

I. Introduction

Viruses are parasites, containing a nucleic acid molecule in the protein coat. They are generally smaller than bacteria and cannot be seen by light microscope. Virus lacks the capacity to thrive and is able to reproduce only within the living cells of a host. They cause infection in all living organisms. Viruses depend on its host for propagation and multiplication of their genome. They depend on biological agent like insect, nematode, mites for their transmission. Virus is also known as small infectious agent. They only can replicates in the living cells of other organism. Through plasmodesmata virus may be transported from one cell to another cell and to distant parts of the plant by phloem. Plants seldom die due to virus infestation but in several cases they affect the plants severely resulting to reduce yield as well as reduce the quality of the product (Dietzgen *et al.*, 2016; Jones, 2021).

Among the various biotic and abiotic factors responsible for poor crop yield, diseases caused by viruses are prominent that lead to loss of several million rupees worldwide. Statistically loss incurred by viral diseases comes next to losses caused by insect pests. Plant viruses may cause economic losses by damaging the leaves, stems, roots, fruits, seed or flowers. Severity of the plant viral diseases may vary with locality, the crop variety and from one season to next season (Jiskani, 2007; Verma, 2003).

Geminiviruses (Family *Geminiviridae*) are arthropod-transmitted plant-infecting viruses with closed circular single stranded DNA (cc-ssDNA) genomes (Brown *et al.*, 2012). These viruses are the plant pathogens which are responsible for disease in economically important crops found in most tropical and subtropical regions of the world (Zerbini *et al.*, 2017). On the basis of host range, insect vector and genome organization Geminiviruses have been classified into nine genera, namely *Becurtovirus*, *Begomovirus*, *Capulavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Mastrevirus*, *Topocuvirus* and *Turncurtovirus* (Zerbini *et al.*, 2017). Among the members of Geminiviruses, *Begomovirus* are the most destructive phytopathogens,

which infect dicotyledonous plant species throughout the world (Brown *et al.*, 2015). They are transmitted by whiteflies (*Bemisia tabaci*) and contain either mono-partite (~2.7 kb single genomic component DNA, called DNA-A) or bi-partite genomes (two genomic components, DNA-A and DNA-B, ~2.7 kb each) (Brown *et al.*, 2012; King *et al.*, 2012). Most of the Old-world monopartite begomoviruses are associated with betasatellite and alphasatellite molecules (Briddon and Stanley 2006). Recently, another class of DNA-satellites, called deltasatellite, has also been reported from the New-world (Fiallo-Olivé *et al.*, 2016). Geminiviruses are characterized by their geminate shape particles and circular single-stranded DNA (ssDNA) genomes. The genus *Begomovirus*, the largest and the most economically important genus of the *Geminiviridae* family, encompasses viruses that are exclusively transmitted by the whitefly *Bemisia tabaci* and infect only dicotyledonous plants. Begomoviruses have been identified as significant limitation to the cultivation in a variety of crops, grown in tropical and subtropical regions of the world, over the past 20 years or more (Huang *et al.*, 2013).

Some taxonomic criteria were suggested by the International Committee on Taxonomy of viruses (ICTV) for demarcating the species of begomoviruses according to the dependability and applicability of rules in the large number of specified begomoviruses (Fauquet *et al.*, 2003). Nucleotide sequence comparison has a major role in determination of taxonomic status. In different species and strains of begomoviruses respectively, the identities of 91% and 94% in genome-wide pairwise association study are considered as the demarcation threshold (Brown *et al.*, 2015).

Begomovirus infected plants shows diverse symptoms which are generally divided into three types: (a) vein yellowing, (b) yellow mosaic, and (c) leaf curl. The name of *Begomovirus* obtained from the type member *Bean golden mosaic virus* (BGMV) (Mayo and Pringle, 1998). It constitutes the largest group of plant infecting DNA viruses that infect a wide range of dicotyledonous plants (Malathi, 2017) and consists of more than 420 species infecting various plants throughout the world (Dominguez-Duran *et*

al., 2018; Singh *et al.*, 2020; Martinez-Marrero *et al.*, 2020). The vector (*Bemisia tabaci*) transfers the plant-infecting begomoviruses and extremely reduced the yield of economically important vegetables around the world (Leke *et al.*, 2015).

Begomoviruses are spread widely as a major threat in crop cultivation in the tropical and subtropical regions of India. These single stranded DNA viruses give rise to significant economical losses by causing diseases in several important crops (such as cucurbitaceous, solanaceous, malvaceous vegetable and legume crops) in most parts of the country. The most affected crops are tomato, cucurbits, cotton, chilies, legumes, papaya, okra, and cassava. It has been observed from literature that in the past few decades, the begomoviruses have histrionically emerged in tomato, cucurbits and chili, throughout the country. For this, the major factors responsible are introduction of viruses and susceptible crops or genotypes, recombination in viruses, weather factors, change in vector population and new intensive agricultural practices (Tripathi and Verma, 2017).

Begomoviruses affected the crops like cucurbits, tomato, and cotton in the USA, Caribbean, Mexico, Central America, Brazil, and Venezuela. The above mentioned places have suffered from high incidences of begomoviruses with devastating economic consequences. Crops such as potato, melons, and beans are also affected. In 1990s geminiviruses ruined up to 95% of the tomato yield in the Dominican Republic. In 1991-92 they damaged tomato crop of \$140 million in Florida (Fauquet and Stanley, 2003). In Sudan, epidemics of cotton leaf curl disease (CLCuD) threatened the cultivation of cotton during 1940s and 1950s (Idris and Brown, 2000).

