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Geographical Distribution and Molecular Characterization of *Begomovirus* Infecting Soybean [*Glycine max* (L.) Merr.] in Northern Hills and North Western Plains of India

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ABSTRACT

Background: Yellow mosaic disease (YMD) is one of the most severe and widespread constraint for the cultivation of soybean. Given that no comprehensive data on the status of soybean yellow mosaic disease in Northern Hills and North Western Plains of India is present, a detailed investigation was undertaken for three years to study the prevalence of the disease in the region and to characterize the pathogen.

Methods: Towards *Kharif* end, a three year (2018-2020) farmer field survey (76 villages) was carried out to assess the YMD distribution in the major soybean growing areas Northern Hills and North Western Plains of India. The variability of yellow mosaic virus associated with YMD of soybean was studied based on molecular characterization of partial DNA-A coat protein gene and DNA-B movement protein with subsequent nucleotide sequencing and phylogenetic tree construction.

Result: The results revealed that YMD is prevalent in the plains, whereas the disease was undetectable in the Hills. In the plains, the mean disease incidence ranged from 25.40% in 2019 to 16.78% in 2020. Moreover, disease incidence (r = -0.748) had negative and significant (p<0.0001) correlation with altitude. The phylogenetic studies revealed that the virus inciting the yellow mosaic in soybean in Tarai region had closest relationship with *Mungbean yellow mosaic India virus* (MYMIV). It shared more than 96 per cent sequence identity with other MYMIV isolates reported earlier within the country and abroad and hence, was designated as an isolate of MYMIV.

Key words: MYMIV, Soybean, Survey, Yellow mosaic.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the oldest crops grown and is popularly known as “Miracle Crop or Golden Bean” owing to its multiple uses. The crop is susceptible to many abiotic and biotic stresses which account for huge losses for the farmers. Among the different biotic stresses, the crop is highly susceptible to the whitefly (*Bemisia tabaci* Genn.) transmitted yellow mosaic disease (YMD) (Yadav *et al*., 2018). It is prevalent in almost all the major soybean growing areas and is a serious threat to soybean cultivation, especially, in the Central and the North Western Plain Zones of India (Singh and Aravind, 2019). It has been reported to cause more than 80 per cent yield loss in soybean if plants are infected at an early stage (Amrate *et al*., 2020). In 2015 yellow mosaic epidemic was observed in soybean during R1-R3 stage in Jabalpur, Madhya Pradesh (Silodia *et al.,* 2018). In the pulse crops, yellow mosaic disease is caused by four different virus species belonging to the genera *Begomovirus* (Family: *Geminiviridae*) *viz*., *Mungbean yellow mosaic India virus* (MYMIV)*, Mungbean yellow mosaic virus* (MYMV)*, Dolichos yellow mosaic virus* and *Horsegram yellow mosaic virus* (Quazi *et al*., 2007). Of these, MYMV and MYMIV are mostly reported to be associated with yellow mosaic in soybean (Girish and Usha, 2005; Ramesh *et al*., 2016).

In India, soybean is grown under rainfed condition in diverse agro-ecological zones and among them, agroclimatic conditions in hills are the most challenging and highly variable (Bhartiya *et al.,* 2017). North Western Plains (NWP) and Northern Hills (NH) zones of India are one of the most significant contributors of soybean production in the country. Management of YMD is often linked with control of the vectors by spraying insecticides which often leads to environmental pollution and health hazards (Naik *et al.,* 2023). The exact disease prevalence data is a prerequisite for adoption of stringent disease management strategies especially in the hills as the farmers mainly resort to the non-chemical means of disease management in the region.

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Given that no comprehensive data on the status of soybean yellow mosaic disease in Uttarakhand is present, a detailed investigation was undertaken for three years to study the prevalence of the disease in the region and to characterize the pathogen. The expected outcome of the investigation is updated information on geographic distribution and molecular diversity of the *Begomovirus* inciting YMD of soybean in the region.

