridae, Begomovirus) are economically important pathogens causing serious losses in food crops. The *B. tabaci* geminivirus complex depends on various factors, such as evolution of variants of the viruses, changes in the biology of vectors, movement of infected planting materials, sources of volunteer and weed host plants, introduction of new crops and host susceptibility genes through the exchange of germplasm, changes in cropping systems and climatic factors (Ramappa *et al.,* 1998; Varma and Malathi, 2003). Host range studies with tomato leaf curl virus (ToLCV) (Muniyappa *et al.,* 2000), pumpkin yellow vein mosaic in pumpkin (Maruthi *et al.,* 2007), Hibiscus leaf curl virus in Hibiscus (Rajeshwari *et al.,* 2005) and Croton leaf curl virus in cotton (Mahesh *et al.,* 2010) revealed that begomo viruses have wide host compatibility, however, their infection and further symptoms expression varied between the host plants.

Meager research efforts done to identify the alternate hosts of the virus by biological means in relation to its survival, as it were not properly understood. Hence, the present study was conducted to find out the alternate hosts of the causal virus, based on biological assay in relation to disease spread.

Materials and Methods

Maintenance of yellow mosaic virus culture

Greengram plants showing characteristic mosaic symptoms of irregular green and yellow patches on older leaves and complete yellowing of younger leaves with fewer flowers and small pods with few malformed and discoloured seed was brought from greengram fields to the laboratory of Main Agricultural Research Station, University of Agricultural Sciences, Raichur.

Virus culture was maintained by inoculating virus to healthy two leaf stage greengram seedlings using whiteflies (*B. tabaci*). All procedure was carried out under 40 mesh nylon net protected green house.

Maintenance of whitefly culture

Initially, whiteflies (*B. tabaci*) were collected from greengram crop at Main Agricultural Research Station (MARS), Raichur and the colony was established on freshly grown cotton (*Gossypium hirsutum*) and Brinjal plants grown and maintained in an insect

proof net house. After four weeks of whiteflies release, freshly emerged whiteflies were collected using an aspirator and were transferred to healthy cotton plants grown in an insect proof cages. The colony so developed was referred to be virus free (aviruliferous) colony and further same colony was periodically maintained by frequently introducing healthy cotton plants grown in pots (6 ×10 cm) maintained in an insect proof cages and polyhouse with temperature of 28+ 2°C.

Biological confirmation of alternate hosts of the virus

For the study, healthy seedlings of different cultivated crop plants *viz*., *Nicotiana benthamiana*, Blackgram (*Vigna mungo*), Horsegram (*Macrotyloma uniflorum*), Pigeonpea (*Cajanus cajana*) Soybean (*Glycine max*) and Cowpea (*V. ungiculata*) and weed hosts *viz*., *Acalypha indica*, *Malvestrunm coromandelium, Croton bonplandianum, Euphorbia geniculata, Parthenium hysterophorus, Alternenthera sessile* and *Phyllanthus madraspatensis* were planted on soil mixture of FYM and sand in polythene bags.

The seedlings of respective hosts were cross inoculated with virus using *B. tabaci* at two leaf stage. For inoculation study, healthy whiteflies were collected from culture house and allowed to feed on yellow mosaic infected greengram plant for 24 hrs as an acquisition access period (AAP).

Such whiteflies were collected and inoculated onto test seedlings, which were further allowed to feed on healthy host for 24 hrs of inoculation access period (IAP). The virus inoculated seedlings of the respective hosts were kept in insect proof glass house for symptom expression. Observation was made on per cent transmission, time to initial and final symptoms expression and type of symptoms on each host. For each host, healthy seedlings were maintained in an insect proof cage without inoculation of the virus for comparison studies.

