RESEARCH ARTICLE Legume Research- An International Journal, Volume 45 Issue 1: 122-127 (January 2022)

Identification and Confirmation of Resistance in Mungbean [*Vigna radiata* (L.) Wilczek] Derivatives to Mungbean Yellow Mosaic Virus (MYMV)

B. Madhumitha, K. Eraivan Arutkani Aiyanathan[1], M. Raveendran[2], M. Sudha[2]

10.18805/LR-4437

ABSTRACT

Background: Mung bean Yellow Mosaic Virus (MYMV) is found to be one of the prime viral diseases of mungbean in Tamil Nadu state. Screening for MYMV resistance in field condition always remains a hassle for breeding society. The peculiar MYMV symptoms often failed in the field due to some factors such as environmental changes, whitefly genotypes, host factors *etc*. With the above perspective, the present study aimed to screen the mung bean derivatives against MYMV through a novel *in vitro* agroinoculation technique and further substantiation through whitefly transmission.

Methods: Four interspecific derivatives (VGGRU 1, VGGRU 2, VGGRU 3 and VGGRU 4) generated by making crosses between mungbean VRM (Gg) 1 and rice bean (TNAU RED) along with the susceptible check VRM (Gg) 1 were agroinoculated with the MYMV infectious clone VA 239 (KA30 DNA A + KA27 DNA) and are further substantiated through whitefly transmission studies from the artificially reared whiteflies.

Result: The agroinoculation results revealed that among the four interspecific derivatives, VGGRU 1 was found to be completely resistant to MYMV. The substantiation of the obtained result through whitefly transmission also revealed that 24 h Acquisition Access Period (AAP) and 24 h Inoculation Access Period (IAP) with *Bemisia tabaci* able to cause 65% infectivity in susceptible plant VRM (Gg) 1 and zero infectivity in VGGRU 1 and the results were PCR confirmed for the presence of viral DNA.

Key words: Agroinoculation, Mungbean yellow mosaic virus, Mungbean, Transmission, Whitefly.

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] commonly known as green gram is one of the major fast growing, warm season pulse crop, primarily cultivated for their rich source of quality proteins. In India, mungbean crop is cultivated in many states including Tamil Nadu to an area of around 43 lakh ha with annual production of 20.70 lakh tones and productivity of 481 kg/ha (Anonymous, 2018). Despite of its importance, the substantial constraints in mungbean productivity are primarily due to biotic stresses. Among them, viral diseases are widely devastating and cause heavy yield loss (Paul *et al.,* 2013) and particularly the most important damage amongst the virus is found to be Mungbean Yellow Mosaic Virus (MYMV). MYMV belongs to begomovirus, the largest genus of the family *Geminiviridae* (Dhakar *et al*., 2010), which is characterized by its monopartite or bipartite (DNA-A and DNA-B) genome and are transmitted by white flies (*Bemisia tabaci*) in a circulative and persistent manner (Sidhu *et al*., 2009). In a bipartite genome, the DNA A encodes proteins required for replication, transcription and encapsidation, whereas DNA B encodes proteins required for movement functions (Van Regenmortel *et al.,* 2000). The first occurrence of mungbean yellow mosaic virus in India was spotted by Nariani (1960). A typical MYMV symptom includes the presence of mosaic pattern that exist in the form of alternate green and yellow patches on the leaves, reduction in floral size and production of shrivelled seeds (Habib *et al.,* 2007).

Department of Plant Pathology, Agricultural College and Research Institute, Madurai-625 104, Tamil Nadu, India.

[1]Agricultural College and Research Institute, Killikulam-628 252, Tamil Nadu, India.

[2]Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

Corresponding Author: B. Madhumitha, School of Agriculture and Biosciences, Karunya Institute of Technology and Sciences, Coimbatore-641 114, Tamil Nadu, India. Email: madhu2sucess@gmail.com

How to cite this article: Madhumitha, B., Aiyanathan, K.E.A., Raveendran, M. and Sudha, M. (2022). Identification and Confirmation of Resistance in Mungbean [*Vigna radiata* (L.) Wilczek] Derivatives to Mungbean Yellow Mosaic Virus (MYMV). Legume Research. 45(1): 122-127. DOI: 10.18805/LR-4437.

