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RCA-based Detection of Begomoviruses in Weed Genera Associated with Legumes in Southern Karnataka

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ABSTRACT

Background: Yellow mosaic disease (YMD) caused by begomoviruses transmitted through the insect vector *Bemisia tabaci* poses a serious threat to the production of legume crops.

Methods: Season-long surveys were carried out for YMD occurrence in six different legume crops and associated natural weeds both symptomatic and asymptomatic across the districts of southern Karnataka, India. The samples were analyzed through RCA PCR using specific primer pairs.

Result: Up to 94.1 per cent YMD incidence was recorded and nine weed species were commonly found associated with legume crops. The weeds *viz*., *Ageratum conyzoides, Alternanthera sessilis, Commelina benghalensis* and *Euphorbia geniculata* were abundantly found in the surveyed regions. The weeds were both symptomatic and asymptomatic. Rolling circle amplification coupled polymerase chain reaction method was employed to detect yellow mosaic virus in asymptomatic weeds. Phylogenetic analysis based on the sequences of PCR amplified products of weeds and symptomatic legumes revealed a close clustering of the weed samples with horsegram yellow mosaic virus, legume yellow mosaic virus and mungbean yellow mosaic virus. Overall, our data suggests the role of weed species associated with legume crops as alternative/collateral hosts of begomoviruses and their role in the epidemiology of yellow mosaic disease.

Key words: Alternative weed hosts, Begomoviruses, Leguminous crops, RCA-PCR, YMVD.

INTRODUCTION

Global food production is required to be doubled as the total population is estimated to reach 10 billion by 2050 (Willet *et al*., 2019). Grain legumes are important source of dietary proteins, particularly in India where a significant population (about 43%) are vegetarians. India accounts for about 23.46% of global legume production (17-22 MT) with an average area of 23.9 MH (Rao 1982). Despite such a vast area under legume cultivation, shortage of pulses was encountered at times due to sudden drop in production. Among the various factors, the unexpected burst of Yellow Mosaic Disease caused by begomoviruses accounts for a sudden drop in overall legume yield. In India, approximately an annual yield loss of about USD 300 million was estimated in crops such as blackgram, mungbean and soybean (Varma and Malathi 2003; Hema *et al.,* 2014). Therefore, studies related to the characterization and management of begomoviruses are important towards avoiding loss in overall legume yield.

The begomoviruses belong to family *Geminiviridae* with either monopartite or bipartite genome assembly and transmitted through the whitefly vector, *Bemisia tabaci*, in a circulative persistent manner in dicotyledonous crops. The first report of YMD was from western India in Lima bean and later in mungbean in northern India (Capoor and Varma 1948; Nariani 1960). YMD induces yellowing of veins, yellow mosaic and leaf curling with 100 per cent yield loss (Dasgupta *et al*., 2003; Borah and Dasgupta 2012). The yield loss due to begomovirus infection is not only limited to legumes but also in other crops like tomato, chilli, okra and papaya (Varma and Malathi 2003; Leke *et al*., 2015; Inoue-Nagata *et al*., 2016; Sharma *et al*., 2018).

The weeds of legume fields were reported to serve as alternative hosts and virus reservoirs during the offseason. The weed, *A. conyzoides* was shown to harbor mungbean yellow mosaic India virus and tomato leaf curl virus (Naimuddin *et al*., 2014; Pandey *et al*., 2017). Thus, increasing evidence suggest that the weed plants may serve as permanent source of infection and may have a role in disease epidemiology (Saikia and Muniyappa, 1986; Ansari and Tewari 2005; Wisler and Norris 2005). Therefore, the identification and characterization of weed hosts in the agricultural fields will prove crucial in weed management and as well as in the disease management.

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MATERIALS AND METHODS

The incidence of YMD was assessed by roving surveys carried out in major legume crop growing regions of Tumakuru, Chikkaballapura, Mandya, Chamarajanagara, Bengaluru rural and urban districts during 2017-18. The infected leaf samples with symptoms and asymptomatic weeds around each legume cropping systems were collected for molecular analysis. The per cent disease incidence of YMD was calculated by counting the number of plants infected out of total number of plants.