Results and Discussion

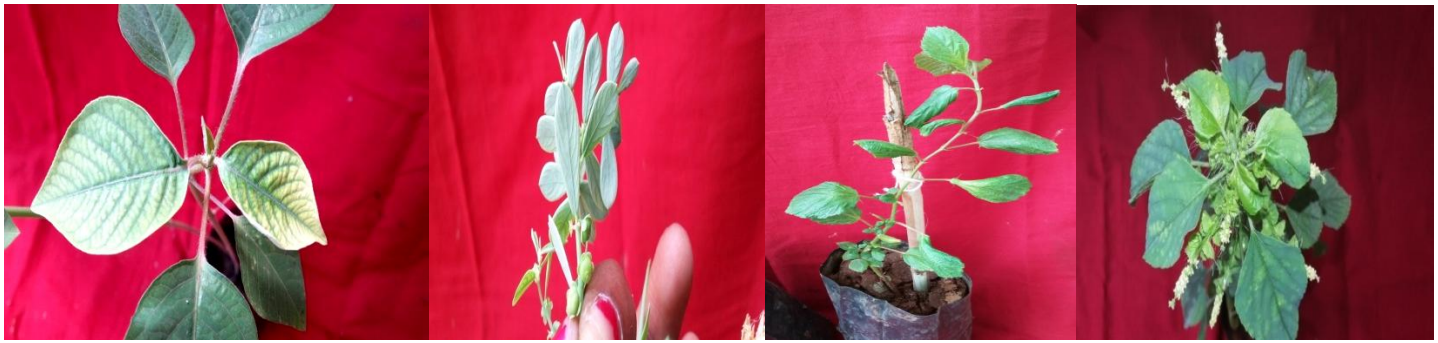
The present investigations on host range studies revealed that, the virus was limited to only six host plants with varied transmission rates of 20 to 50 per cent (Table 1). Crop species infected with MYMV are *Nicotiana benthamiana*, blackgram (*Vigna mungo*), horsegram (*Macrotyloma uniflorum*), soybean (*Glycine max*), pigeonpea (*Cajanus cajana*) and cowpea (*Vigna ungiculata*), which expressed initial symptoms between 17 to 28 days after virus inoculation.

Among seven weed species tested *Croton bonplandianum, Euphorbia geniculata, Phyllanthus madraspatensis, Malvestrunm coromandelium, Acalypa indica and Alternenthera sessile* showed successful transmission between 30 to 60 per cent (Table 2), while *Parthenium hysterophorus* did not show any symptoms. However, virus transmission rates varied from host to host. Weed hosts infected with MYMV expressed the typical initial systemic symptoms of vein clearing within 18 to 24 days. They typically produced the symptoms of vein clearing, light mosaic leaves, leaf puckering, mosaic, distorted leaves and finally exhibited complete yellowing of leaves (Figure 1). While, in *Nicotiana benthamiana* severe leaf curling and distorted symptoms were noticed. This difference in transmission rates and expression of virus symptoms between crops and weeds could be due to preference and also host biochemical compositions of *B. tabaci*, which may interfere with virus multiplications (Colvin *et al.,* 2006; Sharma *et al.,* 2008).

Fig.1 Expression of yellow mosaic virus disease symptoms on crops and weeds upon inoculation through whitefly *B. tabaci*

Greengram *N. benthamiana* **Blackgram** **Horsegram**

Soybean **Pigeonpea** **Cowpea** ***C. bonplandianum***



E. geniculata *P. madraspatensis* *M. coromandelium* *A. indica*

Table.1 Host range of MYMV in crop plants through whitefly (*Bemisia tabaci*)

