I. Introduction

1.1. An overview of plant viruses

Viruses are intracellular and obligate parasites or infectious particles that can infect a wide range of organisms from bacteria to higher plants and animals. A virus particle generally consists of one or more nucleic acid(s) encapsided by protein or lipoprotein layer. A complete virus particle is called a virion. Viruses can replicate only within the host tissue. The virus particles can move within the biomes and able to operate as vehicles of horizontal gene transfer. Virus replication is dependent upon the host's protein synthesizing machinery (Hull, 2002; Van Regenmortel *et al.*, 2000; Sano *et al.*, 2004). Viruses can move from marine to terrestrial environment. However, they lack the protein synthesizing machinery. From that point of view, it appears that viruses are nothing but dead infectious particles. But viruses have been declared as organism after the definition of a virus was broadened to, "A virus is a set of one or more nucleic acid template molecules, normally encased in a protective coat or coats of protein or lipoprotein that is able to organise its own replication only within suitable host cells." (Hull, 2009).

Viruses are one of the most important plant pathogens infecting various plant species worldwide. They can move through plasmodesmata connection to cause infection not only at the point of penetration but also move through the vascular bundle infecting the whole plant. The resulting plant shows various disease symptoms causing lowering of yield both in quality and quantity. The viruses may be transmitted through various vectors, the most likely to be aphids, thrips, whitefly, nematodes etc., or through pollen, vegetative propagation or grafting (Hull, 2002). Plant viruses are one of the major limiting factors among the various factors responsible for low yields in crop productivity. Viral diseases are known to cause million rupees crop losses, which comes next to losses caused by insect pests. Plant virus may damage leaves, stems, roots, fruits, seed or flowers and may cause economic losses by reduction in yield and quality of

plant products. The severity of individual virus diseases may vary with locality, the crop variety and from one season to the next (Jiskani, 2007; Verma, 2003).

Among the plant viruses, RNA viruses are dominant in infecting various plants. Viruses with RNA genome show greater genetic diversity both within and between species. These viruses are diversified into double stranded or single stranded genomes. But the most diversified classes contain positive sense single stranded genomes (Aaziz and Tepfer, 1999; Hull, 2002).

1.2. Historical anecdotes

The earliest written evidence of a plant viral disease was a Japanese poem, written by the Empress Koken in 752 AD and translated by T. Inouy-

"In this village
It looks as if frosting continuously
For, the plant I saw
In the field of summer
The colour of the leaves were yellowing"

The plant has been identified as *Eupatorium lindleyanum*, which was found to be susceptible to *Tobacco leaf curl virus*, which caused a yellowing disease. In Western Europe in the period from about 1600 to 1660 AD, many paintings and drawings were made of tulips that demonstrate flower symptoms of virus disease showing colour breaking (Hull, 2009). The tulip breaking was reported by Charles I Ecluse of Carolus Clusius (Sastry and Saigopal, 2010). Later in 1886, Adolf Mayer, a German agricultural chemist working at Wageningen in the Netherlands, found a mosaic disease of tobacco that could be transmitted to healthy plants when rubbed with the sap that was extracted from plants showing disease symptoms. Later in 1892, the Russian botanist, Dmitrii Ivanowski showed that sap from diseased plants retained its infectivity after passing through a filter that eliminated bacteria. Six years later, the Dutch botanist, Martinus Beijerinck confirmed Ivanowski's results and was the one who used the

term “Virus” (Latin for venom or poisonous fluid) to describe the causal agent of the disease and to distinguish it from bacteria (Randles and Ogle, 1997).

1.3. Earliest reports from India

In India viral diseases are reported from early 1900 AD. Some of them included cardamom mosaic disease at Kanara district of Bombay presidency infecting betel palm, cardamom and pepper (Mollison, 1900); sandal spike disease at Kodagu district of Karnataka infecting sandal (McCarthy, 1900); root wilt at Kerala infecting coconut (Butler, 1908); cotton stenosis infecting cotton (Kottur and Patel, 1920); tristeza disease of citrus at Peshawar infecting orange (Brown, 1920); sugarcane mosaic at Pusa, New Delhi infecting sugarcane (Dasur, 1923); yellow vein mosaic disease at Bombay infecting bhendi (Kulkarni, 1924); clump disease at Chennai infecting groundnut (Sundararaman, 1927); sterility mosaic disease at Pusa, Bihar infecting pigeonpea (Mitra, 1931); leaf curl at Dehradun infecting zinnia elegans (Mathur, 1933); yellow leaf disease of coconut (Varghese, 1934); phyllody (leaf curl) of *Sesamum indicum* (Kashiram, 1930; Pal and Nath, 1935); leaf curl of tobacco (Pal and Tandon, 1937; Pruthi and Samuel, 1937; Pruthi and Samuel, 1939); pansukh of rice (Dastur, 1937); tungro disease of rice (Varma *et al.*, 1999); leaf curl of chillies (Uppal, 1940); mosaic disease of cowpea (Vasudeva, 1942); leaf curl of potato (Pal, 1943); mosaic disease of bottle gourd (Vasudeva and Lal, 1943); melon mosaic (Vasudeva and Pavgi, 1945); cardamom mosaic (Uppal *et al.*, 1945) and tomato leaf curl (Vasudeva and Samraj, 1948). Subsequently the incidence of potyviral diseases were also reported from Bihar (Mishra and Jha, 1955); Madhya Pradesh (Garga, 1963); Uttar Pradesh (Khurana and Bhargava, 1970); Udaipur of Rajasthan and Marathwada region of Maharastra (Surekha *et al.*, 1977), Punjab (Yemewar and Mali, 1980; Cheema and Reddy, 1985) and Andhra Pradesh (Susan, 1985).