MATERIALS AND METHODS

Survey area

A random roving survey was carried out during three consecutive crop seasons of 2018 to 2020 from September to October to know the incidence of yellow mosaic disease in the major soybean growing areas of Northern Hills and North Western Plains of India. The survey route followed a roadmap cutting across the major soybean growing areas in the region. Depending on their geographical location and altitude, the survey locations were divided in to two broad groups *viz*., Hills and Plains (Tarai region). A total of 76 villages (22 in Plains and 54 in Hills) were visited during the survey. From each village two fields were randomly selected for the recording disease parameters.

Disease prevalence

In each field, 25 plants were randomly chosen at all four corners and center for recording disease incidence. Disease severity was recorded on 25 plants per field (five plants each at all four corners as well as center of the field). The disease severity assessment was made using the standard key described by Singh and Singh (2000) and expressed as percent disease index as described by Wheeler (1969). The data on the latitude, longitude and altitude was also recorded at each location. The data recorded during the field survey were used to prepare maps for the prevalence of YMD in the region with the help of ARCGIS version 10.2.2.

DNA extraction and PCR amplification

The molecular characterization was done at Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar, India. The total genomic DNA was extracted from infected leaves by Gem-CTAB method given by Rouhibakhsh *et al.* (2008) with some modifications. Polymerase chain reaction was carried out in 25 µl of reaction mixture containing 1X PCR reaction buffer, 10 µM of each primer (Table 1) , 10 mM of each dNTPs, 2.5 mM of $MgCl_2$ and 2.5 U/µl of Taq DNA polymerase (Genei Laboratories, Bangalore, India). 100 ng of DNA template was used per reaction. The amplification was performed in a thermocycler (Bio-Rad Laboratories, California, USA). The amplified products were separated in 1.5% agarose gel in 0.5X TBE buffer at 60 V and was documented through gel documentation system.

Sequencing and phylogenetic analysis

The PCR products were sequenced at Biologia Research India Pvt Ltd., New Delhi, India. Sequences were compared with other respective viral sequences of the NCBI database using 'Basic Local Alignment Search Tool (BLAST)'. The phylogenic neighbor-joining trees and evolutionary analysis were conducted using MEGA X software package (Tamura *et al*., 2004). Per cent identity matrix and nucleotide alignments were done with Bio-Edit software (Hall 2005). The pair wise sequence identity of the i solate under study with other legume infecting begomoviruses was performed using the Sequence Demarcation Tool (SDT v1.2) (Muhire *et al*., 2014).

Statistical analysis

One-way ANOVA was performed to determine the differences in the foresaid disease and whitefly parameters during the three study years. Significance was considered to be $p<0.05$ for all tests. Disease distribution maps were generated through the Inverse Distance Weighted (IDW) interpolation technique using the ARCGIS (10.3.1) software. All statistical analyses were performed using SPSS 16.0 for Windows.

RESULTS AND DISCUSSION

Disease incidence and severity

The YMD was present in moderate to severe form in all the survey locations in the Tarai region but was not detected in the hills during the three consecutive years of survey. There was significant difference among sites for disease severity ($p = 0.016$) and incidence ($p = 0.005$) during the three years in the Tarai region. The mean disease incidence in the region was 25.40, 20.04 and 16.78 percent in 2019, 2018 and 2020, respectively with an overall mean of 20.84 (Table 2; Fig 1). Highest mean disease incidence and severity was observed in Udham Singh Nagar (24.54% and 20.44%) followed by Dehradun (19.07% and 14.83%) and Nainital Districts.

Table 1: Primers used for amplification of selected regions of yellow mosaic virus infecting soybean.

