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Genetic Diversity and Geographic Distribution of *Bemisia tabaci* Species Complex Infecting Soybean in Northern Hills and North Western Plains of India

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ABSTRACT

Background: The Silverleaf whitefly, *Bemisia tabaci* (Gennadius), is a pest of global significance causing huge economic loss to a wide range of agricultural crops. The pest is regarded as a cryptic species complex which differs in its adaptability to host, fecundity and resistance to insecticides. The expected outcome of the investigation is updated information on prevalence of cryptic species of *B. tabaci* in the major soybean growing areas in the region.

Methods: A roving survey was carried out to assess the occurrence of *B. tabaci* in the major soybean growing areas of Northern Hills and North Western Plain zones during three consecutive crop seasons from 2018 to 2020. The survey locations were divided in to two broad groups *viz.,* Hills and Plains (Tarai region) and a total of 76 villages were visited during the survey. Cryptic species composition was analyzed using the mtCOI sequence through PCR amplification.

Result: The mean whitefly population per plant for the three years in hills and plains were 0.53 and 3.55, respectively. The highest whitefly population was observed in 2019 (1.79 per plant) followed by 2018 (1.50 per plant) and 2020 (1.11 per plant). There was significant ($p<0.0001$) negative correlation ($r = -0.749$) with altitude. Phylogenetic relationships indicated the existence of two cryptic species Asia-1 and Asia-II-1. Of these, Asia-II-1 was found to be prominent especially in the North Western Plain Zone. In the Northern Hill Zone both Asia 1 and Asia-II-1 were detected.

Key words: *Bemisia tabaci*, Cryptic species, Soybean, India.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the oldest crops grown and is popularly known as the “Miracle Crop or Golden Bean” owing to its multiple uses. The crop suffers from much biotic and abiotic stress which limits productivity and account for huge losses for the farmers. Among these, yellow mosaic disease (YMD) and its vector *Bemisia tabaci* are prevalent in almost all the major soybean growing areas and is a major constraint for soybean cultivation (Singh and Aravind, 2019). The whitefly (Aleyrodidae: Hemiptera) is a pest of global significance especially in the tropical and subtropical regions (Cardona, 2005). It is a wide reaching pest and has a broad host spread across many plant families. It has been reported to act as the vector for more than 120 plant viruses of which *Begomoviruses* causing yellow mosaic diseases are the most important ones (Oliviera *et al.*, 2001).

The taxonomic state of the *B. tabaci* species complex and are well-thought-out as a cryptic species complex (de Barro *et al.*, 2011). This has led to the introduction of the concept of biotypes and genetic groups for the unlike populations of *B. tabaci*. These genetic groups differ in their host preferences (Xu *et al.*, 2011), fecundity, virus transmission efficiency (Liu *et al.*, 2009), endosymbionts composition and insecticide resistance (Luo *et al.*, 2010). Since the morphological characteristics cannot be fully depended upon for differentiating the different genetic groups, scientists prefer the use of PCR amplification and sequencing of the conserved region in the mitochondrial cytochrome oxidase I (mtCO1) gene (Frohlich *et al.*, 1999). Among the 34 putative species of *B. tabaci* reported globally, nine have been recognized in India (Hashmi *et al.*, 2018).

Given that extensive study on the genetic group analysis of *B. tabaci* infesting soybean in Northern Hills (NH) and North Western Plains (NWP) zones of India is lacking, the experiment was undertaken to elucidate the cryptic species composition of *Bemisia tabaci* in the region. The expected outcome of the investigation is updated information on the prevalence of cryptic species of whitefly in the major soybean

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growing areas in the region. This will help in streamlining the management strategies against the YMD and its vector.

MATERIALS AND METHODS

Survey

A roving survey was carried out to assess the occurrence of whitefly (*B. tabaci*) vector in the major soybean growing areas of NH and NWP zones during three consecutive crop seasons from 2018 to 2020 from September to October. The survey route followed a roadmap which allowed sampling of the major soybean growing areas in the region. The survey locations were divided in to two broad groups *viz.,* Hills and Plains (Tarai region) depending on their geographical location and altitude. A total of 74 villages (54 in Hills and 20 in Plains) were visited during the survey.