The molecular analysis of the leaf samples was carried out in Plant Virology Laboratory, Department of Plant Pathology, college of Agriculture GKVK, Bengaluru. Total nucleic acid from the leaf samples of legume crops and weeds collected during the surveys was extracted by CTAB method (Maruthi *et al*., 2002). PCR was performed in 25 µL reaction mixture containing 12.5 µL master mix, 2 µL of DNA template, 6.5 µL of sterile distilled water and 2 µL each of forward and reverse primers. The genomic regions of YMV were amplified using begomovirus specific Deng primer pair (5′ TAATATTACCKGWKGVCCSC 3′ and 5′ TGGACYTTRC AWGGBCCTTCACA 3′) (Deng *et al.,* 1994), HgMYV-CP gene specific primer pairs (5′ATGCTTGCAATTAAGTACTT GCA3′ and 5′TAGGCGTCATTAGCCATAGGCA3′) (Anburaj *et al*., 2010) and MYMV-CP gene specific primer pairs (5′ATGGGKTCCGTTGTATGCTTG3′ and 5′GGCGTCATTA GCATAGGCAAT3′) (Naimuddin and Akram, 2010) amplifying around 500 bp and 900 bp regions, respectively.

The RCA was carried out using bacteriophage Φ29 DNA polymerase included in the "IllustraTempliPhi 100 Amplification Kit" (GE Healthcare) to enrich YMV template in samples that failed to amplify with direct PCR. The product obtained by RCA was subjected to PCR to detect trace amount of YMV in the samples. The Deng primers and CP specific primers were used to amplify the RCA product with the expected amplicon size of 500 and 900 bps, respectively. PCR with 2 µL of RCA product and mixture explained above was done. After PCR confirmation, the amplicons were sequenced in both directions using CP gene specific forward and reverse primers at Eurofins Genomics India Private Limited and the sequences were submitted to NCBI database with accession numbers [MK391938-MK391952; MK409375-MK409378].

The sequences of both forward and reverse reactions were aligned and the non-overlapping regions were joined together to compile full length sequence using 'Basic Local Alignment Search Tool (BLAST)' at the National Centre for Biotechnology Information database. The sequences were compared with other respective viral sequences at the NCBI database using BLAST and the multiple sequence alignment was carried out using CLUSTALW2 tool. The phylogenetic Neighbor-Joining tree analysis was conducted using MEGA7.0 software package. Robustness of the tree was determined by bootstrap sampling of multiple sequence alignment with 1000 replications.

RESULTS AND DISCUSSION

Survey and YMD incidence in legume crops

Although, the legume fields chosen for the surveys were at varying stages of growth and showed a large variation in the extent of YMD incidence typical yellow mosaic symptoms were observed in all the surveyed regions (Table 1; Fig 1). Maximum YMD incidence of 64.6% and 86.5% was observed in horsegram fields in *Kharif* 2017 and Summer 2018 respectively while a minimum of 9.0 and 11.1% incidence was observed in field bean. Disease incidence of 27.4%-76.1% was observed in mungbean field. Earlier, maximum disease incidence of YMD in mungbean in Tumakuru was reported 79.54% during *Rabi* season (Manjunath *et al*., 2011) and in New Delhi 35.7% in both pre *Kharif* and *Kharif* seasons (Biswas *et al*., 2015). Further, the total DNA

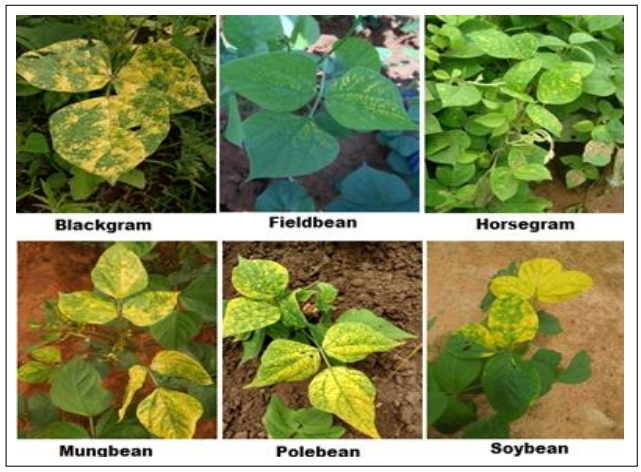

Fig 1: Yellow mosaic symptoms on different legume crops in the fields of southern Karnataka region.

Table 1: Incidence of YMD in legume crops in southern districts of Karnataka.