Primer name	Nucleotide sequence (5′-3′)	Target nucleotide	Product size (bp)	$T_{annealing}$ (°C)	Reference
RUGEMF1	TGTGAGGGACCATGTAAAGTTC	Conserved coat protein gene (AV1)	500	55	Girish and Usha (2005)
RUGEMR1	GCATGAGTACATGCCATATAC				
MYMIV CP-F	GGTCCCCTGATGTCCCTCGTG	Partial coat protein gene (DNA A)	500	55	Chaitanya *et al.* (2017)
MYMIV CP-R	ATGCGTTCTCAGTATGGTTCT				
MYMIV MP-F	GCTATTGTATTGAGCTATGC	Partial movement protein gene (DNA B)	1065	53	Agnihotri *et al.* (2019)
MYMIV MP-R	TGTGTTCCTTTTCAGGGAT				

Similar trend was observed for disease severity as well. Disease incidence (r = -0.748) had negative and significant (p<0.0001) correlation with altitude.

Overall disease incidence and severity for the three years of study varied during different years of survey and from site to site within a year in the Tarai region. This variation in the disease incidence shows that the agro-ecological factors play a critical role in host-pathogen interactions apart from the routine crop management practices. Similar studies on the variation in prevalence of YMD in soybean among the different years of study and locations have been reported from other parts of the country by Silodia *et al.* (2017) and Ashok *et al.* (2018). There was more than 30 per cent disease incidence at Pantnagar (Udham Singh Nagar Dist.) during all the three years of survey and hence, the location can be considered as a hotspot for YMD and can be utilized for varietal screening and development of management strategies. The AICRP soybean annual reports 2017-2019 and Singh and Aravind (2019) have also emphasised Pantnagar to be a hotspot for YMD where the disease is present in moderate to severe form every year irrespective of the date of sowing and other climatic conditions.

Table 2: Prevalence of soybean yellow mosaic disease and its vector in Northern Hills and North Western Plains of India.

District	Mean altitude	No. of locations	Per cent disease incidence				Per cent disease index			
			2018	2019	2020	Mean	2018	2019	2020	Mean
North hill zone										
Almora	1565.62	11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bageshwar	1011.72	5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chamoli	1259.1	7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Champawat	1829.07	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nainital (Hills)	688.02	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pauri garhwal (Hills)	1338.24	7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pithoragarh	1540.95	9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rudraprayag	809.21	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tehri garhwal	1435.44	4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean (Hills)	1275.26		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
North western plain zone										
Pauri garhwal (Plains)	421.74	1	23.20	28.80	13.60	21.87	18.00	23.00	11.00	17.33
Dehradun	451.2	2	19.20	23.20	14.80	19.07	14.50	19.50	10.50	14.83
Nainital (Plains)	398.59	16	15.89	21.31	15.26	17.49	13.64	16.86	13.21	14.57
Udham Singh Nagar	259.43	3	21.87	28.27	23.47	24.53	19.33	24.00	18.00	20.44
Mean	Mean (Plains)	382.74		20.04	25.40	16.78	20.74	16.37	20.84	13.18

