

TRANSMISSION, HOST RANGE AND SYMPTOMATOLOGY STUDIES OF SOYBEAN YELLOW MOSAIC DISEASE THROUGH WHITEFLIES

H.D. Gaikwad^{1*}, C.D. Deokar², S.V. Kolase³, T.K. Narute⁴

¹Department of Plant Pathology and Agril. Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra, India

²College of Agriculture, Dhule, Maharashtra, India

³Department of Plant Pathology and Agril. Microbiology, PGI, MPKV, Rahuri, Ahmednagar, Maharashtra, India

⁴Department of Plant Pathology and Agril. Microbiology, PGI, MPKV, Rahuri, Ahmednagar, Maharashtra, India

Email: harshu.jgd@gmail.com

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Abstract: Soybean (*Glycine max* (L.) Merrill) is a one of the major oil seed crop in the world. The Yellow Mosaic Disease (YMD) of soybean is caused by Mungbean Yellow Mosaic India Virus (MYMIV). The transmission, host range and symptomatology studies were conducted at glasshouse facility of MPKV, Rahuri. For these studies whitefly (*Bemisia tabaci*) was used as vector of this viral disease. In transmission studies of virus indicated that, the 100% transmission rate was recorded, when 15 and 20 whiteflies were used per soybean plant. Mungbean, dolichos bean, black gram, cow pea, cluster bean and horsegram act as host of yellow mosaic disease. The host range studies indicate yellow mosaic of soybean was able to infect these crops through whiteflies under glasshouse condition. In symptomatology studies, soybean test plant, JS-335 developed typical yellow mosaic disease symptoms like irregular bright yellow and green diffused patches on leaves, extensive mosaic and mottling of leaves, chlorosis and reduction in leaf size.

Keywords: Soybean, Yellow mosaic disease, Virus transmission, Whitefly, Virus host range

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill), (2n=40) is a globally important oilseed crop. China is the origin of soybean. Soybean ranks first in the world among other oil seed crops in production. The United States of America is the world's largest soybean producer. Brazil, Argentina, China and India are other top soybean producers in the world (Shahbandeh, 2020). In 2019-2020, Global soybean production was 335.35 million metric tons, around 6.91 percent less than last year's soybean production around the globe. In India, the total harvested area was 12 million hectares and produced 9.30 million metric tons (MT) with 0.78 MT/Ha yield in 2019-20 (World agricultural production, FAS/USDA, 2020). During kharif season of 2019 in Maharashtra, area under sowing was 37.37 lakh Ha. with 39.42 lakh MT production and 1055 Kg/Ha yield. (SOPA Databank, 2019).

Soybean comprising a wide range of nutrient properties is therefore known as a miracle or golden bean with 20 per cent oil and 38 to 43 per cent protein, having biological importance of as meat or fish proteins, and an abundance of amino acids like lysine (5 per cent) and tryptophan (Quayum *et al.*, 1985). The average soybean yield per ha in India is still below average in recent years. In addition to several other causes, weed infestation, crop attacking diseases and invading pests are key elements that are responsible for sub-jacent production. The three major

limitations on soybean production are the infestation of weeds and area-limited abiotic and biotic stresses. Any increase in efficiency can therefore be achieved by resolving these three problems (Meseldžija *et al.*, 2020).

Important disease such as, rust, sclerotium wilt, bud blight, fusarium wilt, bacterial blight, brown leaf spot and over 50 different viruses, which are known to attack, are commonly observed among the biotic stresses of soybean (Nyvall, 1989). In India, the number of known viruses of soybean are lower than 10. The major ones are soybean yellow mosaic virus (Lal *et al.*, 2005, Ramteke *et al.*, 2007), groundnut bud necrosis virus and soybean mosaic virus, which causes bud blight of soybean (Bhat *et al.*, 2002, and Lal *et al.*, 2002).