Submitted: 09-06-2020 **Accepted**: 02-10-2020 **Online:** 21-12-2020

MYMV is found to be one of the prime viral diseases of pulses in Tamil Nadu state. Development of resistant mungbean cultivars against MYMV has long been a major objective in disease resistance breeding programmes. Owing to the fact that MYMV is transmitted by whiteflies, the confirmation of resistance through field screening always remains a hassle. The MYMV symptoms may not always appear in the fields due to some factors such as environmental changes, whitefly genotypes, host factors *etc*., which makes failure in the development of infections in field and also it makes intricate to identify the true resistant lines.

In this perspective and with a view towards developing a reliable laboratory screening protocol for assessing resistance/susceptibility of mungbean accessions against MYMV, Rogers *et al.* (1986) developed a new technique called "Agroinfection" using the Ti plasmid of *Agrobacterium* for viral infection and demonstrated on tomato golden mosaic virus. In this technique, the infectious viral clones are introduced into plants using *A. tumefacians* and this *A. tumefaciens* subsequently deliver the infectious viral DNA from the T-DNA into a plant cell and initiates the infection. Thus, this technique produces a uniform disease outbreak among the test genotypes rather than the natural infestation and acts as a finer tool for the confirmation of resistance. Practicability of using an *in vitro* agroinoculation technique in MYMV screening was demonstrated by various researchers on urdbean, mungbean and soybean (Jacob *et al*., 2003; Usharani *et al*., 2005; Haq *et al*., 2010; Karthikeyan *et al*., 2011; Sudha *et al.,* 2013). With the above leads, the study was carried out for (i) confirmation of resistance in mungbean and rice bean derivatives to MYMV through agroinoculation (ii) assessing the MYMV disease intensity at different day intervals on the infected plants (iii) further validation of the resistance was done with artificial whitefly transmission.

MATERIALS AND METHODS

The following experiments were conducted during the period from 2018 to 2019 at the Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology (CPMB and B), Tamil Nadu Agricultural University, Coimbatore.

Plant materials

A total of four interspecific derivatives (VGGRU 1, VGGRU 2, VGGRU 3 and VGGRU 4) generated by making crosses between mungbean VRM (Gg)1 and ricebean (TNAU RED), obtained from a previous breeding programme made by Dr. M. Pandiyan, Agricultural College and Research Institute, Echankottai, Thanjavur and one susceptible mungbean variety VRM (Gg)1 as check were used in this study.

MYMV construct for agroinoculation

Balaji *et al.* (2004) formerly constructed an infectious clone named VA 239 (KA30 DNA A + KA27 DNA B) which was isolated from the YMV infected black gram leaves collected from Vamban village of Pudukkottai District, Tamil Nadu and mobilized into the *A. tumefaciens* strain C 58. The above mentioned construct was obtained and utilized for agroinoculation in the present study.

Agroinoculation

The experiment on agroinoculation was conducted in the Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore. Agroinoculation was done on surface sterilized overnight sprouted seeds of four interspecific derivatives (VGGRU 1, VGGRU 2, VGGRU 3 and VGGRU 4) and VRM (Gg) 1. *Agrobacterium tumefaciens* strains harbouring the partial tandem repeat clone VA 239 were grown to 1 Optical Density at 600 nm in 2 mL AB minimal medium (pH 7.0) containing the antibiotics like streptomycin (150 mg L^{-1}), spectinomycin (50 mg L^{-1}) and tetracycline (5 mg L^{-1}) at 28°C at 220 rpm. From this, 1 mL of the culture was taken and inoculated into another 50 mL of AB minimal medium (pH -7.0) containing the above mentioned antibiotics and grown to 1 OD at 600 nm at 28°C at 220 rpm. The culture was spinned at 4000 rpm for 10 min at 25°C. The obtained cells were re-suspended in 50 mL of AB minimal medium (pH -5.6) with 100 µL acetosyringone (100 µm). Seed coat of the pre-soaked sprouted seeds was removed by using forceps and pricked around the hypocotyl region and were immediately immersed in the culture of *A. tumefaciens* which carries MYMV construct VA239. After the overnight incubation, seeds were washed with distilled water and sown in pots containing autoclaved sand and vermiculite in the ratio of 1:1. Agroinoculated plants were maintained in a growth chamber at 25°C with 60-70% relative humidity and a photoperiod of 16/18 h, for the proper growth of the plants twice in a week hoagland's solution was applied and the plants were transferred to green house after 15 days for symptom observation. The development of yellow mosaic symptoms on the plant in a given time is considered as susceptible plant and the absence of yellow mosaic symptoms on the plant is scored as resistance against the disease. The percentage of infectivity after agroinoculation was calculated based on the number of infected plants to the portion of the number of plants inoculated.