Whitefly sampling

The whitefly population was recorded on 25 plants per field (five plants each at all four corners as well as center of the field). The whitefly population per plant was assessed by counting the number of whiteflies in three trifoliate leaves one each at top, middle and bottom. The adult whiteflies were collected using a handheld aspirator and the collected specimens were transferred to 2 ml Eppendorf tubes containing 70 percent ethanol and stored at -20°C. A minimum of 50 whiteflies were collected from each location. A total of 24 specimens were collected during the survey.

DNA extraction, PCR analysis and Sequencing

All the laboratory experiments were carried out at the Department of Plant Pathology, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. Total DNA was extracted from whitefly following the method suggested by Alhudaib *et al.* (2014) with some modifications. The PCR analysis was performed using the primer pair C1-J2195 COI (Forward) [TTGATTTTTTGGTCA TCCAG AAGT] and L2-N-3014 (Reverse)[TCCAATG CACTAATCTGCCATATTA] (Frolich *et al.*, 1999). The PCR reaction mixture consisted of 3 µl template DNA, 1 µl each of forward and reverse primer (10 µM), 1 µl BSA (10 mg/ml), 12.5 µl PCR mastermix (Genei Laboratories Pvt Ltd) and sterile distilled water to make up the volume to 25 µl. PCR cycle conditions involved 5 min initial denaturation at 94°C followd by 35 cycles of 94 °C for 1 min, 48 °C for 2 min and 72°C for 1 min and final extension at 72°C for 5 min. The PCR products were electrophoresed in 1.5% agarose gel. The amplification yielded about 880 bp fragment (Fig 1) which were purified and sequenced at Biologia Research Pvt Ltd, New Delhi, India.

Genetic group analysis and phylogeny

Reliable sequences were obtained only for 20 samples. The final data set comprised 20 sequences generated in the study and additional 34 reference sequences obtained from GenBank to represent different *B. tabaci* genetic groups (Table 1). Two species namely *Trialeurodus vaporariorum* (AF418672) and *B. afer* (GU220055) were used as out

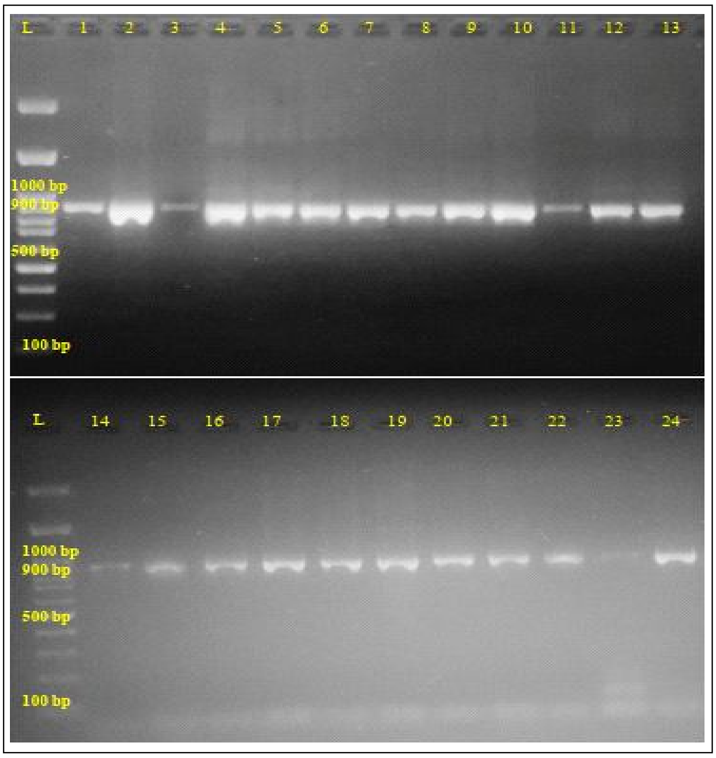

Fig 1: PCR amplification of partial mitochondrial cytochrome oxidase I gene in *Bemisia tabaci.*

groups. The data sets with these 56 sequences were subjected to multiple sequence alignment using the ClustalW algorithm using default parameters. Sequence divergence among the samples was assessed using the Kimura 2-parameter distance model and graphically revealed in a maximum likelihood tree by MEGA X version 10.2.4 (Kimura, 1980; Kumar *et al.*, 2018).