The significant constraints on grain legume production are Yellow Mosaic Diseases (YMD) in India. The yield loss per annum due to YMD of blackgram, mungbean and soybean collectively was evaluated to be \$ 300 million (Varma and Malathi, 2003). In North India, YMD of soybean was first detected in the year 1970s (Nariani, 1960 and Suteri, 1974) and has since been transmitted at unprecedented proportions. Studies based on enzyme-linked immunosorbent assay (ELISA), transmission studies through whitefly and immune-specific electron microscopic (ISEM) have indicated that the etiological virus causing YMD in soybean is a begomovirus of family Geminiviridae. Begomoviruses have distinctive icosahedral

*Corresponding Author

geminata particles which encapside the genome of single-stranded and circular DNA. The most of dicots affected by this virus, and they are transmitted by the whitefly species, *Bemisia tabaci*, Gennadius. They have a monopartite or bipartite genome. In bipartite begomoviruses, DNA A encodes proteins needed for replication, transcription and encapsidation while DNA B encodes proteins necessary for movement functions (van Regenmortel, 2000).

Considering the importance of yellow mosaic disease on economics and physiological functions of soybean, the systematic studies were conducted regarding transmission, host range, symptomatology studies of yellow mosaic virus through whiteflies.

MATERIALS AND METHODS

Symptomatology, host range and transmission of yellow mosaic virus of soybean

In insect-proof glasshouse, disease free seedlings of soybean plants and other 12 plant species were grown. After 24 h acquisition access feeding on yellow mosaic virus infected soybean plant, healthy seedlings at two leaf plant phase were inoculated with viruliferous indigenous whiteflies. The viruliferous whiteflies were fed in insect-proof tubes on disease free test seedlings for 24 h. Inoculated seedlings were placed in insect proof glasshouse for the production of symptoms. The symptoms were noted at different intervals of plant growth phases.

Maintenance of inoculum

Raising healthy soybean seedlings

The soybean seeds of variety JS335 were sown in 15 cm diameter and 30 cm depth pots packed with soil+sand+FYM pot mixture. These healthy seedlings were raised in insect proof cages. Then the seedlings were used as test plants for a various tests after becoming 7 days old later germination.

Yellow mosaic virus culture on soybean

Soybean plants showing conspicuous symptoms of yellow mosaic were collected from naturally infected plants from fields. Whiteflies (*Bemisia tabaci*) were released on infected soybean plants for acquisition of virus for 24 h. The viruliferous whiteflies were collected and later transferred to seven days old healthy soybean seedlings in a glasshouse. The inoculation access period of 24 h was given for transmission of virus. The inoculated plants were kept in insect proof cages for expression of yellow mosaic disease symptoms and they were used as stock culture. The same culture was maintained in a glasshouse through inoculating regularly to the healthy plants of soybean variety JS335 with the viruliferous whiteflies, (*Bemisia tabaci*) for various experiments.

Vector culture

The type culture of *B. tabaci* used for inoculation was maintained on brinjal, cotton and cucumber plants kept in an insect proof cages with size 45 x 45

x 30 cm, fastened muslin cloth on the sides and top with Fevicol™. The front surface was packed with glass, and in the grooves created in the wooden frames could be conveniently moved. Healthy plants were placed into the cages, which were grown in polythene bags (4 x 6 cm). The cages were kept in an insect-free polyhouse. The whiteflies colonized on the lower surface of young leaflets of plants (Plate No. 1a and 1b).

The aspirator assembled of a rubber tube of 40 cm length and a glass tube (30 x 0.5 x 40 cm) was used for collection of whiteflies. The colonized leaves with healthy whiteflies were turned slightly upward and then whiteflies sucked into the glass tube. These accumulated disease free whiteflies were used in this investigation.

Preparation of collection tube for acquisition access feeding by *Bemisia tabaci*

Bottles of plastic or polyvinyl chloride (PVC) with size 20 x 8 cm, curved towards the small opening were taken, the lower section of the bottles was detached using a sharp cutting object (knife) and sealed with muslin cloth. The bottle's narrow opening was cut to a few centimeters higher than the screw cap. To avoid whiteflies fleeing from the bottle during collection, a cotton plug was inserted into the open mouth.

Preparation of micro cages for inoculation of plants

Plastic tubes (7.5 x 2.5 cm) were taken and the bottom of the tube was removed with the help of a sharp cutting object (knife). With muslin cloth, the lower section was sealed, which would help to ensure aeration and prevent the buildup of extra moisture within the cage. A tiny hole (0.5 cm) for releasing the whiteflies was created in the central part of the tube. After introducing young leaflets into the tube, the open end of the tube was filled with cotton.