Legume crops	Districts surveyed for YMD	Disease incidence (%)	
		Kharif 2017	Summer 2018
Blackgram	Bengaluru urban; Chamarajanagara	15.0	59.00
Field bean	Bengaluru urban; Chikkaballapura	9.00	11.10
Horsegram	Bengaluru urban; Chikkabalapura; Mandya	64.6	86.50
Mungbean	Bengaluru urban; Chikkaballapura; Chamrajanagara; Mandya; Tumkur	27.4	76.1
Pole bean	Bengaluru rural; Chikkaballapura; Chamrajanagara	27.3	34.8
Soybean	Bengaluru urban; Tumkuru	17.0	40.3

extracted from the leaf samples were tested for the presence of begomovirus. Using Deng primer pair an amplicon of 500 bp was obtained in PCR reactions of all the crops (Fig 2a). While both the CP gene specific primers resulted in 900 bps size amplicon with RCA enriched DNA samples of all the crops except the field bean samples (Fig 2b). Previously, 900 bp size amplicon was also observed using HgYMV CP gene specific primers in lima bean with symptoms of YMD (Bhagyashree *et al*., 2017).

YMD incidence among associated weeds

Sixty weed samples collected randomly from the surveyed legume fields were identified into 20 different species representing nine different families such as Amaranthaceae, Asteraceae, Commelinaceae, Convolvulaceae, Euphorbiaceae, Fabaceae, Malvaceae, Poaceae and Rubiaceae. The weed samples were categorized as symptomatic and asymptomatic depending on visible YMD symptoms such as yellowing, yellow mosaic, leaf curl, vein clearing, leaf distortion, *etc.* (Table 2). *A. conyzoides, A. sessilis, C. benghalensis* and *E. geniculata* were found most abundantly across all the cropping systems (Fig 3).

In blackgram cropping system, six weed species including three asymptomatic *C. dactylon, E. geniculata* and *P. hysterophorus* were confirmed for the presence of virus with Deng primers. Symptomatic *A. sessilis* and

Table 2: Weed species collected in legume cropping system of southern Karnataka.

Family/Botanical name	Common name	Cropping system	Symptoms observed
I. Amaranthaceae			
a) Achyranthes aspera L.	Chaff flower	Field bean + Horsegram + Mungbean + Polebean + Soybean	Leaf distortion, mosaic, mottling
b) Amaranthus viridis L.	Slender amaranth	Blackgram + Field bean + Mungbean + Polebean + Soybean	Asymptomatic, mosaic
c) Digera arvensis Forsk.	Cotton weed	Field bean	Asymptomatic
II. Asteraceae			
a) Acanthospermum hispidium D.C.	Starbur	Mungbean	Asymptomatic
b) Ageratum conyzoides L.	Whiteweed	Blackgram + Fieldbean + Horsegram + Mungbean + Soybean	Asymptomatic, yellowing, mosaic
c) Alternanthera sessilis (L.) R. Br.	Sessile joyweed	Blackgarm+ Field bean + Horsegram + Mungbean +Polebean	Vein clearing, yellowing
d) Parthenium hysterophorus L.	Congress grass	Blackgram + Mungbean	Asymptomatic
III. Commelinaceae			
Commelina benghalensis L	Tropical spiderwort	Field bean + Horsegram + Mungbean + Polebean + Soybean	Mosaic, yellowing
IV. Convolvulaceae			
Ipomoea purpurea (L.) Roth	Purple morning glory	Horsegram+ Soybean	Mosaic
V. Euphorbiaceae			
Croton bonplandianum L.	Rushfoil and croton	Blackgram + Horsegram + Mungbean + Polebean + Soybean	Yellowing, mosaic, Asymptomatic
Euphorbia geniculata Ortega.	Wild poinsettia	Blackgram + Field bean + Horsegram + Mungbean + Polebean + Soybean	Asymptomatic, Leaf curl, mosaic
VI. Fabaceae			
Cassia tora (L.) Roxb.	Sickle pod	Mungbean + Soybean	Mosaic, yellowing
Crotalaria pallida Aiton	Rattlebox plant	Mungbean + Soybean	Asymptomatic, Mosaic, yellowing
Indigo feratinctoria L.	True indigo	Mungbean	Asymptomatic
Tephrosia purpurea (L.) Pers.	Wild indigo	Mungbean	Asymptomatic
VII. Malvaceae			
Malvastrum coromandalianum (L.) Garcke	Broom weed	Mungbean	Mosaic, Vein clearing
Sida acuta Burm. F.	Wireweed	Field bean + Mungbean	Vein clearing, mosaic
Sida hombifolia L.	Indian hemp	Mungbean + Polebean	Asymptomatic
VIII. Poaceae			
Cynodon dactylon (L.) Pers.	Bermuda grass	Blackgram + Horsegram	Asymptomatic
IX. Rubiaceae			
Borreria articularis (L.f.) F. N. Will.	Button weed	Field bean + Horsegram + Mungbean + Soybean	Yellowing, mosaic, asymptomatic

asymptomatic *A. viridis* did not amplify through PCR with Deng primers but resulted in 500 bp amplicon when subjected for RCA-PCR (Table 3a-c).