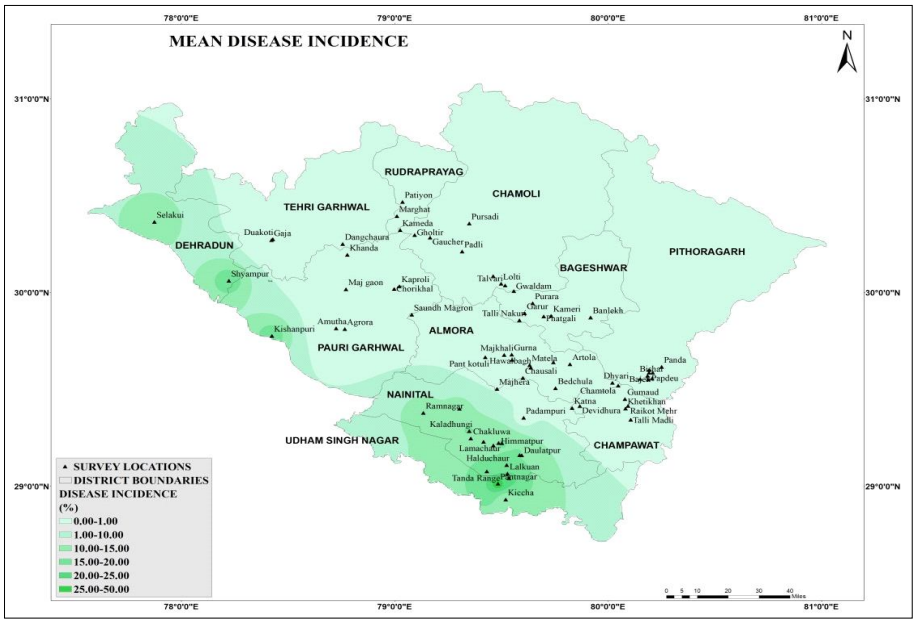

Fig 1: Distribution of soybean yellow mosaic disease in Northern Hills and North Western Plains of India.

Molecular characterization and diversity analysis

PCR with *Begomovirus* DNA A specific primers RUGEMF1 and RUGEMR1 amplified the expected 500 bp amplicon from all the 22 symptomatic samples collected during the survey while no amplification was obtained in asymptomatic healthy leaf samples. The results indicated the presence of whitefly transmitted *Begomovirus*. The PCR amplicons of the Pantnagar isolate were sequenced and sequence analysis showed more than 97 per cent similarity with MYMIV isolates reported in India and abroad. Two different MYMIV specific primers targeting the coat protein gene on DNA A and movement protein in DNA B was used to amplify 500 bp and 1000 bp long amplicons, respectively (Fig 2). The PCR products were purified and sequenced for further analysis. These partial sequences were deposited in NCBI Genebank database under accession number MZ267790 (movement protein) and MZ267791 (coat protein).

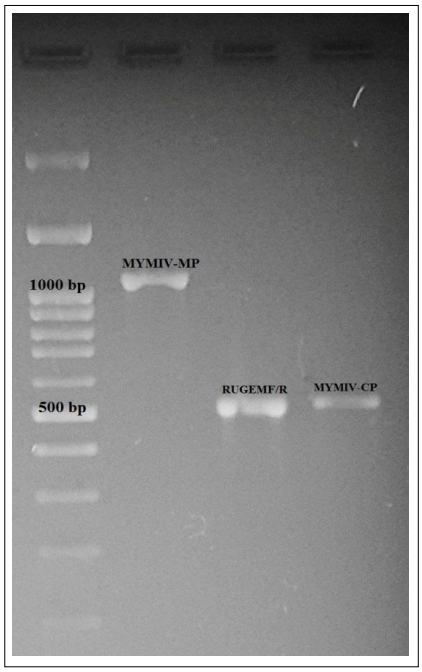

Fig 2: PCR amplification of MYMIV from infected soybean samples using MYMIV-MP-F/R, RUGEMF/R and MYMIV-CP-F/R primers.

BLAST analysis of partial coat protein gene revealed highest sequence identity (97.29%) with MYMIV isolate Mb04 coat protein gene (GQ387504) reported from India which is higher than the 91 per cent similarity required for species delineation of *Begomovirus*. The *Begomovirus* under study shared 79.401, 80.001-80.501 and 72.501-74.701 percent identity with selected isolates of MYMV, HgYMV and DoYMV previously reported (Fig 3). The phylogenetic analysis revealed that the isolate under study formed clad with MYMIV group which showed closest relationship (Fig 4). The blast analysis of partial movement protein gene revealed highest sequence identity (96.48%) with Indian isolate of MYMIV infecting soybean (MT232630). It shared 80.3 to 93.1, 77.0 to 78.8, 67.5-67.9 percent similarity with DNA B of MYMV, HgYMV and DoYMV, respectively. The phylogenetic analysis of partial movement protein gene showed close relationship with MYMIV DNA B components (Fig 5).