Statistical analysis

Descriptive statistics were used to the describe distribution. The whitefly population for the plains was checked for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests. One-way ANOVA was performed to determine the significant differences among whitefly parameters during the three study years. Pearson's correlation coefficient was used to examine the relationships among the continuous variables. Significance was considered to be $p<0.05$ for all tests. The whitefly 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

Whitefly population

There was no significant difference in the whitefly population in different locations over the years of the survey (p=0.326). The highest whitefly population was observed in 2019 (1.79 per plant) followed by 2018 (1.50 per plant) and 2020 (1.11 per plant) (Table 2). The mean whitefly population per plant

Table 1: Reference sequence used for genetic group analysis along with their NCBI accession number and location.

Species	NCBI accession no	Location	Genetic group
Bemisia tabaci	GQ281714	India: Tamilnadu	Asia 1
Bemisia tabaci	HM137326	China: Guangdong	Asia II 1
Bemisia tabaci	AY686088	China: Jiangsu	Asia II 2
Bemisia tabaci	EU192045	China: Guangxi	Asia II 3
Bemisia tabaci	AY686083	China: Guangdong	Asia II 4
Bemisia tabaci	AF418666	India: Banglore	Asia II 5
Bemisia tabaci	HM137338	China: Guangxi	Asia II 6
Bemisia tabaci	DQ116660	India: Tamil Nadu	Asia II 7
Bemisia tabaci	AJ748358	India: Karnataka	Asia II 8
Bemisia tabaci	HM137313	China: Hunan	Asia II 9
Bemisia tabaci	HM137356	China: Guangdong	Asia II 10
Bemisia tabaci	HM590147	India	Asia II 11
Bemisia tabaci	AB440792	Japan: Okinwa	Asia III
Bemisia tabaci	GU086328	Australia	Australia
Bemisia tabaci	GU086325	Indonesia	Indonesia
Bemisia tabaci	HM137315	China: Sichuan	China 1
Bemisia tabaci	AY686072	China: Guangdong	China 2
Bemisia tabaci	EU192050	China: Yunnan	China 3
Bemisia tabaci	AJ550171	Madagascar	Indian Ocean
Bemisia tabaci	AY827596	Italy	Italy
Bemisia tabaci	AB440785	Japan: Kagoshima	Japan 1
Bemisia tabaci	AB240967	Japan: Hiroshima	Japan 2
Bemisia tabaci	EU192049	China: Yunnan	Mediterranean
Bemisia tabaci	AJ748368	India: Karnataka	MEAM- 1
Bemisia tabaci	AJ550177	Reunion	MEAM- 2
Bemisia tabaci	EU760739	Cameroon	Africa
Bemisia tabaci	DQ130053	Belize	New World
Bemisia tabaci	AY057149	Uganda	Sub-Saharan Africa 1
Bemisia tabaci	AY057194	Uganda	Sub-Saharan Africa 2
Bemisia tabaci	AF344257	Cameroon	Sub-Saharan Africa 3
Bemisia tabaci	AF344247	Cameroon	Sub-Saharan Africa 4
Bemisia tabaci	AM040598	Uganda: Mukono	Sub-Saharan Africa 5
Bemisia tabaci	AY903553	Uganda	Uganda
Bemisia tabaci	KJ709461	Pakistan: Islamabad	Pakistan
Bemisia afer	GU220055	-	Out-group
Trialeurodes vaporariorum	AF418672	-	Out-group

