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CITRUS YELLOW MOSAIC: A TRANSMISSIBLE VIRUS OF CITRUS SPIECES IN INDIA

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ABSTRACT
The yellow mosaic disease of citrus is one of the important diseases causing heavy losses in citrus industry. In the present study, an attempt has been made to analyze certain studies on host range and transmission in citrus yellow mosaic virus infecting Rangapur lime, sweet orange and acid lime. The virus disease of citrus was recognized by mosaic symptoms were noticed in sweet orange, Rangapur lime and acid lime plants. Systemically infected Rangapur lime, sweet orange and acid lime leaves showing characteristic yellow mosaic were collected from AICRP on Tropical Fruits (Citrus), Tirupati. During the present investigations major efforts were made on characterization of CYMV and following information have been generated. The virus under study has a narrow host range. Mechanically sap transmissible from rangpur lime to rangpur lime, sweet orange and acid lime among the citrus hosts and to *Canna indica*, maize and sorghum in non-citrus hosts. Pure cultures of the virus were developed by sap transmission and there have been used in experiments. The virus culture was maintained on citrus plants. Studies on graft transmission indicated that T-budding (65.56%) and back patch (61.7%) were the best in per cent transmission than leaf patch (42.5%) grafting. The CYMV transmission from sweet orange to sweet orange and Rangapur lime; Rangapur lime to sweet orange and Rangapur lime; and acid lime to acid lime was found better. The transmission of CYMV was very low when it was from acid lime to sweet orange and rangpur lime and vice versa. The citrus mosaic virus was also transmitted by mealy bug, *Planococcus citri* after 48h acquisition feeding and 4 days inoculation feeding.

INTRODUCTION

Citrus is an important fruit crop in the Semi Arid regions of South India especially in Andhra Pradesh. It occupies third place among fruit crops in India after banana and mango. During the last two decades a number of virus and virus like diseases have been reported on citrus in India by Ahlawat *et al*. (1985 and 1996 a, b). Citrus yellow mosaic disease in India was first described by Murthi and Reddy (1975). It was later studied in detail by Ahlawat *et al*. (1985 and 1996a). Mosaic disease is widely distributed and is a common and severe disease in India especially on sweet orange. It was described as a new graft transmissible disorder in sweet orange characterised by yellow mottling of leaves and yellow fleeking along the veins. The incidence of the disease has been reported to 10-77% (Murthy and Reddy, 1975). A badna virus has been reported to be associated with mosaic disease in India (Ahlawat *et al.,* 1996b).

The use of uncertified scion budlings for planting is an important factor responsible for spread of the disease in non-traditional areas of citrus cultivation besides traditional areas. Due to ignorance and non-availability of certified scion material in huge quantities, the private nurseries collect scion material from farmers' field which is a source of latent infection. So, it is necessary to index mosaic disease and supply of virus free scion material of Sathgudi sweet orange nucellar mother block. Citrus indicator hosts for mosaic diseases takes larger incubation periods to express symptoms. Hence, there is a need to identify non-citrus herbaceous hosts for the disease to use in biological indexing. Different inoculation methods, host range and transmission by vector were studied and the results were reported in the paper.

MATERIALS AND METHODS

Virus isolate: The citrus yellow mosaic isolate used in these studies was a severe one among 19 isolates collected from different orchards in AP. These isolates were multiplied by grafting and by periodical mechanical inoculations on Sathgudi sweet orange, acid lime, Rangapur lime and Jambhiri seedlings and maintained in an insect proof glass house.

Mechanical inoculation: Symptomatic leaves of sweet orange, acid lime and Rangapur lime were collected and maintained in glass house. The leaves were cut into small pieces transferred into a chilled motor and macerated by using 0.01 M phosphate buffer pH 7.0 (With 0.2% of 2- Mercaptoethanol) at the rate of 1g/9mL. The extract was filtered and used for inoculation. Leaves of test plants were immediately inoculated using carborandum (600 mesh) as an abrasive. The test plants used in the experiment were 3 month old seedlings of all four citrus species. The test plants were kept in an insect proof glasshouse for observation.

Insect transmission: Two Aphid species, *Taxoptera citricida* and *A. gossippi* on acid lime and one mealybug species, *Planococcus citri* which are being maintained were used for virus transmission. The Aphid species were tested in non-persistent way where as mealy bug species were tested in

persistent manner. Ten insects of each species were used in inoculation tests/seedlings of sweet orange Like wise 10 seedlings were used for each insect species.

Grafting: Bark patch, T-budding and leaf patch grafting methods were performed to study the transmission of mosaic virus by grafting. The test plants used in the studies were one year old seedlings of sweet orange, Acid lime, Rangapur lime and Jambhiri.

