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RESEARCH ARTICLE

Seed-borne nature of mungbean yellow mosaic India virus in mung bean (*Vigna radiata* L.) occurs due to cultivar and sowing date variation

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Abstract

Mungbean yellow mosaic India virus (MYMIV) is the major viral species responsible for yellow mosaic disease in Northern India, belonging to the genus Begomovirus, family *Geminiviridae*. Recently, a few of the begomovirus species were reported to be seed-transmissible which is a disputed character for begomoviruses. In the present study, the possibility of MYMIV transmission was assessed through seed collected from field-infected mungbean cultivars Pusa1371 and Pusa 9531 using serological, molecular and progeny assays for three continuous seasons with three different sowing dates. Detection of MYMIV from infected seed through direct antigen-coated enzyme-linked immunosorbent assay (DAC-ELISA) showed very low absorbance values in the range of 0.23- 0.30 at 405 nm. Polymerase chain reaction (PCR) using the total DNA from the whole seed resulted in the amplicon size of 602bp and 850bp corresponding to DNA-A and DNA-B regions, respectively. Inconsistent amplification was observed only in the whole seed and seed coat, but not in cotyledons and embryonic axes of the cultivars with the first and second sowing of *Kharif*/ rainy season. No amplification was observed with the seed harvested from the third sowing of *Kharif* 2021 and spring-summer of 2021 and 2022. The radicle region of the seedlings tested negative through PCR with MYMIV-specific primers. No visible symptoms were observed in 1800 raised seedlings from the MYMIV-infected seed of each cultivar. Testing of 1350 susceptible and 450 resistant cultivar seedlings through DAC-ELISA and 225 susceptible and 45 resistant cultivars through PCR showed the absence of MYMIV from the raised seedlings. Based on the results of DAC-ELISA, PCR and grow-out experiments, it was concluded that MYMIV may be seed-borne in nature but not seed-transmitted in the tested mungbean cultivars.

Keywords: Begomovirus, DAC- ELISA, PCR, grow-out test, seed transmission, mungbean yellow mosaic India virus

Introduction

Seed transmission of plant viruses is an important means for the introduction of plant viruses into new geographical areas, where they may become established, spread and can cause epidemics in the presence of suitable vectors and host species (Forster et al. 2001). Moreover, seed transmission is an effective survival strategy for viruses, especially those with narrow host range, infect annual plants and vectored in a non-persistent manner (Johansen et al.1994). The process of virus seed transmission is environmentally influenced and is a consequence of specific interactions between the virus and host physiology (Carroll 1981). Approximately more than one-third of known plant viruses have been reported to seed transmitted in various fiber, food ornamental crops, and weeds (Mink 1983; Matthews 1993; Singh and Mathur 2004; Sastry 2013). Mungbean (*Vigna radiata* L.) is one of the most important pulse crops grown in India. In mungbean, yellow mosaic disease (YMD) is a major concern for mungbean production, especially in India and Southeast

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Asia. The YMD was reported to be caused by four species of begomoviruses, namely, dolichos yellow mosaic virus, horse gram yellow mosaic virus, mungbean yellow mosaic virus (MYMV) and mungbean yellow mosaic India virus (MYMIV) (Malathi and John 2009; Naimuddin et al. 2016). MYMIV is majorly responsible for YMD in central, eastern and northern India (Malathi and John 2009).