for the three years in NH and NWP zones was 0.53 and 3.55, respectively. In NHZ, it ranged from 0.47 in 2020 to 0.63 in 2019; whereas in NWPZ, it ranged from 2.54 in 2020 to 4.16 in 2019. The highest mean whitefly population was observed in Udham Singh Nagar district (3.99 per plant) followed by Nainital (3.46 per plant) and Dehradun (3.45 per plant). The lowest whitefly population was observed in Tehri Garhwal (0.34 per plant) and Chamoli districts (0.37 per plant). Our results corroborate with the findings Ashok *et al.* (2018) who have reported that the whitefly population in soybean varied from year to year and from location to location. During their survey of whitefly in the major soybean growing areas of Rajasthan, they observed that whitefly population ranged from 2.20 to 3.23 whiteflies per plant in 2012 to 2.65 to 3.31 in 2013. The varieties susceptible to YMD had a higher whitefly population. In a fixed plot survey

Table 2: Prevalence of *Bemisia tabaci* in major soybean growing areas of NH and NWP zones of India.

District	Mean altitude	No. of locations	Whitefly population/plant			
			2018	2019	2020	Mean
Northern hill zone						
Almora	1565.62	11	0.52	0.60	0.37	0.52
Bageshwar	1011.72	5	0.38	0.55	0.46	0.47
Chamoli	1259.1	7	0.28	0.42	0.39	0.37
Champawat	1829.07	6	0.55	0.64	0.47	0.55
Nainital (Hills)	688.02	2	0.86	1.04	0.95	0.86
Pauri garhwal (Hills)	1338.24	7	0.49	0.59	0.37	0.48
Pithoragarh	1540.95	9	0.60	0.66	0.48	0.58
Rudraprayag	809.21	3	0.68	0.73	0.51	0.64
Tehri garhwal	1435.44	4	0.33	0.46	0.23	0.34
Mean (Hills)	1275.26		0.52	0.63	0.47	0.53
North western plain zone						
Pauri garhwal (Plains)	421.74	1	3.72	4.48	1.68	3.29
Dehradun	451.2	2	3.96	4.86	1.54	3.45
Nainital (Plains)	398.59	16	3.38	3.46	3.53	3.46
Udham singh nagar	259.43	3	3.72	4.84	3.41	3.99
Mean (Plains)	382.74		3.70	4.41	2.54	3.56
State mean	-	-	1.50	1.79	1.11	1.46

Table 3: Whitefly specimens collected during the survey along with their location, NCBI accession number and genetic group.

SampleID	Location: district	Latitude/Longitude	NCBI accession no.	Genetic group
Bt-kch	Kicha: U.S. Nagar	28°55′56.6″N 79°31′09.8″E	MZ227524	Asia-II-1
Bt-pnt	Pantnagar: U.S. Nagar	29°00′52.9″N 79°29′01.9″E	MZ227525	Asia-II-1
Bt-bdk	Bindukhatta: Nainital	29°02′33.1″N 79°32′07.1″E	MZ227526	Asia-II-1
Bt-glp	Gaulapar : Nainital	29°10′36.5″N 79°35′25.1″E	MZ227527	Asia-II-1
Bt-hld	Haldwani: Nainital	29°13′12.0″N 79°30′38.5″E	MZ227528	Asia-II-1
Bt-lmc	Lamachaur: Nainital	29°13′50.4″N 79°24′55.0″E	MZ227529	Asia-II-1
Bt-tnd	Tanda: Nainital	29°04′38.2″N 79°25′53.3″E	MZ227530	Asia-II-1
Bt-chr	Chorgalia: Nainital	29°07′14.9″N 79°42′01.3″E	MZ227531	Asia-II-1
Bt-cdk	Chandak: Pithoragah	29°36′07.7″N 80°11′27.5″E	MZ227532	Asia-II-1
Bt-shm	Shyampur: Dehradun	30°03′42.0″N 78°13′21.5″E	MZ227533	Asia-II-1
Bt-ktb	Kotabagh: Nainital	28°55′42.1″N 79°47′22.6″E	MZ227534	Asia-II-1
Bt-hdc	Haldichaur: Nainital	29°06′38.1″N 79°31′27.2″E	MZ227535	Asia-II-1
Bt-rmn	Ramnagar: Nainital	29°22′21.1″N 79°08′35.0″E	MZ227536	Asia-II-1
Bt-pdm	Padampuri: Nainital	29°21′16.0″N 79°36′12.5″E	MZ227537	Asia-II-1
Bt-brc	Barechhina: Almora	29°38′25.6″N 79°44′35.3″E	MZ227538	Asia-I
Bt-khm	Khameda: Pauri garhwal	30°19′23.5″N 79°01′30.6″E	MZ227539	Asia-I
Bt-kld	Kaladungi: Nainital	29°17′08.1″N 79°20′54.5″E	MZ227540	Asia-II-1
Bt-slq	Selaqui: Dehradun	30°21′57.4″N 77°52′26.3″E	MZ227541	Asia-II-1
Bt-bpd	Bhupdevpur: Pauri garhwal	29°47′05.8″N 78°23′13.2″E	MZ227542	Asia-II-1
Bt-ksn	Kishanpuri: Pauri garhwal	29°46′38.9″N 78°25′25.2″E	MZ227543	Asia-II-1