In field bean cropping system, presence of virus was confirmed in symptomatic *E. geniculata* by PCR with begomovirus specific primers. However, asymptomatic *A. aspera* and *D. arvensis* showed no amplicon with direct PCR but resulted in 500 bp amplicon after RCA-PCR. Similarly, symptomatic *A. aspera* and *D. arvenensis* did not amplify in direct PCR but showed 500 bp amplicon after RCA-PCR (Table 3a-c).

Six of ten weed samples collected from horsegram fields were symptomatic and remaining four were asymptomatic. Two of the symptomatic weed samples confirmed for the presence of YMV by direct PCR using either Deng primers or CP gene specific primers. In the remaining four symptomatic weed samples, only *A. sessilis* was detected positive for YMV after enrichment of virus template by RCA. Among the four asymptomatic weed samples, two were confirmed for the presence of virus by direct PCR while remaining two did not amplify despite RCA enrichment of virus template (Table 3a-c).

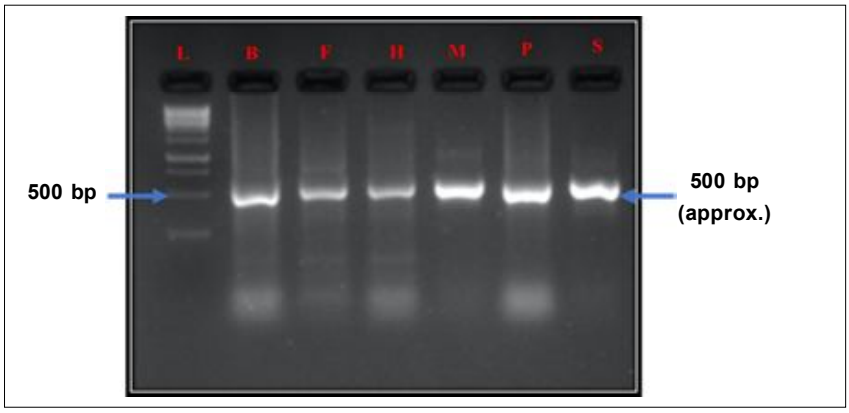

Fig 2a: PCR analysis of legume crop samples using Deng primers.
L: Ladder, B: Blackgram, F: Field bean, H: Horsegram, M: Mungbean, P: Polebean, S: Soybean.

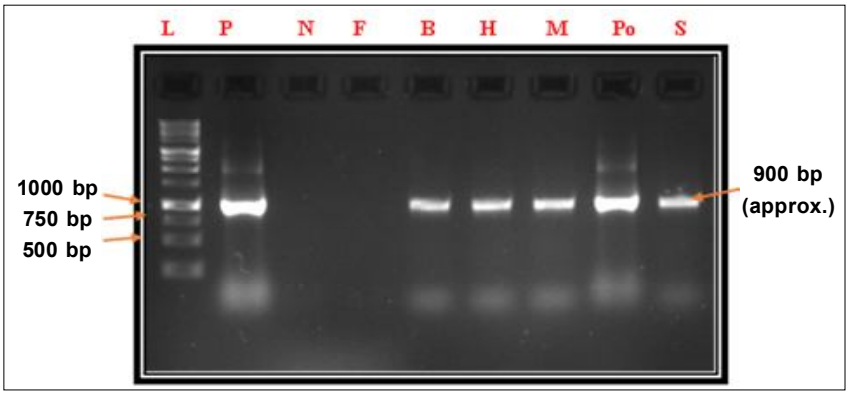

Fig 2b: RCA-PCR analysis of legume crop samples using HgYMV CP gene specific primers
L: Ladder, P: mungbean yellow mosaic virus (Positive control), N: Healthy Horsegram leaf sample (Negative control), F: Fieldbean, B: Blackgram, H: Horsegram, M: Mungbean, Po: Polebean, S: Soybean.