MYMIV belongs to the genus *Begomovirus* of the family *Geminiviridae* (Walker et al. 2019). The species MYMIV have a bipartite genome, consisting of two circular single-stranded DNA components *i.e.,* DNA-A and DNA-B encapsidated separately in twinned icosahedral particles (Fauquet et al. 2003; Fauquet and Stanley 2003; Lefkowitz et al. 2018). DNA-A encodes for genes governing replication, and encapsidation and DNA-B encodes for proteins facilitating intra and intercellular movement. Both DNA-A and DNA-B are essential for infectivity (Malathi et al. 2017). For many years, begomoviruses were believed not to be seed transmissible, as they are confined to the phloem tissue. However, recently seed transmission of begomoviruses *viz.*, bitter gourd yellow mosaic virus in bitter gourd (Manivannan et al. 2019), dolichos yellow mosaic virus in lablab bean (Suruthi et al. 2018), mungbean yellow mosaic virus in urd bean (Kothandaraman et al. 2016), sweet potato leaf curl virus in sweet potato (SPLCV) (Kim et al. 2015), tomato yellow leaf curl virus in tomato (ToYLCV) (Kil et al. 2016), and tomato leaf curl New Delhi virus (ToLCNDV) in chayote (*Sechium edule*) (Sangeetha et al. 2018) and zucchini squash (*Cucurbita pepo*) (Kil et al. 2020) have been reported. Hence, from these observations, it is pertinent to study the possibility of other begomoviruses seed transmission because infected seeds can spread the virus beyond time and space limitations (Fabre et al. 2014). In the present study, the possibility of MYMIV transmission was assessed through seed using various techniques in three consecutive seasons spring-summer 2021, *kharif/*rainy-2021 and spring-summer 2022 with three different dates of sowing.

Materials and methods

Study site and planting materials

The experiment was conducted at the Indian Agricultural Research Institute (IARI), New Delhi, India (lat.28° 38'23" N; long. 77°09'27" E; Elevation 228.61m) for three consecutive seasons in order to study the seed transmission of mungbean yellow mosaic India virus. The present study consisted of three sowing dates at 10 days interval with two mungbean cultivars *viz.,* Pusa 1371 [resistant (R) to MYMIV] and Pusa 9531 [susceptible (S) to MYMIV] in three seasons *i.e.*, spring-summer 2021 (March-June), *kharif/*rainy season 2021 (July-November) and spring-summer 2022 (March-June) (Table 1). A split plot design with four replications for each cultivar with each date of sowing was followed with an inter and intra-row spacing of 30 × 10 cm. Recommended agronomic practices and cultural operations were followed for all the dates of sowing till harvest. However, no insecticide was applied to maintain the optimum Whitefly population for the high inoculum pressure of the MYMIV.

Seed collection and preparation

Initially, the presence of MYMIV in mungbean leaf samples was investigated by performing direct antigen-coated enzyme-linked immunosorbent assay (DAC-ELISA) using african cassava mosaic virus (ACMV) polyclonal antibodies (AC Diagnostics, Fayetteville, USA). The ELISA-positive plants were tagged from both the susceptible and resistant cultivars in three sowings for each season. The total seed from ELISA-positive plants of each sowing was collected, labeled and stored until further use.

Serological detection of MYMIV from mungbean seeds

Around 20 seeds of each cultivar (Pusa 1371 and Pusa 9531) from three different sowings of each season were tested in two replications for the detection of MYMIV *i.e.,*120 seeds of each cultivar/season (360 seeds/cultivar). The MYMIV infected leaf sample was used as a positive control. First, the seeds were surface sterilized with 1% sodium hypochlorite for 1-minute. Followed by rinsing twice with sterile distilled water and allowing to imbibe water for 8 to 10 hours (Sandra et al. 2020). The seeds were then dissected into different parts such as seed coat, cotyledon and embryonic axes. Ten whole seed, ten seed coat, cotyledon and embryonic axes were ground at room temperature in coating buffer (1:1 dilution w/v) containing 2% polyvinylpyrrolidone (PVP, MW 40,000, followed by centrifugation at 12000 rpm for 2 minutes. 200 µL of seed extract was loaded into 96 well polystyrene microtiter plates (NuncTM, Thermo Fisher Scientific, India) and incubated at 37°C for 1-hour. The plates were rinsed thrice for 3 minutes with PBS-T buffer (phosphate buffer saline-Tween 20), followed by the addition of 200µl blocking solution (bovine serum albumin, BSA, 1% w/v in PBS-T) and incubation at 37°C for 1-hour. The plates were rinsed thrice with PBS-T and the commercially available ACMV polyclonal antibody was added to the wells at 1:200 dilution (w/v), followed by incubation at 37°C for 1-hour. The plates were washed thrice with PBS-T and goat anti-rabbit IgG alkaline phosphatase conjugate (Sigma, St. Louis, USA, 1:30,000) was added followed by incubation at 37°C for 1-hour. After rinsing the plates with PBS-T, p-nitrophenyl phosphate (0.5 mg/mL, Sigma, St. Louis, USA) was added and the plates were

Table 1. Different sowing dates considered to study the MYMIV transmission through seeds

Sowing	Spring - Summer season	Kharif/Rainy season
First sowing	15–20th March	15–20th July
Second sowing	25–30th March	25–30th July
Third sowing	5–10th April	5–10th August

held in the dark for 1-hour at 37°C. Absorbance values were determined using an ELISA plate reader at 405 nm (Genetix, India). The samples with absorbance values two times higher than the healthy tissue was considered positive (Clark and Joseph 1984).