Sl. No.	Crop	Total No. of plants tested	Total No. of plants infected	Per cent transmission	Days to first symptom appearance	Days to final symptom appearance	Early symptom appearance	Final symptom appearance
1	*Nicotiana benthamiana*	10	5	50	18-22	42-48	Leaf puckering, curling, mosaic and distorted leaves	Severe distorted leaves and plants become stunted
2	Blackgram (*Vigna mungo*)	10	4	40	21-25	42-45	Dark green mosaic appears on growing trifoliate leaves	Leaves become dark green mosaic with severe deformation and leathery
3	Horsegram (*Macrotyloma uniflorum*)	10	4	40	20-26	40-45	Vein clearing followed by light mosaic	Leaves become severe yellowing and distorted
4	Soybean (*Glycine max*)	10	3	30	17-21	40-45	Leaf puckering, mosaic and distorted leaves	Severely stunted plants no flower setting
5	Pigeonpea (*Cajanus cajana*)	10	2	20	20-28	42-49	Small yellow patches appear on trifoliate leaves which later turn to dark yellow mosaic	Leaves become severe chlorosis and withers
6	Cowpea (*Vigna ungiculata*)	10	3	30	19-24	38-42	Light mosaic leaves	Exhibited severe yellowing and distortion

Table.2 Host range of MYMV on weeds through whitefly (*Bemisia tabaci*) transmission

Sl. No.	Crop	Total No. of plants tested	Total No. of plants infected	Per cent transmission	Days to first symptom appearance	Days to final symptom appearance	Early symptom appearance	Final symptom appearance
1	*Croton bonplandianum*	10	6	60	18-20	42-48	Vein clearing and light to dark patches of yellow mosaic	Complete yellowing, puckering and raised green areas.
2	*Euphorbia geniculata*	10	4	40	21-23	38-42	Vein clearing and yellow mosaic	Complete yellowing of leaves
3	*Parthenium hysterophorus*	10	0	0	0	-	-	-
4	*Phylanthus madraspatensis*	10	4	40	20-24	38-41	Vein clearing and green patches on leaves	Complete yellowing plant
5	*Malvestrum coromandelianum*	10	3	30	17-23	40-45	Vein clearing on trifoliate leaves	Yellowing with distorted and malformed
6	*Acalypha indica*	10	3	30	19-22	42-49	Vein clearing and green patches on trifoliate leaves	Complete yellowing and pods were bleached to yellow
7	*Alternenthera sessile*	10	4	40	21-22	39-41	Vein clearing on trifoliate leaves	Complete dark yellowing

Similarly, tomato leaf curl begomo virus (ToLCV) from tomato was successfully transmitted to weeds *Acanthospermum hispidum, Ageratum conyzoides, Bidens biternata, Conyza stricta, Datura stramonium, Euphorbia geniculata, Oxalis corniculata, P. hysterophorus, Solanum nigrum, Sonchus brachyotis, Stachyterpicta indica* and *Synedrella nodiflora* and tobacco (*N. Benthamiana*) by *B. tabaci* in a varied period of incubation (Ramappa *et al.,* 1998). In addition, leaf curl begomovirus of *Hibiscus* plant found infecting weeds (*Ageratum conyzoides*, *Croton bonplandianum* and *Euphorbia geniculata*) and tobacco species *viz*., *N. benthamiana*, *N. glutinosa*. *N. tabacum* (var. Samsun), cotton and tomato (Rajeshwari *et al.,* 2005). The hosts confirmed with the tomato leaf curl begomovirus in tomato (ToLCV) (Ramappa *et al.,* 1998). Similar host range studies carried with Begomovirus associated symptoms on different crops (Avinash Marwal *et al.,* 2013, Raj *et al.,* 1996, Ramappa, 1993, Muniyappa *et al.,* 1991 and Ahmad and Harwood, 1973).

Host range studies on the virus inoculum of yellow mosaic virus disease on greengram through biological approach revealed that among different crops tested, *Nicotiana benthamiana*, blackgram, horsegram, soybean, pigeonpea and cowpea acted as potential reservoir hosts for virus. Apart from cultivated crops, weed species *viz*., *Croton bonplandianum, Euphorbia geniculata, Phyllanthus madraspatensis, Malvestrunm coromandelium, Acalypa indica* and *Alternenthera sessile* also confirmed as potential source of virus inoculum.

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