Based on the symptoms of begomovirus, vegetable crops (potato, chili, sponge gourd and tomato), legume (black gram) and weed species were collected during 2011-2012 from Bahraich district of Uttar Pradesh in India. For example *Tomato leaf curl New Delhi virus* (ToLCNDV) was found in potato, sponge gourd, chilli and tomato. *Ageratum enation virus* (AEV) was detected in black gram, *C. bonplandianum*, *A. conyzoides* samples (Khan *et al.*, 2014).

Common uncultivated plants, *Croton bonplandianum*, *Acalypha indica* and *Ageratum conyzoides* are found in the cultivated fields of sub-Himalayan north-east Indian plains and may act as reservoirs of crop infecting begomoviruses (Saha *et al.*, 2013). It has been reported by many scientists that weeds serve as reservoir or alternative hosts for begomovirus survival and play a significant role in the epidemiology of diseases. Weeds have also received much attention with regards to the types of begomoviruses they harbor (Green *et al.*, 2006).

From Bolivian weeds, eight Begomovirus species was isolated by Wyant *et al.*, (2011) by applying RCA (rolling-circle amplification) and RFLP (restriction fragment length polymorphism) techniques. Among these eight species, four species were deemed as the distinct new species and for them the names *Solanum mosaic Bolivia virus*, *Sida mosaic Bolivia virus*, and *Abutilon mosaic Bolivia virus* were proposed. The viruses were classified as typical bipartite New-world begomoviruses by phylogenetic analysis.

Ornamental rose plants found, in Faisalabad, Pakistan (*Rosa chinensis*) with the characteristics of highly stunted growth and leaf curling was examined for begomovirus infection, by using rolling circle amplification and PCR methods. A new begomovirus species that infects rose plants was discovered, depending on complete genome sequence homologies with other begomoviruses (Khatri *et al.*, 2014). They proposed a new species name, *Rose leaf curl virus* (RoLCuV), which showed close identity (83 %) with *Tomato leaf curl Pakistan virus*, and 96% identity with *Digera arvensis yellow vein betasatellite* (DiAYVB) while associated with betasatellite. It justified a new isolate for the betasatellite.

Essential oil from *Satureja hortensis* L., *Ocimum basilicum* L. and *Thymus vulgaris* L. were reported to be potential control agents against *Bemisia tabaci* in greenhouse conditions (Aslan *et al.*, 2004). Certain plants within the brassicaceae family such as cabbage can be used to restrict vector populations and virus sources to refuge host plants which are usually susceptible to infection (Rampersad, 2003). Essential oils extracted from *Thymus vulgaris*, *L. patchouli*, *Pogostemon cablin*, and *Corymbia*

citriodora could expeditiously decrease egg hatchability, nymph and pupae survival and oviposition of *B. tabaci* biotype B (Yang *et al.*, 2010).

Crude extract of neem leaf, garlic, mahogany bark, chili pepper fruit, pawpaw dried leaf, and bougainvillea leaf significantly reduced the whitefly vector population and increased fruit production in comparison to control ($P < 0.05$) in Okra. Whitefly population control and reduction of incidences and severities of Okra Leaf Curl Disease (OLCD) was observed by application of neem leaf extract with garlic extract. Significant increase ($P < 0.05$) of fruit yield was also observed in crude extract treated plats in comparison to the untreated plats (Bediako *et al.*, 2014). Meena *et al.*, (2014) produced Geminivirus-free chilli plants through meristem tip culture.

Plants such as *Boerhaavia diffusa*, *Clerodendrum aculeatum* were able to prevent infection of viruses, as virus inhibitors activated systemic resistance in non-infested parts of the plants. Other plants (*Phytolacca americana*, *Mirabilis jalapa* and *Dianthus caryophyllus*) containing ribosome inactivating proteins [RIPs], could defend themselves against virus infections (Waziri, 2015).

Sub-Himalayan plains of North-east India are mainly consisting of seven districts of northern part of West-Bengal and Brahmaputra valley of Assam. Most of these areas have a climate that is suitable for growth of vegetables round the year. Substantial losses in farmers' fields have been experienced due to viral diseases in the study area. Extensive transmission of begomoviruses by white flies (*Bemisia tabaci*) causes formidable biotic constraints of crop production in the area (Saha *et al.*, 2013).

Recently, it has been observed that begomovirus severely affected crop diseases of sub-Himalayan plains of North-east India. In some places, 100% disease incidence was observed. These findings indicated that new variants or strains of begomoviruses might have been occurred, which could break the resistance of the crops. Sufficient data on molecular characterization of begomoviruses are not available. Additionally, the existence of different genetic variants of begomoviruses associated with

different economically important crops in sub-Himalayan plains of North-east India is also not sufficient.

Therefore, cloning, sequencing and analysis of complete genomes of begomoviruses causing disease in economically important crops need to be explored. This exploration will help (1) to identify specific begomoviruses and its strains, (ii) to determine distribution of begomoviruses and its strains, (iii) to understand host-virus interactions, and (iv) to identify resistance in crops against begomoviruses. All these information will help to formulate a long term management strategies in future. Keeping the above-mentioned view, it was thought to be worthwhile, to carry out the present research work with the following objectives.

Objectives

1. Identification of begomoviruses infecting economically important crops of sub-Himalayan plains of North-east India using Polymerase chain reaction (PCR) and Rolling circle amplification.
2. Sequence analysis and determination of variability among begomoviruses and their strains using bioinformatics tools.
3. Eco-friendly management of important *Begomovirus* diseases using botanicals.