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Research Article

WHITEFLY TRANSMITTED YELLOW MOSAIC DISEASE, SEVERE THREAT TO COWPEA PRODUCTION IN ASSAM, INDIA

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Abstract- Yellow mosaic diseases (YMD) in economically important legumes across Southern Asia are majorly caused by four species of geminiviruses (genus Begomovirus, family Geminiviridae). YMD is transmitted by whitefly and causes severe loss to a number of important grain legumes, a rich source of dietary protein. The viruses have limited host range to plants of the family Fabaceae. The efforts to reduce the losses are hampered by limited availability of natural resistance sources and the lack of durable resistance. There exists active genetic interaction between these begomoviruses of the legumes, in the form of both component exchange and classical recombination, but very few studies are there on interaction with viruses that infect other plants. Genetic isolation indicates viruses infecting legumes evolve independently of the begomoviruses of other plant families. This has clear implications for the requirement to develop resistance in legumes, which holds the promise of durability. In our study, we surveyed in 4 districts of Assam, India, at various locations, for severity of viral diseases in cowpea. The morphometric appearances of viral disease in cowpea leaf samples in the field were collected. The genomic DNA were extracted and PCR performed for the detection of viral DNA. The PCR products were cloned and sequenced. The full length obtained sequences were analyzed using NCBI-BLAST and CIUSTL-W program for identification of virus.

Keywords- Cowpea, Mungbean Yellow mosaic India virus, YMD, RCA.

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Introduction

Cowpea (*Vigna unguiculata*) is one of the most important leguminous crops grown by resource poor farmers in the developing world due to its social, economic and dietary importance. The cowpea seeds are highly nutritious with dried grain containing up to 25% protein and 56% carbohydrate [1]. Cowpea is well adapted to high temperatures, drought and poor soils as compared to other crop species [2, 3]. In spite of its importance, cowpea faces numerous production constraints. The major constraints in cowpea production are low yields of traditional varieties, and high susceptibility to viral, fungal and insect damage. Significant proportion of losses is due to virus infection which is estimated between 10 and 100% [4], depending on the virus-host vector relationships as well as the prevailing epidemiological factors. Cowpea plants are often infected by more than one virus, resulting in serious economic losses in its production [5]. Over 20 viruses have been reported to infect cowpea worldwide [6].

In Africa, the economically important viruses of cowpea are cowpea chlorotic mottle virus (CPCMV), cowpea severe mosaic virus (CPSMV), cowpea aphid-borne mosaic virus (CABMV), cucumber mosaic virus (CMV), cowpea mosaic virus (CPMV), cowpea mild mottle virus (CPMMV) and cowpea chlorotic mosaic virus (CCMV) [7]. One of the most important viruses of cowpea is *Cowpea aphid-borne mosaic potyvirus* (CABMV) which belongs to the *Potyviridae* family and causes high yield loss to cowpea. The severest crop loss of 18 - 87% reported in Iran, and a probable 100% loss reported in Nigeria [6]. In India, cowpea is highly susceptible to golden mosaic disease (CGMD) and severe leaf curl disease which are caused by Mungbean yellow mosaic India virus (MYMIV) [8-11].

Geminivirus are small circular, single strand circular DNA plant viruses containing monopatite or bipartite genome of approximately 2.7 kb size encapsidated in a twinned icosahedral particles [12]. They infect a wide range of both monocotyledonous and dicotyledonous plants but each member of the Geminiviridae family has its own specific limited host range. They are classified into four genera: Mastrevirus, Curtovirus, Topocuvirus and Begomovirus, on the basis of the viral vector, host range and their particular genome organization. Geminiviruses transmitted by whitefly *Bemisia tabaci* belong to the largest genus *Begomovirus*, among geminiviridae family have either bipartite genome (Presence of both separate DNA-A and DNA-B being ssDNA) of approximately 2.7 kb size or a monopartitess DNA genome (2.7 kb) [13, 14].The majority of the begomoviruses are bipartite in nature and the genomic components are segmented into DNA-A and DNA-B [15 16].