DNA extraction and PCR analysis

The leaf samples collected from both agroinoculated control and infected plants after symptom expression were subjected to DNA isolation using CTAB method as described by Sudha *et al.* (2009). The DNA was quantified using a Nano-Drop spectrophotometer (ND-1000 Spectro photo meter, NanoDrop Technologies, USA) and quality was checked in 0.8% agarose gel. DNA concentration was normalized to 25 ng/µl for PCR reaction after the necessary quality and quantity checks. The amplification was done using the MYMV coat protein (CP) gene-specific primers FP1 5′GCGGAATTACGATACCGCC3′ and RP1 5′GATGCAT GAGTACATGCC3′ (Richa Maheswari *et al.,* 2014) for both control and agroinoculated plants in MyCycler (BioRAD, USA). A standard volume of 10 µl reaction consists of 6 µl PCR mix 2X SMART master mix (readymade mix of *taq* polymerase, dNTPs and PCR buffer), 1.0 µL of 10 µM primer (forward and reverse each) (First Base, Singapore), 2.0 µL of 25 ng μL^{-1} DNA. The temperature cycles were as follows: 5 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 56°C and 1 min at 72°C. The final elongation step was extended to 10 min at 72°C and finally maintained at 4°C. The amplified products were separated on a 1.2% ethidium bromide pre-stained agarose gel and are visualized on a digital gel documentation and image analysis system (Alpha Imager 1200, Alpha Innotech Corp., USA).

Confirmation of viral DNA in plants

DNA was extracted in ten days interval from both the control and infected plants of susceptible VRM (Gg)1 and resistant VGGRU 1 after the expression of symptoms *i.e.* 25, 35, 45 and 55 days after sowing (DAS) and the infectivity was confirmed by PCR (35 cycles) assay of virus using coat protein primers.

Collection and maintenance of whiteflies

To initiate transmission studies, adults of *Bemisia tabaci* were collected from different fields of cotton and brinjal in Tamil Nadu Agricultural University, Coimbatore and mass cultured in insect-proof glass house using different host plants (cotton, brinjal and bhendi). The whitefly culture was maintained by regular transfer of fresh batch of plants after every six weeks. The new adults after three generations were collected using aspirator and used for transmission studies.

DNA extraction and *mt goi gene* amplification for white fly species confirmation

The total DNA was isolated from the maintained whiteflies through lysis method (Zeidan and Czosnek, 1991) and subjected to amplification using LCOI 1490 forward primer 5′GGTCAACAAATCATAAAGATATTGG 3′ and HCOI 2198 reverse primer 5′ TAAACTTCAGGGTGACCAAAAAATCA 3′ (Folmer *et al.,* 1994) for white fly species confirmation. The PCR was performed with initial denaturation at 94°C for 3 minutes, followed by 40 cycles each consists of 30 secs at 94°C, 40 secs at 53°C, 1 min at 72°C followed by final extension for 20 minutes at 72°C. The PCR products were gel purified and sequenced in Bioserve Biotechnologies (India) Private Limited, Hyderabad.