The coat protein ORF (CP or AV1) is the only *Begomovirus* sequence approved by the International

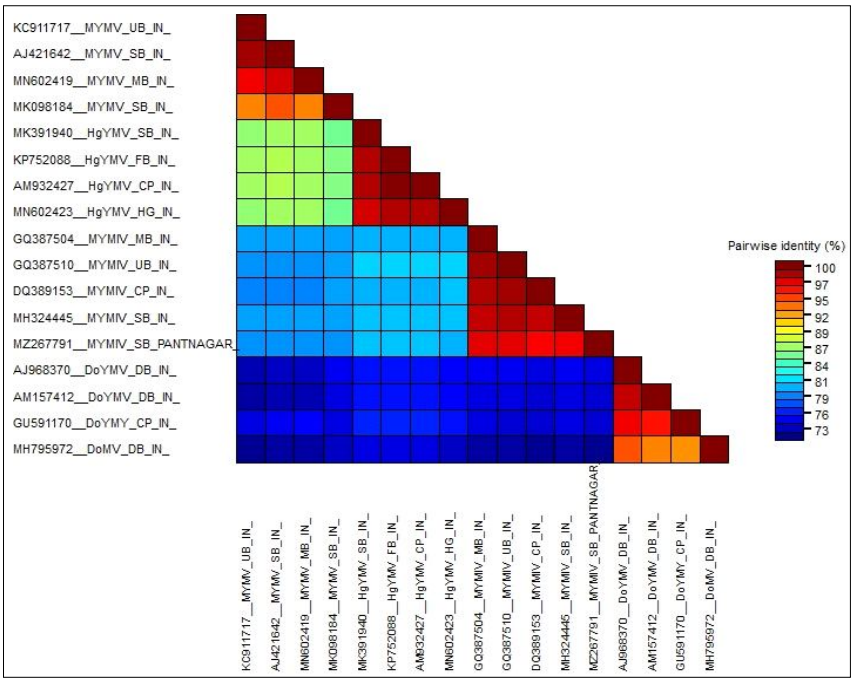

Fig 3: SDT matrix of pairwise identity scores generated by alignment of partial coat protein gene of MYMIV_SB [Pant] with legume infecting *Begomovirus* isolates in Genebank.

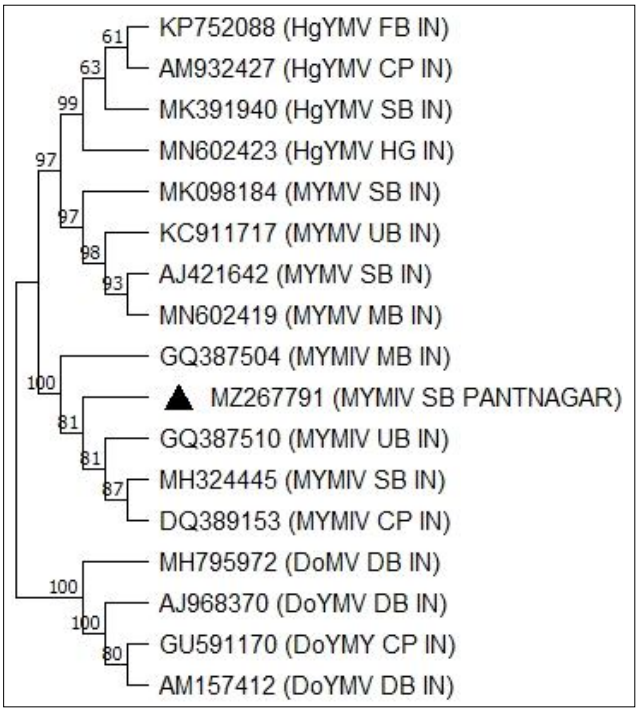

Fig 4: Phylogenetic tree derived from partial coat protein gene sequences of MYMIV_SB [Pant] with other legume infecting *Begomovirus* isolates in Genebank.