Host range: To study host range of CYMV, non-citrus plant species were grown in an earthen pots kept inside an insect proof glasshouse viz., *Arachis hypogaea, Chenopodium amaranticolor, C.quinova, C. murale, Cajanas cajan, Catheranthus rosea, Canna indica, Cicer arietinum, Citrullus lanatus, Commelina diffusa, Cucumis melo, Cucumis pepo, Cymopsis tetragonaloba, Cucurbita maxima, Dolichos lablab, Datura metal, Glycene max, Gomphrena globosa, Helianthus*

Table 1: Host Range of citrus yellow mosaic virus [CYMV]

Sl.No.	Test Plant	No. of plants infected/ No. of plants inoculated	Symptoms	Incubation period	Per cent transmission
1	*Arachis hypogaea*	0/10	—-	—	0
2	*Chenopodium amaranticolor*	0/10	—	—	0
3	*C.quinova*	0/10	—	—	0
4	*C. murale*	0/10	—	—	0
5	*Cajanas cajan*	0/10	—	—	0
6	*Catheranthus rosea*	0/10	—	—	0
7	*Canna indica*	10-10	Chlorotic spots later turned to severe mosaic and vein banding.	14 days	100
8	*Cicer arietinum*	0/10	—	—	0
9	*Citrullus lanatus*	0/10	—	—	0
10	*Commelina diffusa*	0/10	—	—	0
11	*Cucumis melo*	0/10	—	—	0
12	*Cucumis pepo*	0/10	—	—	0
13	*Cymopsis tetragonaloba*	0/10	—	—	0
14	*Cucurbita maxima*	0/10	—	—	0
15	*Dolichos lablab*	0/10	—	—	0
16	*Datura metal*	0/10	—	—	0
17	*Glycene max*	0/10	—	—	0
18	*Gomphrena globosa*	0/10	—	—	0
19	*Helianthus annus*	0/10	—	—	0
20	*Hibiscus esculantus*	0/10	—	—	0
21	*Luffa acutangula*	0/10	—	—	0
22	*Luffa cylindrical*	0/10		—	0
23	*Mimordica chrantia*	0/10		—	0
24	*Nicotiana glutinosa*	0/10	—	—	0
25	*N. tobacum var (Harrison special)*	0/10	—	—	0
26	*Petunia hybrida*	0/10	—	—	0
27	*P. vulgaris*	0/10	—	—	0
28	*Solanam melangena*	0/10	—	—	0
29	*Sorghum bicolour* NTJ-2	0/10	—	—	0
30		10/10	Chlorotic streaks later turned into dark green streaks all along the leaf lamina.	10 days	100
	Sorghum bicolour var. Kurnool		—		
31	*Tridax procumbens*	10/10	—	—	0
32	*Vigna mungo*	0/10	—	—	0
33	*V.sinensis*	0/10	Chlorotic streaks later turned into dark green streaks all along the leaf lamina.	—	0
34	*V.radiata*	0/10		—	0
35	*Zea mays var.* Aswini	0/10		—	0
36	DHM-103	10/10		10 days	100
	DHM-105	1010		″	″
	Harsha	1010		″	″
	Madhuri	10/10		″	″
	Trishula	10/10		″	″
	Varun	10/10		″	″
		10/10		″	″

annus, Hibiscus esculantus, Luffa acutangula, Luffa cylindrical, Mimordica chrantia, Nicotiana glutinosa, N. tobacum Var Harrison special*, Petunia hybrida, P. vulgaris, Solanam melangena, Sorghum bicolour var NTJ-2, Sorghum bicolour var K, Tridax procumbens, Vigna mungo, V.sinensis, V.radiata, Zea mays* varieties, Aswini, DHM-103, DHM-105, Harsha, Madhuri, Trishula and Varun 10 seedlings of each plant species were inoculated as described in sap inoculation previously.

Serodiagnosis: DAC ELISA and dot blot ELISA were performed to check all the inoculated hosts for presence of the virus.

Dac-elisa: Described by Hobbs *et al*. (1987) was adopted to check the presence of virus in inoculated hosts. The leaf samples of inoculated plants ground in carbonate buffer at the rate of 1gm/9mL and 200μL. The extracts were added to each well of the plate and incubated for 1h at 37ºC. The plate was washed 3 times with PBS-T. (Kept 3 minutes interval between each wash). Antiserum dilution of 1:1000 was added to antigen coated wells. The plate was incubated for 1 hour at 37ºC and washed 3 times with PBS-T. The goat anti-rabbit antibodies labelled with ALP dilution of 1:2500 was added to the plate. The plate was incubated at 37ºC for 1h and washed with PBS-T for 3 times. The enzyme specific substrate P-nitro-phenyl phosphate (5mg/10mL of PBS-T) was added to the wells and incubated at room temperature for 30 min. The reaction was terminated by adding 50μL of 3 M NaOH solution to each well. The reactions were read in ELISA reader, Anthos HT1.