Whitefly transmission

Whiteflies from the rearing cage were collected and released into plastic or polyvinyl chloride (PVC) tubes in which, infected branches with the soybean yellow mosaic virus were previously inserted and allowed to feed for 24 h acquisition access period (AAP). Then, the viruliferous whiteflies were released on to healthy soybean plants by using aspirator and were allowed to feed for 24 h inoculation access period (IAP). After inoculation, the plants were sprayed with 0.1 per cent dimethoate to kill all the whiteflies. For the development of symptoms the inoculated plants were maintained in an insect proof glasshouse.

RESULTS AND DISCUSSION

Studies on symptomatology, host range and transmission of yellow mosaic virus through whiteflies

Studies on symptomatology of yellow mosaic virus of soybean

All the test plants of soybean cv. JS-335 inoculated with the virus and developed characteristic symptoms of YMV viz., irregular bright yellow and green diffused patches on leaves, extensive mosaic and mottling of leaves, puckering and raised green areas on leaves, chlorosis and reduction in leaf size, defoliation and flower drop. Infected plant bear few immature and small sized seeds (Plate 2).

Gill and Rataul (1986) reported that the symptoms of soybean yellow mosaic virus appeared on the first trifoliolate leaf in the form of mild scattered small yellow spots alternating with yellow patches. It spreaded to the next trifoliolate leaf and the size of yellow spots gradually increased till the entire leaf became yellow. On cases necrotic centers developed in most of the yellow spots and the yellow color gradually changed into white. Previously similar symptoms of the yellow mosaic disease were reported by Nair and Nene, (1973), Gautam (1990), Verma *et al.* (1992), Varma and Malathi, (2003), Bashir *et al.* (2006), Qazi *et al.* (2007), Akhtar *et al.* (2009), Gazala *et al.* (2013).

Studies on host range of yellow mosaic virus of soybean

Thirteen plant species were inoculated with soybean yellow mosaic virus using a viruliferous whiteflies under the insect-proof glass house, giving 24 h AAP and 24 h IAP. The percentage of soybean yellow mosaic virus transmission was 100 percent on soybean. The dolichos bean, black gram, cow pea, horsegram and cluster bean showed 60, 60, 60, 40 and 40 percent infection respectively and mungbean showed high percent infection among them (80%). The findings showed that mungbean, dolichos bean, black gram, cow pea, cluster bean and horsegram were also found to be infected by the virus causing soybean yellow mosaic, while the virus could not be transmitted to other plant species, namely groundnut, pea, cucumber, bottle gourd, bitter gourd. The ten to fifteen days after inoculation, the virus caused traditional yellow mosaic patches on soybean,

mungbean, dolichos bean, black gram, cow pea, cluster bean and horsegram (Table 1).

Nariani (1960) reported that *Vigna aconitifolia*, *V. radiata*, *V. mungo*, *Glycine max*, *Phaseolus lathyroides* and *Dolichos biflorus* are hosts of mungbean yellow mosaic virus. The similar host range of mungbean yellow mosaic virus was reported earlier by Muniyappa *et al.* (1976), Qazi *et al.* (2007), Yadav *et al.* (2009) and Ramappa and Devamani (2017).

Studies on transmission of yellow mosaic virus of soybean through whiteflies

To determine the optimum number of whiteflies required for 100 percent transmission, seven to eight days of old soybean seedlings were inoculated with varying numbers of *Bemisia tabaci* viz., 1, 5, 10, 15 and 20. The virus could be passed on by a single adult whitefly. The transmission efficiency varied with respect to the number of whiteflies. When 15 and 20 whiteflies were used per plant, a hundred percent transmission was attained. When 1 and 5 whiteflies were inoculated per plant, the percent transmission was 10 and 25, respectively. The transmission of virus ranges from 10-100% depending on the number of viruliferous whiteflies used for transmission when 24 hours of AAP and 24 hours of inoculation were provided (Table 2 and Plate 3).