Total DNA isolation and polymerase chain reaction for MYMIV detection

A total of 360 seeds of each cultivar Pusa 1371 and Pusa 9531 from three different sowings of three seasons were collected and surface sterilized with 1% sodium hypochlorite for 1-minute, followed by rinsing twice with sterile distilled water and allowed to imbibe water for 8 hours. Ten seeds were then dissected into different parts such as seed coats, cotyledons and embryonic axes. A group of ten mature whole seeds, ten seed coats, cotyledon and an embryo were subjected to DNA extraction in two replications, using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thomson 1980). The DNA was quantified using a NanoDrop™2000 spectrophotometer (Thermo Fisher Scientific, USA). 100ng of total DNA of Pusa 9531 and Pusa 1371 was subjected to polymerase chain reaction (PCR) with BM 923F (5'-aatcatgccaaagcggacc-3') and BM 924R (5'-ccatggattgttccttacaa-3') primers, which can amplify only MYMIV DNA-A, but not mungbean yellow mosaic virus (MYMV). After confirmation of MYMIV, further PCR analysis was carried out with the primer pair NS72F(5'-atgagacatttgctaatagtcgtct-3') and NS72R (5'-atggagaattattcaggagcagtcg-3') corresponding to the MYMIV DNA-B region. The PCR was performed in a 25 µL reaction mixture containing 100 ng of seed DNA template, 2.5 µL of 10X PCR buffer, 0.5 µL of 10 mM dNTP mix, 0.125 µL of Taq DNA polymerase (5 U/µL, GeneDireX, India), 2.5 µL of each primer and nuclease-free water to make up the volume. The reaction mixture was subsequently subjected to initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 40 seconds, primer annealing at 56°C (DNA-A) and 51°C (DNA-B) for 1-minute and extension at 72°C for 2 minutes followed by a final extension for 10 min at 72°C. The PCR products were analyzed in 1% agarose gel and sequenced at Delhi University, South Campus, New Delhi, India.

Seed transmission rate of MYMIV

Before conducting a grow-out test, 10 mungbean seeds were allowed to sprout in Petri plates. Thereafter, total DNA was isolated from the radicle region of the MYMIV-infected Pusa 9531 seed. Over 100 ng of total DNA was subjected to PCR using two sets of primer pairs *i.e.*, BM 923F & BM 924R and NS 72F & NS 72R with the same conditions as described above. Later, the seed transmission efficiency of MYMIV-infected mungbean seed was studied through a grow-out test under an insect-proof glass house. For a grow-out test, 200 seeds collected from the MYMIV-infected mungbean cultivars, Pusa 1371 and Pusa 9531 in three sowings *i.e.,*600 seeds of each cultivar/season were potted separately in 6" diameter pots and placed at National Phytotron Facility (NPF), IARI, New Delhi. The seedling grow-out test was carried out with the same number of seeds collected from all three seasons *i.e.*, spring-summer, *kharif* 2021 and spring-summer 2022 (1800 seeds/cultivar for 3 seasons). Germinated seedlings were maintained at 26 to 28°C with a 16/8 hours photoperiod and 70% relative humidity and observed for symptom appearance until 45 days after sowing (DAS). First trifoliate leaves of randomly selected 150 and 50 germinated seedlings from susceptible and resistant cultivars from all three seasons *i.e.,* 450 (S) and 150 (R) were assayed at 20DAS by performing DAC-ELISA [Total of 1350 (S) and 450(R) for 3 seasons]. For PCR analysis, total DNA was isolated from the 25 and 5 leaf samples of susceptible and resistant cultivars from each sowing in three seasons using the CTAB method [total of 225(S) and 45(R) for 3 seasons]. PCR amplification was performed using primer pair NS72F and NS72R with the same PCR conditions as mentioned above.