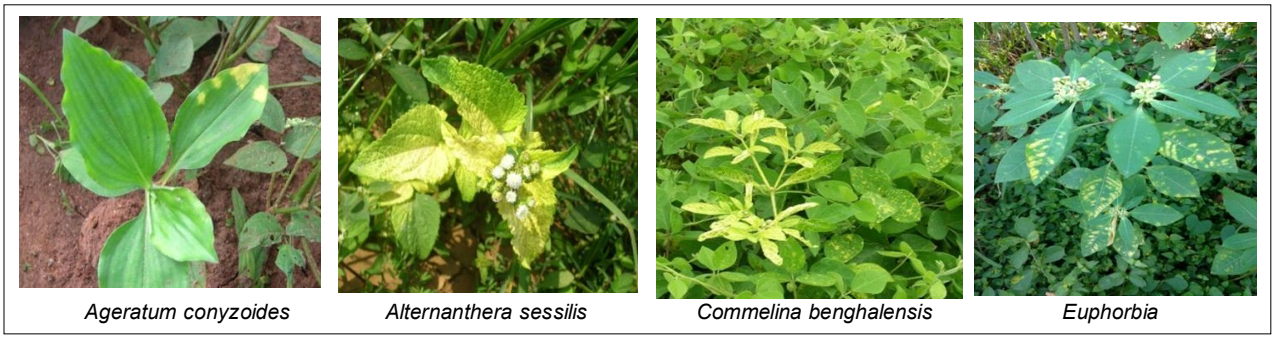

Fig 3: Most common weed hosts collected in legume cropping system of southern Karnataka region.

Table 3a: Direct PCR confirmation of begomovirus from symptomatic weed hosts of legume cropping system.

Cropping system	Symptomatic weed hosts subjected for direct PCR[1,2,3]												
	A.a	*A. c*	*A.s*	*A.v*	*B.a*	*C.b*	*C.t*	*Cr.b*	*C.p*	*E.g*	*I.p*	*M. c*	*S. a*
Blackgram	x	X	Vc$^{-1,2,3}$	x	x	X	x	Y$^{+1,2}$	x	x	x	x	x
Field bean	x	X	Vc^{+1}	M$^{-1,2,3,}$	Y$^{-1,2,3}$	Y, M^{+1}	x	x	x	Lc,M^{+1}	x	x	Vc$^{-1,2,3}$
Horsegram	Ld$^{-1,2,3}$	X	Y,Vc$^{-1,2,3}$	x	x	M$^{+1,2}$	x	Y^{+1}	Y^{-1}	x	M$^{-1,2,3}$	x	x
Mungbean	x	Y,M$^{+1,3}$	Vc$^{-1,3}$	M$^{+1,2}$	x	M$^{+1,2,3}$	M,Y$^{+1,2}$	M $^{+1,3}$	x	M $^{-1,2,3}$	x	M,Vc$^{+1,3}$	M,Vc$^{+1,3}$
Polebean	M,Mo$^{-1,2,3}$	X	Vc,Y$^{+1,2,3}$	x	x	M^{+1}	x	Y$^{+1,2}$	x	x	x	x	x
Soybean	x	Y $^{-1,3}$	x	M $^{-1,2,3}$	M$^{+1,2,3}$	M $^{+1,3}$	M -1,2,3	x	M -1,2,3	M$^{-1,3}$	M$^{-1,2,3}$	x	x

x= No weeds seen in cropping system;

-= No amplification through PCR.

+= Amplification through PCR; [1]Deng primer; [2]HgYMV primer;[3]MYMV primer.

Vc= Vein clearing, Y= Yellowing, M= Mosaic, Lc= Leaf curl, Mo= Mottling,

A.a= A. aspera, A.c= A. conyzoides, A.s= A. sessilis, A.v= A. viridis, B.a= B. articularis, C.b= C.benghalensis, C.t.= C tora, Cr.b= C. bonplandianum, C.p= C.palida. E.g.= E. geniculata, I.p.= I.purpurea, M.c.= M. coromandalianum, S.a= S. acuta,

Table 3b: Direct PCR detection of begomovirus from asymptomatic weed hosts of legume cropping system.

Cropping system	Asymptomatic weed hosts subjected for direct PCR[1,2,3]														
	A.a	*A. c*	*A.h*	*A.v*	*B.a*	*C.d*	*Cr.b*	*C.p*	*D.a*	*E.g*	*I. t*	*M. c*	*P. h*	*S. r*	*T. p*
Blackgram	x	-	x	-	x	+^{1,2,3}	x	x	x	+ ^{1}	x	x	+^{1,2}	x	x
Field bean	-^{1,2,3}	-	x	x	x	x	x	x	-^{1,2,3}	x	x	x	x	x	x
Horsegram	x	+^{1,2}	x	x	-^{1,2,3,}	+^{1,2,3}	x	x	x	-^{1,2,3}	x	x	x	x	x
Mungbean	-^{1,2,3}	x	-^{1,2,3}	x	+^{1,2}	x	x	-^{1,2,3}	x	x	+^{1,3}	x	+^{1,2,3}	+^{1,3}	+^{1,3}
Polebean	x	x	x	-^{1,2,3}	x	x	x	X	x	-^{1,2,3}	x	x	x	+^{1}	x
Soybean	+^{1,2}	x	x	x	x	x	+^{1,2}	x	x	x	x	x	x	x	x

x = No weeds seen in cropping system.