1.4. Host range of RNA viruses infecting plants

Viruses containing RNA as genetic material are responsible for severe disruption to many economically important crops (Fauquet *et al.*, 2005; Poutaraud *et al.*, 2004; Gibbs *et al.*, 2003; Shukla *et al.*, 1994). It is the largest and one of the most rapid growing of the plant virus group. Several reports also support the wide host range for RNA viruses that include both monocots and dicots. Some of the hosts of the RNA viruses are papaya (Mansilla *et al.*, 2013; Singh *et al.*, 2017), tomato (Mathioudakis *et al.*, 2012; Ambros *et al.*, 2017), common bean, cowpea, *Nicotiana benthamiana*, horned melon, summer squash, creeping cucumber, bitter melon (Gonzalez *et al.*, 2002; Barbosa *et al.*, 2016), angled loofah, garden cucumber, oriental pickling melon (Singh *et al.,* 2003), cassava, sweet potato, yams, taro, tannia, elephant foot yam (Babu *et al.,* 2012) and other plants of the family Cucurbitaceae (Mohammed *et al.*, 2012; Piche *et al.*, 2004; Mederos *et al.*, 2017; Nagendran *et al.*, 2017); Chenopodiaceae, Fabaceae and Solanaceae (Bhadramurthy and Bhat, 2009; Maina *et al.*, 2016; Green *et al.*, 2017). RNA viruses have been reported to infect several tree plants also, such as garden roses (Lanley *et al.,* 2011; Babu *et al.,* 2014; Baker *et al.,* 2014; Di Bello *et al.,* 2015) and citrus (Xiao *et al.*, 2017). Hosts of RNA viruses also include cereals like rice (Hull, 1996; Latif *et al.*, 2013; Chong *et al.*, 2015), maize (Chen *et al.*, 2016; Wang *et al.*, 2016a; Adams *et al.*, 2017; Wang *et al.*, 2017a), sugarcane (Thompson and Randle, 2001; Viswanathan *et al.*, 2010; Wang *et al.*, 2017b); wheat (Zhang *et al.*, 2017a) and barley (Bouallegue *et al.*, 2014; Najar *et al.*, 2017).

1.5. Crop diseases in north-east India: The present scenario in the current study area

North-east India is the most eastern part of India and is connected to the east India via a narrow corridor of Siliguri sub-division of West Bengal. The north-east India (Fig. 1.1) comprises of the seven sisters' states (Assam, Meghalaya, Arunachal Pradesh, Nagaland, Manipur, Mizoram and Tripura). In 1990s Sikkim was also included in this area (Taher, 2001; Moral, 1997). Environment of some northern districts of West Bengal are similar to that

of north-east India. It includes the most species enriched hotspot of eastern-Himalaya and Indo-Burma. North-east India is the richest reservoir of genetic variability and diversity of different horticultural crops like fruits, vegetables, spices, ornamental plants along with medicinal and aromatic plants. Considerable diversity exists among the regional horticultural species including variation in plant type, morphological and physiological characteristics, reactions to diseases and pests, adaptability and distribution in the north-east region. The diversity of these crops is mainly managed by the local farmers. Some of the crops are used for medicinal value and others for their edibility. These crops are also a good source of income (Asati and Yadav, 2004). In north-east Indian states, total yearly production of fruits, vegetables, flowers (cut and loose flowers), aromatic and medicinal plants, plantation crops and spices were 8,890,610 metric tons (MT); 31,770,040 MT; 369,160 MT; 2,530 MT, 575,360 MT and 105,740 MT respectively occupying 746,610 hectare (HA); 1,925,240 HA; 61,660 HA; 5,990 HA; 210,740 HA and 334,100 HA area respectively (Horticultural Statistics at a Glance 2017, Govt. of India, http://www.indiaenvironmentportal.org.in/files/file/Horticulture%20At%20a%20Glance%202017.pdf, Website reference 1). These crops are vulnerable to diseases and pests and are seriously affected by different pathogens including fungi, bacteria and viruses. The yield of these horticultural crops is decreasing day by day because of biotic stress. The most prominent and widespread pathogens are viruses which are of high economic importance as they can wipe out the whole productivity of certain area (Ahlawat and Pant, 2003). More so, the unique climatic condition of this region helped the microbes to grow within the respective hosts in an efficient way.