Nariani (1960) first reported that the occurrence of mung yellow mosaic and its transmission by the whitefly *Bemisia tabaci* (Genn.) predominantly and it has been reported to be the vector of similar diseases on *Phaseolus lunatus* L. by Capoor and Varma (1948) and on *Dolichos lablab* by Capoor and Varma (1950a). The similar findings on transmission of yellow mosaic virus of soybean through whiteflies were reported earlier by Ahmad and Harwood (1973), Nair and Nene (1973), Rathi and Nene (1974a, b), Rathi and Nene (1976), Chenulu *et al.* (1979), Singh *et al.* (1998), Kumar (2001), Usharani *et al.* (2004) and Jyothi (2012).

Table 1. Host range studies of yellow mosaic disease of soybean under glasshouse conditions

Sr. No.	Test plant species	No. of plants infected	Percent infection	Incubation period	Symptoms
1.	Soybean (<i>Glycine max</i> (L.) Merr.)	5/5	100%	13-15 days	Chlorotic spots with mosaic pattern
2.	Dolichos bean (<i>Dolichos lablab</i>)	3/5	60%	12-15 days	Mosaic pattern with few chlorotic spots
3.	Groundnut (<i>Arachis hypogea</i>)	0/5	-	-	-
4.	Pea (<i>Pisum Sativum</i>)	0/5	-	-	-
5.	Mungbean (<i>Vigna radiata</i> L.)	4/5	80%	13-15	Chlorotic spots with mosaic appearance
6.	Horsegram (<i>Dolichus biflorus</i> L.)	2/5	40%	14-15	Chlorotic spots
7.	Cow Pea (<i>Vigna unguiculata</i>)	3/5	60%	10-15	Chlorotic spots and mosaic pattern

Sr. No.	Test plant species	No. of plants infected	Percent infection	Incubation period	Symptoms
8.	Cluster bean (<i>C. Tetragonoloba</i> Traub.)	2/5	40%	14-15	Chlorotic spots
9.	Blackgram (<i>Vigna mungo</i>)	3/5	60%	12-15	Chlorosis of leaves and mosaic pattern
10.	Cucumber (<i>Cucumis sativus</i>)	0/5	-	-	-
11.	Bottle gourd (<i>Lagenaria sicerasia</i>)	0/5	-	-	-
12.	Bitter gourd (<i>Momardica charantia</i>)	0/5	-	-	-
13.	<i>Chenopodium quinoa</i>	0/5	-	-	-

Table 2. Determination of number of viruliferous whiteflies required for transmission of yellow mosaic disease of soybean

Whitefly species	Number of whiteflies inoculated per plant	Total number of plants inoculated	Total number of plants infected	Percent transmission (%)
<i>Bemisia tabaci</i>	1	20	2	10
<i>Bemisia tabaci</i>	5	20	5	25
<i>Bemisia tabaci</i>	10	20	11	55
<i>Bemisia tabaci</i>	15	20	20	100
<i>Bemisia tabaci</i>	20	20	20	100

Plates:



Plate 1a. Maintenance of whiteflies on brinjal.



Plate 1b. Maintenance of whiteflies on cucumber.



Plate 2. Symptoms of yellow mosaic disease on soybean plants at glasshouse.



Plate 3. Whiteflies inoculated soybean plants for transmission studies of soybean yellow mosaic disease.

CONCLUSION

The studies revealed that soybean test plant, JS-335 developed typical yellow mosaic disease symptoms like irregular bright yellow and green diffused patches on leaves, extensive mosaic and mottling of leaves, puckering and raised green areas on leaves, chlorosis and reduction in leaf size on virus inoculation via whiteflies. Mungbean, dolichos bean, black gram, cow pea, cluster bean and horsegram act as host of yellow mosaic disease as the yellow mosaic of soybean was able to infect them through whiteflies giving 24 h AAP and 24 h IAP under glasshouse condition. The 100% transmission rate was recorded, when 15 and 20 whiteflies were used per soybean plant and even single whitefly was able to transmit the soybean yellow mosaic disease. Thus the effective management of whiteflies and virus host plants will be helpful to improve quality and yield of soybean.

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