[1]Deng primer; [2]HgYMV primer; [3]MYMV primer.

+ = Weed samples showed positive amplification through PCR.

- = Weed samples no amplification through PCR.

A.a.= A. aspera, A.c= A. conyzoides, A.h.= A.hispidium, A.v.= A. viridis, B.a.= B. articularis, C.d.= C. dactylon, Cr.b.= C. bonplandianum, C.p= C. palida. D.a.= D. arvensis, E.g.= E. geniculata, I.t.= I. feratinctoria, M.c.= M. coromandalianum , P.h.= P. hystserophorus, S.r.= S.rhombifolia, T.p.= T. purpurea.

Out of 17 (9 symptomatic and 8 asymptomatic) weed samples collected from mungbean cropping system, twelve samples detected for the presence of begomovirus through direct PCR using Deng primers. Of the remaining five weed samples subjected for RCA-PCR, four were confirmed for the presence of begomovirus. Of these four, two were asymptomatic such as *A. aspera* and *A. hispidium* and remaining two were symptomatic namely, *A. sessilis* and *E. geniculate* (Table 3a-c).

In the pole bean fields, asymptomatic weed, *S. rhombifolia* was positive for begomovirus by direct PCR while asymptomatic weed species *A. viridis* and *E. geniculata* were confirmed only after RCA-PCR (Table 3a-c).

Out of 10 weed hosts collected from soybean fields, eight were symptomatic and two were asymptomatic. Four of these were confirmed by direct PCR and four were confirmed by RCA-PCR. The symptomatic *A.conyzoides, A. viridis, C. tora* and *E. geniculata* were confirmed for begomovirus presence only by RCA-PCR (Table 3a-c).

The percentage of weed species detected with begomovirus from around blackgram, field bean, horsegram, mungbean, polebean and soybean is 85.71%, 66.67%, 40%, 94.11%, 71.42% and 60% respectively. Overall, out of 60 weed samples collected from six legume cropping systems, 31 weed samples (51.67%) amplified for begomovirus specific product through direct PCR using Deng primers while remaining 29 did not amplify. However, upon enrichment of viral DNA, 16 weed samples out of 29 resulted in expected amplicon (Table 3c, Fig 4).

A maximum of 94.1% of weed samples showed positive for begomovirus in mungbean crop system while a minimum of 40.0% confirmed positive in soybean (Table 4). Moreover, 13 weed species *viz., A. aspera, A.conyzoides, A. hispidium, A. viridis, B. articularis, C. dactylon, C. bonplandianum, D. arvensis, E. geniculata, I. tinctoria, P. hysterophorus, S. rhombifolia* and *T. purpurea* were found asymptomatic alternative weed hosts of begomovirus in legume cropping systems. *A. aspera, A. conyzoides* and *C. bonplandianum* were confirmed as hosts for HgYMV.While, *B. articularis, I.tinctoria,S.rhombifolia* and *T. purpurea* served as alternative hosts for MYMV. Interestingly, *C. dactylon*, *P. hysterophorus* were found hosts for both HgYMV and MYMV.