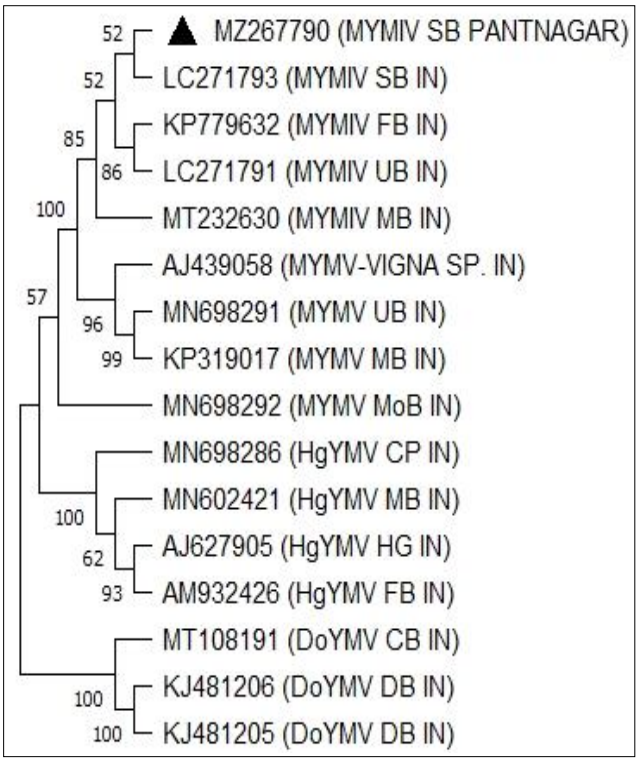

Fig 5: Phylogenetic tree derived from partial movement protein gene sequences of MYMIV_SB [Pant] with other legume infecting *Begomovirus* isolates in Genebank.

Committee on Taxonomy of Viruses for ascertaining the identity of a *Begomovirus* and the sequence comparison has been used to identify and classify geminiviruses (Padidam *et al*., 1995; Brown *et al*., 2001). In our investigation, we have observed more than 96 per cent identity of partial coat protein and movement protein sequences of our isolate with other legume infecting MYMIV isolates. It showed less than 90 per cent sequence identity with other legume infecting begomoviruses *viz*., MYMV, DoYMV and HgYMV. Thus, based on high sequence identities and phylogenetic relationships of partial DNA-A (in particular) and DNA-B genome with MYMIV isolates, the *Begomovirus* isolate under study could be designated as an isolate of MYMIV. These results are in agreement with the earlier published reports of Srinivasaraghavan (2014) who studied *Begomovirus* infecting different pulse crops in Uttarakhand and identified the presence of MYMIV in soybean in Pantnagar. Based on DNA polymorphism and nucleotide diversity of the legume infecting begomoviruses in India, *Begomovirus* inciting yellow mosaic disease in soybean in northern and central parts of the country has been reported to be MYMIV (Girish and Usha, 2005; Ramesh *et al*., 2016). The soybean infecting MYMIV have more than 89 percent identity with other MYMIV isolates (Usharani *et al*., 2004).However, more detailed investigation based on whole genome sequencing of the DNA-A is necessary for the final confirmation of the *Begomovirus* under study as an isolate of MYMIV.

As per the obtained results, it can be concluded that the YMD in soybean is prevalent in North Western Plains of the country and is causing huge economic loss to the farmers in the region. An integrated strategy, using the disease resistant varieties and vector management, has to be deployed for effective management of the disease to effectively manage the disease the region.

CONCLUSION

This study provides an updated status of the YMD and its pathogen in major soybean areas in NH and NWP zones of the country. According to the results obtained, it can be concluded that the YMD in soybean is prevalent in the plains and is causing huge economic loss to the farmers in the region. An integrated strategy, using the disease resistant varieties and vector management, needs to be devised to effectively manage the disease and overcome the threat posed by it in the NWPZ. In lieu with the earlier reports on the presence of *Mungbean yellow mosaic India virus* (MYMIV) inciting the yellow mosaic in the northern parts of the country, the causal agent of the YMD in soybean in Tarai region has also exhibited closest relationship with MYMIV. Hence, the soybean genotypes that have resistance to MYMIV can be deployed in the Tarai region as well.

Conflict of interest

The authors declare that there are no conflicts of interest.

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