White fly transmission studies

Ten to fifteen *B. tabaci* adults were collected in nylon clip cages from the maintained culture with the help of an aspirator and are allowed for starvation. After starvation, the clip cage containing whiteflies were clipped on to the MYMV agro-infected mungbean plants and allowed to feed for an acquisition period of 24h. After 24 h acquisition access period (AAP), *B. tabaci* adults were removed from MYMV agro-infected mungbean plants and transferred into a separate insect free chamber containing healthy VGGRU 1 and VRM (Gg) 1 plants for inoculation access period (IAP) of 24 h. After 24 h of IAP, the *B. tabaci* adults were removed and the plants were sprayed with an insecticide (Dimethoate 30 EC at 1 ml/L) and evaluated for MYMV symptom development 10-20 days later.

Further to substantiate the agroinoculation results, attempt was also made for whitefly screening. Artificial rearing of whitefly was done for obtaining pure MYMV inoculum and also to avoid the mixed infections that are commonly seen during field level.

PCR assay for virus in viruliferous and non-viruliferous whiteflies

Total DNA was extracted from the viruliferous and non-viruliferous whiteflies through lysis method as mentioned above and observed for the presence of MYMV CP gene using polymerase chain reaction (PCR). The PCR reaction mixture includes the MYMV coat protein (CP) gene-specific primers with the same temperature conditions and reaction volume as mentioned above for MYMV infected leaf samples.

RESULTS AND DISCUSSION

Resistance screening of mungbean derivatives against MYMV through agroinoculation

On screening for MYMV resistance through agroinoculation, among the four interspecific derivatives of mungbean (namely VGGRU 1, VGGRU 2, VGGRU 3, VGGRU 4 that are generated by making crosses between mungbean VRM (Gg) 1 and ricebean (TNAU RED), the line VGGRU 1 was found to be highly resistant, while the others along with the susceptible check [VRM (Gg)1] developed yellow mosaic symptoms in their trifoliate leaves from 15 to 25th days after agroinoculation and there was no symptom development in the control (Fig 1).

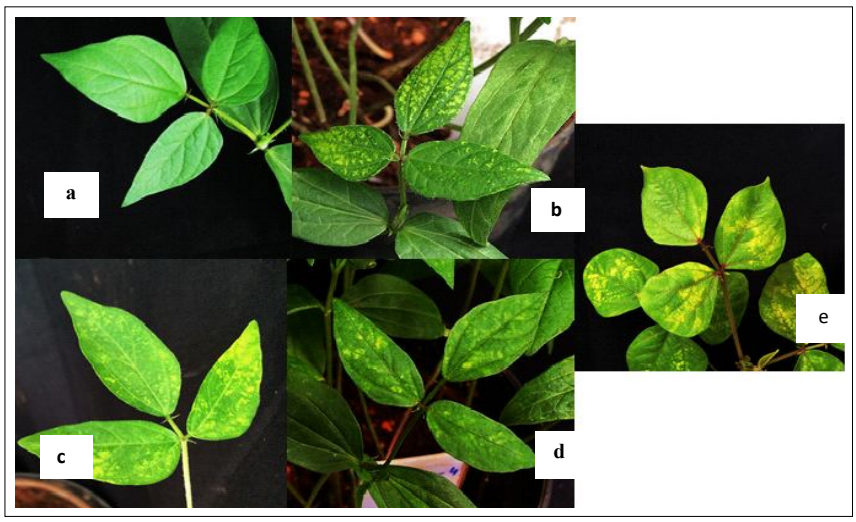

Fig 1: Symptom development in agroinoculated plants.
Agroinoculated plants (a) VGGRU 1(Resistant), (b) VGGRU2, (c) VGGRU 3, (d) VGGRU4, (e) VRM (Gg) 1.