Results and discussion

The viral diseases caused by begomoviruses pose a major challenge in improving the productivity of field and vegetable crops. Begomoviruses are transmitted by whitefly (*B. tabaci*) under natural conditions and; graft transmission and artificial inoculation with an infectious clone under experimental conditions (Stanley 2001). However, recent studies proved the seed transmission in some of the begomoviruses, which necessitated checking the chances for seed transmission in other species. In the present study, an investigation was carried out to search the possibility of MYMIV seed transmission in mungbean cultivars, Pusa 9531 and Pusa 1371 through DAC - ELISA, PCR and grow-out test from the three consecutive seasons of harvested seed.

Confirmation of the MYMIV through DAC- ELISA

Mungbean leaf samples infected with MYMIV showed symptoms of yellow mosaic at the field level (Fig. 1Ba). The leaf samples tested through DAC-ELISA showed absorbance values in the range of 0.74 to 1.60 in susceptible and resistant cultivars. The pods collected from MYMIV-infected plants were short in length, ill filled and the seeds were misshapen, shrunken and shriveled with yellow color patches (Figs. 1Bb-Bd). As few seeds were produced from individual MYMIV-infected plants, the seed from the infected plants was pooled and used for further studies. The mungbean seed tested through DAC-ELISA showed absorbance values in the range of 0.23 to 0.30 in all three sowings of three seasons in both susceptible and resistant cultivars (Table 2). The DAC-ELISA values of whole seed and seed parts are at par with the healthy value, indicating the absence of virus in mungbean seed harvested from MYMIV-infected plants. Generally, uniform virus distribution and the presence of high viral

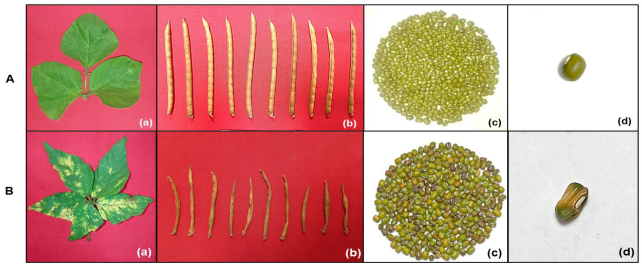

Fig. 1. Effect of mungbean yellow mosaic India virus (MYMIV) on pod formation and seed shape. (A). Leaf, pods and seed material collected from health plants of Pusa 9531 (Aa-d). (B). Leaf, pods and seed material collected from infected plants of cultivar Pusa 9531 (susceptible). (Ba). Infected leaf showing symptoms of severe yellow mosaic patches and mottling; Bb =Shrivelled and ill-filled pods; Bc & Bd= Seeds from infected plants showing brownish discoloration and malformation

concentration in the embryo are the ideal conditions for the detection of the virus in seed through ELISA and PCR to ensure its seed transmission (De Assis Filho and Sherwood 2000). This condition was not observed in the present study, as MYMIV was not detected in all the tissues of the infected mungbean seed consistently even though the seed was tested in a group of ten as suggested for mungbean yellow mosaic virus (Kothandaraman et al. 2016).