Dot blot ELISA: Leaf samples of inoculated plants nitro cellulose were prepared in carbonate buffer. 10μL of each antigen sample was applied on nitro cellulose membrane with the help of a micropipette. The membrane was air dried for 20 minutes and transferred to a blocking solution for 1 hour at room temperature. After incubation the membrane was transferred to blocking solution having 1:1000 diluted antiserum and incubated for 1h at 37ºC. The membrane was washed 3 times with PBS-T (3 min interval between each wash). The membrane was placed in 1:5000 diluted goat antirabbit antibodies labelled with HRP for 1h at room temperature and washed thrice with PBS-T. Substrate solution specific to enzyme is added (DAB System), gently shaked till the development of colour and washed with sterile water. The membrane was dried and the results were recorded.

RESULTS

Symptoms: The virus was mechanically transmitted to all four citrus species of acid lime, sweet orange, Rangapur lime and Jambhiri. Infected plants showed typical bright yellow mosaic symptoms after 60-80 days of inoculation (Table 1).

The test plants inoculated by mealybug species produced typical mosaic symptoms on Sathgudi sweet orange. The symptomatic plants were also confirmed by both DAC-ELISA and Dot-Blot ELISA tests (Table2).

Grafting

Among the three methods of graft inoculation, T-budding and bark patch were best in per cent transmission compared to leaf patch. The results also indicate that CYMV transmission from sweet orange to sweet orange and Rangpurlime; Rangapur lime to sweet orange and Rangpurlime; and acid lime to acid lime was found better (Table3). The transmission of CYMV was very low when it was from acid lime to sweet orange and Rangapur lime and vice versa. Symptoms were observed 3 months after grafting as minute specks of light green colour distributed all over the leaf lamina. In another 5 days, these specks become chlorotic and conspicuous. Typical mosaic symptoms appeared in about a week and became severe.

Host range

Variable symptoms of the disease were observed in four non-citrus hosts out of 42 inoculated. *Canna indica* showed as chlorotic spots after 14 days of inoculation and latter appears to be mosaic after 3 weeks. The developing young leaves showed severe symptoms of mosaic and vein banding symptoms.

In sorghum and maize symptoms were observed 10 days after inoculation as chlorotic streaks on young leaves and latter turned into dark green streaks all along the leaf lamina. All the 4 isolates of acidlime, sweet orange, Rangapur lime and Jambhiri produced same symptoms as *Canna indica*.

DAC and Dot Blot ELISA

All the inoculated hosts showing mosaic symptoms were confirmed by DAC and dot blot ELISA and it reacted positively with citrus mosaic badna virus polyclonal antiserum.

DISCUSSION

Mosaic disease of citrus has been reported from India (Reddy *et al.,* 1972; Murti and Reddy, 1975; Ahlawat *et al.,* 1985). Citrus mosaic disease is widely distributed in India and is of great economic importance to the citrus industry. The presence of the disease in commercial nurseries and supply of uncertified scion budlings suggests inadvertent spread of the disease through contaminated bud wood. The disease was transmitted by sap inoculation to 4 citrus species viz., Rangpurlime, acid lime, sweet orange and rough lemon and three non-citrus species viz., sorghum, maize and *Canna indica*. Aparna *et al*. (2002) this is first report of non citrus herbaceous host to CYMY it was also transmitted by grafting. The sugarcane bacilliform virus (SCBV) was also known to be mechanically transmissible from sugarcane to banana, rice, sugarcane and sorghum (Bauhdia *et al.,* 1993) but not to maize, barley, oat, *Chenopodium quinoa* and *Nicotiana benthamiana*. Similarly, Kalanchoe top spotting badnavirus (KTSV) was also

Table 2: Transmission of CYMV by Insect Vectors

S.No.	Vector	Inoculation host	No. of plants infected/ No. of plants inoculated	Incubation period	Percent transmission
1	*Aphis gossipii*	Sweet orange	0/10	—	0
2	*T. citricida*	Sweet orange	0/10	—	0
3	*Planococcus citri* (Mealy bugs)	Sweet orange	8/10	2 months	80