Symptoms of plant viral diseases have been recognized and documented, although it has recently become possible to identify the causal pathogens. The most severe diseases of cowpea are caused by viruses which represent significant proportion of yield loss with respect to the potential value of the crop in sub-Saharan Africa [17]. Cowpea plants are often infected by more than one virus disease, resulting in serious economic losses in agricultural production.

Materials and Methods

Survey in the field

To investigate the occurrence of viral disease in major agro climatic zones in North-East India, a survey was conducted in four major districts of Assam;

Goalpara, Nalbari, Barpeta and Nagaon during 2012-2013 at different time intervals and field locations. The surveys were undertaken during the month of August-December when the cowpea crop is heavily cultivated by farmers in India. Five distant fields located approximately 5–10 km apart were selected and surveys carried out in each district growing cowpea. The fields of four major districts were considered to determine whether there were variations in virus occurrence amongst districts or fields or across districts or fields. The surveys were carried out when the cowpea crop was at its vegetative growth stage.

Disease assessment of infected plants in the field

Assessment of virus symptoms in cowpea was based on percentage incidence, occurrence and symptom severity. The incidence of disease was calculated by expressing the number of plants with virus symptoms as a percentage of the total number of cowpea plants in each sampled plot area. Disease severity was assessed on cowpea leaves with occurrence of viral symptoms and it was performed visually based on the following standard rating scale: where 1 = 0% (absence of viral disease symptoms) and whenever the index 5 ≥ 60% (very severe symptoms leads to death of the plants).

Infected plants exhibiting stunted growth, severe leaf curling, reduced leaf size, yellow patches and distortion of leaf lamina symptoms were selected for sampling [Fig-1 & 2]. Due to the association of whiteflies with the plants, begomovirus infection was suspected to cause the disease in the samples. The virus infected cowpea plant materials were collected from various field locations and stored for molecular analysis.

Fig-1 Virus infected field survey at various locations in Assam a-b. Nalbari; c-d Goalpara; e-f. Barpeta; g-h. Hazo and i. Nagaon

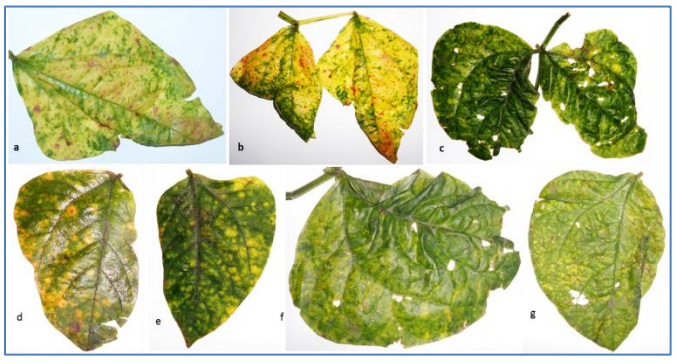

Fig-2 Typical yellow mosaic disease symptoms of cowpea collected from various locations in Assam, India.

Storage of virus infected plant materials and Genomic DNA extraction

Mature and young leaf samples from infected cowpea plants were collected, washed, dried, sealed in zipper bags, and stored at −80°C until used. Total DNA were extracted using Cetyl Trimethyl Ammonium Bromide (CTAB) method of DNA isolation developed for young plant tissues [18] with minor modifications for minimizing phenolic compounds and polysaccharide to get good quality of pure DNA for further processing. Total genomic DNA were electrophoresed on 0.8% agarose gel to check the yield and quality. The gel were stained in ethidium bromide solution 10 mg/ml and photographed.