In the field screening, three lines namely VGGRU 2, VGGRU 3 and VGGRU 4 were found to be resistant based on the rating scale (Table 1) suggested by Singh *et al.* (1988) but developed MYMV symptoms on agroinoculation (Table 2). It is clear from the result that the resistance at field conditions may be due to some factors that restrict the insect transmission. To confirm the results, the agroinoculation experiment was repeated twice and comparable results were obtained and by this technique 50-100% infectivity could be observed. Similar to the above results, Jacob *et al.* (2003) followed a single strain strategy of agroinfection by co-delivery of MYMV DNA A and DNA B from one *Agrobacterium* strain and yielded 100% agroinfection. Usharani *et al.* (2005) conducted infectivity analysis of MYMV through agroinoculation in soybean isolate and obtained infectivity of about 70-95 per cent. Similarly, Sudha *et al.* (2013) reported that the MYMV infectivity on agroinoculated plants ranged between 0-100 per cent even for the field-resistant genotypes. Based on the above result, it is clearly understood that the resistance can be easily identified through reliable agroinoculation technique and the results were further confirmed through PCR to verify the presence of viral DNA in the host genome using oligonucleotide primers that are specific to the MYMV coat protein gene of DNA A. The expected amplicon size of 703 bp was obtained in all the infected samples which showed yellow mosaic symptoms (Fig 2). These results are in accordance with the reports of Usharani *et al*. (2005) and Sudha *et al.* (2013), signifying the presence of viral DNA in agroinoculated symptomatic plants and their absence in asymptomatic plants.

Interestingly, VGGRU1 was found to exhibit higher level of resistance and did not develop any mosaic symptoms

Table 1: Rating scale used for scoring against *Mungbean yellow mosaic virus* (MYMV) (Singh *et al*., 1992).

Rating	Percentage foliage affected	Infection category
1	No visible symptoms or minute yellow specks covering 0.1-5% leaf area	Resistant (R)
3	Mottling of leaves covering 5.1-15% leaf area	Moderately resistant (MR)
5	Yellow mottling and discoloration of 15.1-30% leaf area	Moderately susceptible (MS)
7	Pronounced yellow mottling and discoloration of leaves and pods, reducing in leaf size, stunting of plants, 30.1-75% foliage affected	Susceptible (S)
9	Severe yellow mottling and discoloration of leaves, stunting of plants, failure of flowering and fruit setting 75.1-100% foliar affected	Highly susceptible (HS)

Table 2: Comparison of field screening with agroinoculation screening.

Mungbean derivatives	Field screening		Agroinoculation screening						
	Disease score (Scale)	Disease rating (across replications)	Trail 1		Trail 2		Trail 3		
			No of days for symptom development	Per cent infection	No. of days for symptom development	Per cent infection	No. of days for symptom development	Per cent infection	Disease rating after agro-inoculation
				Mean*		Mean*		Mean*	
VGGRU 1	1	R	-	0.00	-	0.00	-	0.00	R
VGGRU 2	1	R	17	69.3	20	80.5	19	90.7	MS
VGGRU 3	1	R	18	90.2	16	69.6	19	76.1	MS
VGGRU 4	1	R	17	88.3	21	82.7	18	82.7	MS
VRM (Gg) 1	7	S	16	91.4	19	88.2	21	73.4	S

*Mean of two replications.

R- Resistant; MS- Moderately susceptible; S- Susceptible.

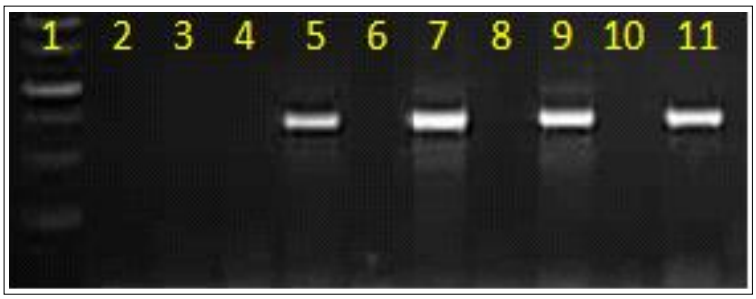

Fig 2: PCR confirmation for agroinoculated lines.

(1)1 kb ladder, (2) VGGRU 1(control), (3) VGGRU 1 (Treated), (4) VGGRU 2 (Control), (5) VGGRU 2 (Treated), (6) VGGRU 3 (control), (7) VGGRU 3 (Treated), (8) VGGRU4 (control), (9) VGGRU4 (Treated), (10)VRM (Gg)1 (control), (11) VRM (Gg)1 (Treated).