Table 3: Transmission of CYMV by Grafting Methods

S. No.	Method of grafting	Source plant [Infected]	Test Plant	No. of test plants	No. of Plants showing symptoms	Per cent transmission
1	T – budding	Rangpurlime	Rangpurlime	10	10	100
			Acid lime	10	4	40
			Sweet orange	10	10	100
		Acid lime	Rangpurlime	10	3	30
			Acid lime	10	10	100
			Sweet orange	10	1	10
		Sweet orange	Rangpurlime	10	9	90
			Acid lime	10	2	20
			Sweet orange	10	10	100
						Mean = 65.56
2	Leaf Patch Grafting	Sweet orange	Rangpurlime	10	3	30
			Acid lime	10	0	0
			Sweet orange	10	7	70
			Jambhiri	10	7	70
						Mean = 42.5
3	Bark patch grafting	Rangpurlime	Rangpurlime	10	10	100
			Acid lime	10	3	30
			Sweet orange	10	9	90
			Jambhiri	10	8	80
		Acid lime	Rangpurlime	10	2	20
			Acid lime	10	9	90
			Sweet orange	10	1	10
			Jambhiri	10	4	40
		Sweet orange	Rangpurlime	10	9	90
			Acid lime	10	2	20
			Sweet orange	10	10	100
			Jambhiri	10	7	70
						Mean = 61.67

mechanically transmissible to Kalanchoe bloss feldiana but not to six other herbaceous plants tested (Lockhart and Ferji, 1988). The banana streak virus (BSV), another member of badna virus group was however; not mechanically transmissible to banana or any other plants tested but was transmitted mechanically from BSV infected banana to sugarcane (Lockhart, 1986). Similarly, *Mimosa bacilliform*virus was not mechanically transmissible to 28 species of test plants (Martin and Kim, 1987). Citrus yellow mosaic virus is also mechanically transmissible from citrus to citrus and sugarcane (Bhaskar Reddy, 1997). It may therefore be concluded that like other badna viruses the present citrus mosaic yellow virus is also mechanically transmissible from citrus to citrus and *Canna indica*, maize and sorghum as non-citrus hosts. These non-citrus herbaceous hosts could be used for biological indexing of CYMV instead of citrus indicator hosts which require more than eight weeks for symptom expression. The results obtained in the graft transmission of citrus mosaic, when bark patches, young and mature leaf bits and buds were used, established that bark bits and buds are the best source of inoculum for the significant transmission. Of these three methods in the present case, T-budding (65.56% and bark patch (61.67%) were the best in per cent transmission than leaf patch (42.5%) grafting. Dakshnamurti also established that the bark bits were the best source of inoculum for the quickest transmission of citrus mosaic. From the data it has been observed that, the CYMV transmission from sweet orange to sweet orange and rangpur lime; rangpur lime to sweet orange and rangpur lime; and acid lime to acid lime were found better. The transmission of CYMV was very low when it was from acid lime to sweet orange and rangpur lime and vice versa. From the above results, we concluded that the compatability will be more in case of T-budding and bark patch grafting methods when compared to leaf patch grafting.

The transmission of the mosaic virus suggests that CYMV is a member of badna virus group as most of these viruses are transmitted by mealy bugs. Eight of the ten definitive members of the badnavirus group - Cacao swollen shoot virus (CSSV), Commelina yellow mottle virus (CoYMV), Banana streak virus (BSV), Kalanchoe top spotting virus (KTSV), Piper yellow mottle virus (PYMV), sugarcane bacilliform virus (SCBV), pine apple bacilliform (PBV) and Scheflera ring spot virus (SRSV) are transmitted by mealy bugs (Lockhart and Olskwski, 1994), the mode of transmission is semi - persistant and non-propagative.

Table 4: Screening of mosaic samples by ELISA

Sample No.	Sample	Healthy (in OD)	Infected sample (in OD)	Buffer control (in OD)
1.	Acid lime	0.069	0.342	0.072
2.	Canna indica	0.0095	0.449	—
3.	Maize	0.02	0.38	—
4.	Rannngpurlime	0.06	0.338	—
5.	Sorghum	0.01	0.399	—
6.	Sweetorange	0.076	0.453	—

Transmission of citrus mosaic virus through citrus aphid, *Toxoptera citricida* was proved by (Reddy *et al.*, 1972) but later several scientists failed to transmit mosaic virus through aphids and mealybugs (Pant and Ahlawat, 1997, Ahlawat, *et al.,* 1996). We attempted insect transmission using aphids (*T. citricida*) and mealybugs (*P.citri*). Aphid species failed to transmit the disease but mealybug species successfully transmitted the disease and produced visible mosaic symptoms on three citrus species viz., Rangapur lime, acid lime and sweet orange. All the inoculated hosts showing mosaic symptoms were confirmed by DAC and dot blot ELISA and reacted positively with citrus mosaic badna virus polyclonal antiserum.

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