MYMIV detection in seeds through PCR

To confirm the DAC-ELISA predictions about MYMIV, total DNA isolated from the whole seed and various seed tissues were subjected to PCR. Initially, PCR was conducted on the whole seed of the susceptible cultivar Pusa 9531 with the MYMIV-specific primers, BM 923F and BM 924R which resulted in the amplification of 602 bp (Fig. 2a). After confirmation of MYMIV, the PCR analysis of mungbean seed samples was carried out with NS72F and NS72R primers. An amplicon size of 850bp was observed only from whole seed and seed coat, but not in cotyledons and embryonic axes from the susceptible cultivar Pusa 9531 with the seed harvested from first and second sowing plants during the *kharif/*rainy season 2021, which might be due to the presence of MYMIV as a contaminant on the seed tissue parts. (Figs. 2b and 2c). Surprisingly, amplification was also observed in the seed coat of the resistant variety with the seed harvested from first sowing, but with inconsistent results (Fig. 2b), which might be due to the breaking of resistance under high MYMIV population under changing climate scenario as consistent amplification was observed from leaf samples in our previous study (Jones 2016; Shobharani et al. 2023). No amplification was observed with the seed harvested from the third sowing of *kharif* season 2021 and spring-summer 2021 and 2022 (Fig. 2d), which might be due to less MYMIV population reaching the reproductive tissues where the whitefly population was also less along with unfavorable weather parameters (Temp: 30-35^{O}C; 36-61% RH) (Cobos et al. 2019; Maury et al. 1987). Naimuddin et al. (2016) also studied the seed-borne nature of MYMIV in mungbean genotype T44 plants and mentioned that MYMIV-specific primers gave amplification from the whole seed. However, experimental evidence was lacking. Later, the seed transmission nature of MYMIV was experimentally proved in yardlong beans (*Vigna unguiculata* subsp. *sesquipedalis* L.) through MYMIV-specific amplification from whole seed, seed coat, cotyledons and young leaves from sprouting seeds (Mulyadi et al. 2021).

Sequencing of the amplified product from the whole seed corresponding to DNA-A region shared 97.38% identity with MYMIV Sehore-1 isolate and the sequence was submitted to the NCBI database (Acc. No. OQ891081).

MYMIV detection in mungbean seedlings

The PCR analysis of total DNA isolated from the radicle region of the sprouted mungbean seeds of the cultivar Pusa 9531 did not show amplification with DNA-A and DNA-B specific primers. Generally, progeny testing is typically more accurate than seed testing for determining virus seed transmission, because only transmissible viruses are found in progeny and the virus titer is also higher than in seeds (De Assis Filho and Sherwood 2000). In the present study, seedlings were raised from infected seeds (grow-out test); no peculiar symptoms were observed in raised seedlings even after 45 DAS from all the season-harvested seeds (Figs. 3b and 3c). Testing of 1800 seedlings from three sowing dates of three seasons through DAC-ELISA, absorbance values were observed to be in the range of 0.25 to 0.40 for tested seedlings raised from the seed of three sowing dates and three seasons (Fig. 4). In PCR analysis, none of the randomly selected seedlings showed amplification either with DNA-A or DNA-B specific primers. The results obtained through both DAC-ELISA and PCR analysis indicated the absence of MYMIV transmission from infected seed to seedlings in the tested cultivars. The present study is in accordance with other reports providing evidence disputing seed transmission of begomoviruses with no detection of seed transmission, namely, SPLCV in sweet potato; ToYLCV in *N. benthamiana* and tomato; ToLCNDV in melon and MYMIV in mungbean (Andreason et al. 2020; Fortes et al. 2023; Perez-Padilla et al. 2020; Rosas-Diaz et al. 2017). Contrary to the present results, MYMV seed transmission was studied in black gram and virus was detected in seedlings up to 32% by PCR though they were symptomless (Kothandaraman et al. 2016).

Based on the findings, it was concluded that the data derived through DAC-ELISA, PCR and seedling grow-out test for three consecutive seasons with three different sowing times do not support the seed transmission nature of MYMIV. The experiments are in progress to screen the other mungbean and soybean cultivars to check the possibility of MYMIV seed transmission; as it generally depends on the genotype and the properties of virus

Table 2. Detection of mungbean yellow mosaic India virus (MYMIV) through direct antigen-coated enzyme-linked immunosorbent assay (DAC-ELISA)