1.6. Objectives

Diseases caused by RNA viruses are affecting the production and quality of a wide range of crops at an alarming rate. Although there are many literature reports describing the incidence of RNA viral diseases all over the world, there is very few information regarding RNA viral disease occurrence

and their management strategies from north-east India. Hence, the present work on **"Molecular detection, diversity analysis and management of some RNA viruses infecting crops in north-east Indian plains"** was undertaken. Development of an effective management strategy to combat crop loss to viral diseases is dependent on a proper understanding of the nature of the pathogen and its distribution in the area (Fig. 1.2). Insect vectors are one of the major components for virus spread and disease severity. Thus, the understanding of vector transmission is prerequisite for management of the viruses and a successful agriculture. Therefore, the present study was undertaken by focusing on the following objectives:

1. Molecular detection of some RNA viruses infecting some economically important crops growing in north-east Indian plains
2. Transmission Electron Microscopic studies of some viruses
3. Sequencing of the detected viral genome
4. Analysis of different virus sequences of the present study with some available sequences in the GenBank
5. Study of insect vectors present in the study area
6. Management of some diseases caused by RNA viruses

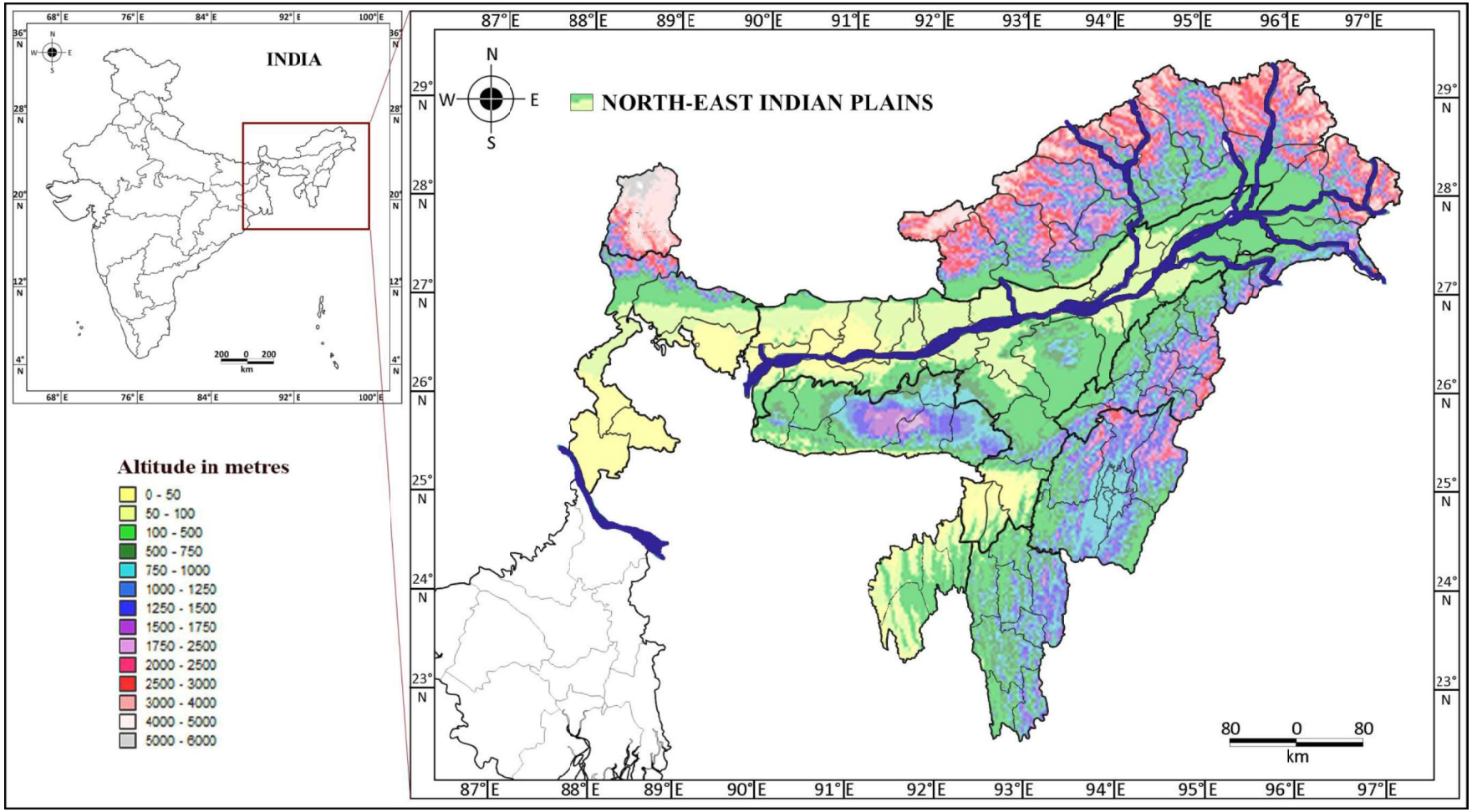

Fig. 1.1: Map of north-east India showing the north-east Indian plains.

Fig. 1.2: Virus infected bottle gourd (a), tomato (b) and cucumber (c) fields of north-east Indian plains. Red circles in the photographs indicate the infected plants in the fields.