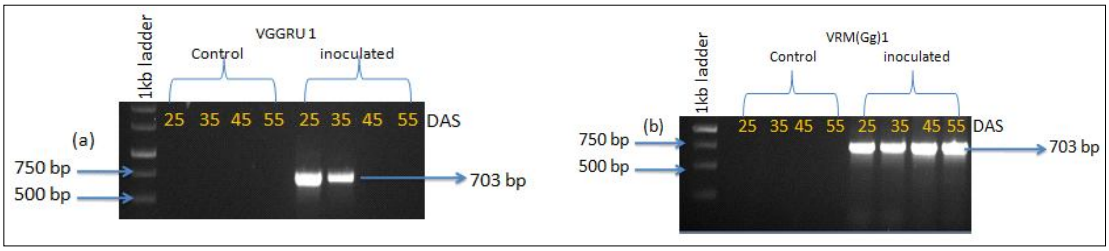

Fig 3: PCR banding pattern in agroinoculated plants at 25, 35, 45 and 55 days after sowing using coat protein primer. (a) Banding pattern of VGGRU 1, (b) VRM (Gg)1.

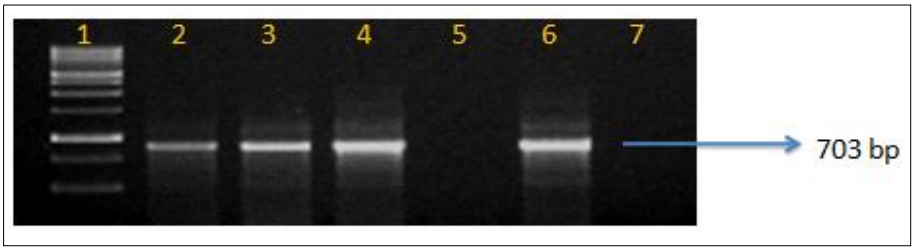

Fig 4: Confirmation for MYMV CP gene in whiteflies. From left to right 1) 1 kb ladder, 2) 10 viruliferous whiteflies, 3) 15 viruliferous whiteflies, 4) 25 viruliferous whiteflies, 5) Non-viruliferous whiteflies, 6) Positive control, 7) Water blank.

Table 3: Transmission efficiency of MYMV by *Bemisia tabaci* in mungbean.

Name of lines	AAP	IAP	No of days taken for symptom development	MYMV Infectivity (%)
VRM (Gg)1	24 h	24 h	19	65.0
VGGRU 1	24 h	24 h	-	0.0

even after 2 months from inoculation. Further confirmation by PCR with CP gene specific primer using DNA samples taken at ten days interval *i.e.* 25 35, 45 and 55 DAS from both resistant (VGGRU1) and susceptible (VRM (Gg)1 showed a visible increase in the intensity of the amplified bands of VRM (Gg)1 from 25 to 55 DAS but the intensity of the bands in VGGRU1 resistant line was comparatively less and reduced to half its intensity at 35 DAS and there was no amplification as the days get older (Fig 3). The presence of viral load was found high in the susceptible plants and the viral load for resistance gradually decreases as plants get matured due to activation of defense genes. Usharani *et al.* (2005) and Kayalvizhi *et al.* (2015) also reported the same results which indicated that viral intensity on the resistant plant gets decreased when the plants get matured (reproductive stage) and it may be due to the expression of defense genes in resistant plants.