Spring - Summer season 2021				
Varieties	Sample/Seed parts	First sowing (20th March)	Second sowing (30th March)	Third sowing (10th April)
Pusa 1371 (Resistant)	Whole seed	0.24	0.24	0.25
	Seed coat	0.23	0.24	0.25
	Cotyledon	0.23	0.24	0.24
	Embryo	0.23	0.23	0.23
Pusa 9531 (Susceptible)	Whole seed	0.26	0.26	0.27
	Seed coat	0.25	0.25	0.26
	Cotyledon	0.23	0.23	0.24
	Embryo	022	0.23	0.23
Positive		0.70		
Healthy		0.22		
Buffer		0.14		
Kharif season 2021				
Varieties	Sample/Seed parts	First sowing (20th July)	Second sowing (30th July)	Third sowing (10th August)
Pusa 1371 (Resistant)	Whole seed	0.26	0.26	0.25
	Seed coat	0.29	0.24	0.24
	Cotyledon	0.25	0.25	0.25
	Embryo	0.24	0.23	0.23
Pusa 9531 (Susceptible)	Whole seed	0.30	0.28	0.25
	Seed coat	0.29	0.24	0.27
	Cotyledon	0.24	0.24	0.23
	Embryo	0.24	0.24	0.24
Positive		1.60		
Healthy		0.22		
Buffer		0.11		
Spring – Summer season 2022				
Varieties	Sample/Seed parts	First sowing (20th March)	Second sowing (30th March)	Third sowing (10th April)
Pusa 1371 (Resistant)	Whole seed	0.24	0.25	0.25
	Seed coat	0.24	0.23	0.25
	Cotyledon	0.23	0.23	0.24
	Embryo	0.24	0.24	0.23
Pusa 9531 (Susceptible)	Whole seed	0.27	0.26	0.28
	Seed coat	0.25	0.25	0.26
	Cotyledon	0.23	0.23	0.24
	Embryo	0.23	0.23	0.23
Positive		0.90		
Healthy		0.23		
Buffer		0.13		

*Data are the average of two replications

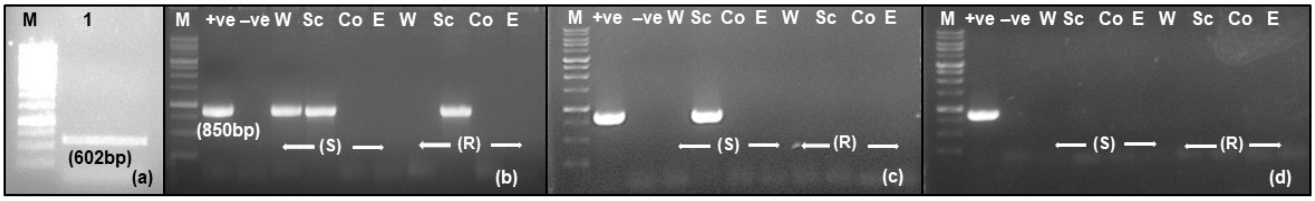

Fig. 2. Detection of MYMIV in infected seed samples of mungbean cultivars, Pusa 1371 and Pusa 9531. a - PCR amplicons (602bp) obtained with CP-specific primer pair BM 923 and BM 924 (Lane 1 -amplification of MYMIV from a group of ten whole seeds of susceptible cultivar Pusa 9531 in *Kharif* 2021. (b-d) - Detection of MYMIV with movement protein (MP) specific primer pair NS72F and NS72R in a group of ten seeds from various seed parts (+ve = Positive; -ve = Negative; W= Whole seed; Sc = Seed coat; Cot= Cotyledons; E= Embryo) in *Kharif* 2021. b, c and d = First, second and third sowing of *kharif* 2021; Lanes: M = GeneRuler 1kb DNA ladder; S=Susceptible cultivar (Pusa 9531) and R=Resistant cultivar (Pusa 1371)

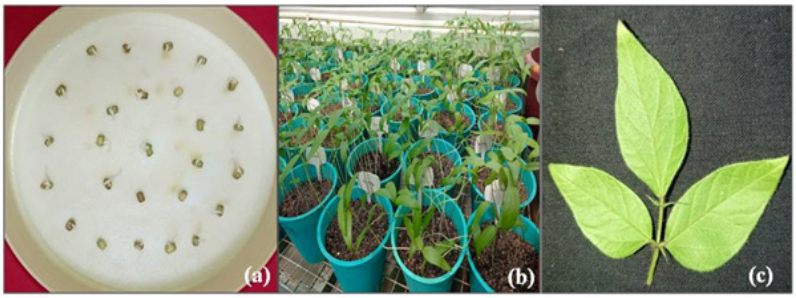

Fig. 3. Testing of MYMIV transmission to progeny seedlings through grow-out test. (a). Sprouting from MYMIV infected seed; (b and c). No visible symptoms on the seedlings raised from MYMIV-infected seed