conducted by Silodia *et al.* (2017), it was observed that the whitefly population per plant increased exponentially from 2 per leaf to 7-25 per leaf during the second week of August.

Whitefly population (r=-0.749) had negative and significant ($p<0.0001$) correlation with altitude. The regression equation for whitefly population vs altitude (x) was $y= -0.011x$ $y= -0.001x+3.420$, where y represents whitefly population per plant. There was a drastic reduction in whitefly population with increase in altitude above 500 m. The *B. tabaci,* the prominent whitefly in soybean, has been reported to be prominent only in areas with altitude 0-400 m whereas in higher altitude (1000-3000m) the predominant whitefly species was *Trialeurodes vaporariorum.* In the areas having altitude between 400-1000 m, both the species of whitefly are present (Cardona 2005). A negative correlation between altitude and YMD incidence and its vector has been observed by Alam *et al.* (2014) as well.

Genetic group analysis

Out of the 20 whitefly isolates collected, 15 where from the Tarai region and the rest five where collected from the hills. The samples were collected from six different districts *viz.,* Udham Singh Nagar [U.S. Nagar] (3 samples), Nainital (10 samples), Dehradun (2 samples), Pauri Garhwal (3 samples), Almora (1 sample) and Pithoragarh (1 sample). The phylogenetic analysis revealed that the whitefly sequences under clustered into two different clades (Fig 2). Out of the 20 whitefly isolates characterized, 18 clustered with the Asia-II-1 and the remaining two isolates clustered with that of Asia-I. All the isolates collected from the Tarai region in North Western Plains zone belonged to the Asia-II-1 group. Out of the five whitefly isolates collected from the Northern Hill Zone, two (Bt-brc and Bt-khm) belonged to the Asia I group while the rest three (Bt-cdk, Bt-bpd and Bt-pdm) clustered with the Asia-II-1 group. Thus all the sequences were assigned to the two cryptic species identified by Dinsdale *et al.* (2010) (Table 3).

The overall mean pair wise distance among the 20 sequences was 0.352. The isolates Bt-bdk and Bt-glp had pair wise distance of 0.0102 from the rest of the Asia-II-1 isolates and 0.203 from that of Asia-1 isolates characterized. The pair wise distance of the isolates that clustered with Asia-II-1 with that of the reference Asia-II-1 ranged from 0.0051 to 0.0152. The Bt-brc and Bt-khm had pair wise distance of 0.127 and 0.132 from the Asia-1 reference sequence respectively. The sequence data generated in the present investigations has been submitted to the NCBI database and the accession numbers have been obtained (MZ227524 to MZ227543).