Whitefly screening

Whitefly transmission experiment was attempted to prove the Koch's postulates for MYMV under controlled conditions during the course of present investigation. The genotype of non-viruliferous whiteflies collected from different fields of cotton and brinjal was identified and confirmed as Asia I type after partial gene sequencing (accession no: MK333460). This genotype results correlated with the findings of Prasanna *et al.* (2015) and Elango *et al.* (2015). The PCR results using MYMV CP primers for both viruliferous and non-viruliferous whiteflies after 24 h of AAP revealed the presence of MYMV amplicons only on the viruliferous whiteflies which contains MYMV in it and there was no amplification in non-viruliferous whiteflies which had no AAP treatment along with the water blank (Fig 4). The transmission experiment was done after whitefly genotype confirmation and the results revealed that 24 hr AAP and 24 hr IAP with *B. tabaci* were able to cause 65% infectivity in susceptible plant VRM (Gg) 1 and zero infectivity in VGGRU 1 (Table 3). The results are in confirmation with the report of Govindan *et al.* (2013), where MYMV DNA fragment of 703 bp was observed only in viruliferous adults of *B. tabaci*. Similarly, Usharani *et al.* (2005) defined the host range of MYMV using both whitefly transmission and agroinoculation. It was found that the vector was able to transmit the virus from agroinoculated plants to the healthy plants.

CONCLUSION

Field level screening for mungbean yellow mosaic resistance often remains a difficulty to the breeding society. The present study was successful in artificial screening for the resistance through a novel technique called agroinoculation and further substantiation through whitefly transmission. Among the four interspecific derivatives tested, VGGRU1 was found be completely resistance against MYMV in both agroinoculation and whitefly transmission experiments comparing with the susceptible check VRM (Gg) 1. In the future prospects, the characterization of complete set of RNA transcripts of VGGRU1 along with VRM (Gg) 1 may be helpful in pulling out the genes responsible for the resistance in VGGRU1 against MYMV-plant interaction.

ACKNOWLEDGEMENT

We acknowledge the Department of Science and Technology, SERB, India for funding and supporting the research programme (YSS/2015/000321) and thankful to Dr K. Veluthambi, Madurai Kamaraj University, Tamil Nadu, India, for providing the *Agrobacterium* strains for our study.

REFERENCES

Anonymous, (2018). Annual Report 2016-17, Ministry of Agriculture and Farmers Welfare (Department of Agriculture, Cooperation and Farmers Welfare), Directorate of Pulses Development, Vindhyachal Bhavan, New Delhi.

Balaji, V., Vanitharani, R., Karthikeyan, A.S., Anbalagan, S., Veluthambi, K. (2004). Infectivity analysis of two variable DNA B components of Mungbean yellow mosaic virus-*Vigna* in *Vigna mungo* and *Vigna radiata*. Journal of Biosciences. 29: 297-308.

Biswas, K.K. and Varma, A. (2000). Identification of variants of mungbean yellow mosaic Gemini virus by host reaction and nucleic acid spot hybridization. Indian Phytopathology. 53(2): 134-141.

Cayal*viz*hi, B., Nagarajan, P., Raveeendran, M., Rabindran, R., Selvam, N.J., Bapu, J.K., Senthil, N. (2015). Unraveling the responses of mungbean (*Vigna radiata*) to mungbean yellow mosaic virus throug 2D-protein expression. Physiological and Molecular Plant Pathology. 90: 65-77.

Dhakar, K., Gupta, V.K., Rathore, M.S., Gaur, R.K. (2010). Virus resistance and gene silencing in plants infected with begomovirus. Journal of Applied Sciences. 10: 1787-1791.

Ellango, R., Singh, S.T., Raina, H., Chaubey, R., Ramamurthy, V.V., Rana, V.S., Naveen, N.C., Asokan, R., Gayatri Priya, N., Mahmood, R., Rajagopal, R. (2015). Distribution of Genetic Groups in India. Environmental Entomology. 44(3): 1-7.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. (1994). DNA primers for amplifcation of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology. 3(5): 294.

Govindan, K., Nagarajan, P., Angappan, K. (2014). Molecular studies on transmission of mung bean yellow mosaic virus (MYMV) by *Bemisia tabaci* Genn. in Mungbean. African Journal of Agricultural Research. 9(38): 2874-2879.

Habib, S., Shad, N., Javaid, A., Iqbal, U. (2007). Screening of *mungbean* germplasm for resistance/tolerance against yellow mosaic disease. Mycopathology. 5: 89-94.