PCR amplification of viral DNA

Total genomic DNA were quantified and 100 ng were used for Rolling circle amplification (RCA) reaction to amplify the viral DNA. A general procedure for detecting circular DNA viruses using multiply-primed RCA were used. Total DNA were extracted from the sample and random hexamer primers are subsequently added. After heat denaturation, the phi 29 polymerase were added in the reaction mixture and then incubated at 30 °C for overnight.

Cloning of viral DNA components and sequencing

RCA amplified concatemer products were digested with five common restriction enzymes EcoRI, PstI, XbaI, EcoRVandSacI (Thermo Scientific, USA). Digested products were resolved on 1% agarose gel run at 110V to separate the corresponding band to ~ 2.7 kb genomes of DNA-A and DNA-B genomes (if present) were purified using NucleoSpin gel and PCR Clean-up (MN- Germany). The 2.7 kb monomers of DNA-A or 2.6 kb of DNA-B were cloned into pTZ57R/T vector. The recombinant clones were confirmed through PCR and restriction digestion. The monomeric full length recombinant clones were purified using SureTrap Plasmid mini Kit (Genetix, India) and sequenced commercially by (Eurofins, Bangalore) company. From each representative samples, the full length sequences were obtained, either by combining the sequencing results of two or three portions of the genomes or sequenced full-length fragments using a primer walking strategy.

Results

Incidence and Severity of Virus Symptoms on Cowpea Crop

The results survey in field showed that there were highly significant ($P < 0.05$) differences in disease incidence and the severity of virus symptoms on cowpea crop in the surveyed districts in fields and the disease incidence was found to be around 60-70%. The disease prevails across the year and there is no significant seasonal variation about the severity of the disease incidence. Similarly, a highly significant interaction effects ($P < 0.05$) were also observed and considered in the fields and the districts. Virus symptoms were encountered in the four districts but with varying incidence levels of virus symptoms. On average, the district of Hajo, Assam had consistently high incidence of virus symptoms in all the fields surveyed. Overall, there was a high disease incidence of 70-80% in Hajo district, followed by Nalbari with 50-60%, with the lowest incidence recorded in the districts of Assam. A slightly lower disease incidence and severity was recorded in all of the fields in the districts during the period of 2012- 2013. Natural multiple infections caused by 4–5 viruses were also observed. Mixed viral infections have biological, epidemiological and economic implications. The mixed infections of viruses may interact synergistically or antagonistically causing changes in the concentration of either or both viruses and consequently causing a new disease.

Confirmation of viral DNA in infected samples

Out of 50 virus infected plant materials, more than 50% of the samples gave viral DNA amplification product of 2.6–2.8 kb after digestion with 5 common restriction enzymes (described in above sections), whether no any bands were observed in control samples. The appearance of 2.7 kb fragment (DNA-A genome) and 2.6 kb (DNA-B genome) confirms the begomovirus infection in the analyzed samples [Fig-3].

Sequence analysis and identification of virus

The full length sequences of DNA-A and DNA-B components were independently subjected to NCBI-BLAST program and Clustl- Omega analysis [Fig-4]. Based on the closest sequence identity of the virus isolates and the length of the sequences, both DNA-A and DNA-B sequences of begomoviruses were downloaded from Gen Bank repository data base with their accession numbers provided by the FASTA output and were fed into ClustalX [19, 20] to determine the variation between the

virus isolates. Our data analysis confirmed the incidence of Mungbean yellow mossaic India Virus (MYMIV) infection in the infected samples of cowpea collected from Hazo, Assam, India. A total of 40% of symptomatic plant leaf samples were detected with a single virus. The most common and widespread virus infection in single association detected from the plant leaf samples was MYMIV with 60% infectivity.