The present investigation has been one of the first exclusive studies on the cryptic species status of the whitefly in soybean in Uttarakhand. The existing reports revealed the presence of nine different cryptic species within the country *viz.,* Asia-1, Asia-II-1, Asia-II-5, Asia-II-7, Asia-II-8, Asia-II-10, ASIA-II-11, MEAM-1 and China-3 (Ellango *et al.*, 2015; Krishnamoorthy *et al.*, 2021). Asia 1 is the predominant cryptic species in Asia and its presence has been detected in seven different countries including India, Pakistan and Bangladesh (Hu *et al.*, 2015). The presence of Asia-II-1 and Asia-1 in the country and exclusively in the soybean

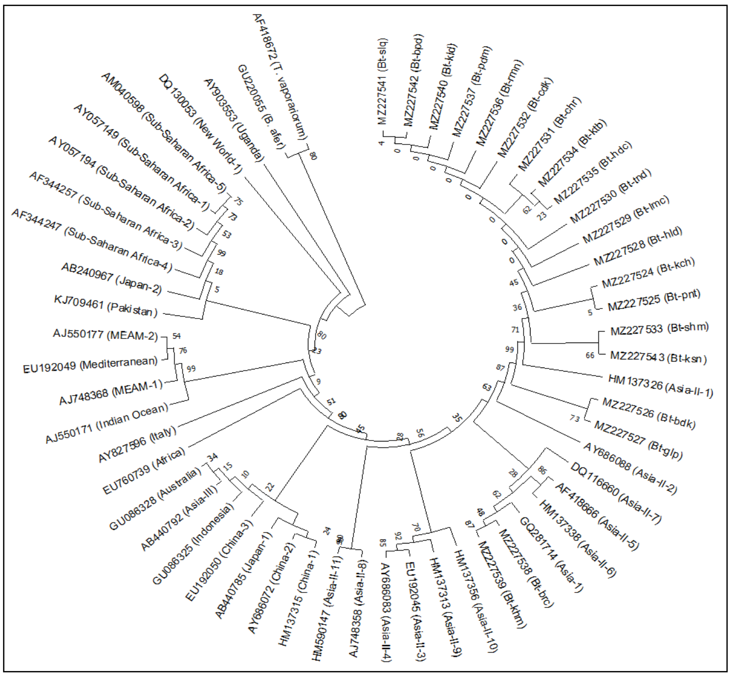

Fig 2: Phylogram of the evaluated *Bemisia tabaci* samples with well-assigned homologous sequences of the *B. tabaci* genetic groups from the NCBI database by means of maximum likelihood (ML) tree method and the Kimura 2-parameter distances of mitochondrial COI sequence.

ecosystems have been reported by Prasanna *et al.* (2015). They identified three different cryptic species namely Asia-1, Asia-II-1 and Asia-II-7; two of which has been detected in our study as well. They have reported Asia-II-1 as the most predominant whitefly cryptic species in soybean in India. But contradictory to our finding of the presence of Asia-II-1 in Pantnagar, they have reported Asia-II-7 to be the predominant cryptic species in the Pantnagar. The presence of the Asia-II-1 in soybean has been reported in New Delhi (Hashmi *et al.* 2018), Uttar Pradesh and Punjab (Ellango *et al.* 2015). The presence of Asia 1 and Asia-II-1 in different crops in the country is also evident from the findings of Chowda-Reddy *et al.* (2012), Roopa *et al.* (2015) and Prabhulinga *et al.* (2021).

CONCLUSION

This study provides an updated status of occurrence and cryptic species composition in the major soybean growing areas of NH and NWP zones of India. According to the results obtained, it can be concluded that *B. tabaci* is prevalent in the NWP zone and cause huge economic loss to the farmers. The findings of the study could be useful information for monitoring the future patterns of whitefly population diversity and displacement in the region. The information revealed by the study could be an important aid to the development of management strategy against the whitefly and YMD in soybean.

Conflict of interest: None.

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