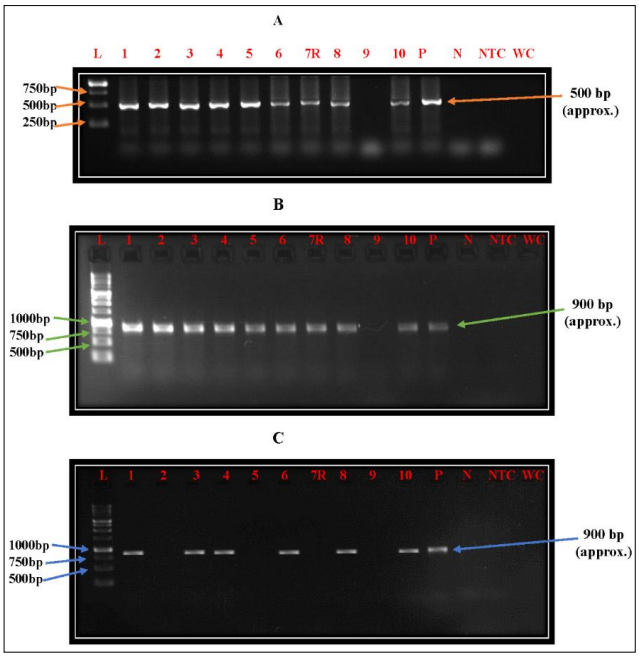

Fig 4: Detection of begomoviruses in weed samples through PCR using three primer pairs A: Deng primer, B: HgYMV specific primers pair, C: MYMV specific primer pair.
L: Ladder, 1: *A. hispidium,* 2: *A. aspera,* 3: *A. conyzoides,* 4: *A. sessilis,* 5: *A.v iridis,* 6: *B. articularis,* 7R: *C. tora* (RCA enriched template), 8: *C. benghalensis*, 9: *C. pallida,* 10: *E. geniculata,* P: Infected mungbean plant (+ve control), N: Healthy plant (-ve control), NT: Non-template control, WC: Water control.

Table 3C: RCA-PCR detection of begomovirus from symptomatic and asymptomatic weed hosts of legume cropping system.

Cropping system	Symptomatic and asymptomatic weed hosts detected through RCA-PCR using Deng primers											
	A.a	*A. c*	*A.h*	*A.s*	*A.v*	*B.a*	*C.p*	*C.t*	*D.a*	*E.g*	*I.P*	*S.a*
Blackgram	x	*-	x	Vc^{+}	*+	x	x	x	x	-	x	x
Field bean	*+	*-	x	-	M^{+}	Y^{-}	x	x	*+	-	x	Vc^{-}
Horsegram	Ld -	-	x	Vc,Y^{+}	x	*-	Y^{-1}	x	x	*-	M^{-}	x
Mungbean	*+	-	*+	Vc^{+}	-	-	*-	-	x	M^{+}	x	-
Polebean	M,Mo^{-}	x	x	-	*+	x	x	x	x	*+	x	x
Soybean	-	Y^{+}	x	x	M^{+}	-	M^{-}	M^{+}	x	M^{+}	M^{-}	x

Y= Yellowing, Vc= Vein clearing, M= Mosaic, Mo= Mottling; Ld= Leaf distortion.

- = No amplification.

+ = Symptomatic plants positive amplification.

x = No weeds seen in cropping system.

*+ = Asymptomatic weed samples showing positive amplification.

*- = Asymptomatic weed samples with no amplification.

A.a.= A. aspera, A.c= A. conyzoides, A.h.= A. hispidium, A.s.= A.sessilis, A.v.= A.viridis, B.a.= B. articularis, C.p= C. palida, C.t.= C.tora, D.a.= D. arvensis, E.g.= E. geniculata, I.p.= I. purpurea, S.a= S. acuta.

It is important to highlight that none of the fields were free from YMD incidence despite several vector control measures were used in the farmer's fields. This underscores the severity of the YMD in the southern Karnataka region. In fact, our findings corroborate several previous studies that have reported the YMD severity in India (Anburaj *et al*., 2010; Naimuddin *et al*., 2014; Rajkumar 2006 and Salam *et al*., 2011).

Phylogenetic tree analysis of amplicon sequences

The sequences of PCR products of 14 weed species and 4 legume crop samples, excluding field bean samples (did not result in PCR product) and pole bean (due to poor quality of the sequencing data), amplified using CP gene specific primers were determined. These sequences were analyzed to construct phylogenetic tree by comparing with 17 reference sequences obtained from NCBI (Fig 5). Phylogenetic

Table 4: Percent detection of begomovirus in weed samples collected during survey in legume cropping system.

Leguminous cropping Systems*	Total number of weed samples collected	No. of weed samples amplified in PCR with Deng primer (a)	No. of weed samples subjected for RCA-PCR with Deng primer	No. of weed samples amplified in RCA-PCR with Deng primer (b)	Total weed samples YMV detected (a+b)	Percentage detection (%) *
Blackgram	7	4	3	2	6	85.71
Field bean	9	3	6	3	6	66.67
Horsegram	10	5	5	1	6	40.00
Mungbean	17	12	5	4	16	94.11
Polebean	7	3	4	2	5	71.42
Soybean	10	2	8	4	6	60.00
Total	60	29	31	16	45	75.00

Note: RCA= Rolling circle amplification, PCR= Polymerase chain reaction. **Kharif* 2017 - summer 2018.