Haq, Q.M.I., Rouhibakhsh, A., Ali, A., Malathi, V.G. (2011). Infectivity analysis of a black gram isolate of Mungbean yellow mosaic virus and genetic assortment with MYMIV in selective hosts. Virus Genes. 42: 429-439.

Jacob, S.S., Vanitharani, R., Karthikeyan, A.S, Chinchore, Y., Thillaichidambaram, P., Veluthambi, K. (2003). *Mungbean yellow mosaic virus*-Vi agroinfection by co delivery of DNA A and DNA B from one *Agrobacterium* strain. Plant Disease. 87: 247-251.

Karthikeyan, A., Sudha, M., Pandiyan, M., Senthil, N., Shobhana, V.G., Nagarajan, P. (2011). Screening of MYMV resistant mungbean [*Vigna radiata* (L.) Wilczek] progenies through agroinoculation. International Journal of Plant Pathology. 2: 115-125.

Nariani, T.K. (1960). Yellow mosaic of mung (*Phaseolus aureus* L.). Indian Phytopathology. 13: 24-29.

Paul, P.C., Biswas, M.K., Mandal, D., Pal, P. (2013). Studies on host resistance of mungbean against *mungbean yellow mosaic virus* of lateritic zone of West Bengal. The Bioscan. 8: 583-587.

Prasanna, H.C., Kanakala, S., Archana, K., Jyothsna, P., Varma, R.K., Malathi, V.G. (2015). Cryptic species composition and genetic diversity within Bemisia tabaci complex in soybean in India revealed by mtCOI DNA sequence. Journal of Integrative Agriculture. 14(9): 1786-1795.

Richa Maheshwari, Panigrahi, G., Angappan, K. (2014). Molecular characterization of distinct YMV (*yellow mosaic virus*) isolates affecting pulses in India with the aid of coat protein gene as a marker for identification. Molecular Biology Reports. 41: 2635-2644.

Richa Maheswari. (2008). DNA finger printing of *mungbean yellow mosaic virus* (MYMV) affecting pulses. M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore, India pp. 48.

Rogers, S.G., Bisaro, D.M., Horsch, R.B., Fraley, R.T., Hoffmann, N.L., Brand, L., Elmer, J.S., Lloyd, A.M. (1986). *Tomato golden mosaic virus* A component DNA replicates autonomously in transgenic plants. Cell. 45(4): 593-600.

Sidhu, J.S., Mann, R.S., Butter, N.S. (2009). Deleterious effects of cotton leaf curl virus on longevity and fecundity of whitefly, *Bemisia tabaci* (Gennadius). Journal of Entomology. 6: 62-66.

Singh, G., Sharma, Y., Kaur, L. (1992). Methods of rating *yellow mosaic virus* of mungbean and urdbean. Plant Disease Research. 7:1-6.

Sudha Manickam. (2009). DNA isolation protocol for *Vigna radiata* with free of phenolics. *Protocol Exchange.* Doi: 10.5281/zenodo.15918.

Sudha, M ., Karthikeyan, A., Nagarajan, P., Raveendran, M., Senthil, N., Pandiyan, M., Angappan, K., Ramalingam, J., Bharathi, M., Rabindran, R., Veluthambi, K., Balasubramanian, P. (2013). Screening of mungbean *(Vigna radiata)* germplasm for resistance to *Mungbean yellow mosaic virus* using agroinoculation. Canadian Journal of Plant Pathology. 35 (3): 424-430.

Usharani, K.S., Sundernath, B., Haq, Q.M.R., Malathi, V.G. (2004). *Yellow Mosaic Virus* infecting soybean in northern India is distinct from species infecting soybean in southern and western India. Current Science. 86: 845-850.

Van Regenmortel, M.H., Fauquet, C.M., Bishop, D.H., Carstens, E.B., Estes, M.K., Lemon, S.M., Wickner, R.B. (2000). Virus Taxonomy: Classification and Nomenclature of Viruses. Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press. New York.

Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology. 3(5): 294.

Zeidan, M. and Czosnek, H. (1991). Acquisition of *tomato yellow leaf curl virus* by the whitefly *Bemisia tabaci.* Journal of General Virology. 72: 2607-2614.