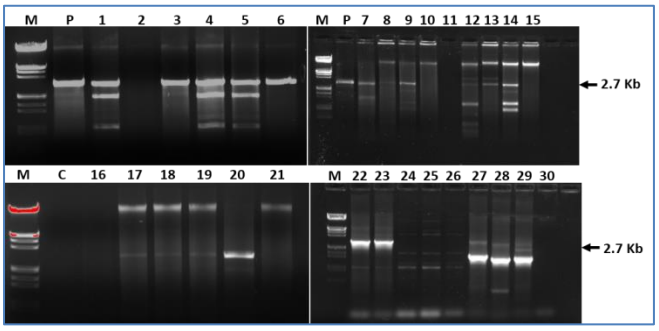

Fig-3 Rolling circle amplification followed by restriction digestion of healthy and infected samples; lane 1- 30 represents RCA of infected samples. The appearance of 2.7 kb and 2.6 kb fragments on digestion with five common restriction enzyme indicates the presence of begomovirus. Lane marked M represents the molecular mass marker Lamda DNA EcoRI+ HindIII. Lane marked C represents control, Lane marked P represents the positive control (pUC19 RCA kit supplied).analysis of cowpea virus infected leaf samples

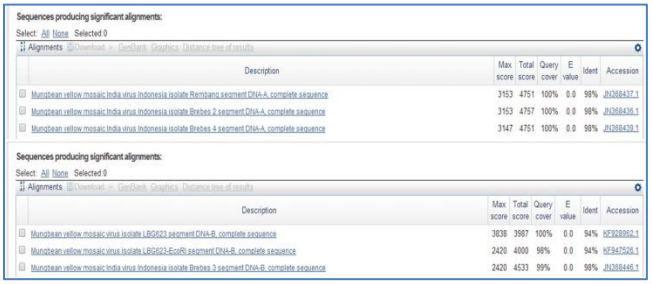

Fig-4 BLAST search analysis of DNA-A and DNA-B of MYMIV of the cowpea virus isolates infecting in Assam.

Discussion

The study established the prevalent identity of cowpea viruses that are significantly important in cowpea growing districts of Assam, India. The widespread distribution of virus infection on cowpea at various locations in the growing districts reported herein and the severity of infection could suggest that the viruses cause huge economical yield losses to cowpea. The results obtained in this study demonstrated that there was a substantial occurrence of viruses during 2012-2013. Considerable variations in incidence and severity of virus symptoms in cowpea among the districts were observed. Among the four districts of Assam surveyed, high incidence of virus symptoms and severity was observed in Hazo and Nalbari districts compared to Goalpara and Barpeta districts. The similar type of viral symptoms was observed in all the fields surveyed in each district, with the exception of a few fields in Nalbari district. In spite of the high virus incidence observed in the cowpea fields, the magnitude ranged from moderate to high severity in all of the districts surveyed. However, there was a lower virus severity recorded in Goalpara than Nalbari district.

The results obtained during surveys showed that virus diseases are widely distributed across the agro-ecological zones in the three districts. However, the extent and source of infection among the surveyed districts varied greatly during the season. The favorable climatic conditions can prolong vector migration, enhance vector population in the cultivated field area and consequently, increase their potential to transmit virus in other related crops. The identified virus disease severities are economically important which can intensify and cause great infection to cowpea crops, resulting from progression of viral disease vector populations in the area and consequently, increase the inoculum level to cause high virus incidence in fields of cowpea.

Conclusion

On the basis of field survey carried out in four district of Assam, we can conclude that MYMIV is the most prevalent pathogen responsible for yellow mosaic disease of cowpea across the state Assam in India. During the August to December months, the period when MYMIV are a severe problem in this crop, since MYMIVs are only vector transmitted, any virus introduced would spread northwards very quickly but may less quickly in southwards.

The diversity of begomovirus may lead to adaptation f cowpea and other similar crops as a new host and as a result the cowpea cultivation in other part of India might be under severe threat. Therefore, some practical intervention measures and development of resistant plants are urgently needed to curb the viral threat.

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Author Contributions

SK carried out the survey and collected the infected samples and performed entire experiments. LS and BT conceived and designed the experiments. LS critically analyzed the data. SK, LS and BT prepared the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest in the present study.

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