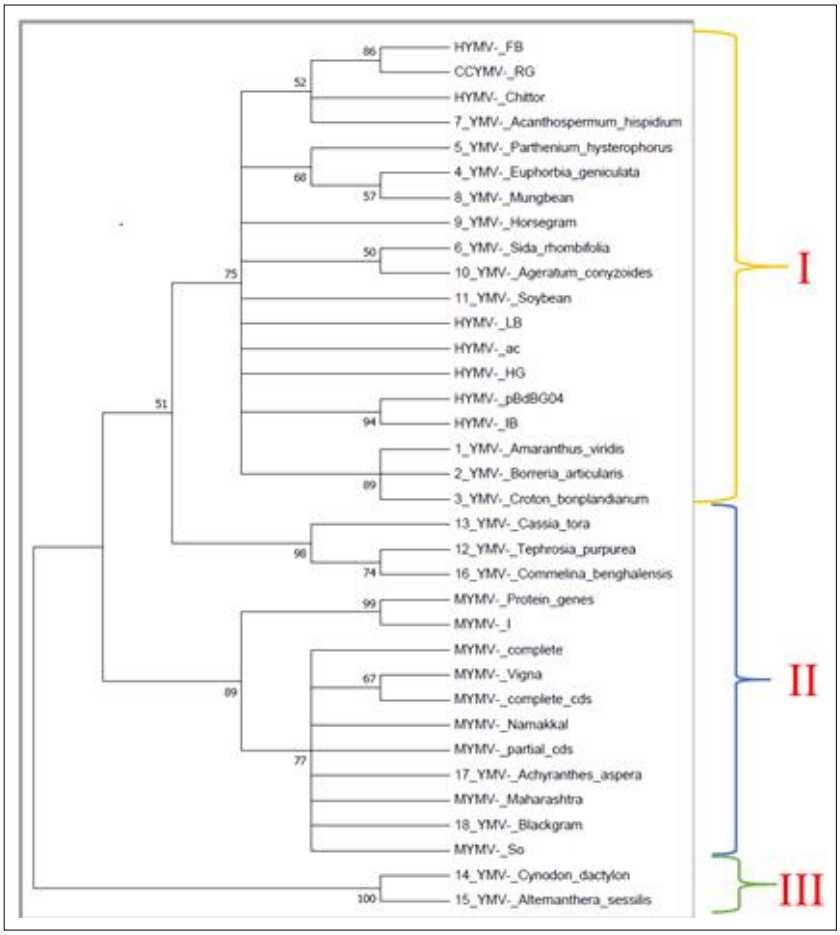

Fig 5: Phylogenetic relationship of begomoviruses using CP gene sequences. Number at nodes indicate the bootstrap percentage scores out of 1000 replicons.

analysis showed that begomoviruses from mungbean, horsegram and soybean belong to same cluster with begomoviruses from 8 weed species and the French bean isolate sequence of horsegram yellow mosaic virus (HgYMV) segment DNA-A complete sequence. Out of four common weed hosts most abundantly occurred in six leguminous crop system, *A. conyzoides* and *E. geniculata* reside in cluster I, *C. benghalensis* in cluster II and *A. sessilis* in cluster III.

The nucleotide identity matrix of CP gene sequences of begomoviruses obtained from 14 weed species and four legume crops showed 31.0 to 100.0 per cent nucleotide identity between crops and weed host samples implying that begomovirus CP gene sequences are closely related (data not shown). *A. conyzoides* and *E. geniculata* showed 98.74% nucleotide identity with French bean isolate HYMV segment DNA-A sequence. *C. benghalensis* showed 87.79% identity with HYMV clone pBdBg04 segment DNA-A sequence. *A. sessilis* showed 97.72% identity with MYMV-Soybean [Madurai] segment DNA A sequence. Thus, our molecular characterization efforts clearly indicated the presence of begomoviruses related to horsegram yellow mosaic virus, HgYMV clone pBdBg04 segment DNA-A and MYMV-Soybean [Madurai] in most legume crops and the weeds associated with the legume crop fields in southern Karnataka regions. It is pertinent to note that *A. conyziodes, C. olitorius* and *A. sessilis*, around mungbean fields were found to harbor MYMIV and were characterized as alternative hosts of MYMIV (Marabi *et al.,* 2017).

CONCLUSION

Legumes constitute an important component of human nutrition as major source of protein. In fact, legume grains can complement cereals as affordable source of dietary protein in human and animal food. Therefore, novel research findings or methods that contribute to effective management of global legume production should further strengthen efforts towards maintaining global food security. The major insights from the current study involving the identification of asymptomatic weed species in the legume fields that carry begomoviruses and the possibility of serving sources of begomovirus inoculum underscores the need to keep legume fields free of weed plants. In fact, our findings suggest that crop rotation with non-leguminous crops, weed management either through appropriate weedicides or manual weeding, removal of volunteer weed plants, whether symptomatic or asymptomatic are very important for effective integrated management of yellow mosaic virus diseases in legume crops.

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