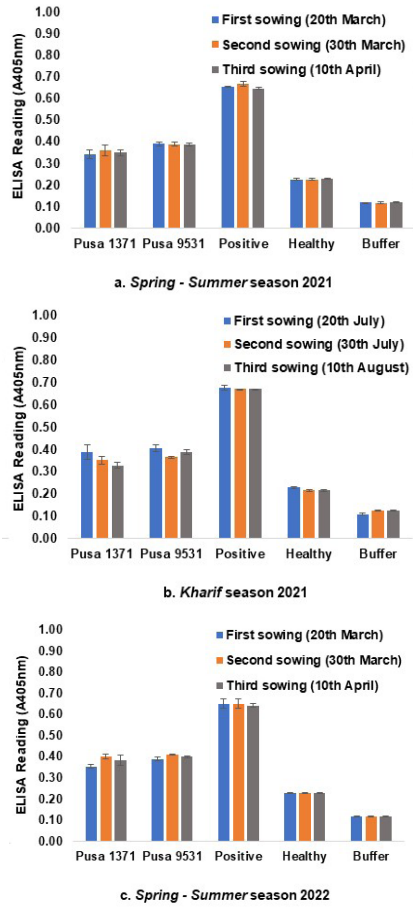

Fig. 4. Detection of mungbean yellow mosaic India virus (MYMIV) from raised seedlings in mungbean cultivars Pusa 1371 and Pusa 9531 through DAC-ELISA, (a). Spring - Summer 2021; (b). *Kharif* 2021 and (c). Spring - Summer 2022

isolates as described in other viruses (Usovsky et al. 2022). Hence, rigorous and comprehensive experimentation will be essential for thorough confirmation of the seed transmission of begomoviruses, so that strict quarantine measures may be undertaken.

Supplementary materials

Supplementary Table S1 is provided, which can be accessed online at www.isgpb.org

Authors' contribution

Conceptualization of research (SKL, NS); Designing of the experiments (SKL, NS, AK); Contribution of experimental materials (HKD, BM, ADM); Execution of field/lab experiments and data collection (SM); Analysis of data and interpretation (SM, SKL, NS); Preparation of the manuscript (SM, NS).

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Supplementary Table 1. Microclimate of the experimental site

Spring – Summer season 2021								
		Temperature (°C)			Relative humidity (%)			Rainfall (mm)
Week	Date	Max.	Min.	Mean	Max.	Min.	Mean	
Week-1	7th- 13th June, 2021	37.8	27.3	33.2	70.7	48.4	59.9	0.8
Week-2	14th - 20th June. 2021	34.9	26.2	30.6	77.1	64.6	71.1	2.6
Week-3	21st - 27th June, 2021	38.3	26.7	32.4	73.6	43.4	58.9	0.0
Week-4	28th – 4thJul, 2021	41.1	28.3	34.7	74.3	43.0	58.9	6.7
Mean		37.8	27.3	33.2	70.7	48.4	59.9	0.8
Kharif season 2021								
		Temperature (°C)			Relative humidity (%)			Rainfall (mm)
Week	Date	Max.	Min.	Mean	Max.	Min.	Mean	
Week-1	7th - 13th Oct, 2021	35.0	21.4	28.2	87.6	51.1	69.6	0.0
Week-2	14th - 20th Oct, 2021	31.5	19.2	25.3	89.6	59.9	75.6	7.8
Week-3	21st - 27th Oct, 2021	30.2	16.1	23.2	85.9	64.9	75.6	5.3
Week-4	28th – 3rd Nov, 2021	29.2	13.5	21.3	91.6	52.7	72.2	0.0
Mean		31.5	17.6	24.5	88.7	57.2	73.3	3.3
Spring - Summer season 2022								
		Temperature (°C)			Relative humidity (%)			Rainfall (mm)
Week	Date	Max.	Min.	Mean	Max.	Min.	Mean	
Week-1	7th- 13th June, 2022	44.0	27.5	35.8	41.1	30.0	36.3	0.0
Week-2	14th - 20th June. 2022	38.0	25.5	31.7	74.0	52.6	63.6	3.3
Week-3	21st - 27th June, 2022	37.6	25.5	31.5	65.0	40.0	52.7	0.0
Mean		39.9	26.2	33.0	60.0	40.9	50.9	1.1