Original Research Article

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

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ABSTRACT

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 cajan) Soybean (Glycine max), Cowpea (V. unigulata) 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.

Keywords

Begomovirus, Host range, Greengram, Whitefly, MYMV.

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 up to 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 begomoviruses 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 weed hosts viz., *Acalypha indica*, *Malvestrum 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 unguiculata*), which expressed initial symptoms between 17 to 28 days after virus inoculation.

Among seven weed species tested *Croton bonplandianum*, *Euphorbia geniculata*, *Phyllanthus madraspatensis*, *Malvestrum 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*.
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 unguiculata*)      | 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                                | -                             | Vein clearing and green patches on leaves                    | -                                                             |
| 4       | *Phyllanthus 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                                       |

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Similarly, tomato leaf curl begomovirus (ToLCV) from tomato was successfully transmitted to weeds Acanthospermum hispidum, Ageratum conyzoides, Bidens biterata, 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 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, Malvestrum coromandelium, Acalypa indica and Alternanthera sessilis also confirmed as potential source of virus inoculum.

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